IMMUNE LANDSCAPE OF KIDNEY PATHOLOGY

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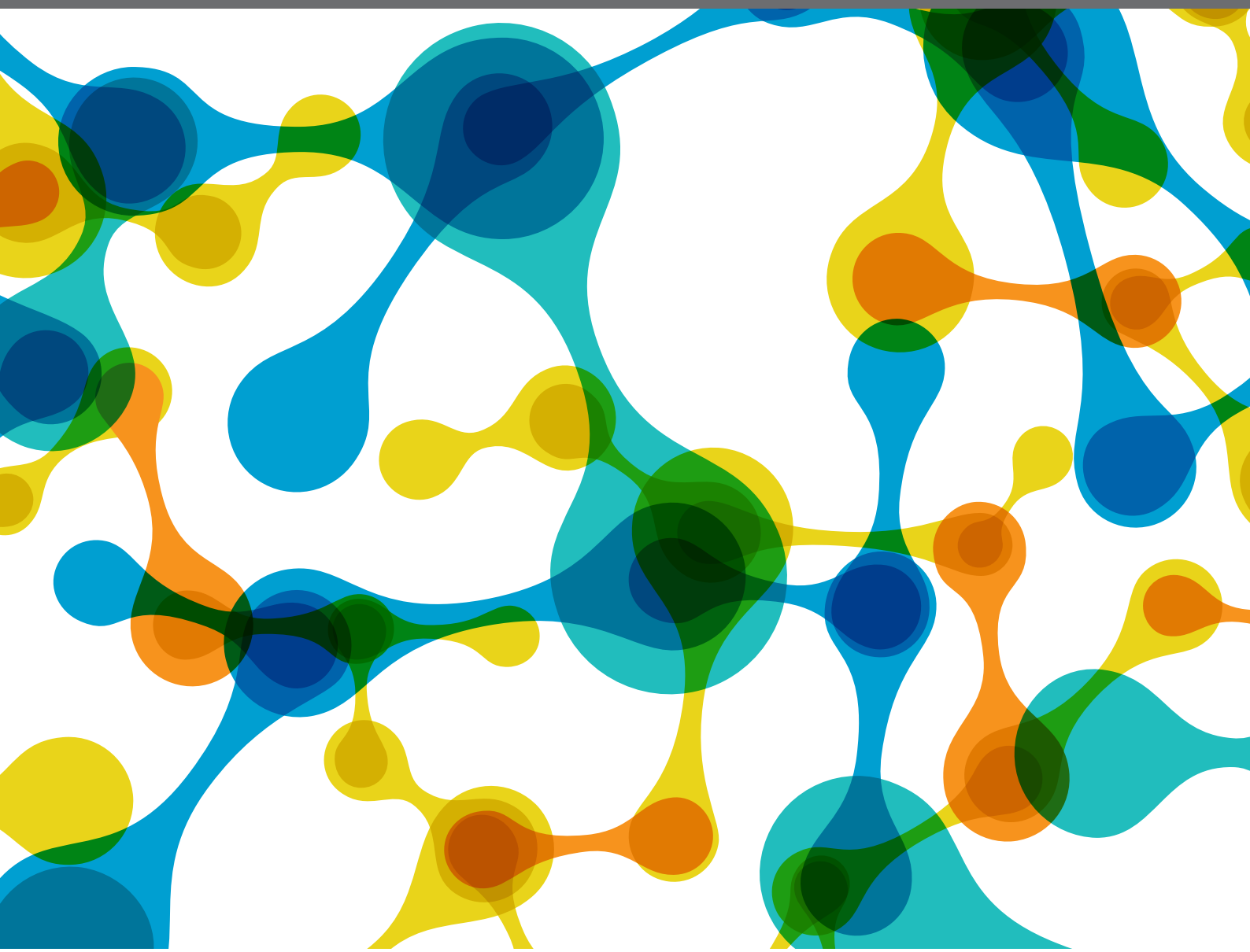

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IMMUNE LANDSCAPE OF KIDNEY PATHOLOGY

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Editorial: Immune Landscape of Kidney Pathology

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Editorial on the Research Topic

Immune Landscape of Kidney Pathology

INTRODUCTION

Kidney disease is an emerging cause of morbidity and mortality. More than 6 million patients worldwide receive renal replacement therapy. The global prevalence of chronic kidney disease (CKD) is between 11.7 and 15.1% of the adult population. Nevertheless, we still lack effective treatments to stop the progression of CKD, which makes it an urgent area with unmet clinical need. CKD is defined as abnormal kidney structure and/or function caused by primary and secondary glomerular diseases (including diabetes, hypertension, autoimmune diseases, etc.). Renal fibrosis is a common feature of CKD and is widely regarded as the main driver of the progression to end-stage renal disease. However, the underlying mechanisms of the renal fibrotic response are complex and still poorly understood. Emerging research shows that unresolved inflammation may be a necessary condition to promote the transition from acute kidney injury to chronic renal fibrosis.

Various white blood cell populations are recruited into injured kidneys and play important roles in pathogen clearance and tissue repair. However, if this inflammatory response does not subside, it will instead promote progressive fibrosis of the damaged kidney. Interestingly, a large number of studies have shown that infiltrating leukocytes, including macrophages, dendritic cells, natural killer cells, and T and B cells, actively promote the transition from renal inflammation to fibrosis (Tang et al., 2020a). In addition, changes in the microenvironment in different kidney compartments also play a key role in the immune response and disease pathogenesis. A better understanding of the immune process in the development of CKD may reveal direct and indirect immunomodulation methods as new therapeutic strategies to prevent the progression of different forms of kidney disease.

Therefore, we initiated this research project co-sponsored by Frontiers in Physiology and Frontiers in Medicine, aiming to bring together research from multiple disciplines, with special attention to immunology, renal physiology and pathology. We invited researchers to share their latest insights into how host immunity and its effectors reshape the kidney microenvironment to achieve the physiological and/or pathogenic effects of diseased kidneys.

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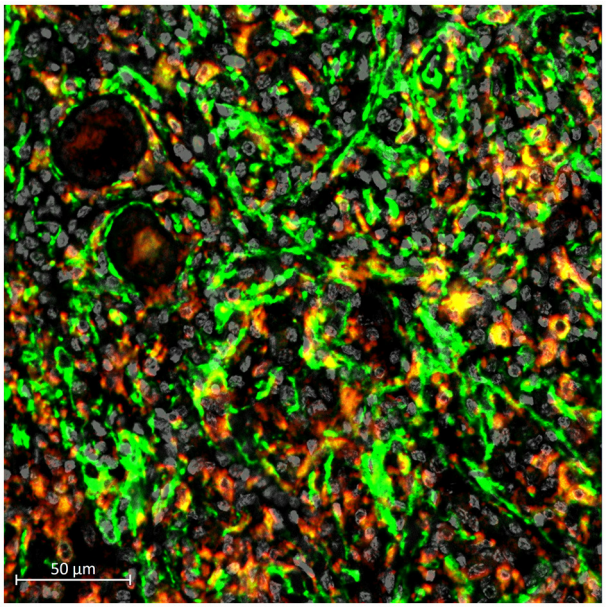

FIGURE 1 | Occurrence of MMT (yellow) in a human kidney with chronic allograft dysfunction, indicating by the presence of macrophage (CD68, red) expressing myofibroblast marker (alpha-SMA, green).

We are very pleased that this Research Topic has been welcomed by basic researchers and clinical scientists from all over the world. A total of 22 high-quality papers have been published, including nine original studies, six reviews, four mini-reviews, one case report and a brief research report. These papers are written by 159 authors from around the world, providing cross-sectional and multi-disciplinary approaches in the latest kidney disease research. Broadly speaking, these papers focus on five core topics: (i) immunodynamics; (ii) pathogenic mechanisms; (iii) advanced research technology; (iv) therapeutic development; and (v) social impact on patients with kidney disease. The following is a brief overview of each study.

Immunodynamics

The kidney is one of the main organs for detoxification in our body. Its failure is an important cause of patient death. In addition, kidney disease is a major contributor to patient death in a wide range of diseases such as diabetes, cancer, bacterial and viral infections (including COVID-19; Tang et al., 2021a; Wang et al., 2021); leading to more than 6 million deaths worldwide each year. Thus, developing a better understanding, and treatment of, kidney disease is critical. Renal fibrosis is a key pathological mechanism in the loss of normal structure and function of the kidney, resulting in progressive kidney damage. Encouragingly, scientists have begun to realize that the over-activation of the immune system is an essential component in this process, and this feature is summarized by Tang et al. in this Research Topic.

Macrophages are a type of immune cell that maintains the health of our kidneys (Tang et al., 2019). They are responsible for detecting, engulfing, and destroying pathogens and unhealthy cells as discussed by Cantero-Navarro et al. Paradoxically, new research finds that macrophages can also accelerate kidney failure as highlighted by a systematic review from Wang et al. A better understanding of the underlying mechanisms can isolate the adverse effects of macrophages from their protective effects. For example, a new phenomenon "macrophage-myofibroblast transition (MMT)" has been identified as a pathway promoting the tissue scarring (**Figure 1**), and dissecting this MMT pathway may identify novel druggable therapeutic targets for kidney fibrosis (Tang et al., 2018a, 2020b).

Changes in the immune landscape are essential components in both disease pathogenesis and tissue repair in states of inflammation, but much remains to be done to fully describe such changes in kidney diseases. Vonbrunn et al. investigated the potential significance of glomerular immune reactivity for allograft survival by analyzing the immune profile of time zero kidney specimens and how this impact clinical outcomes. Albino et al. elucidated how innate immunity contributes to the transition of acute kidney injury to renal fibrosis in a gentamicin-induced renal inflammation model. Furthermore, Rodriguez-Carrio et al. found changes in several novel T cell and monocyte subsets during the progression of chronic kidney disease which were significantly associated with vascular outcomes.

Pathogenic Mechanisms

Unresolved renal inflammation can drive the progression of renal fibrosis, leading to end-stage renal disease. Understanding the mechanisms underlying this unrelenting renal fibrosis is critical for the development of new therapies to halt disease progression. Shao et al. described how a variety of key signaling molecules (e.g., TGF-β1, NF-κB, MAPK, NLRPs, etc.) and epigenetic changes (e.g., DNA methylation, histone modification, and expression of non-coding RNA) contributes to renal inflammation in the pathogenesis of diabetic kidney disease (Tang et al., 2018b; Chung et al., 2021).

Acute kidney injury (AKI) can lead to progressive kidney disease. A number of novel long non-coding RNAs have been identified that contribute to both the development of AKI and in the progression of CKD (Sun et al., 2018; Zhang et al., 2019). Yang et al. revealed that JNK signaling causes aristolochic acid-induced renal tubular cell damage. Zhang et al. identified that lncRNA LRNA9884 enhances the release of inflammatory cytokines through the NF-κB pathway after cisplatin-induced AKI and promotes renal inflammation by binding to the *Ccl2* promotor in the *db/db* mouse model of type-2 diabetes (Zhang et al., 2019).

In C3 glomerulonephritis, the D288G mutation in the gene encoding complement factor I was shown to contribute to C3 deposition in mesangial cells (Song et al.). In renal vasculitis, Tan et al. found that systemic glomerulosclerosis and segmental sclerosis are prognostic and therapeutic markers of IgA vasculitis with nephritis. In addition, new signaling pathways have been identified which act in a cell-type and disease-specific manner in experimental models and kidney patients to promote the progression of kidney disease (Tang et al., 2021a). This basic research work provides examples of how understanding the pathogenesis of kidney disease at the molecular level has the potential to develop precision medicine for kidney disease.

Advanced Research Technology

Understanding the highly dynamic nature of the renal microenvironment during disease development and progression is a major challenge. Encouragingly, there are a number of hallmark analytic technologies have been developed in the last decade, which significantly facilitate and accelerate research into kidney disease in a multidisciplinary manner (Park et al., 2018).

Clusters of regularly spaced short palindromic repeats (CRISPR)—CRISPR-associated protein 9 (Cas9) is an RNA-guided DNA nuclease that has been used to develop simple and efficient techniques to precisely engineer the genome. The CRIPSR-Cas9 system has been widely used to simultaneously delete multiple genes, create conditional alleles, and generate reporter proteins *in vitro* as well as *in vivo* (Higashijima et al., 2017). By using the latest genome editing platform CRISPR/Cas9, Song et al. effectively characterize the mutations of complement factors in a mouse model with C3 glomerulopathy.

Single-cell RNA-sequencing is a breakthrough in biological research for elucidating changes at the single cell level and understanding cell-cell interactions in the complex microenvironment of both physiological conditions and disease development. It is particularly suited to dissect the immunodynamics of kidney disease. In this Research Topic, Zeng et al. systematically summarized the development and application of single-cell RNA-sequencing in kidney immunology. Interestingly, recent work has revealed an unexpected role of macrophage-myofibroblast transition, first identified in kidney fibrosis, in promoting tumor development through tumor-associated macrophage transitioning into cancer-associated fibroblasts in non-small-cell lung carcinoma (Tang et al., 2021b), suggesting an important contribution of tissue fibrotic pathways in cancer. Therefore, we also opened a new platform in Frontiers for sharing the new insights into fibrotic signaling in cancer (https://www.frontiersin.org/research-topics/22920/new-insights-into-fibrotic-signaling-in-cancer).

Therapeutic Development

Several papers in this Research Topic describe therapeutic strategies to inhibit inflammation and immune cell function in kidney disease. Protein kinases are a large family of enzymes that regulate many intracellular signaling processes. For example, spleen tyrosine kinase (SYK) is required for signaling *via* cell surface receptors involved in inflammation, including immunoglobulin receptors. SYK signaling occurs in inflammatory forms of human kidney diseases, and genetic or drug-based SYK inhibition is protective in animal models of crescentic glomerulonephritis and antibody-mediated kidney allograft rejection (Ryan et al., 2016; Ramessur Chandran et al., 2017). Yiu et al. show that SYK is activated in tubular epithelial cells in patients with IgA nephropathy, and that polymeric IgA from patients with IgA nephropathy (but not from healthy controls) activates an inflammatory response in cultured tubular epithelial cells *via* SYK—identifying a SYK-dependent mechanism of tubulointerstitial inflammation.

The JUN amino-terminal kinase (JNK) is a widely expressed enzyme that is highly sensitive to activation by oxidative stress and DNA damage. Activated JNK can phosphorylate protein targets to promote cell necrosis, inflammation and fibrosis (Grynberg et al., 2017). Yang et al. show that the nephrotoxin aristolochic acid—the cause of Chinese herb nephropathy and Balkan nephropathy—induces DNA damage and prominent JNK activation in tubular epithelial cells in mice. Treatment with a JNK inhibitor provided significant protection against tubular necrosis, macrophage infiltration, inflammation and acute renal failure in response to acute high dose AA administration. This further supports therapeutic targeting of JNK to prevent acute kidney injury.

While small molecule drugs are the backbone of current therapies for kidney disease, stem cells and extracellular vesicles are being developed as new potential treatments. Human amniotic epithelial cells (hAEC) are an attractive therapy due to their immunosuppressive capacity, their lack of immunogenicity and their ready availability—being isolated from the human placenta after birth (Al Mushafi et al.). Their immunosuppressive capacity is attributed to secretion of IL-10, TGF-β1, PGE2 and exosomes, while hAEC also act to increase numbers of Tregs and Th2 T cells. Treatment with hAEC suppressed autoantibody production and reduced levels of IL-17 and IFN-g in a mouse model of lupus nephritis (Tan et al., 2018).

Exosomes are a class of small extracellular vesicles excreted by most cell types (Shen et al.). Exosomes carry a cargo of RNA and proteins which, upon uptake into recipient cells, can modulate cell function. Treatment with specific exosome populations can suppress immune-mediated acute and chronic kidney disease models (Eirin and Lerman, 2021). In addition, since the contents of exosomes reflects the cell type of origin, exosomes are being investigated as novel biomarkers in human kidney disease. Furthermore, by manipulating the receptors in the exosome membrane, it is possible to target exosomes to specific cell types—opening up the potential for cell-directed delivery of exosomes carrying biological molecules or drugs to modify disease progression (Shen et al.).

Social Impact

Since the first case in 2019, the COVID-19 pandemic remains an unresolved global issue (Worobey, 2021), among which acute kidney injury is one of the complications of patients infected with the virus (Huang et al., 2020). In fact, social issues not only cause physical harm to humans, but may also affect patients with chronic diseases psychologically.

In this Research Topic, Chan et al. studied the impact of the COVID-19 pandemic on the mental health of patients, revealing a significant impact on the quality of life of patients with chronic kidney diseases receiving dialysis. In addition, Nie et al. conducted a multicentre retrospective cohort study which identified the necessity of kidney biopsy collection for an accurate diagnosis of patients with monoclonal gammopathy. Another retrospective study conducted by Hakroush et al. emphasized the need for histopathological findings in order to make better treatment decisions for critically ill patients who have already exhibited worsening renal function.

SUMMARY

In summary, these papers outline research on the importance of the immune landscape in kidney disease, showing the clinical significance and translational potential of the Research Topic, and providing insights into many exciting research avenues. Our understanding of kidney disease is the immune landscape in kidney pathogenesis continues to grow.

AUTHOR CONTRIBUTIONS

PT and DN-P have made a substantial, direct, and intellectual contribution to the work. HC, YT, and HL edited and approved it for publication.

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REFERENCES

Chung, J. Y., Chan, M. K., Li, J. S., Chan, A. S., Tang, P. C., Leung, K. T., et al. (2021). TGF-beta signaling: from tissue fibrosis to tumor microenvironment. *Int. J. Mol. Sci.* 22:7575. doi: 10.3390/ijms22147575

Eirin, A., and Lerman, L. O. (2021). Mesenchymal stem/stromal cell-derived extracellular vesicles for chronic kidney disease: are we there yet? *Hypertension* 78, 261–269. doi: 10.1161/HYPERTENSIONAHA.121.14596

Grynberg, K., Ma, F. Y., and Nikolic-Paterson, D. J. (2017). The JNK signaling pathway in renal fibrosis. *Front. Physiol.* 8:829. doi: 10.3389/fphys.2017.00829

Higashijima, Y., Hirano, S., Nangaku, M., and Nureki, O. (2017). Applications of the CRISPR-Cas9 system in kidney research. *Kidney Int.* 92, 324–335. doi: 10.1016/j.kint.2017.01.037

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395, 497–506. doi: 10.1016/S0140-6736(20)30183-5

Park, J., Shrestha, R., Qiu, C., Kondo, A., Huang, S., Werth, M., et al. (2018). Single-cell transcriptomics of the mouse kidney reveals potential cellular targets of kidney disease. *Science* 360, 758–763. doi: 10.1126/science.aar2131

Ramessur Chandran, S., Han, Y., Tesch, G. H., Di Paolo, J., Mulley, W. R., Kanellis, J., et al. (2017). Inhibition of spleen tyrosine kinase reduces renal allograft injury in a rat model of acute antibody-mediated rejection in sensitized recipients. *Transplantation* 101, e240–e248. doi: 10.1097/TP.0000000000001826

Ryan, J., Ma, F. Y., Han, Y., Ozols, E., Kanellis, J., Tesch, G. H., et al. (2016). Myeloid cell-mediated renal injury in rapidly progressive glomerulonephritis depends upon spleen tyrosine kinase. *J. Pathol.* 238, 10–20. doi: 10.1002/path.4598

Sun, S. F., Tang, P. M. K., Feng, M., Xiao, J., Huang, X. R., Li, P., et al. (2018). Novel lncRNA Erbb4-IR promotes diabetic kidney injury in db/db mice by targeting miR-29b. *Diabetes* 67, 731–744. doi: 10.2337/db17-0816

Tan, B., Yuan, W., Li, J., Yang, P., Ge, Z., Liu, J., et al. (2018). Therapeutic effect of human amniotic epithelial cells in murine models of Hashimoto's thyroiditis and Systemic lupus erythematosus. *Cytotherapy* 20, 1247–1258. doi: 10.1016/j.jcyt.2018.04.001

Tang, P. C., Chung, J. Y., Xue, V. W., Xiao, J., Meng, X. M., Huang, X. R., et al. (2021b). Smad3 promotes cancer-associated fibroblasts generation *via* macrophage-myofibroblast transition. *Adv. Sci.* 2021:e2101235. doi: 10.1002/advs.202101235

Tang, P. C., Zhang, Y. Y., Chan, M. K., Lam, W. W., Chung, J. Y., Kang, W., et al. (2020a). The emerging role of innate immunity in chronic kidney diseases. *Int. J. Mol. Sci.* 21:4018. doi: 10.3390/ijms21114018

Tang, P. M., Nikolic-Paterson, D. J., and Lan, H. Y. (2019). Macrophages: versatile players in renal inflammation and fibrosis. *Nat. Rev. Nephrol.* 15, 144–158. doi: 10.1038/s41581-019-0110-2

Tang, P. M., Zhang, Y. Y., Hung, J. S., Chung, J. Y., Huang, X. R., To, K. F., et al. (2021a). DPP4/CD32b/NF-kappaB circuit: a novel druggable target for inhibiting CRP-driven diabetic nephropathy. *Mol. Ther.* 29, 365–375. doi: 10.1016/j.ymthe.2020.08.017

Tang, P. M., Zhang, Y. Y., Mak, T. S., Tang, P. C., Huang, X. R., and Lan, H. Y. (2018b). Transforming growth factor-beta signalling in renal fibrosis: from Smads to non-coding RNAs. *J. Physiol.* 596, 3493–3503. doi: 10.1113/JP274492

Tang, P. M., Zhang, Y. Y., Xiao, J., Tang, P. C., Chung, J. Y., Li, J., et al. (2020b). Neural transcription factor Pou4f1 promotes renal fibrosis *via* macrophage-myofibroblast transition. *Proc. Natl. Acad. Sci. U. S. A.* 117, 20741–20752. doi: 10.1073/pnas.1917663117

Tang, P. M., Zhou, S., Li, C. J., Liao, J., Xiao, J., Wang, Q. M., et al. (2018a). The proto-oncogene tyrosine protein kinase Src is essential for macrophage-myofibroblast transition during renal scarring. *Kidney Int.* 93, 173–187. doi: 10.1016/j.kint.2017.07.026

Wang, W., Chen, J., Hu, D., Pan, P., Liang, L., Wu, W., et al. (2021). SARS-CoV-2 N protein induces acute kidney injury *via* Smad3-dependent G1 cell cycle arrest mechanism. *Adv. Sci.* 2021:e2103248. doi: 10.1002/advs.202103248

Worobey, M. (2021). Dissecting the early COVID-19 cases in Wuhan. *Sci.* 374:1202–1204. doi: 10.1126/science.abm4454

Zhang, Y. Y., Tang, P. M., Tang, P. C., Xiao, J., Huang, X. R., Yu, C., et al. (2019). LRNA9884, a novel Smad3-dependent long noncoding RNA, promotes diabetic kidney injury in db/db mice *via* enhancing MCP-1-dependent renal inflammation. *Diabetes* 68, 1485–1498. doi: 10.2337/db18-1075

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Global Glomerulosclerosis and Segmental Glomerulosclerosis Could Serve as Effective Markers for Prognosis and Treatment of IgA Vasculitis With Nephritis

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Background: This study was aimed at investigating the clinical significance and curative effect of global glomerulosclerosis (GS) and segmental glomerulosclerosis (S) in adult-onset IgA vasculitis with nephritis (IgAV-N) patients since there was no consensus pathological grading method for adult IgAV-N.

Methods: A total of 188 biopsy-proven IgAV-N patients were prospectively identified. Patients were separately assigned to GS0/GS1/GS2 group and S0/S1/S2 based on the scores of global glomerulosclerosis and segmental glomerulosclerosis (0% /0–15% />15%, respectively).

Results: GS0, GS1, and GS2 occurred in 56.4, 29.2, and 14.4% of the adult-onset IgAV-N, respectively. Patients in GS2 group tended to have the most serious renal deterioration and the highest levels of blood pressure. IgAV-N patients were also divided into S0 group (64.4%), S1 group (20.7%), and S2 group (14.9%), where no obvious differences in baseline data were noted. K–M curves indicated that GS2 group had the worst renal outcome ($P = 0.05$) while there seemed to be no significant differences between GS0 group and GS1 group. In addition, no remarkable differences in primary outcome were found among S0 group, S1 group, and S2 group though the prognosis of S2 group tended to be the worst. However, the prognosis of S0/S1 group was markedly better than that of S2 ($P = 0.04$). The discrimination of poor prognosis could be improved by adding the pathological indicators of global glomerulosclerosis and segmental glomerulosclerosis. Most importantly, immunosuppressive treatment might be a superior alternative in IgAV-N patients without sclerosis scores or with lower level of sclerosis scores. But addition of immunosuppression was not recommended in patients with higher sclerosis scores.

Conclusions: Global glomerulosclerosis and segmental sclerosis might be used for management and treatment of adult-onset IgAV-N.

Keywords: IgA vasculitis with nephritis, global glomerulosclerosis, segmental glomerulosclerosis, treatment, prognosis

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INTRODUCTION

IgA vasculitis (IgAV), also named Henoch-Schönlein purpura, is a common form of systemic vasculitis that can cause abdominal pain, gastrointestinal bleeding, aching joints and renal damage. IgAV is a self-limited systemic disorder. But it can cause chronic kidney disease (CKD) when it affects the kidneys, which we name IgA vasculitis with nephritis (IgAV-N). Renal deterioration is the most serious complication of IgAV and is also a determinant factor of adverse prognosis (1). It has been acknowledged that proteinuria and hematuria cannot always reflect the renal damage accurately. Renal biopsy has high value for clinical decision-making and prognosis. Since IgAV-N was more common in children, few researches have investigated which pathological classification is suitable for adult-onset IgAV-N (2, 3). Therefore, a pathological classification for adult-onset IgAV-N that can be applied to predicting prognosis and guiding treatment, needs to be produced.

The guidelines published by the Kidney Disease: Improving Global Outcomes (KDIGO) indicated that angiotensin-converting-enzyme inhibitor (ACEI)/angiotensin receptor blockers (ARB) and corticosteroids were recommended for IgAV-N patients based on the clinical manifestations instead of pathological indicators, and the combination therapy of immunosuppressants and steroids is under debate (4–6). Emerging studies have demonstrated that the usage of immunosuppressants is significantly correlated to clinical remission of IgAV-N (7, 8). However, it is not clear exactly what kind of IgAV-N patients will benefit the most from the immunosuppressive therapy.

As a chronic progressive kidney disease, IgAV-N can result in end-stage renal disease (ESRD), in which chronic impairments such as chronic fibrosis and sclerosis are common. Theoretically, the appearance of global glomerulosclerosis and segmental sclerosis in kidney, which are typical chronic kidney injury, may predict a poor prognosis (9). However, few studies have proved it in the adult-onset IgAV-N. At the same time, few articles have proposed a targeted treatment option for the pathological types of IgAV-N. Hence, this study aims to elucidate the clinical significance of global glomerulosclerosis and segmental sclerosis on renal outcomes in adult patients with IgAV-N and to prescribe a course of treatment based on degree of renal sclerosis.

METHODS AND METHODS

Subjects

We prospectively recruited 209 IgAV-N patients from October 2010 to June 2017 in West China Hospital, Sichuan University. A definitive diagnosis of IgAV-N was based on typical clinical manifestations and renal biopsies, according to the American College of Rheumatology (ACR) guidelines (10). The age of all individuals enrolled had to be more than 14 years old at the first onset of IgAV-N. The exclusion criteria included insufficient clinicopathological data for pathological classification (with $<$ 8 glomeruli in the renal biopsy sample) and/or other systemic diseases like diabetes, hepatitis, systemic lupus erythematosus (SLE), HIV infection and so on. All the participants were followed up regularly by reexaminations in our hospital for at least 6 months unless they reached the endpoint. Finally, 188 adult-onset IgAV-N patients were analyzed in the study. This was an observational study that was approved by the Ethics Committee of West China Hospital of Sichuan University. Participants kept informed by face-to-face interviews and written informed consent were obtained.

Clinical Parameters and Treatments

The clinical indicators we recorded included symptoms and signs (edema, joint pain, abdominal pain and bloody stool), systolic blood pressure, diastolic blood pressure, proteinuria, urine red blood cell, serum albumin, serum creatinine, and estimated glomerular filtration rate (eGFR). Nephrotic syndrome was defined as massive proteinuria ($>$3.5 g/24 h) and hypoproteinemia ($\leq$30 g/L). Hypertension was diagnosed as the resting blood pressure $\geq$140/90 mmHg.

The options of treatment modalities were determined by both the attending doctors and the patients, which was not interfered by the researchers since this was an observational study. Three commonly used treatments included supportive medical care with full dose angiotensin-converting-enzyme inhibitor (ACEI) or angiotensin receptor blockers (ARB), steroid therapy (0.5–1 mg/kg daily and tapered down within 6–8 months) with optimal dose of ACEI/ARB, and immunosuppressants (mycophenolate mofetil, cyclophosphamide, or azathioprine) combined with corticosteroids.

Pathology Data and Groups

The pathological evaluation of this experiment was completely blind. If the patients with IgA vasculitis (purpura with or without abdominal pain, gastrointestinal bleeding, or aching joints) were manifested with hematuria, proteinuria and/or renal failure, the renal biopsy was performed by the supervising physicians. The renal biopsies were evaluated by a professional pathologist and an experienced clinician in our medical centers. All researchers did not participate in this pathological evaluation.

The pathological classification including mesangial proliferation (M0/M1, absent/present), endocapillary proliferation (E0/E1, absent/present), segmental glomerulosclerosis (S), tubular atrophy or interstitial fibrosis (T0/T1, absent/present), and crescent injury (C0/C1, absent/present) was primarily based on the updated Oxford classification (3). Global glomerulosclerosis (GS) was also considered in the study and was defined as glomerular impairment with more than 50% of any one glomerulus manifested as scarring lesion or hyaline deposition (9). The definition of segmental glomerulosclerosis was sclerosis or adhesion in part but not the entire glomerulus, where capillary lumina were obliterated by matrix (11).

Previous studies have proved that IgA nephropathy (IgAN) patients with glomerulosclerosis $>$ 25% of glomeruli have quite a bad prognosis (9). If it was grouped by 25% in our cohort, the distribution of patients was severely unbalanced, which was not suitable for reasonable statistical analysis. In order to increase the predictive sensitivity of this indicator, we grouped patients of different pathological types based on sample size

and clinical experience, which was not exactly the same as Oxford classification. Segmental glomerulosclerosis was scored by percentages (S0/S1/S2, no segmental glomerulosclerosis/>0% of glomeruli but ≤15% of glomeruli/>15% of glomeruli). Patients with or without global glomerulosclerosis were divided into three groups (GS0/GS1/GS2, 0%/0–15%/>15% of glomeruli).

Clinical Outcomes and Remission

The primary outcome consisted of end-stage renal disease (ESRD) defined as e-GFR <15 mL/min per 1.73 m2 or receiving maintenance renal replacement treatment, a 60% decline in the e-GFR and/or death. The secondary outcomes included complete remission (24 h-proteinuria ≤0.3 g, with no hematuria or impaired renal function); partial remission (24 h-proteinuria ≤1 g, with or without recurrent hematuria, or proteinuria decline > 50%); and no response (24 h-proteinuria >1.0 g or the decrease level of eGFR >10%).

Statistical Analysis

Numbers with frequencies were used in statistical descriptions of nominal and grade variables while continuous variables were presented as mean ± standard deviation (homoscedastic) or median with interquartile ranges (heteroscedastic). Student's *t*-test, Wilcoxon test, ANOVA, or non-parametric Mann–Whitney *U*-test were selectively used to analyze continuous variables according to groups and data distribution. Pearson's chi-squared test or Fisher's exact test was adopted for categorical variables. Pearson correlation analysis or Spearman correlation analysis was used to explore the relationship among pathological indicators. Kaplan-Meier estimates was constructed to compute the proportions of endpoint in different groups and multivariate Cox regression analysis was established to identify the unfavorable factors for long-term renal outcome of IgAV-N, where hazard ratios (HRs), and confidence intervals (CIs) were used. Receiver operating characteristic (ROC) with area under curves (AUC) were inducted to measure the prediction accuracy. All tests were two-tailed and it was considered to be significant as the $p < 0.05$.

RESULTS

Demographic and Clinical Characteristics

A total of 209 adult-onset IgAV-N patients diagnosed by clinical signs and renal biopsy were included in this study. Of them, 18 patients were excluded because of missing or the presence of other systemic diseases and 3 were excluded because of inadequate pathological biopsy specimens as < 8 glomeruli in the renal biopsy sample. Accordingly, the study cohort finally consisted of 188 patients. The mean age was 30.9 ± 15.2 years at the time of biopsy. The follow-up time was 27.06 ± 20.09 months on average. Patients were further categorized into different groups according to glomerulosclerosis.

Grouped by global glomerulosclerosis, GS0, GS1, and GS2 occurred in 56.4, 29.2, and 14.4% of the adult-onset IgAV-N, respectively (**Table 1**). It was noted that global glomerulosclerosis scores increased with age ($P < 0.01$). The extrarenal symptoms, especially joint and abdominal involvement, were more prevailing in patients without global glomerulosclerosis ($P < 0.01$). Patients with higher rates of global glomerulosclerosis tended to have more serious renal deterioration and higher levels of blood pressure. Given that the differences in clinicopathological characteristics between GS0 and GS1 were relatively small, we considered GS0 and GS1 as a group GS0/GS1. Patients with lower scores of global glomerulosclerosis (GS0/GS1 group) seemed to have a milder illness with significantly higher level of eGFR, and lower levels of serum creatinine and blood pressure (both SBP and DBP).

IgAV-N patients were also divided into S0 group (64.4%), S1 group (20.7%) and S2 group (14.9%), based on the scores of segmental glomerulosclerosis (**Table 2**). No evident differences were observed in clinical manifestations and laboratory indexes except that patients without segmental glomerulosclerosis were more likely to suffer from gastrointestinal symptoms ($P = 0.06$). Similarly, S0 group and S1 group were treated as the same group (S0/S1). A relatively higher level of serum creatinine was found in patients in S2 group, compared with S0/S1 group ($P = 0.06$), while no other differences in baseline data were noted.

Pathological Findings

As shown in **Table 1**, the proportion of endocapillary proliferation were much lower in GS2 group ($P = 0.03$), whereas the proportion of tubular atrophy or interstitial fibrosis were extremely higher ($P < 0.01$), compared with GS0 group and GS1 group. **Table 2** reveals the pathological findings of patients with different scores of segmental glomerulosclerosis and indicates that patients in S0/S1 group had higher rates of endocapillary proliferation ($P = 0.05$). Therefore, we speculated that there was a certain correlation between pathological impairments.

Then a Spearman correlation analysis was conducted (**Table 3**). It could be easily found that mesangial proliferation was positively related to segmental glomerulosclerosis and tubular atrophy/interstitial fibrosis ($r = 0.16$, $P = 0.03$). Endocapillary proliferation was inversely correlated to tubular atrophy/interstitial fibrosis and global glomerulosclerosis ($r = -0.17$, $P = 0.02$; $r = -0.17$, $P = 0.01$, respectively) while had a positive correlation with crescents ($r = 0.22$, $P < 0.01$). Most remarkably, global glomerulosclerosis and tubular atrophy/interstitial fibrosis had a strong correlation ($r = 0.48$, $P = 0.01$).

Renal Survival

A total of 13 patients finally reached the endpoint during their follow-up period and 9 of them progressed to ESRD. **Figure 1** shows the renal survival based on classification of global glomerulosclerosis. It was worth noting that since no patients were followed for more than 50 months in GS2 group, the survival curve dropped sharply at 50 months. It was indicated that GS2 group had the worst renal outcome (**Figure 1A**, $P = 0.05$) while there seemed to be no significant differences between GS0 group and GS1 group. So, we merged the two groups and found that the renal survival of GS0/GS1 group was much better than that of GS2 group (**Figure 1B**, $P = 0.01$),

TABLE 1 | Clinicopathological manifestations of IgAV-N patients at baseline, grouped by the percentage of global glomerulosclerosis.

	Global glomerulosclerosis				Global glomerulosclerosis		
Variables	**GS0**	**GS1**	**GS2**	***P***	**GS0/GS1**	**GS2**	***P***
Numbers (%)	106 (56.4)	55 (29.2)	27 (14.4)	–	161 (85.6)	27 (14.4)	–
Male (%)	54 (50.9)	24 (43.6)	9 (33.3)	0.23	78 (48.3)	9 (33.3)	0.21
Age (years)	19.0 (16.0–38.3)	37.0 (21.0–50.0)	43.0 (22.0–55.0)	**<0.01**	29.5 ± 14.6	39.3 ± 16.8	**<0.01**
Interval from disease onset to biopsy (months)	1.0 (0.7–5.0)	2.0 (0.68–7.0)	4.5 (1.0–12.0)	0.13	2.0 (0.7–6.0)	4.5 (1.0–12.0)	0.06
Clinical symptoms							
Skin purpura (%)	96 (90.6)	54 (98.2)	23 (85.2)	0.09	150 (93.2)	23 (85.2)	0.24
Edema (%)	35 (33.0)	24 (43.6)	13 (48.1)	0.22	59 (36.6)	13 (48.1)	0.29
Abdominal pain (%)	38 (35.8)	10 (18.2)	5 (18.5)	**0.03**	48 (29.8)	5 (18.5)	0.26
Bloody stools (%)	17 (16.0)	7 (12.7)	0 (0)	**0.05**	24 (14.6)	0 (0)	**0.03**
Joint pain (%)	27 (25.5)	10 (18.2)	1 (3.7)	**0.03**	37 (23.0)	1 (3.7)	**0.02**
SBP (mmHg)	120.0 (110.0–128.3)	123.0 (113.0–135.0)	131.0 (115.0–155.0)	**<0.01**	120.0 (112.0–130.0)	131.0 (115.0–155.0)	**<0.01**
DBP (mmHg)	78.0 (70.8–85.0)	80.0 (71.0–86.0)	82.0 (75.0–95.0)	0.07	78.5 ± 11.7	84.1 ± 12.6	**0.02**
Laboratory index							
Proteinuria (g/24 h)	3.11 ± 3.05	3.03 ± 3.37	3.14 ± 2.61	0.98	3.08 ± 3.15	3.14 ± 2.61	0.93
Alb (g/L)	34.50 ± 8.34	36.35 ± 7.99	35.28 ± 7.39	0.39	35.13 ± 8.25	35.28 ± 7.39	0.93
sCr (umol/L)	64.45 (52.80–84.15)	71.00 (59.00–86.60)	88.00 (66.00–139.00)	**<0.01**	68.00 (56.80–84.95)	88.00 (66.00–139.00)	**0.02**
eGFR (ml/min/1.73 m2)	124.70 (94.35–134.78)	105.40 (87.30–116.80)	71.50 (50.90–110.00)	**<0.01**	109.05 ± 30.82	78.05 ± 38.21	**<0.01**
u–RBC (/HP)	184 ± 435	173 ± 427	180 ± 440	0.99	180 ± 431	180 ± 440	0.98
Pathological features							
M (%)	87 (82.1)	48 (87.3)	25 (92.6)	0.34	135 (83.9)	25 (92.6)	0.27
E (%)	24 (22.6)	7 (12.7)	1 (3.7)	**0.03**	31 (19.3)	1 (3.7)	**0.05**
S (%)	36 (34.0)	22 (40.0)	10 (37.0)	0.76	58 (36.0)	10 (37.0)	1.00
T (%)	22 (20.8)	31 (56.4)	23 (85.2)	**<0.01**	53 (32.9)	23 (85.2)	**<0.01**
C (%)	46 (43.4)	17 (30.9)	10 (37.0)	0.30	63 (39.1)	10 (37.0)	1.00
0	59 (55.7)	38 (69.1)	17 (63.0)		97 (60.3)	17 (63.0)	
0–25%	30 (28.3)	13 (23.6)	6 (22.2)		43 (26.7)	6 (22.2)	
>25%	17 (16.0)	4 (7.3)	4 (14.8)		21 (13.0)	4 (14.8)	
Treatment				**0.02**			**<0.01**
ACEI/ARB	6 (5.7)	4 (7.3)	7 (25.9)	**0.01**	10 (6.2)	7 (25.9)	**<0.01**
Steroids	39 (36.8)	25 (45.5)	11 (40.7)	0.57	64 (39.8)	11 (40.7)	0.54
Immunosuppressor	61 (57.5)	26 (47.3)	9 (33.3)	0.06	87 (54.0)	9 (33.3)	0.06

IgAV-N, IgA vasculitis with nephritis; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALB, albumin; sCr, serum creatinine; eGFR, estimated glomerular filtration rate; u-RBC, the count of uric red blood cell; M, mesangial proliferation; E, endocapillary proliferation; S, segmental glomerulosclerosis; C, crescents; T, tubular atrophy/interstitial fibrosis; GS, global glomerulosclerosis; ACEI, angiotensin-converting-enzyme inhibitor; ARB, angiotensin receptor blockers. The bold values mean statistically significant difference.

indicating the higher the global glomerulosclerosis scores, the worse the prognosis.

The K-M survival analyses of IgAV-N divided by segmental glomerulosclerosis levels is also presented in **Figure 1**. In general, there were no significant differences in primary outcome among S0 group, S1 group, and S2 group though the prognosis of S2 group tended to be the worst ($P = 0.25$). However, the renal outcome of S0/S1 group was remarkably better than that of S2 ($P = 0.04$), suggesting that patients with segmental glomerulosclerosis more than 15% could predict the poor prognosis.

Effects of S and GS on Discrimination of Prognosis

A multivariate COX regression model adjusted for Oxford classification, nephrotic syndrome, and treatment was carried out to evaluate the effects of global glomerulosclerosis and segmental glomerulosclerosis on prognosis. Notably, global glomerulosclerosis and segmental glomerulosclerosis could serve as independent predict markers adjusted for pathological indicators and partial clinical manifestations (HR 3.86, 95% CI 1.00–15.01, $P = 0.05$; HR 7.55, 95% CI 1.66–34.41, $P = 0.01$, respectively, **Table 4**). Moreover, other indicators of Oxford classification such as

TABLE 2 | Clinicopathological manifestations of IgAV-N patients at baseline, grouped by the percentage of segmental glomerulosclerosis.

	Segmental glomerulosclerosis				Segmental glomerulosclerosis		
Variables	**S0**	**S1**	**S2**	***P***	**S0/S1**	**S2**	***P***
Numbers (%)	121 (64.4)	39 (20.7)	28 (14.9)	–	160 (85.1)	28 (14.9)	–
Male (%)	56 (46.3)	15 (38.5)	16 (57.1)	0.33	71 (44.4)	16 (57.1)	0.23
Age (years)	31.2 ± 15.4	31.5 ± 15.6	28.5 ± 14.3	0.67	31.3 ± 15.4	28.5 ± 14.3	0.37
Interval from disease onset to biopsy (months)	7.4 ± 15.9	4.3 ± 5.2	5.9 ± 7.8	0.46	6.7 ± 14.1	5.9 ± 7.8	0.78
Clinical symptoms							
Skin purpura (%)	113 (93.4)	34 (87.2)	26 (92.9)	0.48	147 (91.9)	26 (92.9)	1.00
Edema (%)	50 (41.3)	14 (35.9)	8 (28.6)	0.44	64 (40.0)	8 (28.6)	0.30
Abdominal pain (%)	41 (33.9)	6 (15.4)	6 (21.4)	0.06	47 (29.4)	6 (21.4)	0.50
Bloody stools (%)	19 (15.7)	1 (2.6)	4 (14.3)	0.08	20 (12.5)	4 (14.3)	0.76
Joint pain (%)	25 (20.7)	11 (28.2)	2 (7.1)	0.10	36 (22.5)	2 (7.1)	0.08
SBP (mmHg)	123.8 ± 18.7	121.3 ± 19.9	126.1 ± 17.5	0.58	123.2 ± 19.0	126.1 ± 17.5	0.45
DBP (mmHg)	79.0 (71.5–85.0)	80.0 (71.0–95.0)	81.5 (70.3–94.8)	0.24	80.0 (71.0–85.0)	81.5 (70.3–94.8)	0.18
Laboratory index							
Proteinuria (g/24 h)	3.06 ± 3.28	3.22 ± 2.88	3.05 ± 2.41	0.96	3.10 ± 3.18	3.05 ± 2.41	0.94
Alb (g/L)	34.85 ± 8.19	36.37 ± 8.48	34.80 ± 7.33	0.58	35.22 ± 8.26	34.80 ± 7.33	0.80
sCr (umol/L)	78.52 ± 40.95	82.23 ± 39.21	95.81 ± 48.33	0.15	79.43 ± 40.44	95.81 ± 48.33	0.06
eGFR (ml/min/1.73 m2)	113.80 (89.20–129.70)	109.20 (80.70–132.00)	99.00 (64.13–130.47)	0.22	112.80 (87.58–129.88)	99.00 (64.13–130.47)	0.17
u-RBC (/HP)	193 ± 510	160 ± 254	150 ± 297	0.85	185 ± 460	150 ± 297	0.94
Pathological features							
M (%)	99 (81.8)	35 (89.7)	26 (92.9)	0.10	134 (83.8)	26 (92.9)	0.26
E (%)	25 (20.7)	6 (15.4)	1 (3.6)	0.08	31 (19.4)	1 (3.6)	**0.05**
T (%)	47 (38.8)	16 (41.0)	13 (46.4)	0.74	63 (39.4)	13 (46.4)	0.53
C (%)	53 (43.8)	11 (28.2)	9 (32.1)	0.16	64 (40.0)	9 (32.1)	0.29
0	67 (55.4)	28 (71,8)	19 (67.9)		95 (59.4)	19 (67.9)	
0–25%	31 (25.6)	11 (28.2)	7 (25.0)		42 (26.3)	7 (25.0)	
>25%	23 (19.0)	0 (0)	2 (7.1)		23 (14.4)	2 (7.1)	
GS (%)	50 (41.3)	22 (56.4)	12 (42.9)	0.26	72 (45.0)	12 (42.9)	0.84
Treatment				0.41			0.59
ACEI/ARB	14 (11.6)	1 (2.6)	2 (7.1)	0.27	15 (9.4)	2 (7.1)	1.00
Steroids	48 (39.7)	18 (46.2)	9 (32.1)	0.54	66 (41.3)	9 (32.1)	0.24
Immunosuppressor	59 (48.8)	20 (51.3)	17 (60.7)	0.53	79 (49.4)	17 (60.7)	0.18

IgAV-N, IgA vasculitis with nephritis; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALB, albumin; sCr, serum creatinine; eGFR, estimated glomerular filtration rate; u-RBC, the count of uric red blood cell; M, mesangial proliferation; E, endocapillary proliferation; C, crescents; T, tubular atrophy/interstitial fibrosis; GS, global glomerulosclerosis; ACEI, angiotensin-converting-enzyme inhibitor; ARB, angiotensin receptor blockers.

endocapillary proliferation and tubular atrophy/interstitial fibrosis, and nephrotic syndrome were also available for prognostic prediction.

A survival model comprising the variables in the multivariate COX analysis, was established to further demonstrate the predictive power of global glomerulosclerosis and segmental glomerulosclerosis, which was measured by ROC curves (**Figure 2**). The AUC value of the survival model was 0.895. But when both global glomerulosclerosis and segmental glomerulosclerosis were removed, the AUC value dropped to 0.801. Accordingly, the discrimination of poor prognosis could be improved by adding the pathological indicators of global glomerulosclerosis and segmental glomerulosclerosis.

Treatment and Response

Therapeutic schedules were displayed in relation to pathological classification (**Tables 1**, **2**). **Table 1** reveals that more patients in GS2 group were treated with optimal supportive care (25.9 vs. 6.2%, $P < 0.01$) while immunosuppressive agents were less used (33.3 vs. 54.0%, $P = 0.06$). However, no obvious differences in treatment regimens among S0 group, S1 group, and S2 group ($P > 0.10$).

Considering that the use of immunosuppressants in IgAV-N was highly controversial, we analyzed whether the addition of immunosuppressants to routine treatment was beneficial to the clinical remission of patients with IgAV-N. Based on pathological lesions, the responses to each therapy were

TABLE 3 | Correlation analysis of pathological indicators.

Mesangial proliferation (M)					
$r = 0.07$, $p = 0.34$	**Endocapillary proliferation (E)**				
***r* = 0.16, *p* = 0.03**	$r = -0.14$, $p = 0.07$	**Segmental glomerulosclerosis (S)**			
***r* = 0.16, *p* = 0.03**	***r* = −0.17, *p* = 0.02**	$r = 0.10$, $p = 0.17$	**Tubular atrophy interstitial fibrosis (T)**		
$r = 0.12$, $p = 0.11$	***r* = 0.22, *p* < 0.01**	$r = -0.12$, $p = 0.09$	$r = -0.01$, $p = 0.88$	**Crescent Lesions (C)**	
$r = 0.11$, $p = 0.15$	***r* = −0.17, *p* = 0.01**	$r = 0.06$, $p = 0.43$	***r* = 0.48, *p* < 0.01**	$r = -0.10$, $p = 0.17$	**Global glomerulosclerosis (GS)**

The bold values mean statistically significant difference.

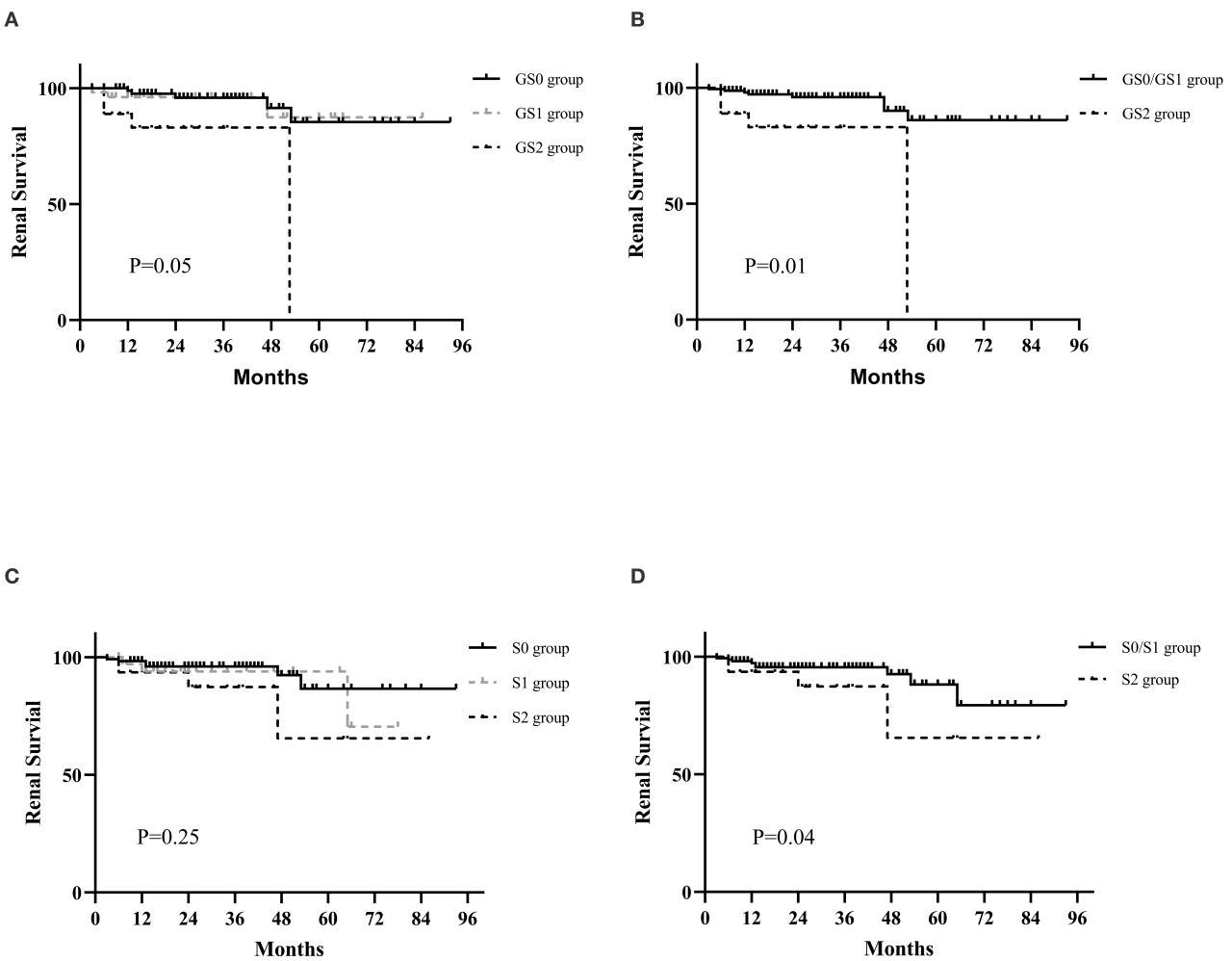

FIGURE 1 | Kaplan-Meier curves for the probability of primary outcomes in IgAV-N patients with different global glomerulosclerosis and segmental glomerulosclerosis scores. **(A)** The renal prognosis of IgAV-N patients among GS0, GS1, and GS2 groups. **(B)** The renal prognosis of IgAV-N patients in GS0/GS1 and GS2 groups. **(C)** The renal outcomes of IgAV-N patients among S0, S1, and S2 groups. **(D)** The renal outcomes of IgAV-N patients in S0/S1 and S2 groups. GS0 group: Patients with 0% global glomerulosclerosis. GS1: Patients with >0 and ≤15% global glomerulosclerosis. GS2: Patients with >15% global glomerulosclerosis. GS0/GS1 group: Patients with ≥0 and ≤15% global glomerulosclerosis. S0 group: Patients with 0% segmental glomerulosclerosis. S1: Patients with >0 and ≥15% segmental glomerulosclerosis. S2: Patients with >15% segmental glomerulosclerosis. S0/S1 group: Patients with ≥0 and ≤15% segmental glomerulosclerosis.

TABLE 4 | Prediction of renal outcomes in IgAV-N carried out by Cox-regression model.

	Univariate analysis		Multivariate analysis	
	HR (95%CI)	***p***	**HR (95%CI)**	***p***
GS2 (vs. GS1/GS2)	4.09 (1.21–13.90)	**0.02**	3.86 (1.00–15.01)	**0.05**
S2 (vs. S1/S2)	3.46 (1.03–11.57)	**0.04**	7.55 (1.66–34.41)	**0.01**
M1 (vs. M0)	2.04 (0.26–25.85)	0.49	0.97 (0.10–9.00)	0.98
E1 (vs. E0)	1.86 (0.55–6.32)	0.32	5.65 (1.04–30.70)	**0.05**
T1 (vs. T0)	3.28 (1.00–10.90)	**0.05**	8.23 (1.68–40.23)	**0.01**
C1 (vs. C0)	1.02 (0.32–3.26)	0.98	0.98 (0.28–3.50)	0.98
NS	5.76 (1.72–19.30)	**0.01**	11.32 (2.89–44.30)	**<0.01**
Immunosuppression	1.22 (0.39–3.85)	0.74	0.61 (0.17–2.14)	0.43

GS, global glomerulosclerosis; S, segmental glomerulosclerosis; M, mesangial proliferation; E, endocapillary proliferation; T, tubular atrophy or interstitial fibrosis; C, crescents; NS, nephrotic syndrome. The bold values mean statistically significant difference.

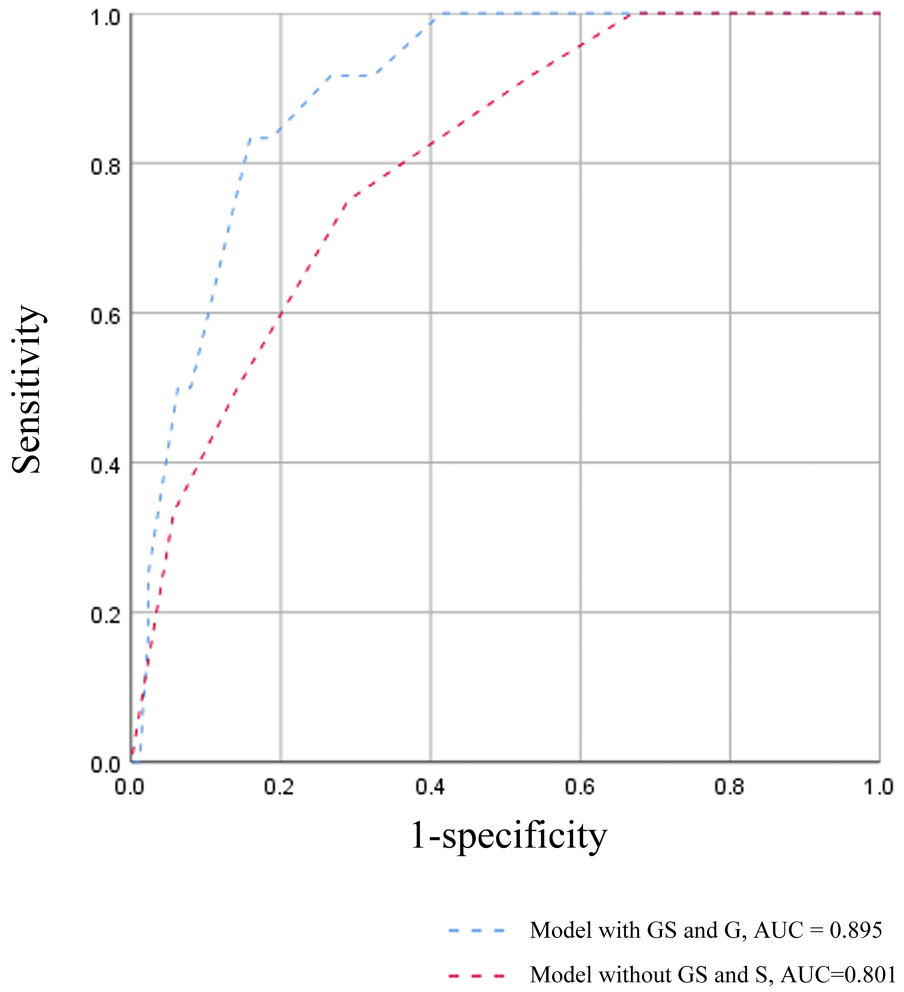

FIGURE 2 | ROC curves for each model in prediction of the poor prognosis.

TABLE 5A | The efficacy of immunosuppressants on clinical remissions of IgAV-N patients with different glomerulosclerosis scores.

	GS0/GS1			GS2			S0/S1			S2		
Response	**non-IT**	**IT**	***P***	**non-IT**	**IT**	***P***	**non-IT**	**IT**	***P***	**non-IT**	**IT**	***P***
CR (%)	13 (17.6)	31 (35.6)	0.01	5 (27.8)	3 (33.4)	1.00	16 (19.8)	26 (32.9)	0.07	2 (18.2)	8 (47.1)	0.12
PR (%)	46 (62.2)	36 (41.4)	0.01	9 (50.0)	4 (44.4)	1.00	47 (58.0)	34 (43.0)	0.08	8 (72.7)	6 (35.3)	0.12
NR (%)	15 (20.2)	20 (23.0)	0.41	4 (22.2)	2 (22.2)	1.00	18 (22.2)	19 (24.1)	0.85	1 (9.1)	3 (17.6)	1.00

GS, global glomerulosclerosis; S, segmental glomerulosclerosis; CR, complete remission; PR, partial remission; NR, no response; IT, treatment with immunosuppressants; non-IT, treatment without immunosuppressants.

TABLE 5B | The efficacy of immunosuppressants on clinical remissions of IgAV-N patients with different glomerulosclerosis scores and endocapillary proliferation.

	GS0/GS1 and E0			GS0/GS1 and E1			S0/S1 and E0			S0/S1 and E1		
Response	**non-IT**	**IT**	***P***	**non-IT**	**IT**	***P***	**non-IT**	**IT**	***P***	**non-IT**	**IT**	***P***
CR (%)	10 (15.6)	24 (36.4)	0.01	3 (30.0)	7 (33.3)	1.00	13 (18.3)	19 (32.8)	0.06	3 (30.0)	7 (33.3)	1.00
PR (%)	44 (62.0)	27 (40.9)	<0.01	2 (20.0)	9 (42.9)	0.26	45 (63.4)	25 (43.1)	0.02	2 (20.0)	9 (42.9)	0.26
NR (%)	10 (15.6)	15 (22.7)	0.30	5 (50.0)	5 (23.8)	0.22	13 (18.3)	14 (24.1)	0.42	5 (50.0)	5 (23.8)	0.22

GS, global glomerulosclerosis; S, segmental glomerulosclerosis; CR, complete remission; PR, partial remission; NR, no response; IT, treatment with immunosuppressants; non-IT, treatment without immunosuppressants.

assessed in **Table 5**. IgAV-N patients receiving steroid combined with immunosuppressant therapy in GS0/GS1 group had a distinctly higher rate of complete remission (35.6 vs. 17.6%, $P = 0.01$). Nevertheless, immunosuppression seemed to have no benefit for complete remission in patients with higher scores of global glomerulosclerosis (33.4 vs. 27.8%, $P = 1.00$). In addition, the differences in the rate of no response between immunosuppressive therapy and treatment without immunosuppression could not be distinguished in either GS0/GS1 group or GS2 group.

Similar results were also found in S0/S1 group and S2 group. The immunosuppressants were more beneficial for patients in S0/S1 group because the complete remission rate was higher (32.9 vs. 19.8%, $P = 0.07$) whereas the addition of immunosuppressors seemed to be unnecessary in patients in S2 group.

Considering that it was unclear if the response to immunosuppression was just based on less chronic disease or the association with more endocapillary hypercellularity, further analysis was carried out. We found that patients without endocapillary hypercellularity in both GS0/GS1 and S0/S1 groups had a better response to immunosuppression agents because of the higher complete remission rate (**Table 5B**). However, for those with endocapillary hypercellularity but no chronic lesions (GS and S), the superiority of immunosuppressive agents did not seem obvious, indicating that sclerosis scores might have guiding significance for the choice of treatment. Therefore, it might be recommended that these two indicators be added to the pathological score of IgAV-N.

DISCUSSION

The International Study of Kidney Disease in Children (ISKDC) classification is the most commonly used histological classification for pediatric patients with IgAV-N and it was shown to be connected with long-term prognosis (2, 12, 13). Due to the heavier pathological damage and poorer outcomes in adults, ISKDC seems to be not sensitive enough to predict the prognosis of adult-onset IgAV-N (10, 14, 15). Since IgAV-N was more common in children, few researches have investigated which pathological classification is suitable for adult-onset IgAV-N. Emerging studies have demonstrated that the indicators of the Oxford classification of IgAN, which shared the similar pathophysiological mechanism with IgAV-N, can indicate the renal outcomes of IgAV-N (14, 16–19). A portion of scholars noted that they could be recommended for clinical practice. Unfortunately, because of the heterogeneity of the research results, this doctrine has not been widely accepted yet. This study was carried out to provide a novel pathological grading scheme to further refine the Oxford classification for adult-onset IgAV-N, which could better predict prognosis and guide treatment.

Our study noted that global glomerulosclerosis (>15% of glomeruli) and segmental glomerulosclerosis (>15% of glomeruli) had a significant correlation with poor prognosis, where more than 3- and 7-fold increased risk were observed, implying that global glomerulosclerosis and segmental glomerulosclerosis could be used as independent prognostic factors.

Global glomerulosclerosis is a kind of the nephrosclerosis that is often regarded as a chronic change. Besides patients with CKD, the kidneys of normal healthy aging may also show signs of nephrosclerosis (20). But the degree of global glomerulosclerosis seems to be more severe in patients with CKD. Several studies have demonstrated that global glomerulosclerosis could predict a worse outcome in patients with IgAN or other CKD (9, 20, 21). However, the role of it remains unknown in IgAV-N since no studies have been performed. In keeping with the above studies, it was uncovered in this study that global glomerulosclerosis was associated with an increased

risk of adverse renal outcomes in adult IgAV-N, especially global glomerulosclerosis >15% of glomeruli. Notably, we also found that global glomerulosclerosis was positively correlated to tubulointerstitial atrophy and fibrosis, consistent with the previous study (22). Further Cox-regression analysis adjusted for clinical symptoms and pathological indicators were performed, and it should be noted that global glomerulosclerosis was an independent predictor (R 3.86, 95% CI 1.00–15.01, $P = 0.05$). Hence, it made a sense to add global glomerulosclerosis to pathology score system of adult IgAV-N.

Segmental glomerulosclerosis was a typical index of Oxford Classification of IgA nephropathy (IgAN), which has been widely accepted to identify the prognosis of IgAN (3, 11, 23). It has been reported that segmental glomerulosclerosis, as a more chronic stage of glomerulonephritis, may develop from the organization of endocapillary inflammatory lesions and/or segmental necrosis, which may lead to sclerosis of kidneys (3). Additionally, segmental glomerulosclerosis may be typically related to podocytopathies (24). Therefore, these could explain why IgAV-N patients with higher rate of segmental glomerulosclerosis had a poorer outcome in our study. However, whether segmental glomerulosclerosis can be used as a prognostic factor of IgAV-N does not reach a consensus. Some studies have proposed that segmental glomerulosclerosis is useful in predicting long-term renal outcome of IgAV-N (16, 19). But K-M analysis in other studies have indicated that it fails to predict the prognosis (25, 26). We speculated that the causes of the inconsistent conclusions may result from the enrolled patients with different severity of segmental glomerulosclerosis. What is noteworthy is that the mainstream classification of segmental glomerulosclerosis scores was the presence or absence (14, 16, 19, 25–28). Patients with higher proportion (>15%) and lower proportion (0–15%) of segmental glomerulosclerosis scores were assigned to the same group in their study. But our study found that the clinicopathological characteristics and prognosis of patients in S0 group and S1 group were similar. Therefore, distinguishing the rate of segmental glomerulosclerosis is more meaningful than distinguishing the presence or absence of it, which could be partially referred to T scores in Oxford Classification (11). In addition, the combination of global and segmental glomerulosclerosis could improve the effect power of survival model (**Figure 2**), which was reasonable to apply it in the clinic.

Previous studies have revealed that the combination treatment of corticosteroids and immunosuppressants has an advantage on IgAV-N remission (7). But unfortunately, the optimal indications for combination therapy have not been well-identified. Results of our study demonstrated that the response to immunosuppression differed greatly, depending on the severity of sclerosis. A higher complete remission rate for combination therapy were found in GS0/GS1 and S0/S1 groups, suggesting that immunosuppressants may be a superior alternative in treating HSPN without chronic changes or with relatively lower level of chronic changes. But for patients with higher scores of global and segmental glomerulosclerosis, adding immunosuppressor to corticosteroids was not necessary because there was no marked difference in response between the two regimens. Thus, global glomerulosclerosis and segmental glomerulosclerosis could serve as effective markers for prognosis and treatment of adult-onset IgAV-N.

There were some limitations of our study. Firstly, this was a single center study with limited sample size and the number of patients in GS2 and S2 group was relatively small. Secondly, the average follow-up time was nearly 30 months that was not long enough and that should be continuous. Thirdly, this was an observational study and no intervention was involved. There was no doubt that the baseline characteristics could not be fully controlled, which might result in potentially biased evaluation of efficacy.

CONCLUSION

Global glomerulosclerosis and segmental sclerosis were independent risk factors of adverse outcome. Immunosuppressive treatment seemed to be a superior alternative in IgAV-N patients without sclerosis scores or with lower level of sclerosis scores. But addition of immunosuppression was not recommended in patients with higher sclerosis scores. Therefore, global glomerulosclerosis and segmental sclerosis might be used for management and treatment of adult-onset IgAV-N.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of West China Hospital of Sichuan University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

WQ and JT: research idea, study design, and data analysis. JT, YX, ZJ, LT, YT, and ZZ: patient enrollment. JT and ZZ: clinical data collection. JT, YX, and PT: data acquisition. JT, YX, and WQ: statistical analysis. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.588031/full#supplementary-material

REFERENCES

1. Pohl M. Henoch-schonlein purpura nephritis. *Pediatr Nephrol.* (2015) 30:245–52. doi: 10.1007/s00467-014-2815-6
2. Jelusic M, Sestan M, Cimaz R, Ozen S. Different histological classifications for henoch-schonlein purpura nephritis: which one should be used? *Pediatr Rheumatol Online J.* (2019) 17:10. doi: 10.1186/s12969-019-0311-z
3. Trimarchi H, Barratt J, Cattran DC, Cook HT, Coppo R, Haas M, et al. Oxford classification of IgA nephropathy 2016: an update from the IgA nephropathy classification working group. *Kidney Int.* (2017) 91:1014–21. doi: 10.1016/j.kint.2017.02.003
4. Chapter 11: Henoch-schonlein purpura nephritis. *Kidney Int Suppl.* (2012) 2:218–20. doi: 10.1038/kisup.2012.24
5. Floege J, Barbour SJ, Cattran DC, Hogan JJ, Nachman PH, Tang SCW, et al. Management and treatment of glomerular diseases (part 1): conclusions from a kidney disease: improving global outcomes (KDIGO) controversies conference. *Kidney Int.* (2019) 95:268–80. doi: 10.36485/1561-6274-2020-24-2-22-41
6. Rovin BH, Caster DJ, Cattran DC, Gibson KL, Hogan JJ, Moeller MJ, et al. Management and treatment of glomerular diseases (part 2): conclusions from a kidney disease: improving global outcomes (KDIGO) controversies conference. *Kidney Int.* (2019) 95:281–95. doi: 10.1016/j.kint.2018.11.008
7. Tan J, Tang Y, Zhong Z, Yan S, Tan L, Tarun P, et al. The efficacy and safety of immunosuppressive agents plus steroids compared with steroids alone in the treatment of henoch-schonlein purpura nephritis: a meta-analysis. *Int Urol Nephrol.* (2019) 51:975–85. doi: 10.1007/s11255-019-02092-7
8. Yu Y, Chen J, Yin H, Deng Z, Xie Y, Yuan Q, et al. Efficacy of steroid and immunosuppressant combined therapy in Chinese patients with henoch-schonlein purpura nephritis: a retrospective study. *Int Immunopharmacol.* (2020) 81:106229. doi: 10.1016/j.intimp.2020.106229
9. Peng W, Tang Y, Tan L, Qin W. Crescents and global glomerulosclerosis in chinese iga nephropathy patients: a five-year follow-up. *Kidney Blood Press Res.* (2019) 44:103–12. doi: 10.1159/000498874
10. Pillebout E, Thervet E, Hill G, Alberti C, Vanhille P, Nochy D. Henoch-schonlein purpura in adults: outcome and prognostic factors. *J Am Soc Nephrol.* (2002) 13:1271–8. doi: 10.1097/01.ASN.0000013883.99976.22
11. Markowitz G. Glomerular disease: updated oxford classification of IgA nephropathy: a new MEST-C score. *Nat Rev Nephrol.* (2017) 13:385–6. doi: 10.1038/nrneph.2017.67
12. Chen JY, Mao JH. Henoch-schonlein purpura nephritis in children: incidence, pathogenesis and management. *World J Pediatr.* (2015) 11:29–34. doi: 10.1007/s12519-014-0534-5
13. Counahan R, Winterborn MH, White RH, Heaton JM, Meadow SR, Bluett NH, et al. Prognosis of Henoch-Schonlein nephritis in children. *Br Med J.* (1977) 2:11–4. doi: 10.1136/bmj.2.6078.11
14. Huang X, Ma L, Ren P, Wang H, Chen L, Han H, et al. Updated Oxford classification and the international study of kidney disease in children classification: application in predicting outcome of Henoch-Schonlein purpura nephritis. *Diagn Pathol.* (2019) 14:40. doi: 10.1186/s13000-019-0818-0
15. Lu S, Liu D, Xiao J, Yuan W, Wang X, Zhang X, et al. Comparison between adults and children with Henoch-Schonlein purpura nephritis. *Pediatr Nephrol.* (2015) 30:791–6. doi: 10.1007/s00467-014-3016-z
16. Cakici EK, Gur G, Yazilitas F, Eroglu FK, Gungor T, Arda N, et al. A retrospective analysis of children with Henoch-Schonlein purpura and re-evaluation of renal pathologies using Oxford classification. *Clin Exp Nephrol.* (2019) 23:939–47. doi: 10.1007/s10157-019-01726-5
17. Li X, Tang M, Yao X, Zhang N, Fan J, Zhou N, et al. A clinicopathological comparison between IgA nephropathy and Henoch-Schonlein purpura nephritis in children: use of the Oxford classification. *Clin Exp Nephrol.* (2019) 23:1382–90. doi: 10.1007/s10157-019-01777-8
18. Xu K, Zhang L, Ding J, Wang S, Su B, Xiao H, et al. Value of the Oxford classification of IgA nephropathy in children with Henoch-Schonlein purpura nephritis. *J Nephrol.* (2018) 31:279–86. doi: 10.1007/s40620-017-0457-z
19. Yun D, Kim DK, Oh KH, Joo KW, Moon KC, Kim YS, et al. MEST-C pathological score and long-term outcomes of child and adult patients with Henoch-Schonlein purpura nephritis. *BMC Nephrol.* (2020) 21:33. doi: 10.1186/s12882-020-1691-5
20. Hommos MS, Zeng C, Liu Z, Troost JP, Rosenberg AZ, Palmer M, et al. Global glomerulosclerosis with nephrotic syndrome; the clinical importance of age adjustment. *Kidney Int.* (2018) 93:1175–82. doi: 10.1016/j.kint.2017.09.028
21. Woo KT, Lim CC, Foo MW, Loh HL, Jin AZ, Chin YM, et al. Chan: 30-year follow-up study of IgA nephritis in a Southeast Asian population: an evaluation of the Oxford histological classification. *Clin Nephrol.* (2016) 86:270–8. doi: 10.5414/CN108891
22. Kawamura T, Joh K, Okonogi H, Koike K, Utsunomiya Y, Miyazaki Y, et al. A histologic classification of IgA nephropathy for predicting long-term prognosis: emphasis on end-stage renal disease. *J Nephrol.* (2013) 26:350–7. doi: 10.5301/jn.5000151
23. Coppo R, Troyanov S, Bellur S, Cattran D, Cook HT, Feehally J, et al. Validation of the Oxford classification of IgA nephropathy in cohorts with different presentations and treatments. *Kidney Int.* (2014) 86:828–36. doi: 10.1038/ki.2014.63
24. Bellur SS, Lepeytre F, Vorobyeva O, Troyanov S, Cook HT, Roberts IS. Evidence from the Oxford classification cohort supports the clinical value of subclassification of focal segmental glomerulosclerosis in IgA nephropathy. *Kidney Int.* (2017) 91:235–43. doi: 10.1016/j.kint.2016.09.029
25. Kim CH, Lim BJ, Bae YS, Kwon YE, Kim YL, Nam KH, et al. Using the Oxford classification of IgA nephropathy to predict long-term outcomes of Henoch-Schonlein purpura nephritis in adults. *Mod Pathol.* (2014) 27:972–82. doi: 10.1038/modpathol.2013.222
26. Inagaki K, Kaihan AB, Hachiya A, Ozeki T, Ando M, Kato S, et al. Clinical impact of endocapillary proliferation according to the Oxford classification among adults with Henoch-Schonlein purpura nephritis: a multicenter retrospective cohort study. *BMC Nephrol.* (2018) 19:208. doi: 10.1186/s12882-018-1009-z
27. Tan J, Tang Y, Xu Y, Yan S, Xu Y, Tan L, et al. The clinicopathological characteristics of Henoch-Schonlein purpura nephritis with presentation of nephrotic syndrome. *Kidney Blood Press Res.* (2019) 44:754–64. doi: 10.1159/000501459
28. Zhong ZX, Tan JX, Tang Y, Tan L, Pei GQ, Qin W. Crescent lesions are not a predictive factor in adult-onset Henoch-Schonlein purpura nephritis. *Clin Exp Med.* (2019) 19:449–56. doi: 10.1007/s10238-019-00567-6

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Long Non-coding RNA LRNA9884 Promotes Acute Kidney Injury via Regulating NF-kB-Mediated Transcriptional Activation of MIF

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Acute kidney injury (AKI) is one of the most common complications affecting hospitalized patients associated with an extremely high mortality rate. However, the underlying pathogenesis of AKI remains unclear that largely limits its effective management in clinic. Increasing evidence demonstrated the importance of long non-coding RNAs (lncRNAs) in the pathogenesis of AKI, because of their regulatory roles in transcription, translation, chromatin modification, and cellular organization. Here, we reported a new role of LRNA9884 in AKI. Using experimental cisplatin-induced AKI model, we found that LRNA9884 was markedly up-regulated in the nucleus of renal tubular epithelium in mice with AKI. We found that silencing of LRNA9884 effectively inhibited the production of inflammatory cytokines MCP-1, IL-6, and TNF-α in the mouse renal tubular epithelial cells (mTECs) under IL-1β stimulation *in vitro*. Mechanistically, LRNA9884 was involved into NF-κB-mediated inflammatory cytokines production especially on macrophage migration inhibitory factor (MIF). Collectedly, our study suggested LRNA9884 promoted MIF-triggered the production of inflammatory cytokines via NF-κB pathway after AKI injury. This study uncovered LRNA9884 has an adverse impact in AKI, and targeting LRNA9884 might represent a potential therapeutic target for AKI.

Keywords: lncRNA, inflammation, AKI, NF-κB, macrophage migration inhibitory factor

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INTRODUCTION

Acute kidney injury (AKI) is defined as a sudden deterioration in kidney function over a short period of time. Studies have reported that 3.2–21% of hospitalized patients and up to 50% of patients admitted to intensive care units develop AKI, with a mortality rate ranging from 40 to 60%. If there are other comorbidities with AKI, the incidence and mortality rates can be as high

as 30 and 80%, respectively (Varrier et al., 2015; Agarwal et al., 2016). Studies found that AKI significantly prolonged the length of a patient's stay in hospital and also significantly increased overall inpatient expenses (about $24 billion annually in the United States) (Silver et al., 2017). The cost required to treat AKI is comparable to treating other serious conditions such as stroke, pancreatitis and pneumonia. Taken together, these findings highlight the need for new therapies to treat this serious condition.

Long non-coding RNAs (lncRNAs) are a class of RNA molecules longer than 200 nucleotides but lacking protein-coding potential. These are transcribed from the mammalian genome and have emerged as important regulators of transcription, translation, chromatin modification and cellular organization. Compared to protein coding RNAs, lncRNAs have higher specificity to disease conditions, making them promising diagnostic and prognostic biomarkers as well as therapeutic targets. Our previous studies demonstrated that lncRNA (Erbb4-IR) induced renal fibrosis via Smad3-Smad7 pathway in the mouse UUO-induced kidneys (Feng et al., 2018), nevertheless, under diabetic conditions Erbb4-IR improved renal inflammation via miR-29b (Sun et al., 2018). Meanwhile, increasing evidence suggested that lncRNAs may be a potential target for treatment of acute and chronic kidney diseases (Mercer et al., 2009; Tang et al., 2018b). Kato et al. (2016) reported that a chemically modified oligonucleotide targeting lnc-MGC inhibited cluster microRNAs, glomerular extracellular matrix (ECM) and hypertrophy in the early stages of diabetic nephropathy. Li et al. (2017) demonstrated that the expression of lncRNA MALAT1 antagonized the inhibitory effect of miR-23c on hyperglycemia-induced cell pyroptosis in HK2 cells. However, the functional and pathogenic roles of lncRNAs in kidney diseases are still largely unclear and remain to be further elucidated.

Our previous study identified 21 novel Smad3-dependent lncRNAs were participated in renal inflammation and fibrosis from experimental mouse kidney disease models (Zhou et al., 2014). Among them, we demonstrated that targeting Erbb4-IR may represent a novel therapy for inhibiting progression of renal fibrosis (Feng et al., 2018; Sun et al., 2018), whereas Arid-IR may related to the renal inflammation (Zhou et al., 2015). Recently, we further uncovered that LRNA9884, one of the 21 Smad3-dependent lncRNAs, was involved in chronic diabetic kidney injury of db/db mice (Zhang et al., 2019b). Nevertheless, its implication in AKI is still unknown. Therefore, in the present study we further explored the potential role of LRNA9884 in AKI by using our clinical-related cisplatin-induced AKI mouse model (Lv et al., 2017; Li et al., 2018a). Interestingly, we found that LRNA9884 was evoked in the cisplatin-injured in mice especially on the nucleus of renal tubular epithelium. Mechanistically, we found that inflammatory cytokine IL-1β was capable for triggering LRNA9884 expression in the mouse renal tubular epithelial cells (mTECs) *in vitro*. More importantly, we finally identified the essentialness of LRNA9884 in NF-kB-mediated renal inflammation by regulating the production of pathogenic effector macrophage migration inhibitory factor (MIF) at genomic level. Thus, LRNA9884 may represent as a potential therapeutic target for AKI management.

MATERIALS AND METHODS

Animal Model

C57BL/6J mice were inoculated with cisplatin at a dose of 20 mg/kg (Sigma-Aldrich, St. Louis, MO, United States) by intraperitoneal injection to develop an experimental model of AKI. All mice were sacrificed by intraperitoneal injection of ketamine/xylene. To verify the model, the kidneys were harvested 3 days after cisplatin treatment and were subsequently processed for hematoxylin-eosin (H&E) and Periodic Acid Schiff (PAS) staining for histological studies. All studies were approved by the Animal Experimentation Ethics Committee of The Chinese University of Hong Kong and the experimental protocols were carried out in accordance with approved guidelines (Lv et al., 2017; Li et al., 2018a).

Cell Culture

Immortalized murine renal proximal tubular epithelial cells (mTECs) were cultured in DMEM/F-12 (Gibco, Carlsbad, CA, United States), supplemented with 10% FBS (Gibco) and 1% antibiotic/antimycotic solution (Life Technologies, Grand Island, NY, United States). mTECs, a gift from Dr. Jeffrey B. Kopp, NIH, is immortalized murine kidney proximal tubular epithelial cells. To determine if LRNA9884 expression could be induced *in vitro*, mTECs were stimulated with inflammation inducing factors such as IL-1β (10 μg/ml; R&D Systems, Minneapolis, MN, United States), TNF-α (10 ng/ml; R&D Systems, Minneapolis, MN, United States), LPS (100 ng/ml; L2630, Sigma-Aldrich, United States), C reactive protein (CRP) (10 μg/ml; source), and Transforming Growth Factor β (TGF-β) (5 ng/ml, source) at 0.5 h. Subsequently, these samples were employed to examine LRNA9884 expression using real-time PCR (Tang et al., 2018a; Zhang et al., 2019b). To inhibit NF-κB activity, cells were pretreated with the NF-κB inhibitor Bay 11-7085 at a dose of 10 μM, (sc-202490; Santa Cruz Biotechnology, Santa Cruz, CA, United States) for 2 h before Il-1β stimulation.

In situ Hybridization

LRNA9884 expression in the AKI kidney was detected using *in situ* hybridization (ISH), as previously described (Feng et al., 2018; Sun et al., 2018; Zhang et al., 2019b). After fixation in 4% (w/v) paraformaldehyde with 1% (v/v) DMSO, the kidney sections were rehydrated, permeabilised, pre-hybridized, and eventually hybridized. During the last phase, the sections were hybridized with a locked nucleic acid–digoxigenin labeled LRNA9884 probe (5′-ACTTGAAGGGTCCAGAAGAGAT-3′) (Exiqon, Vedbaek, Denmark) or negative control scramble probe (5′-GTGTAACACGTCTATACGCCCA-3′) (Exiqon). The sections were incubated with anti-digoxigenin antibody (11093274910, Roche Diagnostics, Indianapolis, IN, United States) conjugated to alkaline phosphatase and developed

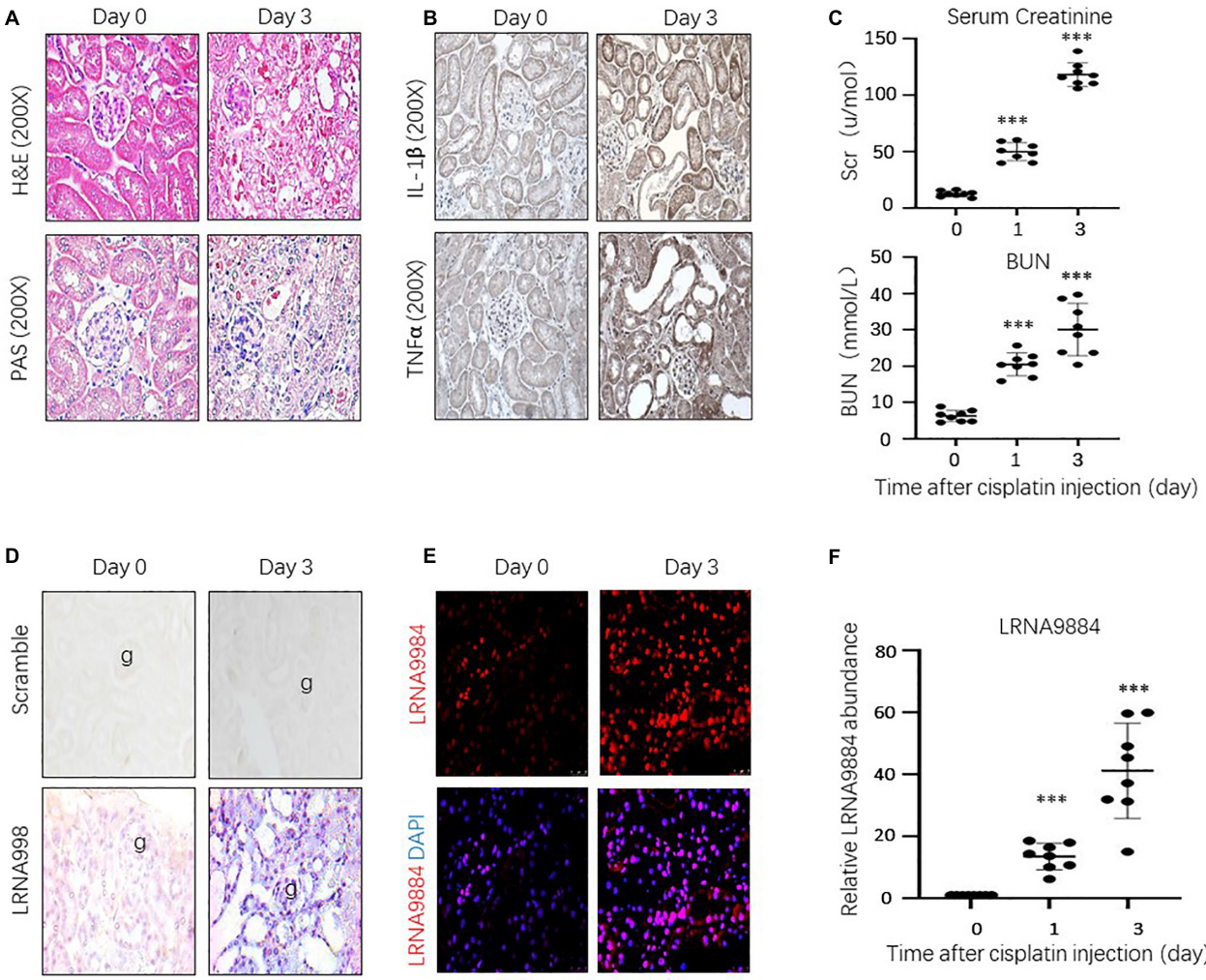

FIGURE 1 | LRNA9884 and inflammatory cytokine expression in mice with cisplatin-induced AKI. **(A)** H&E staining and PAS staining shows changes in renal histology after AKI injury at day 0 and day 3. **(B)** Immunohistochemistry (IHC) of IL-1β and TNF-α expression (200× magnification). **(C)** Serum creatinine and blood urea nitrogen (BUN) of AKI mice. **(D,E)** ISH and FISH detects LRNA9884 expression at day 0, and day 3 after AKI injury (200× magnification). **(F)** Real-time PCR shows expression of LRNA9884 in mice kidneys. Bar represents the mean ± SEM for groups of eight mice. ***$P < 0.001$ vs. mice at day 0.

with phosphate/nitroblue tetrazolium (SigmaAldrich, St. Louis, MO, United States).

Fluorescence *in situ* Hybridization and Immunofluorescence Assay

The cells and the kidney sections were fixed with 4% (w/v) paraformaldehyde, washed three times with 1 × PBS for 5 min, and washed once with distilled water. Prehybridization was performed at 37°C for 4 h in an incubator. The concentration of the hybrid solution were set with different gradients (5, 10, 50, and 100 μM) of probes. Hybridization was conducted overnight at 42°C in the incubator. After hybridization, sections were washed by gradient SSC, dripped with DAPI and sealed. All the equipment were disinfected with DEPC water. The fluorescence *in situ* hybridization (FISH) kit used in this method was purchased from Guangzhou Ruibo (C10910; RiboBio), the probe sequence was identical to the *in situ* hybridization probe, preceded by cy3-fluorescence labeling (Sun et al., 2018).

Western Blotting Analysis

Protein from the renal tubular epithelium was extracted and western blot analysis was performed as previously described (Tang et al., 2017, 2018c; Li et al., 2020). The antibodies used in this study included phospho- NF-κB/p65 (Ser536, CST), NF-κB/p65 (CST), TNF-α, IL-1β, MCP-1, β-actin (Santa Cruz), and LI-COR IRDye 800-labeled secondary antibodies (Rockland Immuno-chemicals). The detection of specific signals was performed employing Odyssey infrared imaging system (LI-COR Biosciences, Lincoln, NE, United States) and quantified using Image J software (National Institutes of Health)[1]. The ratio

[1]http://www.imagej.nih.gov/ij/

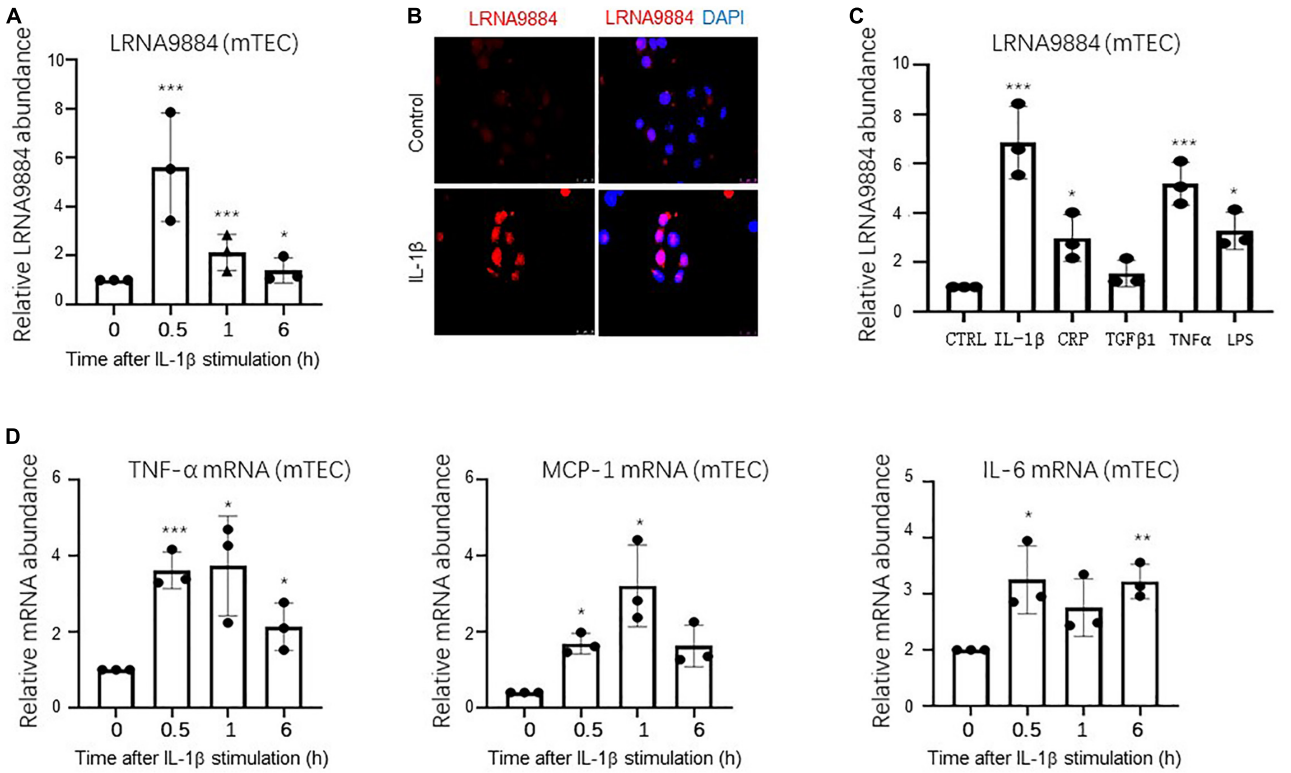

FIGURE 2 | IL-1β triggers LRNA9884 and inflammation cytokines in mTECs. **(A)** Real-time PCR showed that IL-1β (10 ng/ml) induced LRNA9884 expression in mTECs in a dose-dependent manner. **(B)** FISH assay detected an increase in LRNA9884 expression after IL-1β treatment, primarily localized in the nucleus of mTECs. **(C)** Real-time PCR shows LRNA9884 expression after IL-1β, CRP, TGF-β, TNFα, and LPS treatment in mTECs. **(D)** Real-time PCR shows TNF-α, MCP-1 and IL-6 expression after IL-1β treatment. Bars represent the mean ± SEM for at least three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. time 0 h.

for the protein detected was normalized against β-actin and the results were expressed as the mean ± standard error of the mean ± (SEM) (Wang et al., 2018).

RNA Extraction and Quantitative Real-Time PCR

Total RNA was extracted from cells and real-time PCR was performed using an Option 2 instrument (Bio-Rad, Hercules, CA, United States) with IQ SYBR green supermix reagent (Bio-Rad, Hercules, CA, United States). The primers used in this study were mouse mRNA MCP-1, IL-1β, TNF-α, LRNA9884 and MIF as described in **Supplementary Table 1**. β-actin housekeeping gene was used as the internal control. Results were expressed by fold changes to controls as individual dots pattern.

Enzyme-Linked Immunosorbent Assay

We collected medium from stimulated mTEC cells to detect inflammatory and pro-inflammatory cytokines production by using an enzyme-linked immunosorbent assay kit. TNF-a, MCP-1, and IL-6 were measured with a Quantikine ELISA Kit (R&D Systems) according to the product protocols (Zhang et al., 2019a).

Statistical Analysis

All the data are expressed as the mean ± SEM. Statistical analyses were performed using two-way analysis of variance as appropriate. Tests were performed with GraphPad Prism 5 (GraphPad Software, La Jolla, CA, United States). A *P*-value <0.05 was considered to be a significant finding.

RESULTS

LRNA9884 Is Highly Expressed in the Cisplatin-Induced AKI *in vivo*

The experimental model of AKI was developed using C57BL/6J mice treated with cisplatin (20 mg/kg) (Lv et al., 2017). Histological examination revealed loss of brush border of renal epithelial cells, lumen dilation of renal tubular system, and cytoplasmic vacuolar degeneration and necrosis of tubular epithelium in the AKI model compared to the control group. Serum creatinine and urea nitrogen were increased in the AKI mice form day 1. These results indicated that the AKI model was successfully implemented (**Figures 1A,C**). Inflammatory cytokines were up-regulated in the AKI kidneys such as IL-1β and TNFα, indicated that activating inflammatory signals involved in cisplatin-induced AKI (**Figure 1B**). Furthermore,

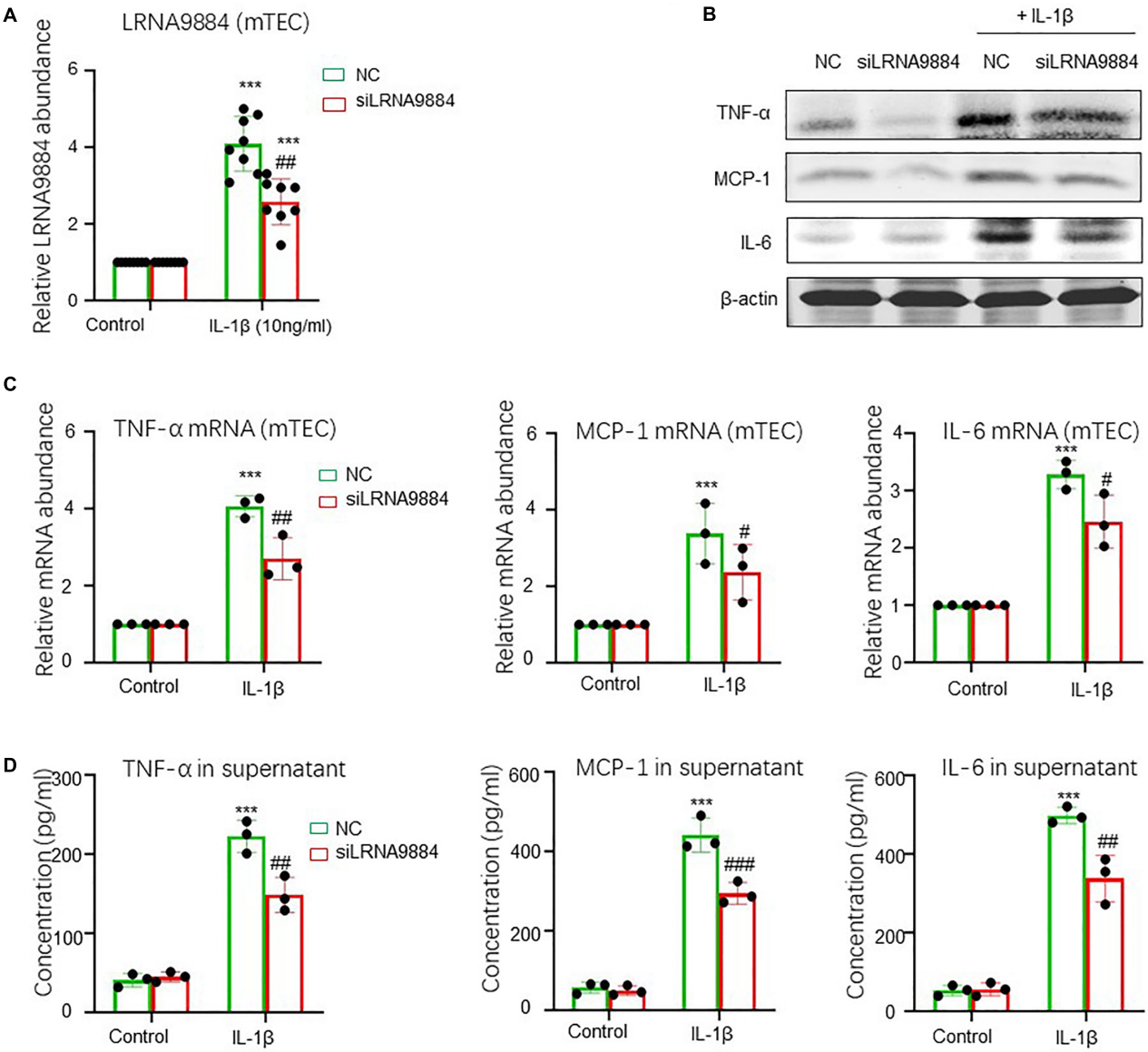

FIGURE 3 | Silencing of LRNA9884 blocks inflammatory cytokine expression. **(A)** Real-time PCR results shows LRNA9884 expression level for siLRNA9884 and nonsense-treated control (NC) after IL-1β-induced in mTECs. **(B)** Western blot shows expression of TNF-α, MCP-1 and IL-6 in IL-1β-induced mTECs. **(C)** Real-time PCR shows TNF-α, MCP-1 and IL-6 expression in NC and siLRNA9884 mTECs for control and IL-1β groups. Bars represent the mean ± SEM for at least three independent experiments. **(D)** ELISA shows TNF-α, MCP-1 and IL-6 expression in NC and siLRNA9884 mTECs for control and IL-1β groups. $^{***}P < 0.001$ vs. Control group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. NC group.

ISH and *FISH* examination of renal tubular epithelial cells harvested from the cisplatin-induced AKI model showed that expression levels of LRNA9884 increased gradually with AKI progression. Moreover, it indicated that LRNA9884 expression was mainly located in the nuclei of renal tubular epithelial cells (**Figures 1D,E**). Quantitative real-time PCR analysis of LRNA9884 expression revealed that AKI mice models had a greater intra-nuclear amount of LRNA9884 compared to the control group on the first day after cisplatin injection ($P < 0.001$). Additionally, the LRNA9884 expression on the third day was greater than on day one ($P < 0.001$) (**Figure 1F**). These results demonstrated that LRNA9884 expression was elevated and localized in the nuclei of tubular epithelia cells from the AKI kidney.

LRNA9884 Is a Positive Feedback to the IL-1β Driven Renal Inflammation *in vitro*

In vitro analysis of cultured mTECs stimulated with IL-1β showed that LRNA9884 expression increased significantly after IL-1β (10 ng/ml) stimulation for half hour ($P < 0.001$) (**Figure 2A**). Moreover, FISH further indicated that the IL-1β induced LRNA9884 was predominantly located in the

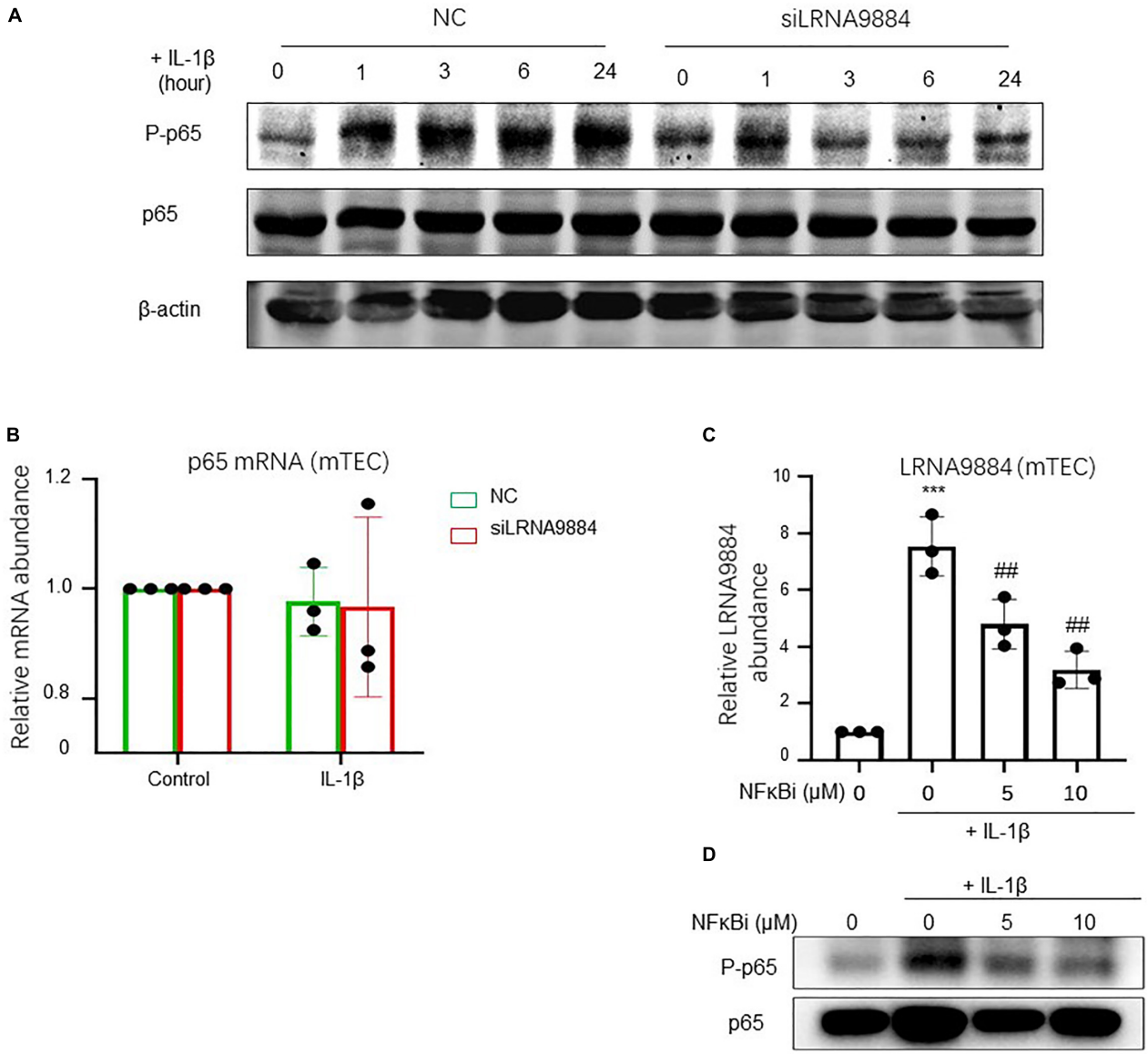

FIGURE 4 | LRNA9884 regulates NF-κB mediated inflammatory actions. **(A)** Western blot shows phosphorylation and total NF-kB in NC and siLRNA9884 groups. **(B)** Real-time PCR shows total NF-kB expression (p65) in control and in IL-1β-induced mTECs groups. **(C)** Real-time PCR shows LRNA9884 expression after inhibition of NF-kB phosphorylation in mTECs in a dose-dependence. **(D)** Western blot shows phosphorylation and total NF-kB after inhibition of NF-kB phosphorylation in mTECs in a dose-dependence. Bars represent the mean ± SEM for at least three independent experiments. $^{***}P < 0.001$ vs. Control group, $^{\#\#}P < 0.01$ vs. IL-1β-induced group.

nucleus (**Figure 2B**), which was consistent with the *in vivo* results obtained from ISH (**Figure 1B**). It was further explored whether other stimuli besides IL-1β could induce an increase in the expression of LRNA9884. Interestingly, our results showed that TNF-α, LPS, CRP, and TGF-β could also trigger LRNA9884 expression compared to the negative control group (CTRL) (**Figure 2C**), but IL-1β was the greatest extend ($P < 0.001$). In addition, we found that IL-1β induced a high expression of various inflammatory factors such as tumor necrosis factor (TNF-α), monocyte chemotactic protein (MCP-1) and interleukin 6 (IL-6) (**Figure 2D**), which may also contribute to the LRNA9884 expression at certain degree. More importantly, we investigated the role of LRNA9884 in IL-1β driven renal inflammation via siRNA-mediated silencing (siLRNA9884) in mTECs *in vitro* (**Figure 3A**). Interestingly, both western blotting, real-time PCR and ELISA detected that silencing of LRNA9884 significantly suppressed the IL-1β-induced production of inflammatory cytokines including TNF-α, MCP-1 and IL-6 in mTECs compared to the nonsense-treated (NC) group (**Figures 3B–D**), indicating the importance of LRNA9884 in the progression of renal inflammation.

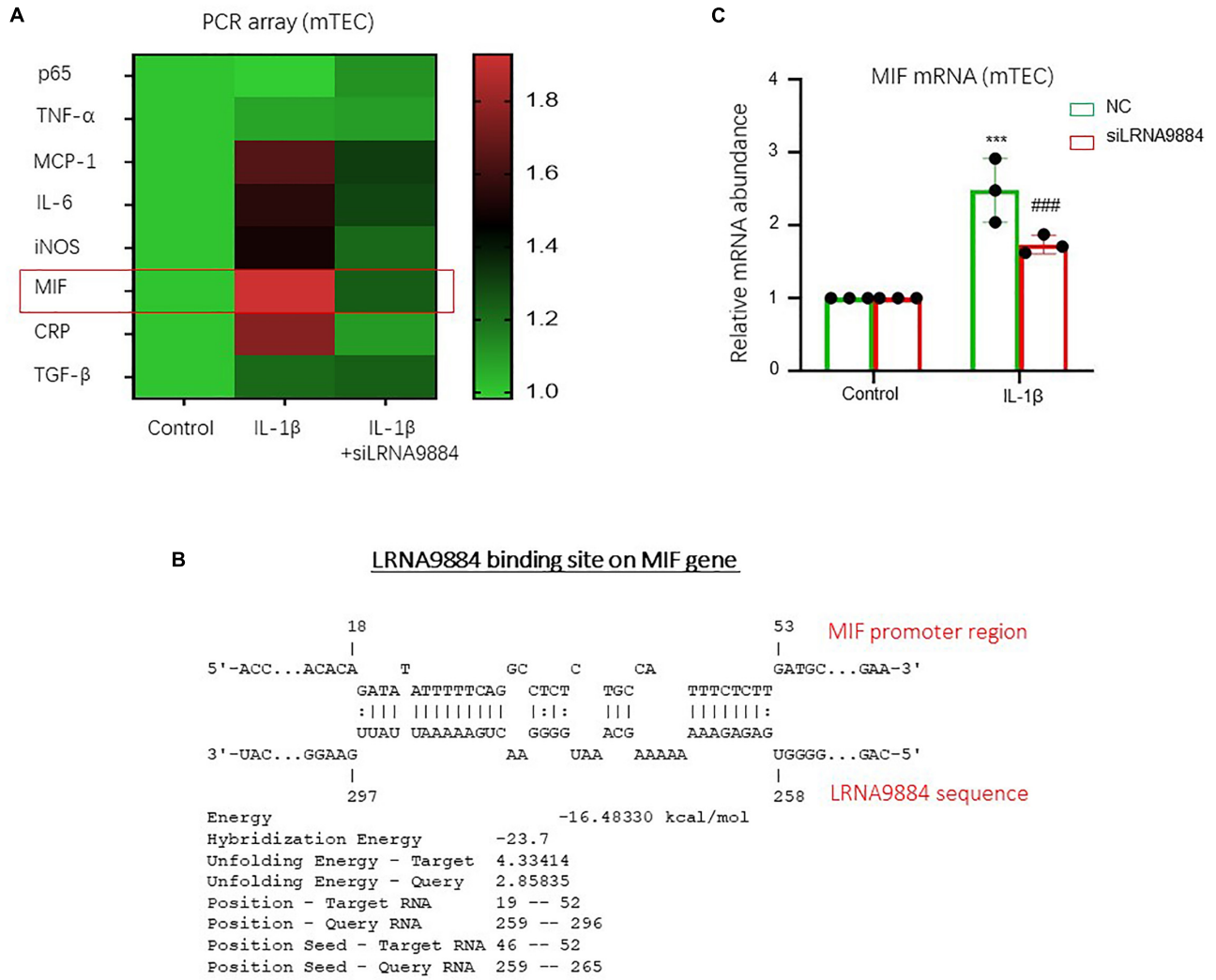

FIGURE 5 | LRNA9884 regulates MIF at transcriptional level. **(A)** Real-time PCR array shows inflammatory markers expression after knockdown of LRNA9884 in mTEC. **(B)** Predicted binding site of LRNA9884 on the genomic sequence of MIF. **(C)** Real-time PCR results show MIF expression ratios in control and IL-1β mTECs groups. $^{***}P < 0.001$ vs. CTRL group, $^{###}P < 0.001$ vs. NC group.

LRNA9884 Is Essential for NF-κB Mediated Inflammatory Cytokines Production

NF-κB signaling is one of the important pathways for renal inflammation. Therefore, inactivation of the NF-κB pathway in AKI mice was studied by downregulating LRNA9884 production as well as using a NF-κB inhibitor (BAY 11-7082). Western blot showed that NF-κB phosphorylation was decreased after LRNA9884 expression levels were reduced (**Figure 4A**). Nevertheless, real-time PCR did not show changes in total NF-κB expression after silencing LRNA9884 (**Figure 4B**); hence LRNA9884 might only affect NF-κB phosphorylation but not its transcription. As a consequence, LRNA9884 expression was later studied following inhibition of NF-κB phosphorylation via real-time PCR. This analysis indicated that LRNA9884 expression was decreased by inhibition of NF-κB in a dose-dependent manner ($P < 0.01$) (**Figure 4C**). These findings suggested that there is a reciprocal regulation between LRNA9884 and NF-κB, in which NF-κB regulates LRNA9884 expression and LRNA9884 regulates NF-κB phosphorylation.

LRNA9884 Promotes NF-κB-Induced MIF via Transcriptional Regulation

As previously described, LRNA9884 was involved in the downstream of NF-κB pathway (Zhang et al., 2019b), but the underlying mechanism is still largely unclear. Here, by conducting PCR array, we revealed that expression of an important pathogenic cytokine MIF was highly affected by the loss of LRNA9884 in mTECs under IL-1β-stimulation (**Figure 5A**). Interestingly, we found a direct LRNA9884 binding site on the promoter region of MIF using Freiburg RNA Tool (**Figure 5B**). We confirmed that MIF expression was significantly

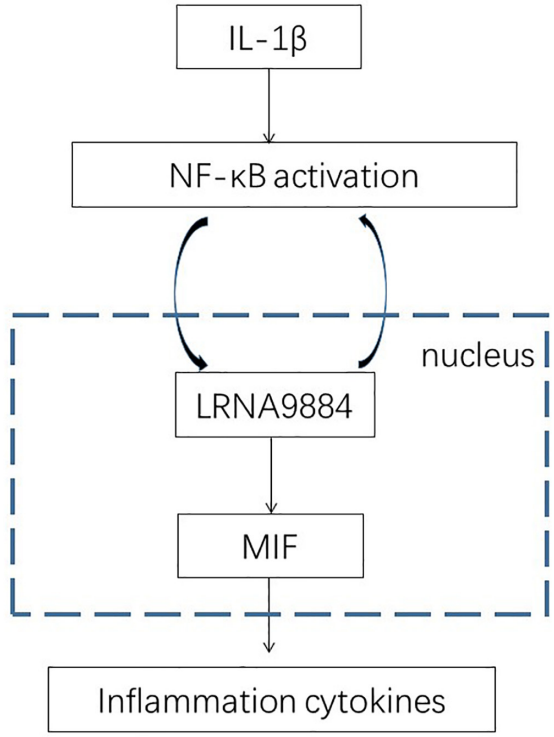

FIGURE 6 | Schematic diagram of LRNA9884 in NF-kB/MIF axis regulation. In AKI, IL-1β triggers NF-kB signaling, which in turn upregulates LRNA9884 in the nucleus, and which binds on the promoter region of MIF genome therefore enhancing MIF transcription at genomic level. LRNA9884 will positively promote IL-1β/NF-κB signaling therefore further promoting the inflammatory response.

downregulated in renal tubular epithelial cells due to silencing of LRNA9884 ($P < 0.001$) (**Figure 5C**). Thus, LRNA9884 may promote NF-κB-mediated renal inflammation via enhancing MIF production at genomic level (**Figure 6**).

DISCUSSION

AKI can gradually progress into chronic renal failure due to persistent inflammation and progressive fibrosis, in which significant amounts of inflammatory and pro-inflammatory cytokines are released causing disease progression (Kinsey et al., 2008; Chawla et al., 2011). When tissue injury occurs in AKI, renal tubular epithelial cells injury, which leads to the release of pro-inflammatory factors from the cells to enhance the permeability of vascular endothelia. Then effector cells involved in immune responses such as macrophages extravasate to the injury site (Bonventre and Zuk, 2004; Friedewald and Rabb, 2004) to trigger the production of an inflammatory cascade, which further aggravates damage to the renal tubular epithelial cells. Therefore, effective inhibition of the production and secretion of various inflammatory factors in the initial stages of AKI may be the key to prevent AKI from progressing into chronic kidney disease.

Long non-coding RNAs have been suggested to play roles in the pathogenesis of various kidney diseases. For instance, LRNA9884 has been shown to be a promoting factor of diabetic kidney injury (Zhang et al., 2019b). In this study, it was found that LRNA9884 exerts a functional role in the pathogenesis of cisplatin-induced AKI. Cisplatin induced nephrotoxicity in mice are most prominently used to study AKI (Ramesh and Ranganathan, 2014). In our study, the expression of LRNA9884 was found to be significantly increased in renal tubular epithelial cells *in vivo*. Furthermore, *in vitro* analysis indicated that the increase in LRNA9884 expression occurs at early stages of AKI development since LRNA9884 expression incremented significantly after only 30 min upon IL-1β stimulation rather than cisplatin stimulation *in vitro* (**Supplementary Figure 1**). Increasing evidences showed IL-1β activation exacerbates cisplatin-induced AKI by activating inflammatory signals (Yan et al., 2017; Privratsky et al., 2018). Our results indicated LRNA9884 was relevant to inflammatory signaling. Thus, it was hypothesized that LRNA9884 might be involved in the inflammatory response of renal tubular epithelial cells during AKI. The present study revealed that LRNA9884 exhibits high expression levels in tubular epithelial cells after their stimulation with various inflammatory mediators. Meanwhile, we found IL-1β significantly up-regulated LRNA9884 expression compared other inflammatory stimulators *in vitro*. Moreover, it demonstrated that downregulation of LRNA9884 leads to declined production of various inflammatory mediators. Consequently, LRNA9884 may work as a potential regulator in the production of a cocktail of inflammatory factors. Lastly, it determined that LRNA9884 was confined within the nuclei of renal tubular epithelial cells rather than to their cytoplasm.

NF-κB is one of the most critical nuclear transcription factors present in virtually all animal cell types. NF-κB participates in classic inflammatory stimulation pathways, which are initiated by the activation of various external stimuli such as CRP, IL-1β, hypoxia induction, and among others. NF-κB phosphorylation initiates a response within 5 min of stimulation (Lawrence, 2009). Interestingly, we found that IL-1β might upregulate LRNA9884 via a P65-dependent mechanism. It is because the phosphorylated P65 can migrate into the nucleus and promote the transcription of LRNA9884. Thus, the AKI-included IL-1β can increase LRNA9884 expression in renal epithelial cells via activating P65 during renal inflammation. More importantly, inflammation-related RNA-sequence screening showed that the increased expression of MIF was LRNA9884-specific. MIF is an upstream pro-inflammatory factor and mainly functions as an activator of the inflammatory cascade, as a promoter of macrophage migration and as an initiator of T cell activation (Lan et al., 2018; Li et al., 2018a,b, 2019). Current study found that LRNA9884 has a binding motif in the MIF promoter region, thereby decreasing LRNA9884 levels may downregulate MIF expression. Our mechanistic study of LRNA9884 identified MIF might be as the direct downstream target of LRNA9884 in renal epithelial cells during IL-1β-mediated renal inflammation. LRNA9884 directly bind on the promoter region of MIF for enhancing its production from renal epithelial cells at transcriptional level, therefore further promoting the renal

inflammation of the AKI-kidney. However, prospective studies are recommended to determine how LRNA9884 behaves and functions in human patients suffering from AKI. Similarly, they are suggested to conduct *in vivo* experiments and to use specific kidney silencing of LRNA9884.

In conclusion, the present study has provided evidence that injured renal tubular epithelial cells release inflammatory cytokines in AKI mice model, which upregulate LRNA9884 expression by activating phosphorylation of NF-κB pathway. This pathway subsequently upregulates MIF, triggering the production of a storm of other inflammatory response, which in turn aggravate renal tubular damage (**Figure 6**). These findings suggest that LRNA9884 has an adverse impact in AKI and targeting LRNA9884 represents a potential therapy for alleviating inflammation response in AKI kidneys.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by Animal Experimentation Ethics Committee of The Chinese University of Hong Kong.

AUTHOR CONTRIBUTIONS

YZ and PT designed the research and performed cellular experiments. YN assisted animal experiments. YZ, PT, and YN analyzed results and wrote the manuscript. CG revised the manuscript. X-RH maintained animal availability. CY and H-YL guided and designed the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2020.590027/full#supplementary-material

Supplementary Figure 1 | Cisplatin take no effect on LRNA9884 expression and silencing of LRNA9884 has no response to cisplatin induced apoptosis in mTECs.

Supplementary Figure 2 | Quantitative analysis of western blot in mTECs.

Supplementary Table 1 | Primer sequences (mouse).

REFERENCES

Agarwal, A., Dong, Z., Harris, R., Murray, P., Parikh, S. M., Rosner, M. H., et al. (2016). Cellular and molecular mechanisms of AKI. *J. Am. Soc. Nephrol.* 27, 1288–1299.

Bonventre, J. V., and Zuk, A. (2004). Ischemic acute renal failure: an inflammatory disease? *Kidney Int.* 66, 480–485. doi: 10.1111/j.1523-1755.2004.761_2.x

Chawla, L. S., Amdur, R. L., Amodeo, S., Kimmel, P. L., and Palant, C. E. (2011). The severity of acute kidney injury predicts progression to chronic kidney disease. *Kidney Int.* 79, 1361–1369. doi: 10.1038/ki.2011.42

Feng, M., Tang, P. M., Huang, X. R., Sun, S. F., You, Y. K., Xiao, J., et al. (2018). TGF-beta mediates renal fibrosis via the Smad3-Erbb4-IR long noncoding RNA axis. *Mol. Ther.* 26, 148–161. doi: 10.1016/j.ymthe.2017.09.024

Friedewald, J. J., and Rabb, H. (2004). Inflammatory cells in ischemic acute renal failure. *Kidney Int.* 66, 486–491. doi: 10.1111/j.1523-1755.2004.761_3.x

Kato, M., Wang, M., Chen, Z., Bhatt, K., Oh, H. J., Lanting, L., et al. (2016). An endoplasmic reticulum stress-regulated lncRNA hosting a microRNA megacluster induces early features of diabetic nephropathy. *Nat. Commun.* 7:12864.

Kinsey, G. R., Li, L., and Okusa, M. D. (2008). Inflammation in acute kidney injury. *Nephron. Exp. Nephrol.* 109, e102–e107.

Lan, H., Wang, N., Chen, Y., Wang, X., Gong, Y., Qi, X., et al. (2018). Macrophage migration inhibitory factor (MIF) promotes rat airway muscle cell proliferation and migration mediated by ERK1/2 and FAK signaling. *Cell Biol. Int.* 42, 75–83. doi: 10.1002/cbin.10863

Lawrence, T. (2009). The nuclear factor NF-kappaB pathway in inflammation. *Cold Spring Harb. Perspect. Biol.* 1:a001651.

Li, C., Xue, V. W., Wang, Q. M., Lian, G. Y., Huang, X. R., Lee, T. L., et al. (2020). The Mincle/Syk/NF-kappaB signaling circuit is essential for maintaining the protumoral activities of tumor-associated macrophages. *Cancer Immunol. Res.* 8:canimm.0782.2019.

Li, J., Tang, Y., Tang, P. M. K., Lv, J., Huang, X. R., Carlsson-Skwirut, C., et al. (2018a). Blocking macrophage migration inhibitory factor protects against cisplatin-induced acute kidney injury in mice. *Mol. Ther.* 26, 2523–2532. doi: 10.1016/j.ymthe.2018.07.014

Li, J., Zhang, J., Xie, F., Peng, J., and Wu, X. (2018b). Macrophage migration inhibitory factor promotes Warburg effect via activation of the NFkappaB/HIF1alpha pathway in lung cancer. *Int. J. Mol. Med.* 41, 1062–1068.

Li, J. H., Tang, Y., Lv, J., Wang, X. H., Yang, H., Tang, P. M. K., et al. (2019). Macrophage migration inhibitory factor promotes renal injury induced by ischemic reperfusion. *J. Cell Mol. Med.* 23, 3867–3877. doi: 10.1111/jcmm.14234

Li, X., Zeng, L., Cao, C., Lu, C., Lian, W., Han, J., et al. (2017). Long noncoding RNA MALAT1 regulates renal tubular epithelial pyroptosis by modulated miR-23c targeting of ELAVL1 in diabetic nephropathy. *Exp. Cell Res.* 350, 327–335. doi: 10.1016/j.yexcr.2016.12.006

Lv, L. L., Tang, P. M., Li, C. J., You, Y. K., Li, J., Huang, X. R., et al. (2017). The pattern recognition receptor, Mincle, is essential for maintaining the M1 macrophage phenotype in acute renal inflammation. *Kidney Int.* 91, 587–602. doi: 10.1016/j.kint.2016.10.020

Mercer, T. R., Dinger, M. E., and Mattick, J. S. (2009). Long non-coding RNAs: insights into functions. *Nat. Rev. Genet.* 10, 155–159.

Privratsky, J. R., Zhang, J., Lu, X., Rudemiller, N., Wei, Q., Yu, Y. R., et al. (2018). Interleukin 1 receptor (IL-1R1) activation exacerbates toxin-induced acute kidney injury. *Am. J. Physiol. Renal* 315, 682–691.

Ramesh, G., and Ranganathan, P. (2014). Mouse models and methods for studying human disease, acute kidney injury (AKI). *Methods Mol. Biol.* 1194, 421–436. doi: 10.1007/978-1-4939-1215-5_24

Silver, S. A., Long, J., Zheng, Y., and Chertow, G. M. (2017). Cost of acute kidney injury in hospitalized patients. *J. Hosp. Med.* 12, 70–76. doi: 10.12788/jhm.2683

Sun, S. F., Tang, P. M. K., Feng, M., Xiao, J., Huang, X. R., Li, P., et al. (2018). Novel lncRNA Erbb4-IR promotes diabetic kidney injury in db/db mice by targeting miR-29b. *Diabetes Metab. Res. Rev.* 67, 731–744. doi: 10.2337/db17-0816

Tang, P. M., Tang, P. C., Chung, J. Y., Hung, J. S. C., Wang, Q. M., Lian, G. Y., et al. (2018a). A novel feeder-free system for mass production of murine natural killer cells in vitro. *J. Vis. Exp*. 9:56785.

Tang, P. M., Zhang, Y. Y., and Lan, H. Y. (2018b). LncRNAs in TGF-beta-driven tissue fibrosis. *Noncoding RNA* 4:26. doi: 10.3390/ncrna4040026

Tang, P. M., Zhou, S., Li, C. J., Liao, J., Xiao, J., Wang, Q. M., et al. (2018c). The proto-oncogene tyrosine protein kinase Src is essential for macrophage-myofibroblast transition during renal scarring. *Kidney Int.* 93, 173–187. doi: 10.1016/j.kint.2017.07.026

Tang, P. M., Zhou, S., Meng, X. M., Wang, Q. M., Li, C. J., Lian, G. Y., et al. (2017). Smad3 promotes cancer progression by inhibiting E4BP4-mediated NK cell development. *Nat. Commun.* 8:14677.

Varrier, M., Forni, L. G., and Ostermann, M. (2015). Long-term sequelae from acute kidney injury: potential mechanisms for the observed poor renal outcomes. *Crit. Care* 19:102. doi: 10.1186/s13054-015-0805-0

Wang, Q. M., Tang, P. M., Lian, G. Y., Li, C., Li, J., Huang, X. R., et al. (2018). Enhanced cancer immunotherapy with Smad3-silenced NK-92 cells. *Cancer Immunol. Res.* 6, 965–977. doi: 10.1158/2326-6066.cir-17-0491

Yan, W., Xu, Y., Yuan, Y., Tian, L., Wang, Q., Xie, Y., et al. (2017). Renoprotective mechanisms of Astragaloside IV in cisplatin-induced acute kidney injury. *Free Radic. Res.* 51, 669–683. doi: 10.1080/10715762.2017.1361532

Zhang, Y. Y., Tan, R. Z., Zhang, X. Q., Yu, Y., and Yu, C. (2019a). Calycosin ameliorates diabetes-induced renal inflammation via the NF-kB pathway in vitro and in vivo. *Med. Sci. Monit.* 25, 1671–1678. doi: 10.12659/msm.915242

Zhang, Y. Y., Tang, P. M., Tang, P. C., Xiao, J., Huang, X. R., Yu, C., et al. (2019b). LRNA9884, a novel Smad3-dependent long noncoding RNA, promotes diabetic kidney injury in db/db mice via enhancing MCP-1-dependent renal inflammation. *Diabetes Metab. Res. Rev.* 68, 1485–1498. doi: 10.2337/db18-1075

Zhou, Q., Chung, A. C., Huang, X. R., Dong, Y., Yu, X., and Lan, H. Y. (2014). Identification of novel long noncoding RNAs associated with TGF-beta/Smad3-mediated renal inflammation and fibrosis by RNA sequencing. *Am. J. Pathol.* 184, 409–417. doi: 10.1016/j.ajpath.2013.10.007

Zhou, Q., Huang, X. R., Yu, J., Yu, X., and Lan, H. Y. (2015). Long noncoding RNA Arid2-IR is a novel therapeutic target for renal inflammation. *Mol. Ther.* 23, 1034–1043. doi: 10.1038/mt.2015.31

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frontiers in Medicine

CASE REPORT
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Monoclonal Gammopathy of Renal Significance: Clinical and Histological Efficacy of a Bortezomib-Based Regimen

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Monoclonal Gammopathy of Renal Significance (MGRS) is a group of heterogeneous disorders characterized by renal dysfunction secondary to the production of a monoclonal immunoglobulin by a nonmalignant B cell or plasma cell clone. We report the clinical and histological outcomes of two patients with biopsy-proven MGRS: one patient showed membranoproliferative glomerulonephritis with monoclonal k-light chain and C3 deposits, the second patient showed immunotactoid glomerulopathy. Both patients were treated with a 9-month chemotherapy protocol including bortezomib, cyclophosphamide, and dexamethasone. Renal biospy was repeated after 1 year. The estimated glomerular filtration rate (eGFR) increased from 22.5 (baseline) to 40 ml/min per 1.73 m2 after 12 months, then to 51.5 ml/min per 1.73 m2 after 24 months; proteinuria decreased from 4.85 (baseline) to 0.17 g/day after 12 months, then to 0.14 g/day after 24 months. Repeat renal biopsies showed a dramatic improvement of the glomerular proliferative lesions and near complete disappearance of the immune deposits. A bortezomib-based treatment proved very effective and was well-tolerated in the two patients presenting with clinically and histologically aggressive MGRS.

Keywords: monoclonal gammopathy of renal significance (MGRS), C3 glomerulonephritis (C3GN), Immunotactoid glomerulonephritis, bortezomib, glomerulonephritis

INTRODUCTION

The term monoclonal gammopathy of renal significance (MGRS) was proposed for the first time in 2012 by the International Kidney and Monoclonal Gammopathy Research Group (IKMG) to describe patients with renal injury secondary to a plasma cell clone who do not present the criteria for multiple myeloma or a lymphoproliferative disorder (1). The hematologic disorder is generally consistent with monoclonal gammopathy of undetermined significance (MGUS) (2).

In 2017 the IKMG updated the term MGRS and redefined it as a clonal proliferative disorder that produces a nephrotoxic monoclonal immunoglobulin and does not meet previously defined haematologic criteria for the treatment of a specific malignancy. Furthermore, the IKMG recommended kidney biopsy for the correct diagnosis of MGRS-related disease (3). Renal disease can result from the direct deposition of nephrotoxic monoclonal immunoglobulin (MIg) or its light- or heavy-chain fragments in various renal tissue compartments, and includes common disorders, such as cast nephropathy, amyloidosis, and MIg deposition diseases, as well as rarer disorders, such as immunotactoid glomerulopathy, proliferative GN with MIg deposits, light-chain proximal tubulopathy, and the rare entities of crystal-storing histiocytosis and crystalglobulinemia. MGRS can also result from indirect mechanisms and manifests as C3 glomerulopathy or thrombotic microangiopathy without tissue MIg deposits (4).

Effective chemotherapy regimens as well as stem cell transplantation are widely used for renal complications of myeloma kidney and amyloid light-chain amyloidosis (5, 6). Specific therapeutic protocols for MGRS are far less well-defined and some reluctance exists among hematologists to treat this disorder. However, both the progressive nature of MGRS and its tendency to recur after kidney transplantation strongly suggest—at least in patients with severe disease—treatment which should target the pathologic clone responsible for the production of the nephrotoxic MIg (7).

Herein we report the clinical outcome and the histological renal evolution of two patients with two different monoclonal Ig-associated diseases at renal biopsy who were treated with a combination regimen including bortezomib, dexamethasone, and cyclophosphamide.

CASE PRESENTATION

Case 1

A 59 year old white female with a history of essential hypertension treated with ramipril was referred to us for a second opinion by another hospital where she had undergone renal biopsy for renal failure (serum creatinine level 1.5 mg/dl), proteinuria (2.9 g/d), and microscopic dysmorphic hematuria. The renal biopsy had shown membranoproliferative glomerulonephritis with C3 and k-light chain deposits involving glomerular capillary walls and the mesangium. Unfortunately, the histological specimen was not suitable for adequate electron microscopy study and full immunofluorescence analysis.

At admission to our hospital she referred asthenia and showed mild lower limb edema. Laboratory investigations showed (**Table 1**): reduced estimated glomerular filtration rate (eGFR) (35 ml/min per 1.73 m2), severe proteinuria (2.7 g/d) and mild microscopic hematuria; normal calcemia, immunoglobulin and complement levels; negative cryoglobulins; normocytic anemia (hemoglobin, 9.4 g/dl); negative serum immunofixation with moderately increased serum k/λ free light chain concentration (k/λ ratio = 11.5), and scanty amounts of k free light chain in urine immunofixation.

TABLE 1 | Clinical, histological, and laboratory features of patients.

	Patient 1	Patient 2
Age (yr)	59	71
Sex	Female	Male
Kidney histology	PGNMID	ITGN
Bone marrow plasma cells (%)	7	6
Baseline eGFR (ml/min per 1.73m^2)	35	10
12-month eGFR (ml/min per 1.73m^2)	49	31
24-month eGFR (ml/min per 1.73m^2)	57	46
Baseline proteinuria (g/day)	2.7	7.0
12-month proteinuria (g/day)	0.09	0.26
24-month proteinuria (g/day)	0.05	0.23
Baseline Hemoglobin (g/dL)	9.4	10.5
12-month Hemoglobin (g/dL)	12.8	13.1
24-month Hemoglobin (g/dL)	13.5	17.0
Baseline serum Calcium (mmol/L)	2.2	1.9
12-month serum Calcium (mmol/L)	2.5	2.3
24-month serum Calcium (mmol/L)	2.5	2.3
Baseline k/λ sFLC (mg/l)	369/32	89/41
12-month k/λ sFLC (mg/l)	23/16	16/14
24-month k/λ sFLC (mg/l)	31/17	19/16

yr, year; PGNMID, proliferative glomerulonephritis with monoclonal Ig deposits; ITGN, immunotactoid glomerulonephritis; eGFR, estimated glomerular filtration rate; k/λ sFLC, k/λ serum free light chain concentration.

A bone marrow aspirate and biopsy were performed and a real-time ultrasound-guided percutaneous renal biopsy was repeated.

Bone marrow aspirate and biopsy were examined by light microscopy, immunohistochemistry, and flow cytometry, and only showed 7% polyclonal plasma cells.

Renal tissue specimens were examined by light microscopy, immunofluorescence, and electron microscopy. Light microscopy showed membranoproliferative glomerulonephritis with nodular mesangial expansion, and infiltrating mononuclear and polymorphonuclear leukocytes (**Figures 1A–C**). Immunofluorescence showed positive staining for C3 (3+) and k-light chain (3+) involving glomerular capillary walls and the mesangium, with negative staining for λ-light chain and for heavy chain, performed both on frozen material (**Figures 2A–C**) and on formalin-fixed paraffin embedded tissue after protease digestion (not shown). Ultrastructural

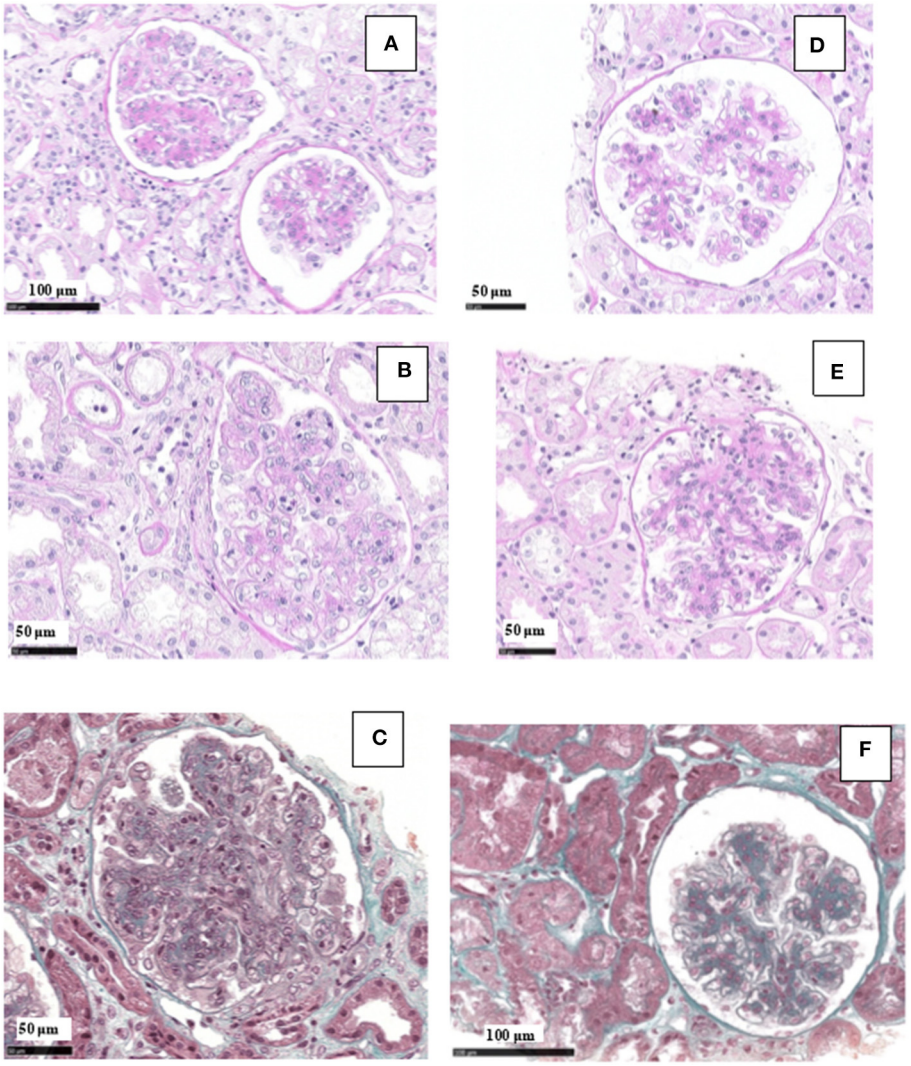

FIGURE 1 | Light microscopy findings in patient #1: first biopsy showed membranoproliferative glomerulonephritis with focal mesangial nodules, and endocapillary mononuclear and polymorphonuclear leukocytes (**A**, PAS; **B**, PAS; **C**, Masson Trichrome). The repeat biopsy showed a remarkable reduction of the membranoproliferative lesions with disappearance of the capillary leukocyte infiltration and persistent mesangial expansion (**D**, PAS; **E**, PAS; **F**, Masson Trichrome).

evaluation highlighted subendothelial and mesangial electron dense deposits, with no deposits along the tubular basement membranes (**Figures 3A–C**).

The patient was treated with 6 cycles (cycle length = 28 days) of cyclophosphamide-bortezomib-dexamethasone regimen (CBD, also referred to as CyBorD or VCD) and received: cyclophosphamide 350 mg per os on days 1, 8, 15 + bortezomib 1.3 mg/m2 subcutaneously on days 1, 8, 15, 22 + dexamethasone 20 mg per os on days 1, 8, 15. Then, he received 3 cycles (cycle length = 28 days) of bortezomib-dexamethasone regimen (VD): bortezomib 1.3 mg/m2 subcutaneously on days 1, 8, 15, 22 + dexamethasone 20 mg per os on days 1, 8, 15. She also received acyclovir and trimethoprim-sulfamethoxazole prophylaxis for 12 months. No adverse effects related to the cytotoxic therapy were observed.

Renal biopsy was repeated after 12 months which showed: a remarkable reduction of the membranoproliferative lesions with disappearance of the capillary leukocyte infiltration and persistent mesangial expansion (**Figures 1D–F**); substantial negative staining for C3 and k and λ-light chain (**Figures 2D–F**); complete disappearance of the subendothelial and mesangial deposits on ultrastructural examination (**Figures 3D–F**).

The laboratory investigations at 12 and 24 months showed substantial renal function improvement, and normalization of proteinuria, serum k/λ free light chain concentration, and hemoglobin levels (**Table 1**).

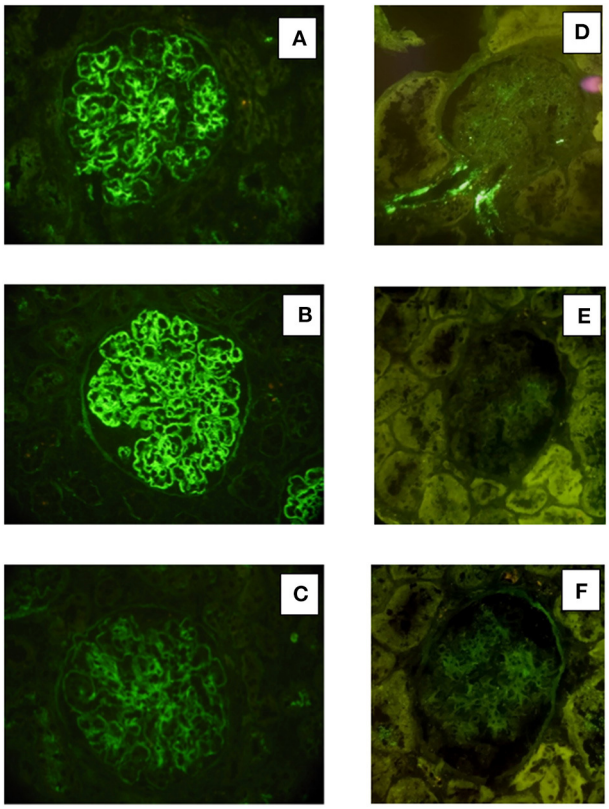

FIGURE 2 | Immunofluorescence findings in patient #1: the first biopsy showed bright positivity (3+) for C3 **(A)** and k-light chain **(B)** involving the glomerular capillary walls and the mesangium, while it resulted negative for λ-light chain **(C)**. The repeat biopsy showed substantial negative staining for C3 **(D)** and k and λ -light chain **(E, F)**.

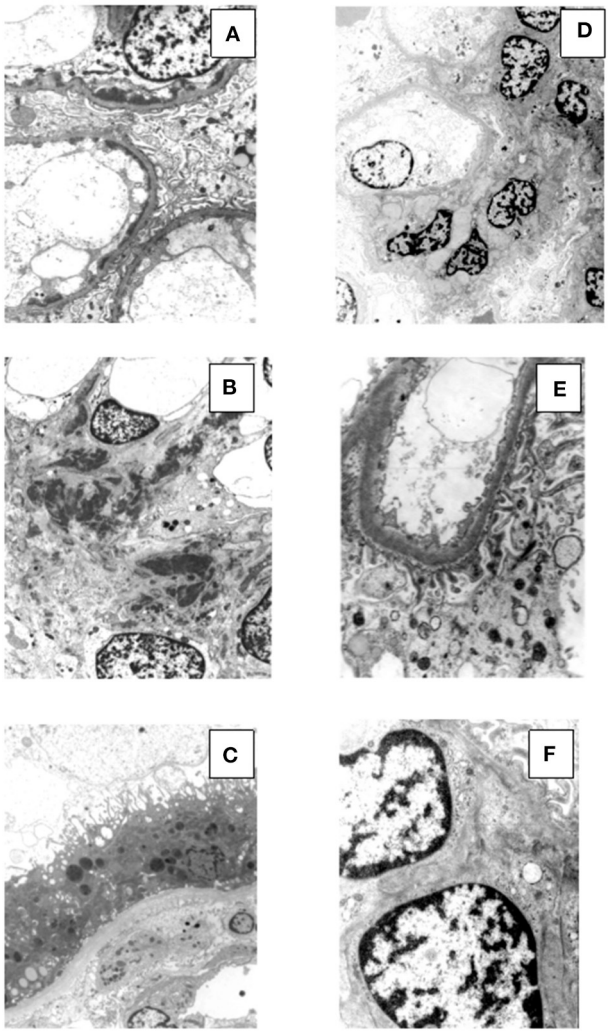

FIGURE 3 | Electron microscopy findings in patient #1: the first biopsy showed subendothelial (**A**, original magnification, x3900) and mesangial (**B**, original magnification, x2950) electron dense deposits, while no deposits were seen along the tubular basement membranes (**C**, original magnification, x2950). The repeat biopsy showed complete disappearance of the subendothelial and mesangial deposits. (**D**, original magnification, x2200; **E**, original magnification, x6610; **F**, original magnification, x8900).

Case 2

A 71 year old man with a history of recent onset hypertension presented to the hospital for evaluation of moderate renal failure (eGFR = 37 ml/min per 1.72 m2) and proteinuria (1.5 g/d) discovered 4 months earlier.

On presentation, he was mildly tachypneic (oxygen saturation 90% while breathing ambient air) and had moderate lower limb edema. Laboratory investigations showed (**Table 1**): severely reduced eGFR (10 ml/min per 1.73 m2), nephrotic proteinuria (7.0 g/d) and microscopic hematuria with red blood cell casts in the urine sediment; mild hypocalcemia; normal complement levels; negative cryoglobulins; moderately reduced total protein and immunoglobulin G levels; normocytic anemia (hemoglobin, 10.5 g/dl); a small amount (<10%) of λ monoclonal immunoglobulin on serum immunofixation (k/λ ratio = 2.1), with negative urine immunofixation.

Real-time ultrasound-guided percutaneous renal biopsy was performed, followed by bone marrow aspirate and biopsy.

Renal tissue specimens were examined by light microscopy, immunofluorescence, and electron microscopy. Light microscopy showed membranoproliferative glomerulonephritis with nodular mesangial expansion, occasional protein thrombi and numerous endocapillary infiltrating mononuclear and polymorphonuclear leukocytes (**Figures 4A–C**). Immunofluorescence demonstrated positive, coarsely granular, staining for C3 (3+), IgG (2+), C1q (1+) and λ-light chain (3+) involving glomerular capillary walls and, occasionally, the mesangium, with negative staining for k-light chain (**Figures 5A–C**). Ultrastructural evaluation showed large subendothelial, intramembranous, and mesangial electron dense deposits composed of microtubules (27–30 nm) with hollow centers, organized in parallel arrays (**Figures 6A–C**) consistent with immunotactoid glomerulonephritis.

Bone marrow aspirate and biopsy were examined by light microscopy, immunohistochemistry, and flow cytometry, and only showed 6% λ-restricted plasma cells.

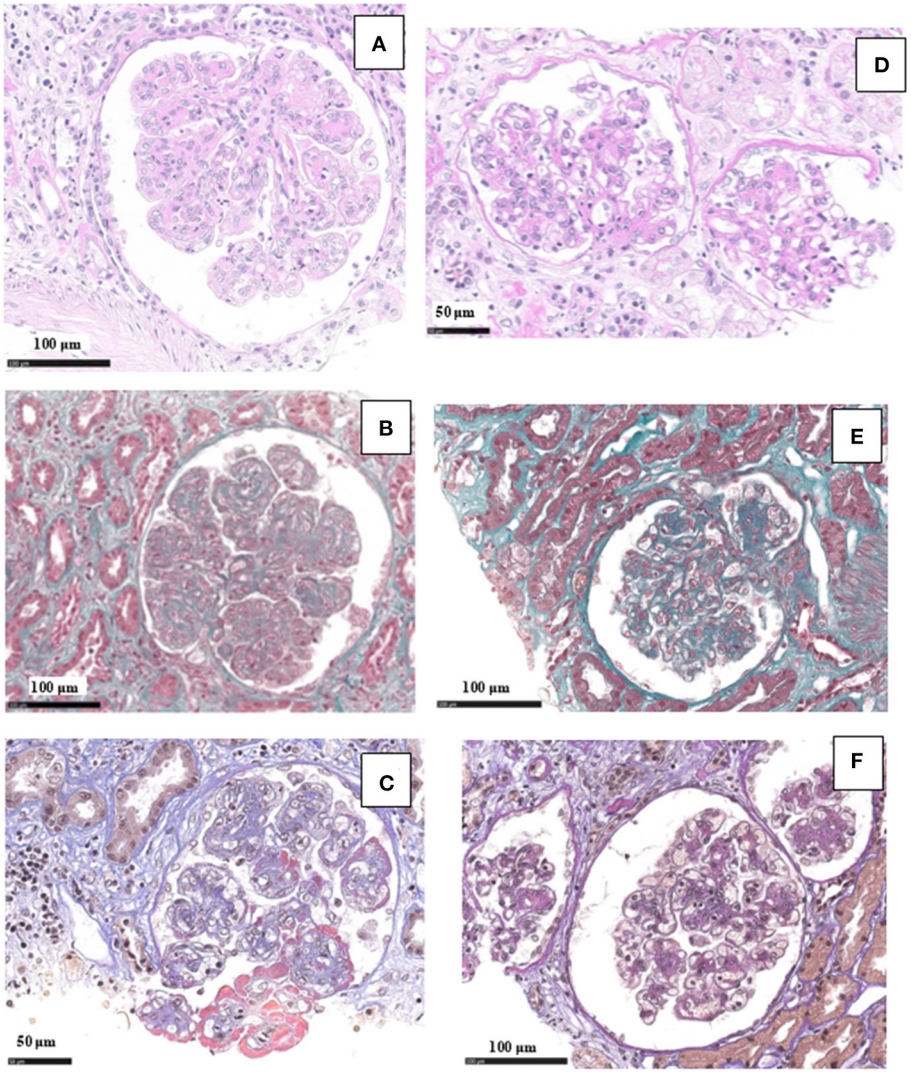

FIGURE 4 | Light microscopy findings in patient #2: the first biopsy showed membranoproliferative glomerulonephritis with nodular mesangial expansion, and infiltrating mononuclear and polymorphonuclear leukocytes (**A**, PAS; **B**, Masson Trichrome; **C**, wide deposits along the glomerular basement membranes appear pink on AFOG staining). The repeat biopsy showed a remarkable reduction of the membranoproliferative lesions with disappearance of the capillary leukocyte infiltration and persistent mesangial expansion (**D**, PAS; **E**, Masson Trichrome; **F**, small mesangial deposits on AFOG staining).

The patient was treated with four cycles (cycle length = 28 days) of cyclophosphamide-bortezomib-dexamethasone regimen and received: cyclophosphamide 200 mg per os on days 1, 8, 15 + bortezomib 1.3 mg/m2 subcutaneously on days 1, 8, 15, 22 + dexamethasone 20 mg per os on days 1, 8, 15. Then, he received 5 cycles (cycle length = 28 days) of bortezomib-dexamethasone regimen: bortezomib 1.3 mg/m2 subcutaneously on days 1, 8, 15, 22 + dexamethasone 20 mg per os on days 1, 8, 15. He also received isoniazid prophylaxis for 6 months due to a positive QuantiFERON-TB Gold test result and acyclovir prophylaxis for 12 months. No adverse effects related to the cytotoxic therapy were observed.

Renal biopsy was repeated after 12 months and showed a significant reduction of the membranoproliferative lesions, disappearance of the capillary leukocyte infiltration and of the protein thrombi, and persistent but less evident nodular mesangial expansion (**Figures 4D–F**). On immunofluorescence there was less evident glomerular capillary wall and mesangial staining for C3 (2+) with complete disappearance of λ-light chain deposition (**Figures 5D,E**). Electron microscopy demonstrated nearly complete disappearance of the subendothelial, intramembranous and mesangial electron dense, microtubular deposits (**Figures 6D–F**).

The laboratory investigations at 12 and 24 months showed substantial renal function improvement, and normalization of proteinuria, serum k/λ free light chain concentration, and calcium levels (**Table 1**).

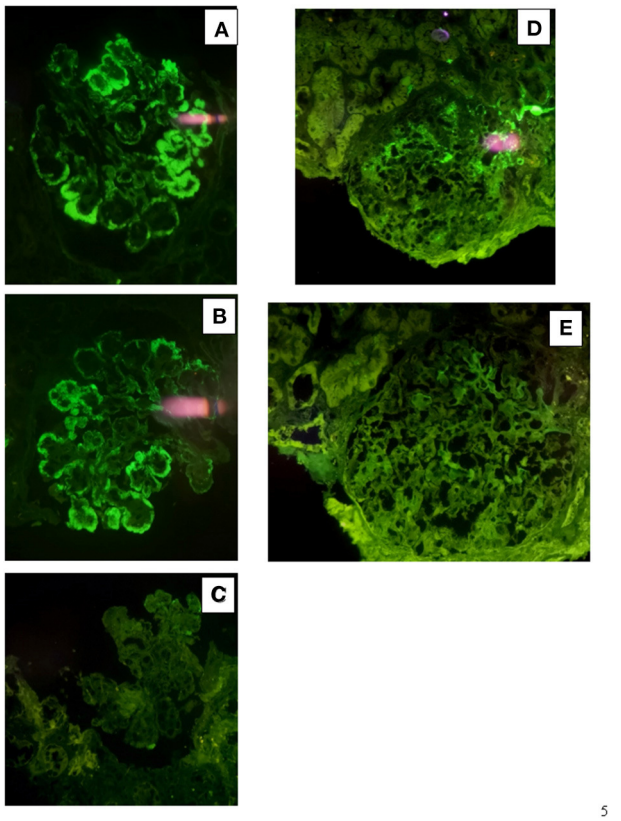

FIGURE 5 | Immunofluorescence findings in patient #2: the first biopsy showed positive, coarsely granular, staining for C3 (3+), IgG (2+/3+, **A**), C1q (1+) and λ-light chain (3+, **B**) involving glomerular capillary walls and, occasionally, the mesangium, while it resulted negative for k-light chain **(C)**. The repeat biopsy showed less evident glomerular capillary wall and mesangial staining for C3 (2+, **D**) with complete disappearance of λ-light chain deposition **(E)**.

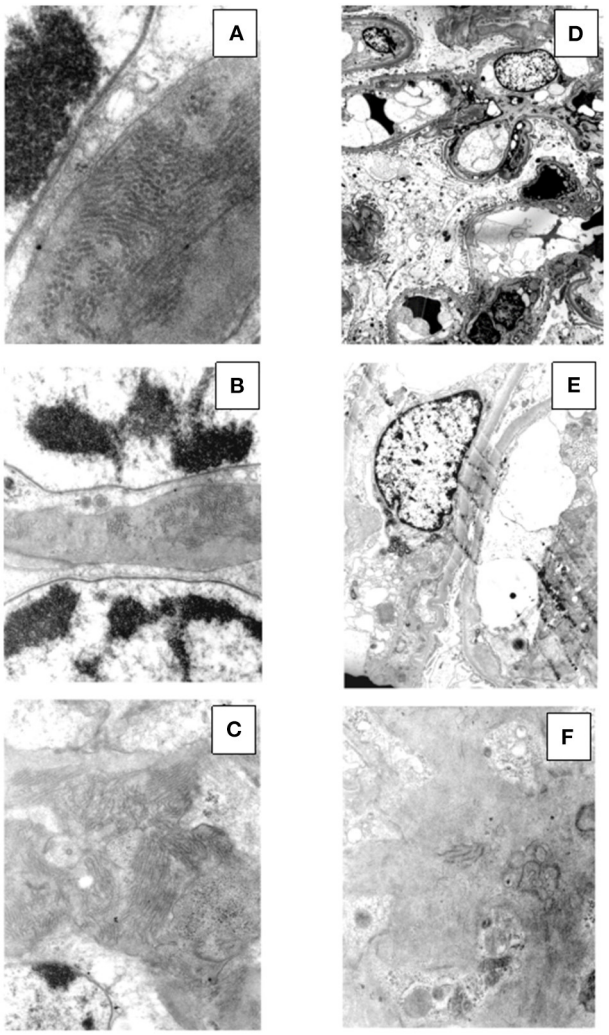

FIGURE 6 | Ultrastructural findings in patient #2: the first biopsy showed large subendothelial, intramembranous, and mesangial electron dense deposits composed of microtubules (27–30 nm) with hollow centers, organized in parallel arrays (**A**, original magnification, x39000; **B**, original magnification x15500; **C**, original magnification, x15500). The repeat biopsy showed the nearly complete disappearance of the subendothelial, intramembranous and mesangial electron dense, microtubular deposits (**D**, original magnification, x1650; **E**, original magnification, x3900; **F**, original magnification, x11500: small occasional residual deposits).

DISCUSSION

The clinical presentation of MGRS is broad and encompasses variable combinations of proteinuria, hematuria, acute or chronic renal insufficiency, hypertension, and hypocomplementemia (2, 4). Similarly, the histological patterns of kidney involvement in MGRS may be extremely complex and heterogeneous and can result from the direct deposition of nephrotoxic monoclonal immunoglobulin or its fragments as well as from indirect mechanisms. Therefore, according to the IKMG recommendations, kidney biopsy is mandatory for a correct diagnosis (3). Once an MGRS is diagnosed or suspected on the basis of renal pathology a detailed hematologic workup is essential to link the presence of the paraprotein to the associated renal disease and consequently to better define the most adequate therapy (7).

In this report, we present two cases of very aggressive clinical and histological MGRS successfully treated with a combined chemotherapy regimen. Renal biopsy carried out on the first patient showed proliferative glomerulonephritis with monoclonal k-light chain and C3 deposits. It should be emphasized that the texture of the staining was granular and not linear as observed in monoclonal immunoglobulin deposition disease (MIDD). This case represents monoclonal immunoglobulin-associated proliferative glomerulonephritis characterized by the presence of only monotypic light chain without associated heavy chain. The monoclonal immunoglobulin often includes heavy-chain IgG, less commonly IgM or rarely IgA, with k or λ-light chain restriction. Rarely

only heavy or light chain may be evident (8). Very recently a series of 17 patients with proliferative glomerulonephritis with deposition of monoclonal immunoglobulin light chain only (PGNMID-light chain) has been described (9). In this series, by immunofluorescence, deposits were composed of restricted light chain (kappa in 71% of cases) and C3, as in our patient. Furthermore, patients with PGNMID-light chain showed higher frequency of a detectable pathogenic plasma cell clone and of abnormal serum free light chain compared to PGNMID with IgG deposits, supporting in these cases the necessity of bone marrow evaluation, including flow cytometry and immunohistochemical analysis (10). The second patient showed immunotactoid glomerulopathy. Both patients presented with renal failure and subnephrotic or nephrotic proteinuria, both had <10% plasma cells in their bone marrow biopsy, and neither of them had myeloma-defining events. In one patient a scanty amount of k free light chain was detected in urine immunofixation, and in the other a small amount of λ monoclonal immunoglobulin was found on the serum immunofixation. The case report regarding the first patient has been published in part in the Giornale Italiano di Nefrologia (11).

The treatment of MGRS is directed at the underlying B-cell or plasma cell clones and is based on a combination of various chemotherapy agents, aiming to preserve kidney function and prevent recurrence after kidney transplantation (7, 12–14). Autologous stem cell transplant may benefit selected patients (14). No formal guidelines exist for this disease and different regimens have been described. However, expert opinion/consensus-based treatment decisions can guide clinical practice (7, 15–18).

Based on the good results reported in different histological patterns of MGRS (7, 12–20), we decided to employ a bortezomib-based regimen, directed against the pathologic clone. Bortezomib is a proteasome inhibitor with a non-renal metabolism. Other proteasome inhibitors are currently available, but bortezomib has the most robust data in the treatment of MGRS. Therefore, both patients received a 9-month course of combined bortezomib-based chemotherapy. It is worth noting that patient 2 showed rapid improvement of renal function and proteinuria, so we decided to only administer four cycles of cyclophosphamide (instead of six) to reduce the risk of tuberculosis reactivation, due to his positive QuantiFERON-TB Gold test result.

The chemotherapy regimen resulted in substantial renal functional improvement, complete normalization of proteinuria and disappearance of serum and urine clones in both patients. We decided to repeat the renal biopsy after 12 months to evaluate the histological evolution of the kidney injury and to define the need for further treatment. The repeat biopsies actually showed a dramatic improvement of the proliferative lesions and the complete disappearance of the capillary wall and mesangial deposits, suggesting a very good histological response to the regimen we employed, therefore no further chemotherapy was prescribed.

The laboratory investigations at 24 months confirmed complete biochemical remission. No adverse effects were reported.

These two cases suggest that targeting plasma cell clones responsible for producing a paraprotein causing kidney injury may be an appropriate strategy when treating patients with MGRS, regardless of the histological pattern of renal disease. It is worth noting that while treatment with bortezomib-based regimens is commonly adopted in monoclonal gammopathy associated proliferative glomerulonephritis (7, 12–20), such regimens are only occasionally reported in MGRS with an immunotactoid pattern (21), where anti-CD20 therapy is frequently adopted as many cases are related to a B cell proliferative disorder. In our two patients the bortezomib-based regimen was probably very effective at least in part because of the presence of a mildly increased number of plasma cells in bone marrow biopsies.

A major strength of this report is that it is the first to include MGRS repeat renal biopsies at the end of therapy. Re-examination of histology may be useful in providing insight into the evolution of kidney injury, as well as in guiding the decision about further therapeutic approaches or, as in our two patients, in supporting discontinuation of therapy.

MGRS may be a progressive disease that, if untreated, can lead to end stage renal failure and recur after renal transplantation (2, 13, 15, 20). No randomized controlled trials exist to guide the optimal approach to therapy, but bortezomib-based regimens are frequently used.

In our two patients with aggressive renal disease, combination therapy of bortezomib, cyclophosphamide and dexamethasone proved to be extremely effective and safe.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

GQ, MM, and DR were responsible for the research idea and study design. AB was responsible for the histological evaluation. MPa, MPi, and AV were responsible for the hematological follow-up and data analysis/interpretation. GDV and EDS were responsible for data acquisition. RF and MF were responsible for revising the article. Each author contributed important intellectual content during manuscript drafting or revision, accepts personal accountability for the author's own contributions and agrees to ensure that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

REFERENCES

1. Leung N, Bridoux F, Hutchison CA, Nasr SH, Cockwell P, Fermand J-P, et al. Monoclonal gammopathy of renal significance: when MGUS is no longer undetermined or insignificant. *Blood.* (2012) 120:4292–5. doi: 10.1182/blood-2012-07-445304
2. Bridoux F, Leung N, Hutchison CA, Touchard G, Sethi S, Fermand J-P, et al. Diagnosis of monoclonal gammopathy of renal significance. *Kidney Int.* (2015) 87:698–711. doi: 10.1038/ki.2014.408
3. Leung N, Bridoux F, Batuman V, Chaidos A, Cockwell P, D'Agati VD, et al. The evaluation of monoclonal gammopathy of renal significance: a consensus report of the International Kidney and Monoclonal Gammopathy Research Group. *Nat Rev Nephrol.* (2019) 15:45–59. doi: 10.1038/s41581-018-0077-4
4. Sethi S, Rajkumar SV, D'Agati VD. The complexity and heterogeneity of monoclonal immunoglobulin-associated renal diseases. *J Am Soc Nephrol.* (2018) 29:1810–23. doi: 10.1681/ASN.2017121319
5. Hutchison CA, Xiong F, Mollee P. The treatment of paraprotein-related kidney disease. *Curr Opin Nephrol Hypertens.* (2017) 26:477–83. doi: 10.1097/MNH.0000000000000369
6. Rosner MH, Perazella MA. Acute kidney injury in the patient with cancer. *Kidney Res Clin Pract.* (2019) 38:295–308. doi: 10.23876/j.krcp.19.042
7. Fernand JP, Bridoux F, Kyle RA, Kastritis E, Weiss BM, Cook MA, et al. How I treat monoclonal gammopathy of renal significance (MGRS). *Blood.* (2013) 122:3583–90. doi: 10.1182/blood-2013-05-495929
8. Sethi S, Fervenza FC, Rajkumar SV. Spectrum of manifestations of monoclonal gammopathy-associated renal lesions. *Curr Opin Nephrol Hypertens.* (2016) 25:127–37. doi: 10.1097/MNH.0000000000000201
9. Nasr SH, Larsen CP, Sirac C, Theis JD, Domenger C, Chauvet S, et al. Light chain only variant of proliferative glomerulonephritis with monoclonal immunoglobulin deposits is associated with a high detection rate of the pathogenic plasma cell clone. *Kidney Int.* (2020) 97:589–601. doi: 10.1016/j.kint.2019.10.025
10. Bhutani G, Nasr SH, Said SM, Sethi S, Fervenza FC, Morice WG, et al. Hematologic characteristics of proliferative glomerulonephritides with nonorganized monoclonal immunoglobulin deposits. *Mayo Clin Proc.* (2015) 90:587–96. doi: 10.1016/j.mayocp.2015.01.024
11. Manes M, Radin E, Pellu' V, Molino AA, Gabrielli D, Parodi E, et al. Monoclonal gammopathy of renal significance and membranoproliferative glomerulonephritis: a complex relationship with promising therapeutic opportunities. *G Ital Nefrol.* (2019) 36:2019–vol4.
12. Rosner MH, Adeani A, Yanagita M, Glezerman IG, Leung N. Paraprotein-related kidney diasease: diagnosing and treating monoclonal gammopathy of renale significance. *Clin J Am Soc Nephrol.* (2016) 11:2280–7. doi: 10.2215/CJN.02920316
13. Hogan JJ, Weiss BM. Bridging the divide: an Onco-Nephrologic approach to the monoclonal gammopathies of renal significance. *Clin J Am Soc Nephrol.* (2016) 11:1681–91. doi: 10.2215/CJN.03160316
14. Batko K, Malyszko J, Jurczyszyn A, Vesole DH, Gertz MA, Leleu X, et al. The clinical implication of monoclonal gammopathies: monoclonal gammopathy of undetermined significance and of renal significance. *Nephrol Dial Transplant.* (2019) 34:1440–52. doi: 10.1093/ndt/gfy259
15. Jain A, Haynes R, Kothari J, Khera A, Soares M, Ramasamy K. Pathophysiology and management of monoclonal gammopathy of renal significance. *Blood Adv.* (2019) 3:2409–23. doi: 10.1182/bloodadvances.2019031914
16. Ravindran A, Fervenza FC, Smith RJH, Sethi S. C3 glomerulopathy associated with monoclonal Ig is a distinct subtype. *Kidney Int.* (2018) 94:178–86. doi: 10.1016/j.kint.2018.01.037
17. Gumber R, Cohen JB, Palmer MB, Kobrin SM, Vogl DT, Wasserstein AG, et al. A clone-directed approach may improve diagnosis and treatment of proliferative glomeruloinephritis with monoclonal immunoglobulin deposits. *Kidney Int.* (2018) 94:199–205. doi: 10.1016/j.kint.2018.02.020
18. Chauvet S, Frémeaux-Bacchi V, Petitprez F, Karras A, Daniel L, Burtey S, et al. Treatment of B-cell disorder improves renal outcome of patients with monoclonal gammopathy-associated C3 glomerulopathy. *Blood.* (2017) 129:1437–47. doi: 10.1182/blood-2016-08-737163
19. Sethi S, Rajkumar SV. Monoclonal gammopathy-associated proliferative glomerulonephritis. *Mayo Clin Proc.* (2013) 88:1284–93. doi: 10.1016/j.mayocp.2013.08.002
20. Amaador K, Peeters H, Minnema MC, Nguyen TQ, Dendooven A, Vos JMI, et al. Monoclonal gammopathy of renal significance (MGRS): histopathologic classification, diagnostic workup, and therapeutic options. *Neth J Med.* (2019) 77:243–54.
21. Nasr SH, Fidler ME, Cornell LD, Leung N, Cosio FG, Sheikh SS, et al. Immunotactoid glomerulopathy: clinicopatholohic and proteomic study. *Nephrol Dial Transplant.* (2012) 27:4137–46. doi: 10.1093/ndt/gfs348

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REVIEW
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Integrin, Exosome and Kidney Disease

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Integrins are transmembrane receptors that function as noncovalent heterodimers that mediate cellular adhesion and migration, cell to cell communication, and intracellular signaling activation. In kidney, latency associated peptide-transforming growth factor β (TGF-β) and soluble urokinase plasminogen activator receptor (suPAR) were found as the novel ligands of integrins that contribute to renal interstitial fibrosis and focal segmental glomerular sclerosis glomerulosclerosis (FSGS). Interestingly, recent studies revealed that integrins are the compositional cargo of exosomes. Increasing evidence suggested that exosomal integrin played critical roles in diverse pathophysiologic conditions such as tumor metastasis, neurological disorders, immunology regulation, and other processes. This review will focus on the biology and function of exosomal integrin, emphasizing its potential role in kidney disease as well as its implications in developing novel therapeutic and diagnosis approaches for kidney disease.

Keywords: exosome, integrin, extracellular vesicle, kidney disease-diagnosis, kidney disease-therapy

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INTRODUCTION

Integrins are transmembrane receptors that function as noncovalent heterodimers. There are 24 distinct integrin receptors that can recognize and bind to multiple ligands such as extracellular matrix (ECM) proteins, thereby mediating cell adhesion and intracellular signaling (Moreno-Layseca et al., 2019). Other novel ligands include latency associated peptide-transforming growth factor β (L-TGF-β) and soluble urokinase plasminogen activator receptor (suPAR) were found to bind to integrin and participated in the pathogenesis of kidney disease. Moreover, activated integrins on diverse kidney cells in pathological conditions participated in macrophage and fibroblast activation which played important roles in diverse kidney diseases.

Exosomes are small extracellular vesicles (EVs) secreted by nearly all types of cells which are originally considered to be the garbage bins of cells to excrete unwanted materials (Johnstone et al., 1987). Recently, accumulating studies have demonstrated that exosomes participated in crosstalk between cells and also mediated communications between organs. Moreover, exosomes can serve as vectors of therapeutics and facilitate disease diagnosis in a noninvasive way (Kalluri and LeBleu, 2020). Interestingly, integrins are revealed as the important compositional components of exosomes which take responsibility for those novel functions of exosomes.

The diverse pathophysiological roles of exosomal integrins varied from guiding the homing of exosomes (Hoshino et al., 2015), signal transmission (Kalappurakkal et al., 2019), causing phenotype transition of recipient cells (Lu et al., 2018) to cell adhesion (Genschmer et al., 2019) and migration (Sung et al., 2015). Although studies have revealed essential roles of exosomal integrin in oncology, neurology, and immunology, its role in kidney pathophysiology

remains unclear. Thus, exploring the role of exosomal integrin in kidney disease would be helpful in understanding the mechanism of kidney disease and identifying novel diagnosis and treatment strategies. Here, we review the biology and functions of integrin as well as integrin carried by exosomes. Pathophysiologic roles of exosomal integrin in diverse diseases are also discussed, especially the role and potential applications in therapy and diagnosis of kidney diseases.

BIOLOGY AND FUNCTION OF INTEGRIN

Structure and Endocytic Trafficking of Integrin

Integrins are transmembrane heterodimers which express conservatively in almost all cell types. Integrin family was initially discovered on immune cells and mediates leukocyte extravasation by binding to intercellular cell adhesion molecule-1 (ICAM-1) on vascular endothelial cells (capturing intravenous immune cells) (Dustin, 2019). Integrins display three distinct conformations (bent, extended close, and extended open), while the activity is usually observed in the extended conformation (Campbell et al., 2020). Integrins can be categorized into 24 subtypes formed by 18 types of α subunits and eight types of β subunits. Among them, integrin αv, α6, and β1 are known for pairing with diverse subunits (Moreno-Layseca et al., 2019). Each integrin subunit contains a large extracellular domain, single-time transmembrane domain, and often rather short cytoplasmic domain (Humphries et al., 2006). Studies have shown that certain integrin subtypes are expressed on specific tissue or cell or bind to certain types of cells. For example, integrin β6 is expressed in a few subset of epithelial cells (Breuss et al., 1993). Integrin α6β1 and integrin α6β4 targeted to lung fibroblast while integrin αvβ5 targeted liver Kupffer cell (Hoshino et al., 2015). However, the dominant subtype and the abundance of integrin in a specific cell type could change under certain injury conditions. For example, the dominant integrin subtype in podocyte is α3β1 (Kreidberg et al., 1996), while it changes into αvβ3 under focal segmental glomerulosclerosis (FSGS) situation (Hayek et al., 2017).

Although the expression levels of integrin are quite stable in certain cells and tissues, they are continuously trafficking from cytoplasm to surface membrane by diverse complex pathways including the Rab family of small GTPase (Moreno-Layseca et al., 2019). This process includes integrin endocytosis into early endosome which then traffic to late endosome and recycle to the cell surface, or alternatively transport to multivesicular bodys (MVBs) and subsequent lysosome for degradation (Rainero and Norman, 2013). Generally, majority of endocytic integrins travel back to the cell surface while small fractions target to degradation (De Franceschi et al., 2015). Interestingly, integrin endocytic trafficking process shares a common intracellular structure, MVBs, with exosome (Rainero and Norman, 2013), thus integrins could also be transported *via* exosome which has been demonstrated in recent studies. A study using gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses also revealed a correlation between integrin signaling and exosome secretion (Zhang et al., 2020).

Ligands of Integrin

Tremendous efforts have been invested in integrin ligand discovery, the well-known integrin ligands belong to ECM proteins, newly identified ligands include L-TGF-β compound and suPAR (Humphries et al., 2006; Campbell et al., 2020; Hayek et al., 2020). According to the binding motif on the ligand, integrins can be classified into five types, among which, the most common type is RGD-binding integrins which belong to αv integrins. Thus, studies have been using RGD peptide to inhibit integrin αv subtype binding (Hoshino et al., 2015). Although multiple ligands have been discovered, most of which are non-specific ligands that can bind to more than one types of integrins and mediate cell-cell adhesion in integrin A-ECM-integrin B format (Sung et al., 2015). This suggests that in studying of integrin function under certain conditions, not only specific ligands but also the existence of other integrin subtypes should be considered.

Biological Functions of Integrins

Integrins bare different biological functions according to their diverse localizations throughout the body including cellular adhesion and migration, regulation of cellular phenotypes, cell to cell communication, and intracellular signaling activation (**Table 1**).

Integrins were first reported as adhesion molecule in the immune system (Springer, 1990), which represented the basic function of integrins. Many studies have revealed that integrins mediated adhesion between cells or cell to ECM. In kidney, tubular epithelial cells bind to each other on the lateral surface through integrins and bind to ECM on the basal surface by integrins as well (Glynne et al., 2001). Integrins and ECM interaction is also important for cells that underwent polarization during differentiation. Studies have showed that integrin β1 connects ECM and cytoskeletal protein on one side of the cell, which then forms the basal membrane of cells during polarization, such as epithelial cells and endothelial cells (Lee and Streuli, 2014; Moreno-Layseca et al., 2019). Besides, dysregulation of integrin or redistribution have a great impact on cellular apical and basal polarization under injury or cancerization (Glynne et al., 2001; Liu et al., 2018a).

Moreover, integrins are associated with certain cellular phenotype and function under pathological conditions. In contrast to associated nephropathy, integrin αvβ6 increased in injured tubular cells, while remains at low baseline level in normal tubules. Interestingly, injured tubular cells with high integrin were able to bind with suPAR which caused further damage (Hayek et al., 2020). Integrins also participate in regulation of cell cycle in numerous pathways as determined by various *in vitro* and *in vivo* studies. Integrin β1, β3, and other subtypes have been well demonstrated to be involved in cellular proliferation (Panchatcharam et al., 2010; Moreno-Layseca and Streuli, 2014; Raven et al., 2017). Integrins mediate the local niche signal which forms spatial checkpoints that enable cells progress into S phase to proliferate. Reversely, some integrin subtypes or isoforms could prevent cells from progressing into cell cycle, thus inhibiting proliferation, such as integrin α6Bβ6 in colon cancer cells (Dydensborg et al., 2009).

Ligand-integrin binding leads to signaling activation intracellularly, including focal adhesion kinase (FAK), RhoA

TABLE 1 | Expression and function of major integrin subtypes.

Integrin subtype	Expression	Function	Reference
αvβ3	podocytes, endothelial, and cancer stem cells	Bind to suPAR, promote FSGS, cancer progression	Hayek et al., 2017; Nieberler et al., 2017
αvβ6	epithelial cells and tumor cells	Activate TGF-β1, tumor progression, and metastasis	Breuss et al., 1993; Nieberler et al., 2017
αvβ8	kidney glomerular mesangial cells, brain, and placenta	Activate TGF-β1, inhibit cell growth, spreading, and focal contact formation	Cambier et al., 2000; Campbell et al., 2020
β1	multiple cell types	Cell adhesion, maintain cell polarity, regulate cell proliferation, and cell cycle	Liu et al., 2018a; Kormann et al., 2020
α3β1	kidney tubular epithelial cells, glomerular endothelial cells, and podocytes	Kidney development and cell anchorage	Kreidberg et al., 1996; Glynne et al., 2001
α4β1	reticulocytes	Blood vascular related disease progression	Rieu et al., 2000
α5β1	endothelial cells and cancer stem cells	Vascular morphogenesis, cancer, and metastasis	Zovein et al., 2010; Nieberler et al., 2017
α6β1	ureteric bud	Maintains the structural integrity of the kidney collecting system	Viquez et al., 2017
α6β4	cancer cells and epithelial cells	Lung organotropic metastasis	Hoshino et al., 2015

signaling, and Glycosylphosphatidylinositol-anchored proteins (GPI-APs) nanoclustering (Kalappurakkal et al., 2019). These signals can then activate downstream processes. For example, integrin αvβ3 can regulate angiogenesis (Danhier et al., 2012) by promoting HIF-1α expression and subsequent endothelial-mesenchymal transition (EndoMT; Fan et al., 2018). Importantly, recent studies suggested that the immobilization of integrin-binding ligand and integrin conformation was essential to integrin activation (Kalappurakkal et al., 2019; Campbell et al., 2020).

INTEGRINS AS COMPOSITIONAL CARGO OF EXOSOMES

Exosomes belong to small EVs with the size of less than 200 nm. It is excreted into extracellular space and can transfer mRNA, miRNA, lipid, and protein to receptor cells, therefore, mediating crosstalk with neighbor and remote cells (Valadi et al., 2007; Paolicelli et al., 2019; Lv et al., 2020). Exosomes share most of the compositions from the parent cells with certain cargoes selectively sorting into the vesicles. Multiple proteomic studies on exosomes have revealed that proteins that usually present include both membranous protein and luminal protein (van Niel et al., 2018). Notably, integrins are the commonly identified exosome related proteins sorted from the parent cell. Integrin can be transported by exosomes in tumor (Quaglia et al., 2020), the central nervous system (Zhang et al., 2020) or the immune system (Genschmer et al., 2019). As the common compositional cargo of exosomes, integrins were identified as one of the critical functional cargoes of exosomes in different pathophysiological conditions.

PATHOPHYSIOLOGIC ROLES OF INTEGRINS TRANSPORTED BY EXOSOMES

Exosomal integrin is a versatile form that functions actively in different pathophysiological conditions (**Table 2**) which attributed to guiding the homing of exosomes, signal transmission, phenotype transition of the recipient cells, and cell adhesion and migration (**Figure 1**). Here, the roles of exosomal integrins in tumor, neurological disorders, immunology, and other diseases were discussed.

Tumor Formation, Progression and Metastasis

In recent years, the role of exosomes in mediating tumor formation and progression has been well studied (Li et al., 2019), integrins are among the important cargoes contributing to the process. It is reported that integrin αvβ3 containing small EVs from prostate cancer cells was transferred to recipient cancer cells which induced aggressive phenotype changes (Quaglia et al., 2020). Cancer cell migration was proved to be mediated by autocrine secretion of exosomes. Fibrosarcoma cells-derived exosomal integrins and fibronectin forms adhesion assembly that mediated adhesion between cell and ECM, therefore, promoted cell motility with specific directions (Sung et al., 2015). Fibronectin was coated on exosome through binding with exosomal integrin, which then anchor to cell membrane on one side and ECM on the other and contributed to cellular adhesion (Sung et al., 2015). Moreover, it is demonstrated that exosomes transfer integrin αvβ6 from cancer cells to monocytes, which promoted M2 monocyte polarization and prostate cancer progression (Lu et al., 2018).

Besides, integrins on tumor-derived exosomes can determine organotropic metastasis by forming tumor microenvironment (TME) in specific organ tissues as they uptake the exosomes (Hoshino et al., 2015). They demonstrated the specific integrin subtype directed organotropic metastasis for the first time, such as integrins α6β4 and α6β1 mediated lung metastasis, while integrin αvβ5 mediated liver metastasis (Hoshino et al., 2015). The targeting properties of exosomal integrin were attributed to the activation of the Src-S100A4 axis (cancer associated genes) by exosomal ITGα6β4 in lung fibroblasts during pre-metastatic niche formation (Hoshino et al., 2015). These findings indicated the great therapeutic potential by targeting certain integrin subtype that was involved in tumor metastasis.

TABLE 2 | Diverse functions of exosomal integrin.

Diseases/process	Pathophysiologic role of exosomal integrin	Reference
Oncology		
Tumor metastasis	Organotropic metastasis and tumor microenvironment formation	Hoshino et al., 2015
Prostate cancer	Cell migration and induce integrin expression through uptake of exosomes	Fedele et al., 2015
Prostate cancer	Macrophage polarization and transferring αvβ6 integrin from cancer cells to monocytes through exosomes	Lu et al., 2018
Prostate cancer	Determined cargo loading of exosomes which promoted cancer cell formation	Quaglia et al., 2020
Fibrosarcoma	Promoted cell migration	Sung et al., 2015
Neurology		
Demyelination	Oligodendrocyte precursor cells proliferation	Zhang et al., 2020
CNS diseases	Therapeutic protein delivery, exosome uptake, and spread of viral proteins to the brain	Yuan et al., 2017
Pulmonary		
COPD	Mediated exosome adhesion to extracellular matrix	Genschmer et al., 2019
Gut		
lymphocyte homing	Exosomal integrin α4β7 target high endothelial venule (HEV) endothelial cells causing diminish in lymphocyte homing niche	Myint et al., 2020

Neurological Disorders

Exosomal integrins participate in the development of neurological disorders and are active in multiple trans-cellular communication processes. Proteomic analysis proved that integrin αvβ3 was upregulated in interleukin-1β (IL-1β) stimulated astrocyte-derived EVs (ADEV) and significantly increased uptake of ADEV in neurons, while integrin αvβ3 blocking partially suppressed this process (You et al., 2020). Exosomal integrins also contributed to the recovery of central nervous system (CNS) degenerative diseases, which was associated with the proliferation of oligodendrocyte precursor cells (OPCs; Zhang et al., 2020). Moreover, in therapeutic studies, macrophage derived exosome contained the integrin subtype lymphocyte function-associated antigen 1 (LFA-1). This facilitated macrophage derived exosome to overcome the blood-brain barrier and deliver therapeutic protein cargos specifically to treat CNS diseases (Yuan et al., 2017).

Immunology Regulation and Others

Integrin was first discovered as adhesion molecules in immune cells that mediated extravasation (Springer, 1994). Similar to forming TMV for tumor metastasis, exosomal integrins were also involved in immune cell homing (Myint et al., 2020). Study showed that integrin α4β7 on T cell-derived exosomes guided the exosomes homing to the intestine through binding to mucosal addressin cell adhesion molecule-1 (MAdCAM-1; Mora et al., 2003). On the other hand, integrin α4β7-expressing T cell exosomes could suppress MAdCAM-1 expression which, therefore, inhibited subsequent lymphocyte homing to the gut (Park et al., 2019).

Besides, activated polymorphonuclear leukocyte (PMN) derived exosomes were capable of targeting ECM through MAC-1 (αMβ2 integrin). This caused activation of neutrophil elastase (NE) that was coated on exosomes and lead to ECM degradation (Genschmer et al., 2019). Moreover, during reticulocyte maturation, integrin α4β1, that expressed commonly on the surface of reticulocyte, was cleared from the reticulocyte through exosome secretion. This reduced the risk of blood circulation complications, such as sickle-cell anemia, caused by integrin α4β1 on reticulocytes (Rieu et al., 2000).

INTEGRINS IN KIDNEY DISEASES

Integrins Expression in Renal Cells

Studies have revealed that integrins are expressed on various types of cells in the kidney including tubular epithelial cell (TECs) (Zhu et al., 2020), fibroblast (Bon et al., 2019), and podocyte (Hayek et al., 2017).

Tubular epithelial cells are the primary cellular component of kidney which is susceptible to diverse injuries (Liu et al., 2018b). TECs express αv and β1 integrins under normal conditions (Bon et al., 2019), while integrin αv, β1, and β6 are the dominant subtypes with injury (Hayek et al., 2020). *ITGβ6* (gene of integrin β6) was rarely identified in normal TECs but rapidly increased in the format of αvβ6 subtype under injury. Moreover, a study of clinic kidney biopsy concluded that integrin β6 was elevated in the distal tubules in diverse diseased and transplanted kidney (Trevillian et al., 2004). Highly expressed β1 integrins are known to be involved in epithelial cell polarization which traffic from basal membrane to apical membrane under injury (Glynne et al., 2001). This could result in detachment of TECs from basal ECM and impairment of polarization, which caused further injury of tubules and dysregulation of cell secretion, since integrins played a key role in delivering molecules to the right subcellular compartments (Moreno-Layseca et al., 2019).

Fibroblasts are one of the main cellular components in renal interstitial fibrosis, they can migrate to damaged site, transform into myofibroblasts, and produce ECM. Fibroblasts normally express integrin α1, α4, α5, and β1 and turn into integrin α5, β1, and αv under fibrosis situations (Norman and Fine, 1999), among which integrin αv was the dominant type (Bon et al., 2019). Integrin α5 facilitates fibroblasts migration through binding to ECM (Lobert et al., 2010). Interestingly, integrin αv expressed by fibroblasts binds to latent-TGF-β and stimulates subsequent tissue fibrosis (Henderson et al., 2013). This relation between integrin and fibroblasts also presents in other organs such as colon (Peng et al., 2018), skin (van Caam et al., 2020), lung, liver (Reed et al., 2015), and pancreatic duct (Cavaco et al., 2019).

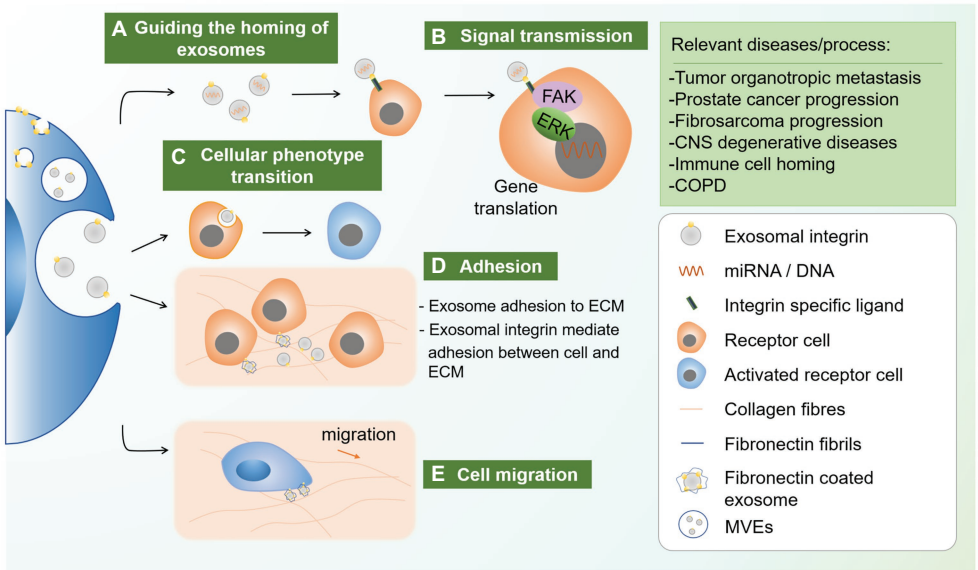

FIGURE 1 | Novel functions of exosomal integrin. Exosomal integrin played diverse roles in different disease conditions including guiding the homing of exosomes, signal transmission, cellular phenotype transition, and cellular adhesion and migration. (a) Exosome cargoes such as miRNA and protein can be delivered to neigbor and distant cells, while specific type of exosomal integrin can guide the vesicles to specific cells through integrin-ligand recognition. (b) Integrin-ligand interatction could activate intracellular signals, for example FAK and ERK-1/2, which initiate relevant gene translation. (c) Apart from intracellular signaling, endocytosis of specific exosomal integrin could also cause cellular phenotype transition of the receipient cells. (d) Exosomal integrin mediated exosome adhesion to extracellular matrix (ECM) through integrin-ECM binding. (e) Moreover, ECM, for example, fibronectin, could be coated on exosome through a process involving endocytosis of integrin αvβ1-fibronectin complex which then sorted into MVEs. FN-coated exosomes secreted and bind to collagen fibrils, which can then coupled to cellular integrin receptors. This adhesion ensures the stable adhesion to ECM during migration. CNS, central nervous system; COPD, chronic obstructive pulmonary diseases; MVEs, multivesicular endosomes.

Podocytes are special for their foot processes and integrin α3 plays a critical role in its maturation. *In vivo* study showed that the mutation of murine integrin α3 gene caused abnormal kidney and lung development (Kreidberg et al., 1996). Studies reported that activated β3 integrin on podocytes could initiate FSGS pathology in a suPAR-APOL1-integrin αvβ3 tripartite complex dependent manner. The underlie mechanisms included autophagosomes formation, actin cytoskeleton dysregulation, and cell detachment (Wei et al., 2011).

Novel Roles of Integrins in Kidney Disease

Recent studies have showed that integrins bind with novel molecules and drive subsequent signaling pathways, including TGF-β and suPAR. Distinct integrins bind with latent-TGF-β which activates TGF-β and downstream signals, such as Smad2/3. These signals can promote interstitial fibrosis in chronic kidney disease (CKD; Meng et al., 2016) and suppress TEC proliferation after injury in acute kidney injury (AKI; Yang et al., 2019). It was demonstrated that the increased TGF-β signaling was initiated in the early stage of AKI which continuously expressed during recovery stage. TGF-β expression in the tubules was companied by integrin β6 and lead to subsequent interstitial fibrosis (Geng et al., 2012). In this regard, integrins may play a prominent role in AKI to CKD transition by activation of TGF-β.

Unlike ECM or TGF-β, suPAR is not stabilized, it is the released version of the podocyte urokinase receptor (uPAR), which function as the cellular receptor for urokinase. suPAR exists in the circulatory system and its increased concentration is associated with acute (Hayek et al., 2020) and chronic kidney injuries (Hayek et al., 2017). Several studies have reported that suPAR primarily binds with β3 integrin on the surface of podocytes (Wei et al., 2011) by way of a tripartite complex of suPAR-APOL1 risk variants-integrin β3 (Hayek et al., 2017). Meanwhile, suPAR bind to TECs through integrin β6 under injured conditions and activated Rac1, which bound to SRp40 at the 5′ end of exon 7 in versican pre-mRNA. Versican then resulted in subsequent fibroblast activation and promoted interstitial fibrosis by activating the CD44/Smad3 pathway (Han et al., 2019). Moreover, suPAR could bind to integrin β1 and β2 which promoted inflammation and tumor progression (Simon et al., 2000).

Potential Function of Exosomal Integrins in Kidney Disease

Studies from our group and others have demonstrated that TEC released exosomes mediated cross-talk with fibroblasts (Guan et al., 2020) and macrophages (Lv et al., 2020) which contributed to renal inflammation and fibrosis. However, the traveling direction of TEC exosomes to specific cells remains largely unknown. Since integrins are the common compositional cargoes of exosomes, it is reasonable to speculate that integrin may be critical for directing the fate of the exosomes. Indeed,

our study showed that integrin αLβ2 (LFA-1) and α4β1 (VAL-4) on exosomes enabled them to adhere to the inflamed kidney (Tang et al., 2019). Thus, integrin on exosomes may be critical for guiding the traveling of TECs exosomes and mediated the cross-talk with specific recipient cells. Besides, due to the critical role of integrins such as integrin αvβ6 and β1 on TECs, integrin carried by exosomes may play an important role in interstitial inflammation and fibrosis. Moreover, podocytes express integrin β3 that binds with soluble particle suPAR (Wei et al., 2011), thus, podocyte may secrete exosomes with β3 integrins and meditate cellular communication in kidney disease.

Since integrin intracellular trafficking shares multiple pathways with exosome packing and releasing, integrin may also be involve in exosome generation (Rainero and Norman, 2013). Knockdown of integrin β4 decreased the concentration of exosomes in the cultured OPCs supernatant and the capacity to proliferate, while supplement of exosomes reversed this capacity (Zhang et al., 2020). This strongly indicated the critical role of integrin in exosome generation and function. Therefore, the role of integrins in exosomes release and cargo loading for kidney cells under pathologic conditions deserve further investigation.

Hence, as the compositional cargo, integrins carried by exosome may mediate specific cell-crosstalk which participate in the pathophysiological process of the kidney.

INTEGRINS CARRIED BY EXOSOMES IN THE TARGET THERAPY AND DIAGNOSIS OF DISEASE

Currently, exosome has been demonstrated as the promising engineered nanocarriers in therapy of disease due to its low immunogenicity, biological barrier permeability, and intrinsic targeting properties (Tang et al., 2020). Studies have showed that exosomal integrins may contribute to the properties of targeting delivery of exosomes. Integrins naturally expressed on exosomes could be used to realize target exosome therapy. Recently, it was found that macrophage-derived EV migrated toward inflamed endothelial cells which was mediated by integrin αLβ2 and integrin α4β1 on EVs in kidney (Tang et al., 2019) and brain (Yuan et al., 2017), respectively. Proteomic analysis of macrophage-derived micro vesicle (MV) carried with dexamethasone revealed that integrin αLβ2 (LFA-1) and α4β1 (VAL-4) express distinctly on the surface, which could efficiently direct MV to the inflamed kidney through recognizing ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1) (Tang et al., 2019).

Since exosomal integrins are important for tumor metastasis, it might hold promise in targeted drug delivery for tumor (Qiao et al., 2020). It is demonstrated that non-small cell lung cancer cells could specifically uptake breast cancer (MDA-MB-231) cell-derived exosomes (231-Exo), which was loaded with mRNA-126 that successfully inhibited lung metastasis *in vivo* (Nie et al., 2020). This organotropic process was depended on integrin β4-exosome that specifically targeted surfactant protein C (SPC) on cancer cells. Moreover, EVs can be engineered to express integrin for target therapy. For example, a study used click chemistry method to conjugate integrin αvβ3-specific cRGD peptides to the surface of exosomes. The results showed the engineered exosome efficiently targeted to injured areas in the brain (Tian et al., 2018).

Based on the discovery of disease-specific integrin by various exosome proteomic studies, detection of different types of integrin carried by exosomes could be novel biomarkers of diseases. It was found that integrin is among the top 100 protein in urinary EVs proteomic studies including AKI, FSGS, autosomal dominant polycystic kidney disease (ADPKD), etc. (Merchant et al., 2017). According to proteomic analysis, integrin on urinary exosomes showed strong correlation with kidney diseases. For example, integrin signaling was identified as the most canonical represented signaling pathways correlated with inherited glomerular diseases by way of ingenuity pathway analysis (Hogan et al., 2014).

CONCLUSIONS AND PERSPECTIVES

Exosomal integrin played diverse roles in different disease conditions *via* mediating intercellular crosstalk. Integrins are essential for normal cellular adhesion and polarization, while specific pathogenic subtypes of integrins have the potential to trigger renal inflammation and fibrosis *via* activating TGF-β, epithelial-mesenchymal transition (EMT) signaling, FAK and mitogen-activated protein kinases (MAPKs). However, the role of exosomal integrin in kidney disease remains largely unknown. Exosomal integrin may contribute to the injury and repair processes of kidney disease as the novel format of integrin *via* mediating cellular communication and downstream signaling activation. In addition, integrins may also hold the potential to participate in intracellular exosome secretion and cargo loading which may provide a promising approach for engineering of exosome for diagnosis and therapeutic purpose.

The guiding effect of specific exosomal integrin was demonstrated in tumor or immune cells. Despite that integrin can direct the destination of exosomes, the underlie mechanism require further investigation. Nevertheless, the guiding effect of exosomal integrin provided an important pathway for developing target therapy for kidney diseases. Further investigation in the role of diverse exosomal integrin subtypes in cellular communication may allow the construction of specific targeting exosome for precise treatment of kidney disease.

AUTHOR CONTRIBUTIONS

A-RS wrote the manuscript. L-LL conceived the concept and contributed to the writing of the manuscript. All authors contributed to the literature review and approved the submitted version.

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REFERENCES

Bon, H., Hales, P., Lumb, S., Holdsworth, G., Johnson, T., Qureshi, O., et al. (2019). Spontaneous extracellular matrix accumulation in a human in vitro model of renal fibrosis is mediated by alphaV integrins. *Nephron* 142, 328–350. doi: 10.1159/000499506

Breuss, J. M., Giuelt, N., Lu, L., Sheppard, D., and Pytela, R. (1993). Restricted distribution of integrin P6 mRNA in primate epithelial tissues. *J. Histochem. Cytochem.* 41, 1521–1527. doi: 10.1177/41.10.8245410

Cambier, S., Mu, D. Z., O'Connell, D., Boylen, K., Travis, W., Liu, W. H., et al. (2000). A role for the integrin alphavbeta8 in the negative regulation of epithelial cell growth. *Cancer Res.* 60, 7084–7093.

Campbell, M. G., Cormier, A., Ito, S., Seed, R. I., Bondesson, A. J., Lou, J., et al. (2020). Cryo-EM reveals integrin-mediated TGF-beta activation without release from latent TGF-beta. *Cell* 180, 490.e416–501.e416. doi: 10.1016/j.cell.2019.12.030

Cavaco, A. C. M., Rezaei, M., Caliandro, M. F., Lima, A. M., Stehling, M., Dhayat, S. A., et al. (2019). The interaction between Laminin-332 and alpha 3 beta 1 integrin determines differentiation and maintenance of CAFs, and supports invasion of pancreatic duct adenocarcinoma cells. *Cancer* 11:20. doi: 10.3390/cancers11010014

Danhier, F., Le Breton, A., and Preat, V. (2012). RGD-based strategies to target alpha(v) beta(3) integrin in cancer therapy and diagnosis. *Mol. Pharm.* 9, 2961–2973. doi: 10.1021/mp3002733

De Franceschi, N., Hamidi, H., Alanko, J., Sahgal, P., and Ivaska, J. (2015). Integrin traffic—the update. *J. Cell Sci.* 128, 839–852. doi: 10.1242/jcs.161653

Dustin, M. L. (2019). Integrins and their role in immune cell adhesion. *Cell* 177, 499–501. doi: 10.1016/j.cell.2019.03.038

Dydensborg, A. B., Teller, I. C., Groulx, J. F., Basora, N., Pare, F., Herring, E., et al. (2009). Integrin alpha 6B beta 4 inhibits colon cancer cell proliferation and c-Myc activity. *BMC Cancer* 9:223. doi: 10.1186/1471-2407-9-223

Fan, C. S., Chen, W. S., Chen, L. L., Chen, C. C., Hsu, Y. T., Chua, K. V., et al. (2018). Osteopontin-integrin engagement induces HIF-1 alpha-TCF12-mediated endothelial-mesenchymal transition to exacerbate colorectal cancer. *Oncotarget* 9, 4998–5015. doi: 10.18632/oncotarget.23578

Fedele, C., Singh, A., Zerlanko, B. J., Iozzo, R. V., and Languino, L. R. (2015). The αvβ6 integrin is transferred intercellularly via exosomes. *J. Biol. Chem.* 290, 4545–4551. doi: 10.1074/jbc.C114.617662

Geng, H., Lan, R., Singha, P. K., Gilchrist, A., Weinreb, P. H., Violette, S. M., et al. (2012). Lysophosphatidic acid increases proximal tubule cell secretion of profibrotic cytokines PDGF-B and CTGF through LPA2- and Galphaq-mediated rho and alphavbeta6 integrin-dependent activation of TGF-beta. *Am. J. Pathol.* 181, 1236–1249. doi: 10.1016/j.ajpath.2012.06.035

Genschmer, K. R., Russell, D. W., Lal, C., Szul, T., Bratcher, P. E., Noerager, B. D., et al. (2019). Activated PMN exosomes: pathogenic entities causing matrix destruction and disease in the lung. *Cell* 176, 113.e115–126.e115. doi: 10.1016/j.cell.2018.12.002

Glynne, P. A., Picot, J., and Evans, T. J. (2001). Coexpressed nitric oxide synthase and apical ×1 integrins influence tubule cell adhesion after cytokine-induced injury. *J. Am. Soc. Nephrol.* 12, 2370–2383.

Guan, H., Peng, R., Mao, L. K., Fang, F., Xu, B., and Chen, M. (2020). Injured tubular epithelial cells activate fibroblasts to promote kidney fibrosis through miR-150-containing exosomes. *Exp. Cell Res.* 392:112007. doi: 10.1016/j.yexcr.2020.112007

Han, R., Hu, S., Qin, W., Shi, J., Hou, Q., Wang, X., et al. (2019). C3a and suPAR drive versican V1 expression in tubular cells of focal segmental glomerulosclerosis. *JCI Insight* 4:e122912. doi: 10.1172/jci.insight.122912.

Hayek, S. S., Koh, K. H., Grams, M. E., Wei, C., Ko, Y. -A., Li, J., et al. (2017). A tripartite complex of suPAR, APOL1 risk variants and αvβ3 integrin on podocytes mediates chronic kidney disease. *Nat. Med.* 23, 945–953. doi: 10.1038/nm.4362

Hayek, S. S., Leaf, D. E., Samman Tahhan, A., Raad, M., Sharma, S., Waikar, S. S., et al. (2020). Soluble urokinase receptor and acute kidney injury. *N. Engl. J. Med.* 382, 416–426. doi: 10.1056/NEJMoa1911481

Henderson, N. C., Arnold, T. D., Katamura, Y., Giacomini, M. M., Rodriguez, J. D., McCarty, J. H., et al. (2013). Targeting of alpha(v) integrin identifies a core molecular pathway that regulates fibrosis in several organs. *Nat. Med.* 19, 1617–1624. doi: 10.1038/nm.3282

Hogan, M. C., Johnson, K. L., Zenka, R. M., Charlesworth, M. C., Madden, B. J., Mahoney, D. W., et al. (2014). Subfractionation, characterization, and in-depth proteomic analysis of glomerular membrane vesicles in human urine. *Kidney Int.* 85, 1225–1237. doi: 10.1038/ki.2013.422

Hoshino, A., Costa-Silva, B., Shen, T. L., Rodrigues, G., Hashimoto, A., Tesic Mark, M., et al. (2015). Tumour exosome integrins determine organotropic metastasis. *Nature* 527, 329–335. doi: 10.1038/nature15756

Humphries, J. D., Byron, A., and Humphries, M. J. (2006). Integrin ligands at a glance. *J. Cell Sci.* 119, 3901–3903. doi: 10.1242/jcs.03098

Johnstone, R. M., Adam, M., Hammond, J. R., Orr, L., and Turbide, C. (1987). Vesicle formation during reticulocyte maturation—association of plasma-membrane activities with released vesicles (exosomes). *J. Biol. Chem.* 262, 9412–9420.

Kalappurakkal, J. M., Anilkumar, A. A., Patra, C., van Zanten, T. S., Sheetz, M. P., and Mayor, S. (2019). Integrin mechano-chemical signaling generates plasma membrane nanodomains that promote cell spreading. *Cell* 177, 1738.e1723–1756.e1723. doi: 10.1016/j.cell.2019.04.037

Kalluri, R., and LeBleu, V. S. (2020). The biology, function, and biomedical applications of exosomes. *Science* 367:eaau6977. doi: 10.1126/science.aau6977

Kormann, R., Kavvadas, P., Placier, S., Vandermeersch, S., Dorison, A., Dussaule, J. C., et al. (2020). Periostin promotes cell proliferation and macrophage polarization to drive repair after AKI. *J. Am. Soc. Nephrol.* 31, 85–100. doi: 10.1681/ASN.2019020113

Kreidberg, J. A., Donovan, M. J., Goldstein, S. L., Rennke, H., Shepherd, K., Jones, R. C., et al. (1996). Alpha 3 beta 1 integrin has a crucial role in kidney and lung organogenesis. *Development* 122, 3537–3547.

Lee, J. L., and Streuli, C. H. (2014). Integrins and epithelial cell polarity. *J. Cell Sci.* 127, 3217–3225. doi: 10.1242/jcs.146142

Li, K. Y., Chen, Y. H., Li, A., Tan, C. L., and Liu, X. B. (2019). Exosomes play roles in sequential processes of tumor metastasis. *Int. J. Cancer* 144, 1486–1495. doi: 10.1002/ijc.31774

Liu, B.-C., Tang, T.-T., Lin-Li, L., and Lan, H.-Y. (2018b). Renal tubule injury: a driving force toward chronic kidney disease. *Kidney Int.* 93, 568–579. doi: 10.1016/j.kint.2017.09.033

Liu, B. B., Zheng, X., Meng, F. F., Han, Y. W., Song, Y. W., Liu, F. F., et al. (2018a). Overexpression of beta 1 integrin contributes to polarity reversal and a poor prognosis of breast invasive micropapillary carcinoma. *Oncotarget* 9, 4338–4353. doi: 10.18632/oncotarget.22774

Lobert, V. H., Brech, A., Pedersen, N. M., Wesche, J., Oppelt, A., Malerod, L., et al. (2010). Ubiquitination of alpha 5 beta 1 integrin controls fibroblast migration through lysosomal degradation of fibronectin-integrin complexes. *Dev. Cell* 19, 148–159. doi: 10.1016/j.devcel.2010.06.010

Lu, H., Bowler, N., Harshyne, L. A., Craig Hooper, D., Krishn, S. R., Kurtoglu, S., et al. (2018). Exosomal alphavbeta6 integrin is required for monocyte M2 polarization in prostate cancer. *Matrix Biol.* 70, 20–35. doi: 10.1016/j.matbio.2018.03.009

Lv, L. L., Feng, Y., Wu, M., Wang, B., Li, Z. L., Zhong, X., et al. (2020). Exosomal miRNA-19b-3p of tubular epithelial cells promotes M1 macrophage activation in kidney injury. *Cell Death Differ.* 27, 210–226. doi: 10.1038/s41418-019-0349-y

Meng, X. M., Nikolic-Paterson, D. J., and Lan, H. Y. (2016). TGF-beta: the master regulator of fibrosis. *Nat. Rev. Nephrol.* 12, 325–338. doi: 10.1038/nrneph.2016.48

Merchant, M. L., Rood, I. M., Deegens, J. K. J., and Klein, J. B. (2017). Isolation and characterization of urinary extracellular vesicles: implications for biomarker discovery. *Nat. Rev. Nephrol.* 13, 731–749. doi: 10.1038/nrneph.2017.148

Mora, J. R., Bono, M. R., Manjunath, N., Weninger, W., Cavanagh, L. L., Rosemblatt, M., et al. (2003). Selective imprinting of gut-homing T cells by Peyer's patch dendritic cells. *Nature* 424, 88–93. doi: 10.1038/nature01726

Moreno-Layseca, P., Icha, J., Hamidi, H., and Ivaska, J. (2019). Integrin trafficking in cells and tissues. *Nat. Cell Biol.* 21, 122–132. doi: 10.1038/s41556-018-0223-z

Moreno-Layseca, P., and Streuli, C. H. (2014). Signalling pathways linking integrins with cell cycle progression. *Matrix Biol.* 34, 144–153. doi: 10.1016/j.matbio.2013.10.011

Myint, P. K., Park, E. J., Gaowa, A., Kawamoto, E., and Shimaoka, M. (2020). Targeted remodeling of breast cancer and immune cell homing niches by exosomal integrins. *Diagn. Pathol.* 15:38. doi: 10.1186/s13000-020-00959-3

Nie, H., Xie, X., Zhang, D., Zhou, Y., Li, B., Li, F., et al. (2020). Use of lung-specific exosomes for miRNA-126 delivery in non-small cell lung cancer. *Nanoscale* 12, 877–887. doi: 10.1039/c9nr09011h

Nieberler, M., Reuning, U., Reichart, F., Notni, J., Wester, H. J., Schwaiger, M., et al. (2017). Exploring the role of RGD-recognizing integrins in cancer. *Cancer* 9:116. doi: 10.3390/cancers9090116

Norman, J. T., and Fine, L. G. (1999). Progressive renal disease: fibroblasts, extracellular matrix, and integrins. *Exp. Nephrol.* 7, 167–177. doi: 10.1159/000020597

Panchatcharam, M., Miriyala, S., Yang, F., Leitges, M., Chrzanowska-Wodnicka, M., Quilliam, L. A., et al. (2010). Enhanced proliferation and migration of vascular smooth muscle cells in response to vascular injury under hyperglycemic conditions is controlled by beta 3 integrin signaling. *Int. J. Biochem. Cell Biol.* 42, 965–974. doi: 10.1016/j.biocel.2010.02.009

Paolicelli, R. C., Bergamini, G., and Rajendran, L. (2019). Cell-to-cell communication by extracellular vesicles: focus on microglia. *Neuroscience* 405, 148–157. doi: 10.1016/j.neuroscience.2018.04.003

Park, E. J., Prajuabjinda, O., Soe, Z. Y., Darkwah, S., Appiah, M. G., Kawamoto, E., et al. (2019). Exosomal regulation of lymphocyte homing to the gut. *Blood Adv.* 3, 1–11. doi: 10.1182/bloodadvances.2018024877

Peng, C., Zou, X. Q., Xia, W. Y., Gao, H. J., Li, Z. Q., Liu, N. Q., et al. (2018). Integrin alpha v beta 6 plays a bi-directional regulation role between colon cancer cells and cancer-associated fibroblasts. *Biosci. Rep.* 38:12. doi: 10.1042/bsr20180243

Qiao, L., Hu, S. Q., Huang, K., Su, T., Li, Z. H., Vandergriff, A., et al. (2020). Tumor cell-derived exosomes home to their cells of origin and can be used as Trojan horses to deliver cancer drugs. *Theranostics* 10, 3474–3487. doi: 10.7150/thno.39434

Quaglia, F., Krishn, S. R., Daaboul, G. G., Sarker, S., Pippa, R., Domingo-Domenech, J., et al. (2020). Small extracellular vesicles modulated by alphaVbeta3 integrin induce neuroendocrine differentiation in recipient cancer cells. *J. Extracell. Vesicles* 9:1761072. doi: 10.1080/20013078.2020.1761072

Rainero, E., and Norman, J. C. (2013). Late endosomal and lysosomal trafficking during integrin-mediated cell migration and invasion. *BioEssays* 35, 523–532. doi: 10.1002/bies.201200160

Raven, A., Lu, W. Y., Man, T. Y., Ferreira-Gonzalez, S., O'Duibhir, E., Dwyer, B. J., et al. (2017). Cholangiocytes act as facultative liver stem cells during impaired hepatocyte regeneration. *Nature* 547, 350–354. doi: 10.1038/nature23015

Reed, N. I., Jo, H., Chen, C., Tsujino, K., Arnold, T. D., DeGrado, W. F., et al. (2015). The alpha(v)beta(1) integrin plays a critical in vivo role in tissue fibrosis. *Sci. Transl. Med.* 7:288ra79. doi: 10.1126/scitranslmed.aaa5094

Rieu, S. P., Minard, C. G., Rabesandratana, H., Sainte-Marie, J., and Vidal, M. (2000). Exosomes released during reticulocyte maturation bind to fibronectin via integrin α4β1. *Eur. J. Biochem.* 267, 583–590. doi: 10.1046/j.1432-1327.2000.01036.x

Simon, D. I., Wei, Y., Zhang, L., Rao, N. K., Xu, H., Chen, Z. P., et al. (2000). Identification of a urokinase receptor-integrin interaction site—promiscuous regulator of integrin function. *J. Biol. Chem.* 275, 10228–10234. doi: 10.1074/jbc.275.14.10228

Springer, T. A. (1990). Adhesion receptors of the immune system. *Nature* 346, 425–434. doi: 10.1038/346425a0

Springer, T. A. (1994). Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 76, 301–314. doi: 10.1016/0092-8674(94)90337-9

Sung, B. H., Ketova, T., Hoshino, D., Zijlstra, A., and Weaver, A. M. (2015). Directional cell movement through tissues is controlled by exosome secretion. *Nat. Commun.* 6:7164. doi: 10.1038/ncomms8164

Tang, T. T., Lv, L. L., Wang, B., Cao, J. Y., Feng, Y., Li, Z. L., et al. (2019). Employing macrophage-derived microvesicle for kidney-targeted delivery of dexamethasone: an efficient therapeutic strategy against renal inflammation and fibrosis. *Theranostics* 9, 4740–4755. doi: 10.7150/thno.33520

Tang, T. T., Wang, B., Lv, L. L., and Liu, B. C. (2020). Extracellular vesicle-based nanotherapeutics: emerging frontiers in anti-inflammatory therapy. *Theranostics* 10, 8111–8129. doi: 10.7150/thno.47865

Tian, T., Zhang, H. X., He, C. P., Fan, S., Zhu, Y. L., Qi, C., et al. (2018). Surface functionalized exosomes as targeted drug delivery vehicles for cerebral ischemia therapy. *Biomaterials* 150, 137–149. doi: 10.1016/j.biomaterials.2017.10.012

Trevillian, P., Paul, H., Millar, E., Hibberd, A., and Agrez, M. V. (2004). αvβ6 integrin expression in diseased and transplanted kidneys. *Kidney Int.* 66, 1423–1433. doi: 10.1111/j.1523-1755.2004.00904.x

Valadi, H., Ekstrom, K., Bossios, A., Sjostrand, M., Lee, J. J., and Lotvall, J. O. (2007). Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat. Cell Biol.* 9, 654–659. doi: 10.1038/ncb1596

van Caam, A., Aarts, J., van Ee, T., Vitters, E., Koenders, M., van de Loo, F., et al. (2020). TGF beta-mediated expression of TGF beta-activating integrins in SSc monocytes: disturbed activation of latent TGF beta? *Arthritis Res. Ther.* 22:42. doi: 10.1186/s13075-020-2130-5

van Niel, G., D'Angelo, G., and Raposo, G. (2018). Shedding light on the cell biology of extracellular vesicles. *Nat. Rev. Mol. Cell Biol.* 19, 213–228. doi: 10.1038/nrm.2017.125

Viquez, O. M., Yazlovitskaya, E. M., Tu, T., Mernaugh, G., Secades, P., McKee, K. K., et al. (2017). Integrin alpha6 maintains the structural integrity of the kidney collecting system. *Matrix Biol.* 57–58, 244–257. doi: 10.1016/j.matbio.2016.12.003

Wei, C., El Hindi, S., Li, J., Fornoni, A., Goes, N., Sageshima, J., et al. (2011). Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis. *Nat. Med.* 17, 952–960. doi: 10.1038/nm.2411

Yang, Q., Ren, G. L., Wei, B., Jin, J., Huang, X. R., Shao, W., et al. (2019). Conditional knockout of TGF-betaRII/Smad2 signals protects against acute renal injury by alleviating cell necroptosis, apoptosis and inflammation. *Theranostics* 9, 8277–8293. doi: 10.7150/thno.35686

You, Y., Borgmann, K., Edara, V. V., Stacy, S., Ghorpade, A., and Ikezu, T. (2020). Activated human astrocyte-derived extracellular vesicles modulate neuronal uptake, differentiation and firing. *J. Extracell. Vesicles* 9:1706801. doi: 10.1080/20013078.2019.1706801

Yuan, D., Zhao, Y., Banks, W. A., Bullock, K. M., Haney, M., Batrakova, E., et al. (2017). Macrophage exosomes as natural nanocarriers for protein delivery to inflamed brain. *Biomaterials* 142, 1–12. doi: 10.1016/j.biomaterials.2017.07.011

Zhang, W., Zhang, X., Zhang, L., Xu, D., Cheng, N., Tang, Y., et al. (2020). Astrocytes increase exosomal secretion of oligodendrocyte precursor cells to promote their proliferation via integrin beta4-mediated cell adhesion. *Biochem. Biophys. Res. Commun.* 526, 341–348. doi: 10.1016/j.bbrc.2020.03.092

Zhu, H., Liao, J., Zhou, X., Hong, X., Song, D., Hou, F. F., et al. (2020). Tenascin-C promotes acute kidney injury to chronic kidney disease progression by impairing tubular integrity via alphavbeta6 integrin signaling. *Kidney Int.* 97, 1017–1031. doi: 10.1016/j.kint.2020.01.026

Zovein, A. C., Luque, A., Turlo, K. A., Hofmann, J. J., Yee, K. M., and Becker, M. S., et al. (2010). Beta1 integrin establishes endothelial cell polarity and arteriolar lumen formation via a Par3-dependent mechanism. *Dev. Cell* 18, 39–51. doi: 10.1016/j.devcel.2009.12.006

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BRIEF RESEARCH REPORT
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Histopathological Findings Predict Renal Recovery in Severe ANCA-Associated Vasculitis Requiring Intensive Care Treatment

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Renal involvement is a common and severe complication of AAV as it can cause ESRD. Histopathological subgrouping and ARRS are helpful to predict long-term ESRD in patients with AAV. Because a subgroup of critically ill patients with severe AAV present with deterioration of kidney function requiring RRT at admission, we here aimed to evaluate histopathological findings and predictive value of Berden's histopathological subgrouping and ARRS for severity of AKI and requirement of RRT during the short-term clinical course in critically ill patients requiring intensive care treatment and predictors for short-term renal recovery in patients requiring RRT. A subgroup of 15/46 (32. 6%) AAV patients with biopsy-proven AAV required RRT during the short-term course of disease, associated with requirement of critical care treatment. While histopathological subgrouping and ARRS were associated with requirement of acute RRT, presence of global glomerular scarring was the strongest predictor of failure to recover from RRT after initiation of remission induction therapy. This new aspect requires further investigation in a prospective controlled setting for therapeutic decision making especially in this subgroup.

Keywords: autoimmune diseases, systemic vasculitis, inflammation, ANCA-associated vasculitis, acute kidney injury, renal replacement therapy, intensive care treatment

INTRODUCTION

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a systemic vasculitis, which most frequently presents as microscopic polyangiitis (MPA) or granulomatosis with polyangiitis (GPA) (1). Renal involvement is a common and severe complication of AAV as it can cause end-stage renal disease (ESRD) or death (2, 3). Histopathological subgrouping into four classes (focal, crescentic, mixed, and sclerotic) as defined by Berden et al. was proposed to predict long-term renal survival rates poorest in the sclerotic class (sclerotic glomeruli above 50%) (4). Unlike Berden's classification, Brix et al. suggested the ANCA renal risk score (ARRS) by incorporation of the baseline glomerular filtration rate (GFR) to the histopathological findings (percentage of normal glomeruli, tubular atrophy/interstitial fibrosis) to predict ESRD in patients with AAV (5). Histopathological subgrouping and ARRS were both established for predicting long-term ESRD over years, but a subgroup of severe AAV presents with acute kidney injury

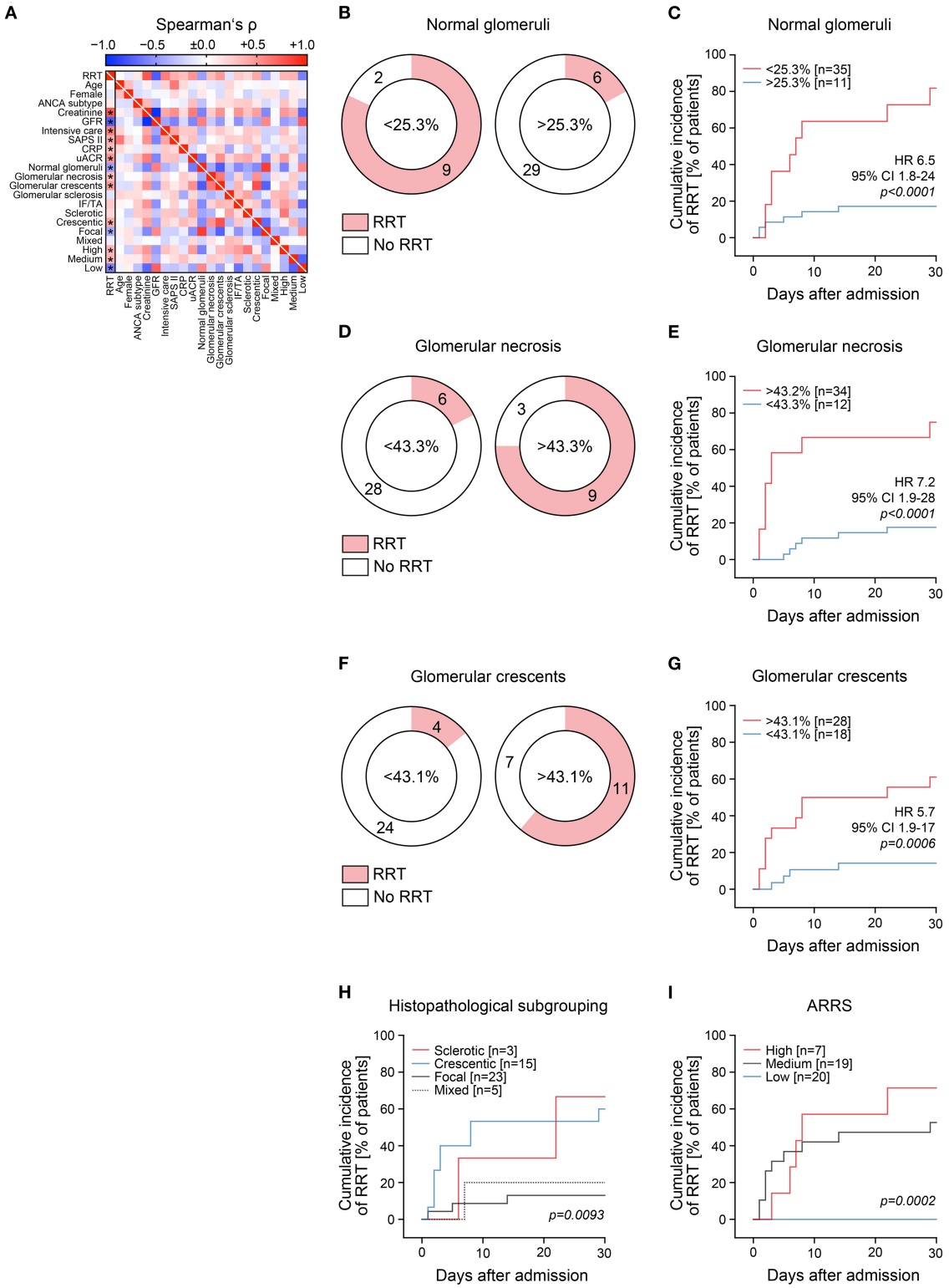

FIGURE 1 | Histopathological findings associate with severity of acute kidney injury in severe AAV. (**A**) Association between requirement of RRT within 30 days after admission; clinical/laboratory and histopathological findings is shown by a heat map reflecting mean values of Spearman's ρ, asterisks indicate $p < 0.05$. (**B–G**) Cutoff points on the ROC that maximized Youden's index were used for cumulative incidence of RRT within 30 days after admission for each parameter. (**H,I**) Histopathological subgrouping and ARRS for successful recovery from RRT within 30 days are shown. Analysis was performed using log rank (Mantel–Cox) testing. ANCA, anti-neutrophil cytoplasmic antibodies; ARRS, ANCA renal risk score; CI, confidence interval; CRP, C-reactive protein; GFR, glomerular filtration rate (CKD-EPI); HR, hazard ratio; IF/TA, interstitial fibrosis/tubular atrophy; RRT, renal replacement therapy; SAPS II, simplified acute physiology score II; uACR, urinary albumin/creatinine ratio.

(AKI) required renal replacement therapy (RRT) during the initial course of the disease (4, 5). Since severity of AKI, requirement of RRT, and short-term renal recovery in critically ill patients are associated with disease severity and clinical course of disease, predictors for RRT requirement and renal recovery after initiation of remission induction therapy are of relevance (6). Therefore, we here aimed to evaluate the histopathological findings and predictive value of Berden's histopathological subgrouping and ARRS for severity of AKI and requirement of RRT during the short-term clinical course in critically ill patients requiring intensive care treatment. In addition, we sought to identify predictors for short-term renal recovery in patients requiring RRT.

METHODS

Study Population

A total number of 46 patients with biopsy-proven AAV at the University Medical Center Göttingen were retrospectively included between 2015 till 2020. While no formal approval was required for the use of routine clinical data, a favorable ethical opinion was granted by the local Ethics committee (no. 4/8/19). A detailed Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) flowchart of patient disposition is shown in **Supplementary Figure 1A**.

Definitions

At admission, the Birmingham Vasculitis Activity Score (BVAS) version 3 was calculated as described previously (7). The simplified acute physiology score (SAPS) II and estimated mortality rates were calculated according to the published guidelines (8). Requirement of intensive care treatment was defined at admission and calculated by the time between admission to the intensive care unit (ICU) or intermediate care unit (IMC) and relocation to the non-ICU/non-IMC medical ward; all patients required critical care treatment > 24 h. RRT was performed intermittently in all cases. Indications of RRT included serum creatinine $\geq$ 500 μmol/L, severe electrolyte and acid–base abnormalities, volume overload, and encephalopathy. RRT was terminated when the glomerular filtration rate (GFR) according to CKD-EPI surpassed 15 mL/min/1.73 m^2 and there was no hyperkalemia, heart failure, edema, and encephalopathy. Short-term course of disease was defined within 30 days after admission; short-term renal recovery was defined as successful recovery from RRT within 30 days after RRT initiation.

Renal Histopathology

Renal pathologists (SH and PS) evaluated the biopsies. Within a kidney biopsy specimen, each glomerulus had to be scored separately for the presence of necrosis, crescents, and global sclerosis (**Supplementary Figure 2A**). Consequently, the percentage of glomeruli with any of these features was calculated as a fraction of the total number of glomeruli in the biopsy. Apart from these categories, degree of interstitial fibrosis/tubular atrophy (IF/TA) was quantified. Based on these scorings, histopathological subgrouping according to Berden et al. (focal, crescentic, mixed, or sclerotic class) and ARRS according to Brix et al. (low, medium, or high risk) were performed (4, 5).

Remission Induction Therapy

Glucocorticoids (GCs) were administered either as intravenous pulse therapy or orally with a tapering schedule. Plasma exchange (PEX) was administered during the induction period at the discretion of treating physicians. Rituximab (RTX) was administered as four intravenous doses at 375 mg/m^2 every week; RTX was not administered within 48 h before PEX treatment. Cyclophosphamide (CYC) was administered as three intravenous doses up to 15 mg/kg every 2 weeks and every 3 weeks thereafter, adjusted for age and renal function. Combination therapy was administered as four intravenous doses at 375 mg/m^2 RTX every week and two intravenous doses at 15 mg/kg CYC every 2 weeks. On the discretion of treating physicians, choice of remission induction therapy was dependent on previous regimens and individual patients, more likely to choose RTX in younger patients with toxicity being the main reason for this choice (9). Prophylaxis to prevent pneumocystis (carinii) jiroveci infection was administered according to local practice.

Statistical Methods

Variables were tested for normal distribution using the Shapiro–Wilk test. Non-normally distributed continuous variables are expressed as median and interquartile range (IQR); categorical variables are presented as frequency and percentage. Statistical comparisons were not formally powered or prespecified. For group comparisons, the Mann–Whitney *U*-test was used to determine differences in medians. Non-parametric between-group comparisons were performed with Pearson's chi-square test. To establish a cutoff for each parameter, the ability of prognostic factors to discriminate groups was evaluated by receiver operator curves (ROC) and the area under the curve (AUC), as well as sensitivity and specificity for prediction. An AUC of 1.0 indicates perfect concordance, an AUC of 0.50 would indicate that the ability of prognostic factors to discriminate groups is no better than chance. Sensitivity and specificity were based on selection of the cutoff point on the ROC that maximized Youden's index (sensitivity+specificity-1), comparison of survival curves was performed with log rank (Mantel–Cox) testing (10). Data analyses were performed with GraphPad Prism (version 8.4.0 for MacOS, GraphPad Software, San Diego, California, USA).

RESULTS

During the short-term course of the disease, 15/46 (32.6%) patients presented with severe AKI requiring RRT within 30 days after admission (**Supplementary Figure 1A**). Requirement for RRT was associated with disease severity reflected by significantly higher SAPS II at admission, level of intensive care treatment longer than 24 h, prolonged requirement

TABLE 1 | Characteristics of patients: requirement of RRT vs. no RRT within 30 days after admission.

	RRT ($n = 15$)	No RRT ($n = 31$)	*P*-value
Median age (IQR)—years	66 (50–76)	63 (53–74)	*0.9676*
Female sex—no. (%)	5 (33.3)	14 (45.2)	*0.4450*
ANCA subtype MPO/PR3—no. (%)	9/6 (60/40)	15/16 (48.4/51.6)	*0.4598*
History of vasculitis—no. (%)	1 (6.7)	5 (16.1)	*0.3717*
Renal injury			
Median serum creatinine (IQR)—μmol/L	567 (438–652)	155 (99–280)	***<0.0001***
Serum creatinine ≥ 500 μmol/liter—no. (%)	10 (66.7)	0 (0)	***<0.0001***
Median GFR (IQR)—mL/min/1.73 m^2	8.4 (5.8–9.6)	32.2 (15.3–60.9)	***<0.0001***
Extrarenal manifestations			
Pulmonary hemorrhage—no. (%)	2 (13.3)	4 (12.9)	*0.9676*
Skin involvement—no. (%)	2 (13.3)	6 (19.4)	*0.6135*
Disease activity			
Median BVAS (IQR)—points	18 (18–20)	17 (14–21)	*0.2031*
Median SAPS II at admission (IQR)—points	31 (24–35)	23 (19–30)	***0.0085***
Intensive care treatment—no. (%)	14 (93.3)	9 (29)	***<0.0001***
Median intensive care treatment (IQR)—days	5 (3–10)	0 (0–3)	***<0.0001***
Median CRP (IQR)—mg/L	73.6 (49.3–174)	37 (10.8–89)	***0.0382***
Median uACR (IQR)—mg/g	839 (678–2,246)	243 (100–481)	***0.0006***
Renal histology			
Median total glomeruli (IQR)—no.	15 (10–18)	17 (11–28)	*0.5814*
Median normal glomeruli (IQR)—no.	3 (1–9)	10 (5–14)	***0.0044***
Median normal glomeruli (IQR)—%	20 (11.1–45.5)	58.8 (37–80)	***0.0013***
Median glomerular necrosis (IQR)—no.	7 (0–15)	2 (1–5)	*0.0726*
Median glomerular necrosis (IQR)—%	46.7 (0–80)	12.5 (3.6–28)	***0.0284***
Median glomerular crescents (IQR)—no.	8 (5–15)	2 (1–11)	***0.0389***
Median glomerular crescents (IQR)—%	54.6 (33.3–80)	27.3 (0–42.4)	***0.0009***
Median glomerular sclerosis (IQR)—no.	0 (0–5)	2 (0–3)	*0.4074*
Median glomerular sclerosis (IQR)—%	0 (0–33.3)	9.4 (0–23.1)	*0.3438*
Median IF/TA (IQR)—%	30 (10–50)	15 (7.5–30)	*0.0839*
Follow-up			
Median follow-up (IQR)—days	386 (165–715)	300 (81–609)	*0.5779*
Death—no. (%)	1 (6.7)	2 (6.5)	*0.9779*
RRT—no. (%)	4 (26.7)	1 (3.2)	***0.0166***

For group comparisons, the Mann–Whitney U-test was used to determine differences in medians. Non-parametric between-group comparisons were performed with Pearson's chi-square test. Bold indicates statistically significant values at group level. ANCA, anti-neutrophil cytoplasmic antibodies; BVAS, Birmingham Vasculitis Activity Score; CRP, C-reactive protein; GFR, glomerular filtration rate (CKD-EPI); IF/TA, interstitial fibrosis/tubular atrophy; IQR, interquartile range; No., number; MPO, myeloperoxidase; PR3, proteinase 3; RRT, renal replacement therapy; RTX, rituximab; SAPS II, simplified acute physiology score II; uACR, urinary albumin/creatinine ratio.

of intensive care treatment, higher levels of C-reactive protein, and urinary albumin/creatinine ratio (uACR, **Figure 1A** and **Table 1**). We next scored each glomerulus for the presence of necrosis, crescents, and global sclerosis (**Supplementary Figure 2A**). Renal histology revealed that requirement of RRT during the short-term course of disease was associated with active glomerular lesions reflected by lower number of normal glomeruli, increased glomerular necrosis, and crescents (**Figure 1A** and **Table 1**). ROC analysis confirmed an association between RRT requirement and amounts of normal glomeruli (AUC 0.7860, $p = 0.0018$), glomerular necrosis (AUC 0.6989, $p = 0.0302$), and crescents (AUC 0.7946, $p = 0.0013$, **Supplementary Figures 3A–C**). Renal survival analysis for cumulative incidence of RRT by using the cutoff point that maximized the combined sensitivity and specificity (Youden's index) of each parameter confirmed the poorest short-term renal outcome in patients with lower number of normal glomeruli, increased glomerular necrosis, and crescents (**Figures 1B–G**). Histopathological subgrouping into sclerotic and crescentic classes along with ARRS high-risk classification were associated with RRT requirement during short-term disease course (**Table 1** and **Figures 1H,I**), both considered to also show poorest long-term renal survival rates (4, 5).

After initiation of remission induction therapy, we next analyzed predictors for short-term renal recovery reflected by successful recovery from RRT within 30 days after RRT initiation. After one dropout due to death at day 21 after admission not included in the further analysis, 9/14 (64.3%) critically ill patients that required RRT during the course of disease recovered within 30 days after initiation of RRT (**Supplementary Figure 1A**). Among all parameters analyzed,

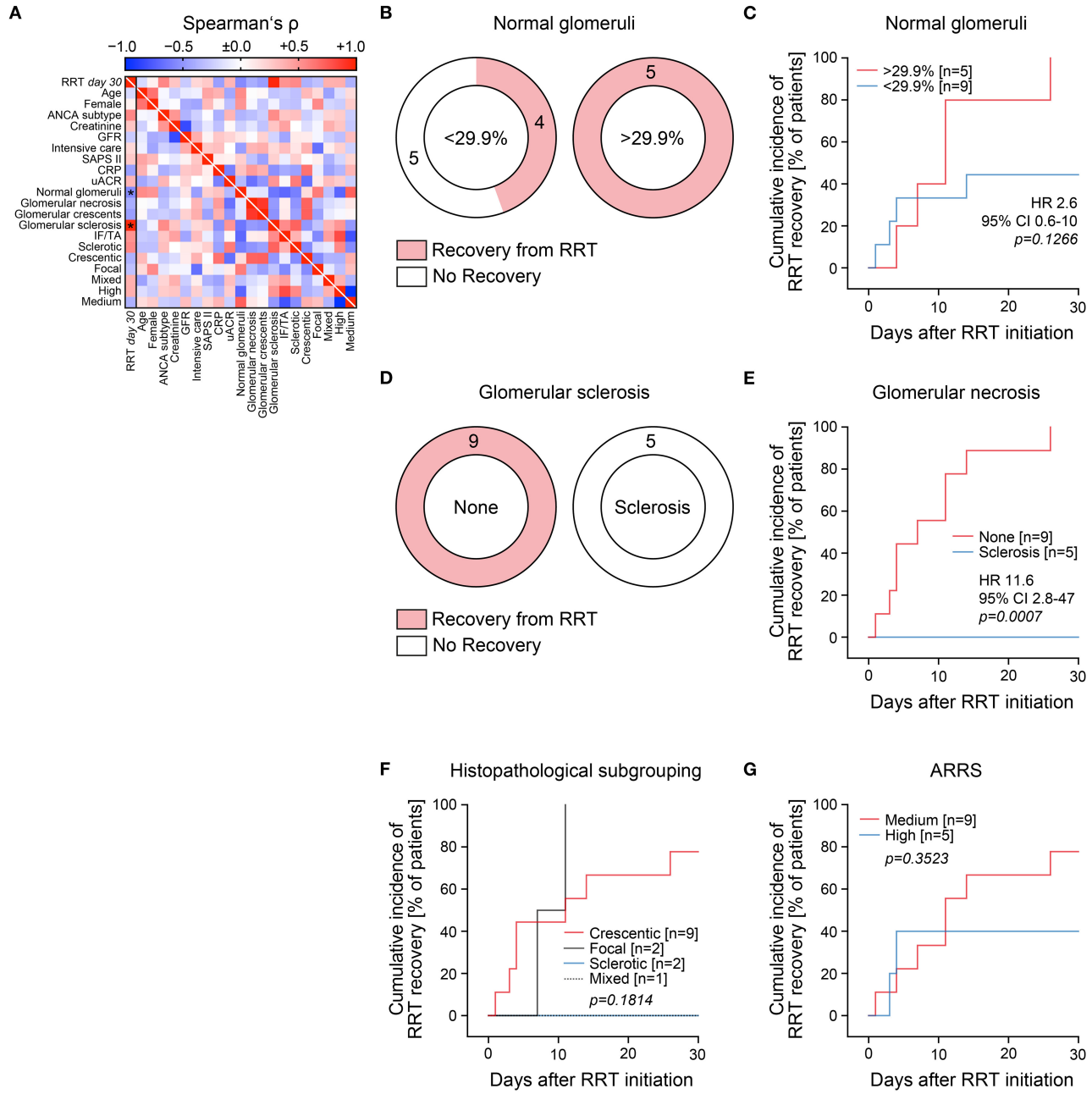

FIGURE 2 | Global glomerular sclerosis associates with failure to recover from RRT in critically ill patients. **(A)** Association between successful recovery from RRT within 30 days after RRT initiation; clinical/laboratory and histopathological findings are shown by heat map reflecting mean values of Spearman's ρ, asterisks indicate $p < 0.05$. (**B–E**) Cutoff points on the ROC that maximized Youden's index were used for cumulative incidence of successful recovery from RRT within 30 days after RRT initiation for each parameter. (**F,G**) Histopathological subgrouping and ARRS for successful recovery from RRT within 30 days after RRT initiation are shown. Analysis was performed using log rank (Mantel–Cox) testing. ANCA, anti-neutrophil cytoplasmic antibodies; ARRS, ANCA renal risk score; CI, confidence interval; CRP, C-reactive protein; GFR, glomerular filtration rate (CKD-EPI); HR, hazard ratio; IF/TA, interstitial fibrosis/tubular atrophy; RRT, renal replacement therapy; SAPS II, simplified acute physiology score II; uACR, urinary albumin/creatinine ratio.

short-term recovery from RRT after initiation of remission induction therapy was associated with higher numbers of unaffected glomeruli, whereas presence of sclerotic glomeruli was the strongest negative predictor (**Figure 2A** and **Table 2**). ROC analysis confirmed superiority of global glomerular sclerosis to predict failure for short-term renal recovery (AUC

TABLE 2 | Characteristics of patients requiring RRT: short-term recovery vs. no recovery from RRT within 30 days.

	Recovery ($n = 9$)	No recovery ($n = 5$)	*P*-value
Median age (IQR)—years	66 (52.5–73.5)	58 (43–73)	*0.5400*
Female sex—no. (%)	3 (33.3)	2 (40)	*0.8030*
ANCA subtype MPO/PR3—no. (%)	4/5 (44.4/55.6)	5/0 (100/0)	***0.0376***
History of vasculitis—no. (%)	0 (0)	1 (20)	*0.1638*
Renal injury			
Median serum creatinine (IQR)—μmol/L	567 (423–640)	643 (473–900)	*0.3636*
Serum creatinine ≥ 500 μmol/liter—no. (%)	6 (66.7)	4 (80)	*0.5967*
Median GFR (IQR)—mL/min/1.73 m^2	8.4 (6.3–9.7)	8 (4.6–9.7)	*0.3816*
Extrarenal manifestations			
Pulmonary hemorrhage—no. (%)	0 (0)	2 (40)	***0.0404***
Skin involvement—no. (%)	2 (22.2)	0 (0)	*0.2549*
Disease activity			
Median BVAS (IQR)—points	18 (18–19)	18 (16.5–23)	*0.7657*
Median SAPS II at admission (IQR)—points	30 (24–33.5)	35 (22–50)	*0.3771*
Intensive care treatment—no. (%)	8 (88.9)	5 (100)	*0.4392*
Median intensive care treatment (IQR)—days	4 (3.5–7)	8 (2–23)	*0.5400*
Median CRP (IQR)—mg/L	77.3 (68.1–148)	49.3 (24–120)	*0.1119*
Median uACR (IQR)—mg/g	839 (520–1,143)	2,849 (547–3,736)	*0.2398*
Renal histology			
Median total glomeruli (IQR)—no.	16 (9.5–22.5)	15 (9.5–32)	*0.9171*
Median normal glomeruli (IQR)—no.	4 (2–8.5)	1 (0–7.5)	*0.2048*
Median normal glomeruli (IQR)—%	35.3 (11.4–50.9)	6.7 (0–22.2)	***0.0490***
Median glomerular necrosis (IQR)—no.	8 (3.5–18.5)	3 (0–21)	*0.3751*
Median glomerular necrosis (IQR)—%	64.7 (21.9–88.6)	20 (0–59.1)	*0.1748*
Median glomerular crescents (IQR)—no.	8 (6.5–18.5)	5 (3–21.5)	*0.3801*
Median glomerular crescents (IQR)—%	64.7 (49.2–88.6)	45.5 (22.9–62.4)	*0.1064*
Median glomerular sclerosis (IQR)—no.	0 (0–0)	7 (3.5–9.5)	***0.0005***
Median glomerular sclerosis (IQR)—%	0 (0–0)	46.7 (18.7–100)	***0.0005***
Median TA/IF (IQR)—%	20 (10–37.5)	30 (25–75)	*0.0784*
Remission induction therapy			
Intravenous steroid pulse—no. (%)	6 (66.7)	5 (100)	
Oral GCs—no. (%)	9 (100)	5 (100)	
PEX—no. (%)	8 (88.9)	3 (60)	
Median sessions of PEX (IQR)—no.	5 (4–6)	5 (5–8)	
RTX—no. (%)	1 (11.1)	2 (40)	
CYC—no. (%)	5 (55.6)	3 (60)	
RTX/CYC—no. (%)	3 (33.3)	0 (0)	
Long-term survival			
Median follow-up (IQR)—days	505 (280–880)	326 (37–920)	*0.2977*
Death—no. (%)	0 (0)	0 (0)	
RRT—no. (%)	0 (0)	3 (60)	***0.0088***

For group comparisons, the Mann–Whitney U-test was used to determine differences in medians. Non-parametric between-group comparisons were performed with Pearson's chi-square test. Bold indicates statistically significant values at group level. ANCA, anti-neutrophil cytoplasmic antibodies; BVAS, Birmingham Vasculitis Activity Score; CRP, C-reactive protein; CYC, cyclophosphamide; GCs, glucocorticoids; GFR, glomerular filtration rate (CKD-EPI); IF/TA, interstitial fibrosis/tubular atrophy; IQR, interquartile range; No., number; MPO, myeloperoxidase; PEX, plasma exchange; PR3, proteinase 3; RRT, renal replacement therapy; RTX, rituximab; SAPS II, simplified acute physiology score II; uACR, urinary albumin/creatinine ratio.

1.000, $p = 0.0027$, **Supplementary Figures 4A,B**), further supported by renal survival analysis for RRT requirement (**Figures 2B–E**). In contrast, histopathological subgrouping and ARRS both failed to identify patients that successfully recovered from RRT during short-term disease course (**Figures 2F,G** and **Table 2**). While only limited follow-up data was available, renal outcome within 30 days significantly correlated with long-term renal survival rates (**Table 2**).

DISCUSSION

Histopathological subgrouping and ARRS are helpful for risk stratification to predict long-term renal survival rates (4, 5). While these observations have been validated in independent cohorts, severity of AKI with requirement of RRT and short-term renal recovery is important especially in critically ill cases of AAV (11, 12). Therefore, we here aimed to evaluate histopathological findings and predictive value of Berden's histopathological

subgrouping and ARRS for the requirement of RRT during short-term clinical course in critically ill patients requiring intensive care treatment. Requirement of RRT during the short-term course of disease was associated with active glomerular lesions reflected by lower number of normal glomeruli, increased glomerular necrosis, and crescents. Berden's histopathological subgrouping into sclerotic and crescentic classes along with ARRS high-risk classification identified patients at risk for RRT requirement during the short-term disease course, both considered to also show the poorest long-term renal survival rates (4, 5). In contrast, presence of global glomerular sclerosis was superior to histopathological subgrouping and ARRS and the strongest negative predictor for recovery from RRT during the short-term course of disease in critically ill patients with AAV requiring intensive care treatment. Glomerular sclerosis is only included in Berden's histopathological subgrouping in cases with affected glomeruli above 50%, predicting long-term renal survival rates poorest in the sclerotic class. In their study, Berden et al. classified 13/100 (13%) patients in the sclerotic class (4). In contrast, only 3/46 (6.5%) patients could be classified as sclerotic class in our cohort. Possibly, a more acute and aggressive onset of severe AAV requiring RRT early at disease onset in our cohort resulted in fewer sclerotic glomeruli, as also reported in other cohorts (13). However, our observation that presence of sclerotic glomeruli associates with failure to recover from RRT in critically ill patients may implicate underestimation of glomerular sclerosis when histopathological subgrouping is used in this subgroup of severe AAV. This is in line with previous reports of renal recovery in patients requiring RRT over a 3-month period of time (14). Since severity of AKI, requirement of RRT, and short-term renal recovery in critically ill patients are associated with disease severity and clinical course of disease, this emphasizes the novelty and great relevance of our findings (6).

The main limitations of our study are being retrospective, different regimens of remission induction, the small patient number, and limited data on long-term renal survival rates. Nevertheless, our observations support that global glomerular sclerosis associates with failure to recover from RRT after initiation of remission induction therapy in cases of severe AAV requiring intensive care treatment. This new aspect requires further investigation in a prospective controlled setting for therapeutic decision making especially in this subgroup.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics committee of the University Medical Center Göttingen, Germany (no. 4/8/19). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SH and BT conceived the study, collected and analyzed data, and co-wrote the first draft. DT collected and analyzed the data. SH and PS evaluated the histopathological findings. PK and MZ participated in the construction and editing of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.622028/full#supplementary-material

REFERENCES

1. Watts RA, Scott DG. Epidemiology of the vasculitides. *Curr Opin Rheumatol.* (2003) 15:11–6. doi: 10.1097/00002281-200301000-00003
2. Pettersson EE, Sundelin B, Heigl Z. Incidence and outcome of pauci-immune necrotizing and crescentic glomerulonephritis in adults. *Clin Nephrol.* (1995) 43:141–9.
3. Hruskova Z, Stel VS, Jayne D, Aasarod K, De Meester J, Ekstrand A, et al. Characteristics and outcomes of granulomatosis with polyangiitis (Wegener) and microscopic polyangiitis requiring renal replacement therapy: results from the European renal association-European dialysis and transplant association registry. *Am J Kidney Dis.* (2015) 66:613–20. doi: 10.1053/j.ajkd.2015.03.025
4. Berden AE, Ferrario F, Hagen EC, Jayne DR, Jennette JC, Joh K, et al. Histopathologic classification of ANCA-associated glomerulonephritis. *J Am Soc Nephrol.* (2010) 21:1628–36. doi: 10.1681/ASN.2010050477
5. Brix SR, Noriega M, Tennstedt P, Vettorazzi E, Busch M, Nitschke M, et al. Development and validation of a renal risk score in ANCA-associated glomerulonephritis. *Kidney Int.* (2018) 94:1177–88. doi: 10.1016/j.kint.2018.07.020
6. Bagshaw SM, Laupland KB, Doig CJ, Mortis G, Fick GH, Mucenski M, et al. Prognosis for long-term survival and renal recovery in critically ill patients with severe acute renal failure: a population-based study. *Crit Care.* (2005) 9:R700–9. doi: 10.1186/cc3879

7. Mukhtyar C, Lee R, Brown D, Carruthers D, Dasgupta B, Dubey S, et al. Modification and validation of the Birmingham vasculitis activity score (version 3). *Ann Rheum Dis.* (2009) 68:1827–32. doi: 10.1136/ard.2008.101279
8. Le Gall JR, Lemeshow S, Saulnier F. A new simplified acute physiology score (SAPS II) based on a European/North American multicenter study. *JAMA.* (1993) 270:2957–63. doi: 10.1001/jama.270.24.2957
9. Forbess LJ, Griffin KW, Spiera RF. Practice patterns of ANCA-associated vasculitis: exploring differences among subspecialties at a single academic medical centre. *Clin Exp Rheumatol.* (2014) 32(3 Suppl 82):S48–50.
10. Le CT. A solution for the most basic optimization problem associated with an ROC curve. *Stat Methods Med Res.* (2006) 15:571–84. doi: 10.1177/0962280206070637
11. Nohr E, Girard L, James M, Benediktsson H. Validation of a histopathologic classification scheme for antineutrophil cytoplasmic antibody-associated glomerulonephritis. *Hum Pathol.* (2014) 45:1423–9. doi: 10.1016/j.humpath.2014.02.017
12. van Daalen EE, Wester Trejo MAC, Goceroglu A, Ferrario F, Joh K, Noel LH, et al. Developments in the histopathological classification of ANCA-associated glomerulonephritis. *Clin J Am Soc Nephrol.* (2020) 15:1103–11. doi: 10.2215/CJN.14561119
13. Hilhorst M, Wilde B, van Breda Vriesman P, van Paassen P, Cohen Tervaert JW, Limburg Renal R. Estimating renal survival using the ANCA-associated GN classification. *J Am Soc Nephrol.* (2013) 24:1371–5. doi: 10.1681/ASN.2012090912
14. Xu PC, Chen T, Wu SJ, Yang X, Gao S, Hu SY, et al. Pathological severity determines the renal recovery for anti-myeloperoxidase antibody-associated vasculitis requiring dialysis at disease onset: a retrospective study. *BMC Nephrol.* (2019) 20:287. doi: 10.1186/s12882-019-1487-7

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ORIGINAL RESEARCH
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c-Jun Amino Terminal Kinase Signaling Promotes Aristolochic Acid-Induced Acute Kidney Injury

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Aristolochic acid (AA) is a toxin that induces DNA damage in tubular epithelial cells of the kidney and is the cause of Balkan Nephropathy and Chinese Herb Nephropathy. In cultured tubular epithelial cells, AA induces a pro-fibrotic response *via* the c-Jun amino terminal kinase (JNK) signaling pathway. This study investigated the *in vivo* role of JNK signaling with a JNK inhibitor (CC-930) in mouse models of acute high dose AA-induced kidney injury (day 3) and renal fibrosis induced by chronic low dose AA exposure (day 22). CC-930 treatment inhibited JNK signaling and protected from acute AA-induced renal function impairment and severe tubular cell damage on day 3, with reduced macrophage infiltration and expression of pro-inflammatory molecules. In the chronic model, CC-930 treatment inhibited JNK signaling but did not affect AA-induced renal function impairment, tubular cell damage including the DNA damage response and induction of senescence, or renal fibrosis; despite a reduction in the macrophage pro-inflammatory response. In conclusion, JNK signaling contributes to acute high dose AA-induced tubular cell damage, presumably *via* an oxidative stress-dependent mechanism, but is not involved in tubular atrophy and senescence that promote chronic kidney disease caused by ongoing DNA damage in chronic low dose AA exposure.

Keywords: acute kidney injury, chronic kidney disease, inflammation, c-Jun amino terminal kinase, macrophage, renal fibrosis, senescence

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INTRODUCTION

Chinese herbal medicine is widely used for the prevention, treatment, and cure of a wide range of diseases (Yang et al., 2018). However, some traditional herbal medicines are toxic to the kidney such as aristolochic acids (AAs). Kidney damage associated with herbal medicines includes acute kidney injury, chronic kidney disease, nephrolithiasis, and bladder cancer (Yang et al., 2018). AA was identified as the nephrotoxin responsible for Balkan Nephropathy where the toxin was derived from weeds growing in fields that contaminated flour used in baking (Jadot et al., 2017). Indeed, the wide-spread recognition of kidney damage caused by this nephrotoxin has led to the term, aristolochic acid nephropathy (AAN).

Aristolochic acid is taken up by tubular epithelial cells of the kidney *via* the organic anion transporter OAT1/3 (Bakhiya et al., 2009). Toxicity to tubular epithelial cells is the hallmark

of AAN which features atrophy and loss of tubules with extensive interstitial fibrosis with an inflammatory infiltrate, although glomeruli are largely spared (Vanherweghem et al., 1993). Analysis of 300 cases of Chinese Herb Nephropathy identified both acute kidney injury and more slowly progressive chronic kidney disease, which were associated with high or low levels of AA ingestion, respectively (Yang et al., 2012). Consistent with clinical findings, animal studies have shown that administration of a single high dose of AA can induce acute kidney injury with tubular necrosis, while repeated administration of low doses of AA leads to chronic kidney disease with tubular atrophy and interstitial fibrosis (Zhou et al., 2010a,b).

Upon uptake into cells, AA binds to DNA causes AA-DNA adducts which can result in a A:T→T:A transversion, inducing the DNA damage response and leading to cancer development over time (Jadot et al., 2017). In addition, studies using cultured tubular epithelial cells have shown that treatment with AA induces high levels of reactive oxygen species (ROS), and that AA-induced cell death can be suppressed using anti-oxidant approaches (Yu et al., 2011; Jadot et al., 2017). Animal studies support a mechanism of AA-induced ROS in tubular cell damage (Li et al., 2012), emphasizing the importance of targeting the response to oxidative stress in preventing the nephrotoxic effects of AA.

The c-Jun amino terminal kinase (JNK) enzyme is exquisitely sensitive to ROS and is known as a stress-activated protein kinase (Grynberg et al., 2017). Treatment of cultured tubular epithelial cells with AA leads to activation of the JNK enzyme, and the addition of a JNK inhibitor compound can inhibit AA-induced production of TGF-β1, upregulation of α-SMA, increased collagen I expression and cell cycle arrest (Yang et al., 2010; Zhou et al., 2010a; Rui et al., 2012). One study has used Western blotting to identify increased phosphorylation of the JNK enzyme in a model of AAN (Chang et al., 2020); however, the location of JNK activation and the functional significance of JNK activation in AAN remain unknown. Therefore, the aim of this study was to determine the pathological role of JNK signaling in both acute and chronic forms of AA-induced renal injury.

MATERIALS AND METHODS

Animals

Male C57BL/6J mice of 8–12 weeks of age were obtained from the Monash Animal Research Platform (Clayton, VIC, Australia). Animal studies were approved (MMCB/2017/21) by the Monash Medical Centre Animal Ethics Committee and performed according to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Aristolochic Acid-Induced Acute Kidney Injury

Groups of seven or eight male mice were given a single intraperitoneal injection of 5 mg/kg aristolochic acid (Sigma-Aldrich, Castle Hill, NSW, Australia) dissolved in saline and killed 3 days later. Groups of AA-injected mice were: (i) treated with 75 mg/kg CC-930 (supplied by Celgene Corporation, San Diego, CA, United States) by twice daily oral gavage starting 1 h before AA injection and continuing until being killed on day 3 (termed CC-930 AA), (ii) treated with vehicle only (0.5% carboxymethylcellulose in water) by twice daily oral gavage (termed vehicle AA), or (iii) did not receive any treatment (termed AA or untreated AA). The 75 mg/kg BID dosing of CC-930 was based on a previous study (Reich et al., 2012). In addition, one group of normal mice (no experimentation) was used as controls. Blood was collected at the time of killing. Urine samples were collected the day before killing. Serum and urine creatinine levels and urine total protein levels were measured using a Duppon ARL Analyzer at the Department of Clinical Biochemistry, Monash Health.

Aristolochic Acid-Induced Chronic Kidney Injury

Groups of 9 or 10 male mice were given intraperitoneal injections of 2 mg/kg AA every second day from day 0 until being killed on day 22. Groups of AA-injected mice were treated with 75 mg/kg CC-930, or vehicle alone, by twice daily oral gavage starting 1 h before AA injection and continuing until being killed on day 22. Normal mice (no experimentation) were used as controls.

Histology

Slices of kidney tissue were fixed in 4% neutral-buffered formalin or methylcarn fixative and processed for embedding in paraffin. Periodic acid-Schiff (PAS) plus hematoxylin staining was performed on 2 μm sections of formalin-fixed kidney tissue. Acute tubular injury was scored in the day 3 AA model as follows. Tubular cross-sections across the entire cortex were analyzed under high power (×400) and scored as normal or damaged. The definition of tubular damage included one or more of the following: tubular dilation or atrophy, loss of brush border, loss of tubular nuclei, and cast formation. Scoring was performed on blinded slides.

Immunohistochemistry

Immunostaining with the F4/80 antibody to detect macrophages (Bio-Rad, Gladesville, NSW, Australia), with a goat anti-collagen IV antibody (Southern Biotechnology, Birmingham, AL, United States) and with rabbit anti-α-SMA (Abcam, Melbourne, VIC, Australia) was carried out on 4 μm sections of methylcarn-fixed tissue using an avidin-biotin complex system with horseradish peroxidase and the substrate diaminobenzidine. Immunostaining with rabbit antibodies to phospho-c-Jun Ser63, phospho-Histone 2A.X Ser139, and CDKN1A (all from Cell Signaling, San Diego, CA, United States) was carried out on 4 μm sections of formalin-fixed sections after antigen retrieval (some sections had a PAS counterstain) as previously described (Flanc et al., 2007). The area of collagen IV staining was assessed by point counting of the entire cortex under medium

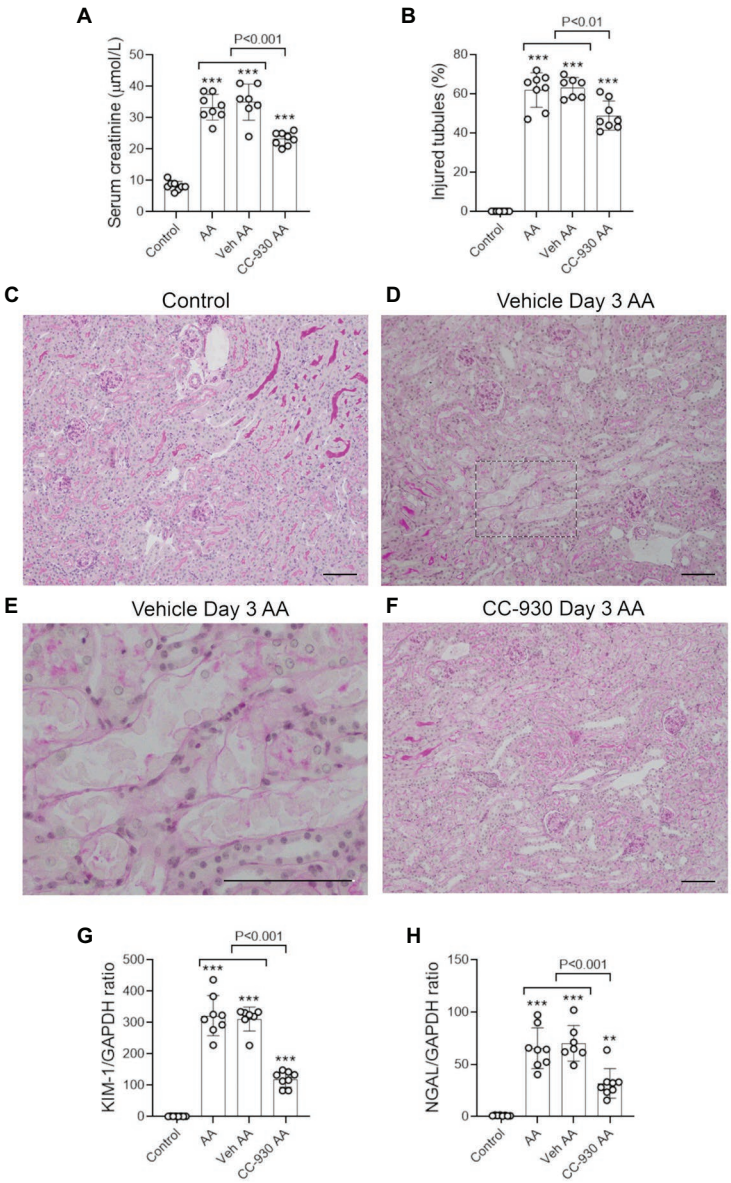

FIGURE 1 | Renal function and tubular damage on day 3 of aristolochic acid (AA)-induced acute kidney injury. AA-injected mice were either untreated (AA), or treated with vehicle (Veh AA) or CC-930 (CC-930 AA) and compared to normal controls. **(A)** Serum creatinine levels. **(B)** Graph of tubular damage score. **(C–F)** Periodic acid-Schiff (PAS) staining of kidney sections. **(C)** Kidney structure in normal control. **(D)** Vehicle treated AA showing damaged tubules with loss of brush border, loss of tubular nuclei and sloughing of cells into the lumen. **(E)** High power view of the image in D. **(F)** CC-930 treated AA shows less severe tubular damage compared to vehicle treated. Bars represent 100 μm. Reverse transcription PCR (RT-PCR) analysis of mRNA levels for the tubular damage markers; **(G)** KIM-1/HAVCR1 and **(H)** NGAL/LCN2. One-way ANOVA with Tukey's multiple comparison test. $^{**}p < 0.01$ and $^{***}p < 0.001$ vs. control.

power (x200) with a minimum of 1,200 points evaluated. Scoring was performed on blinded slides.

Western Blotting

Frozen kidney samples were homogenized in 0.5 ml lysis buffer and processed for Western blotting as previously described (Ma et al., 2014). Blots were probed for phospho-c-Jun and α-tubulin as the loading control using goat anti-rabbit Alexa Fluor 680 or donkey anti-mouse IRDye 800 secondary antibodies (Molecular Probes) and detected using the Odyssey Infrared Image Detecting System (LICOR). Densitometry analysis used ImageJ software, NIH.

Real Time Polymerase Chain Reaction

RNA extraction from frozen kidney tissue, cDNA synthesis, and PCR reactions on a StepOne Real-Time PCR System (Applied Biosystems, Foster City, CA, United States) were performed as previously described (Hou et al., 2018). Taqman primer/probes

for NOS2 and α-SMA have been described previously (Ma et al., 2011), and other primer/probes were purchased from Applied Biosystems. The comparative Ct (ΔCt) method was used to quantify the relative amount of mRNA which was normalized against the internal *Gapdh* mRNA control (Applied Biosystems).

Statistical Analysis

Data are shown as mean ± SD. Analysis used one-way ANOVA with Tukey's multiple comparison test, except for analysis of groups of two which used the student's *t*-test. Analysis was performed using GraphPad Prism 8.0 (San Diego, CA, United States).

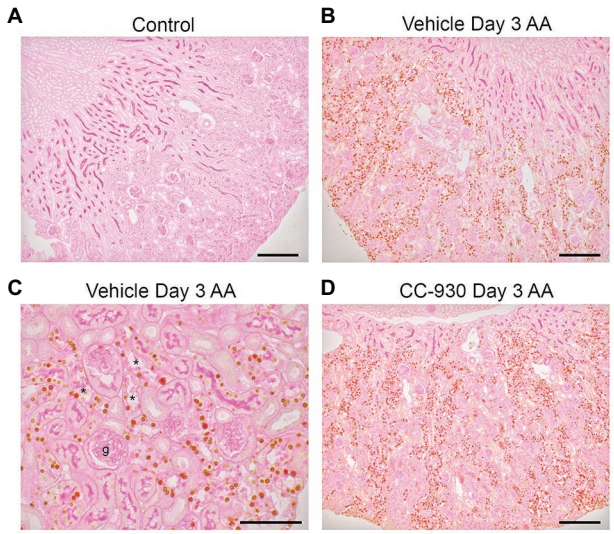

FIGURE 2 | Immunostaining for the DNA damage marker, phospho-Histone 2A.X Ser[139], on day 3 of AA-induced acute kidney injury. **(A)** No staining is seen the normal control kidney. **(B)** Many tubular nuclei show p-H2A.X staining in the cortex in vehicle treated day 3 AA. **(C)** High power view of vehicle treated day 3 AA shows staining in both damaged (*) and intact tubules, but no staining is seen in the glomerular tuft (g). **(D)** A similar pattern of stained for p-H2A.X in tubular nuclei is seen in CC-930 treated day 3 AA. Sections have PAS counterstain. Bars represent 200 μm **(A,B,D)**, or 100 μm **(C)**.

RESULTS

Acute Kidney Injury on Day 3 After AA Administration

A single injection of 5 mg/kg AA resulted in an acute loss of kidney function, as shown by a 4-fold increase in serum creatinine levels (**Figure 1A**). Compared to normal (control) kidney, PAS staining showed significant tubular damage in vehicle and untreated groups on day 3. Tubular damage featured loss of the brush border, loss of tubular nuclei and sloughing of cells into the tubular lumen (**Figures 1B–E**). Cellular damage was most obvious in the proximal portion of the tubule. There was also a marked increase in the mRNA levels of the tubular damage markers, Kidney Injury Molecule 1 (KIM1/HAVCR1) and Lipocalin 2 (LCN2/NGAL) in untreated and vehicle treated AA groups on day 3 (**Figures 1G,H**).

Aristolochic acid is known to cause DNA damage by inducing adducts (Jadot et al., 2017). DNA damage rapidly induces phosphorylation of the H2A.X variant which is required for checkpoint-mediated cell cycle arrest and DNA repair (Yuan et al., 2010). While no phospho-H2A.X Ser[139] staining was evident in control kidney, many tubular cells showed strong nuclear staining for p-H2A.X in vehicle treated and untreated day 3 AA. High power shows p-H2A.X staining in remaining nuclei in damaged tubules, although tubules without obvious damage also showed staining.

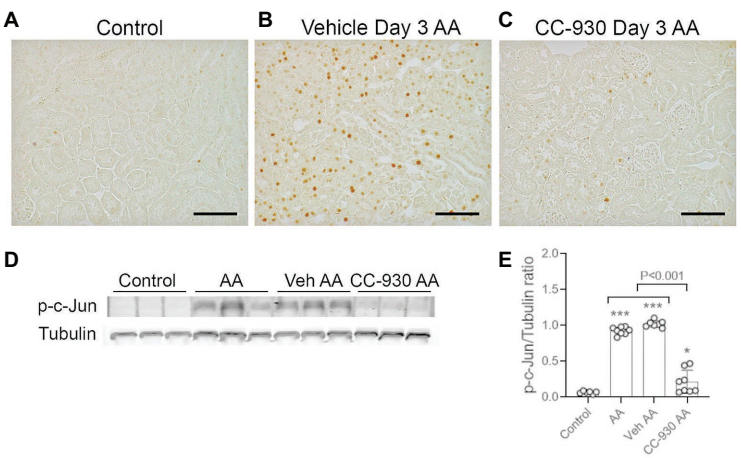

FIGURE 3 | c-Jun amino terminal kinase (JNK) signaling on day 3 of aristolochic acid (AA) induced acute kidney injury. AA injected mice were either untreated (AA), or treated with vehicle (Veh AA) or CC-930 (CC-930 AA) and compared to normal controls. **(A-C)** Immunohistochemistry staining for phospho-c-Jun Ser[63]. **(A)** Normal control kidney shows only very occasion p-c-Jun+ cells. **(B)** Vehicle treated AA shows large numbers of p-c-Jun+ nuclei, predominantly in tubular epithelial cells, including in damaged tubules. **(C)** CC-930 treated AA showing a dramatic reduction in p-c-Jun+ cells. Bars represent 100 μm. **(D)** Western blot of kidney lysates for p-c-Jun and reprobed for α-tubulin. **(E)** Graph showing quantification of Western blots. One-way ANOVA with Tukey's multiple comparison test. $^{*}p < 0.05$ and $^{***}p < 0.001$ vs. control.

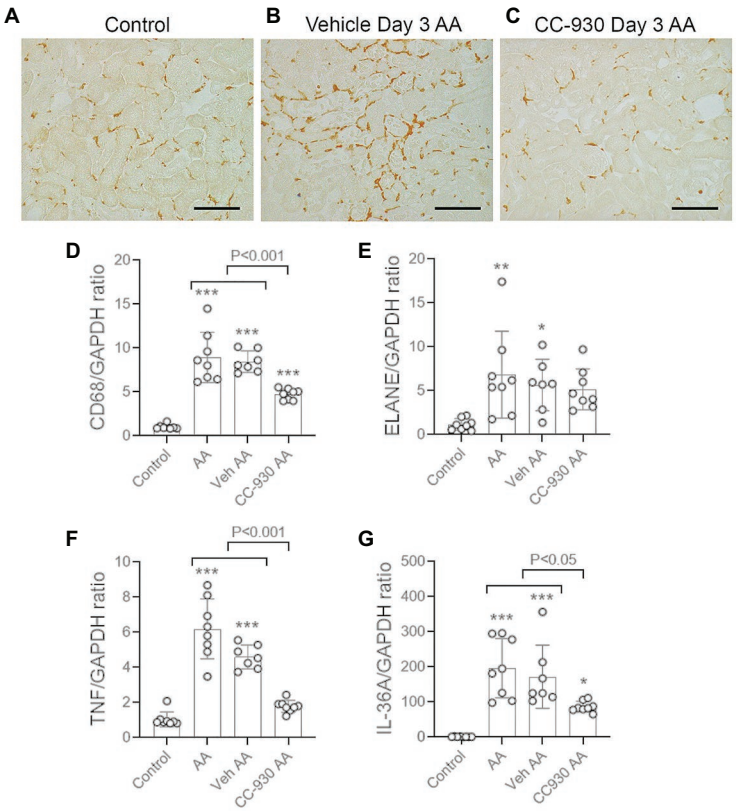

FIGURE 4 | Macrophage infiltration and inflammation on day 3 of AA-induced acute kidney injury. AA injected mice were either untreated (AA), or treated with vehicle (Veh AA) or CC-930 (CC-930 AA) and compared to normal controls. **(A-C)** Immunohistochemistry staining for F4/80+ macrophages. **(A)** Normal control kidney shows a regular network of resident kidney macrophages. **(B)** Vehicle treated AA shows a significant increase in F4/80+ macrophages, mainly around damaged tubules. **(C)** CC-930 treated AA showing a reduction in the number of infiltrating macrophages. Bars represent 100 μm. **(D–G)** RT-PCR analysis of kidney tissue for: **(D)** CD68; **(E)** ELANE; **(F)** TNF, and **(G)** IL-36A. One-way ANOVA with Tukey's multiple comparison test. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ vs. control.

Of note, very few cells in glomeruli or in the early proximal tubule were stained (**Figures 2A–C**).

c-Jun amino terminal kinase is the only enzyme that phosphorylates c-Jun at Serine 63, allowing detection of p-c-Jun Ser^{63} as a surrogate marker of JNK activation (Pulverer et al., 1991; Minden et al., 1994; Ma et al., 2007). Only very occasional cells were stained for p-c-Jun in normal mouse kidney. By contrast, many p-c-Jun+ cells are seen in the kidney of vehicle and untreated groups on day 3 after AA (**Figures 3A,B**). The p-c-Jun staining is prominent in all parts of the nephron, including damaged tubules and tubules without obvious histologic damage. Some interstitial p-c-Jun+ cells are also evident, whereas few p-c-Jun+ cells were evident in glomeruli. Western blotting shows a strong band for p-c-Jun in vehicle and untreated day 3 AA groups, which was barely detected in normal kidney (**Figures 3D,E**).

Inflammation was evident on day 3 after AA administration in vehicle and untreated groups. Immunostaining showed a focal infiltrate of F4/80+ macrophages in areas of tubular damage (**Figures 4A,B**). This was consistent with the 8-fold increase in mRNA levels of the macrophage marker, CD68 (**Figure 4D**). There was a significant, but variable, neutrophil infiltrate as shown by mRNA levels for neutrophil elastase/ELANE (**Figure 4E**). Leukocyte infiltration was associated with up-regulation of inflammatory cytokines TNF and IL-36α (**Figures 4F,G**).

Effect of CC-930 Treatment on Acute Kidney Injury on Day 3 in the AA Model

CC-930 treatment gave protection from acute kidney injury as shown by a significant reduction in serum creatinine levels compared to the untreated and vehicle treated groups (**Figure 1A**). CC-930 treatment also significantly reduced tubular damage as shown by scoring of PAS stained sections and by the mRNA levels of KIM-1 and NGAL (**Figures 1B,F–H**). As expected, CC-930 treatment did not affect AA-induced DNA damage, as shown by a lack of effect upon p-H2A.X staining (**Figure 2D**). However, this protection against acute kidney injury was associated with a profound reduction in JNK signaling as demonstrated by reductions in c-Jun phosphorylation using both immunohistochemistry and Western blotting (**Figures 3C–E**).

CC-930 treatment significantly reduced the macrophage infiltrate based on F4/80 immunostaining and CD68 mRNA levels (**Figures 4C,D**), and reduced mRNA levels of TNF and

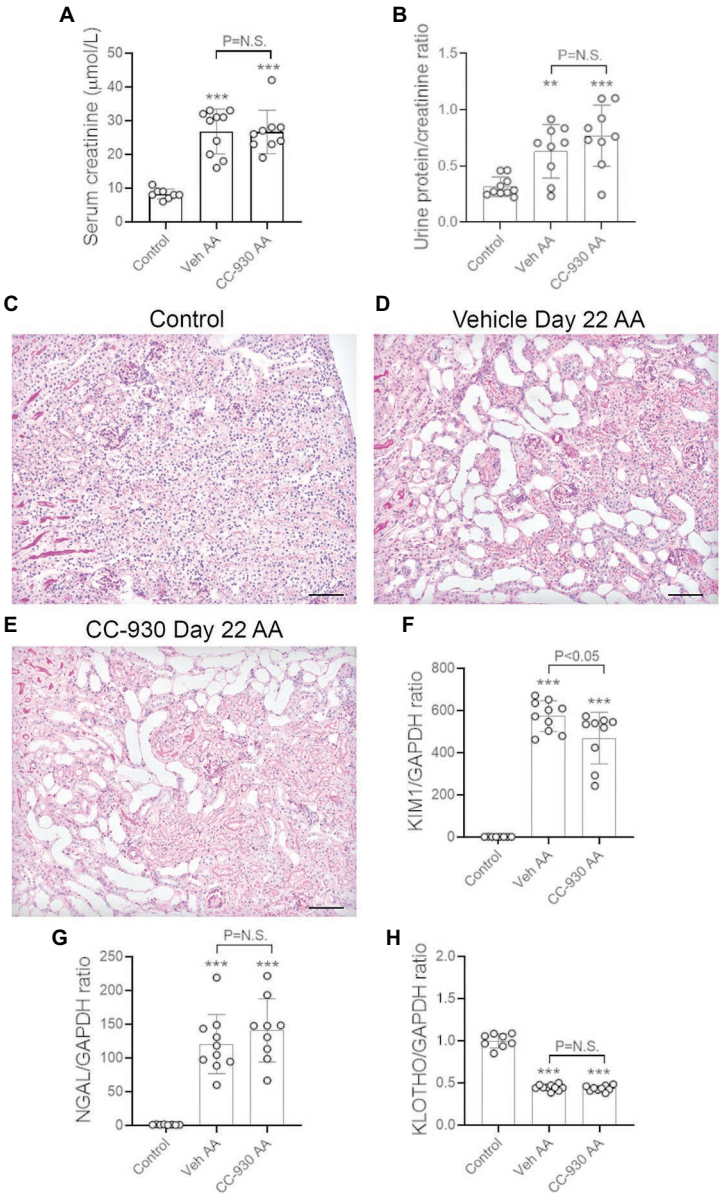

FIGURE 5 | Renal function and tubular damage on day 22 of aristolochic acid (AA) induced chronic kidney disease. Vehicle and CC-930 treated AA groups were compared to normal controls. **(A)** Serum creatinine levels. **(B)** Urinary protein excretion measured as a ratio of mg total protein/mol creatinine. **(C)** PAS staining of normal control kidney. **(D)** PAS staining of vehicle treated AA shows marked tubular atrophy, interstitial cell infiltration and occasional tubular casts, while glomeruli are largely normal. **(E)** PAS staining of CC-930 treated AA shows similar chronic tubular damage to that in vehicle treated. Bars represent 100 μm. RT-PCR analysis of: **(F)** KIM-1/HAVCR1, **(G)**, NGAL/LCN2, and **(H)** α-Klotho mRNA levels. One-way ANOVA with Tukey's multiple comparison test. $^{**}p < 0.01$ and $^{***}p < 0.001$ vs. control. N.S., not significant.

IL-36α (**Figures 4F,G**). However, the CC-930 did have a significant effect upon the variable neutrophil infiltrate (**Figure 4E**).

Characterization of Chronic Kidney Disease Induced by Repeated Administration of AA

A model of chronic tubulointerstitial disease was induced by repeated administration of a lower dose of AA (2 mg/kg) every second day from day 0 until being killed on day 22. This resulted in impaired kidney function in vehicle treated mice as shown by a 3-fold increase in serum creatinine over that of normal mice (**Figure 5A**), and a modest increase in urinary protein excretion (**Figure 5B**). Histologic analysis showed marked tubular atrophy, accumulation of cells in the interstitial space, and some tubular casts. By contrast, glomeruli remained largely normal (**Figures 5C,D**). Substantial tubular damage was also indicated by a marked increase in mRNA levels of tubular damage markers

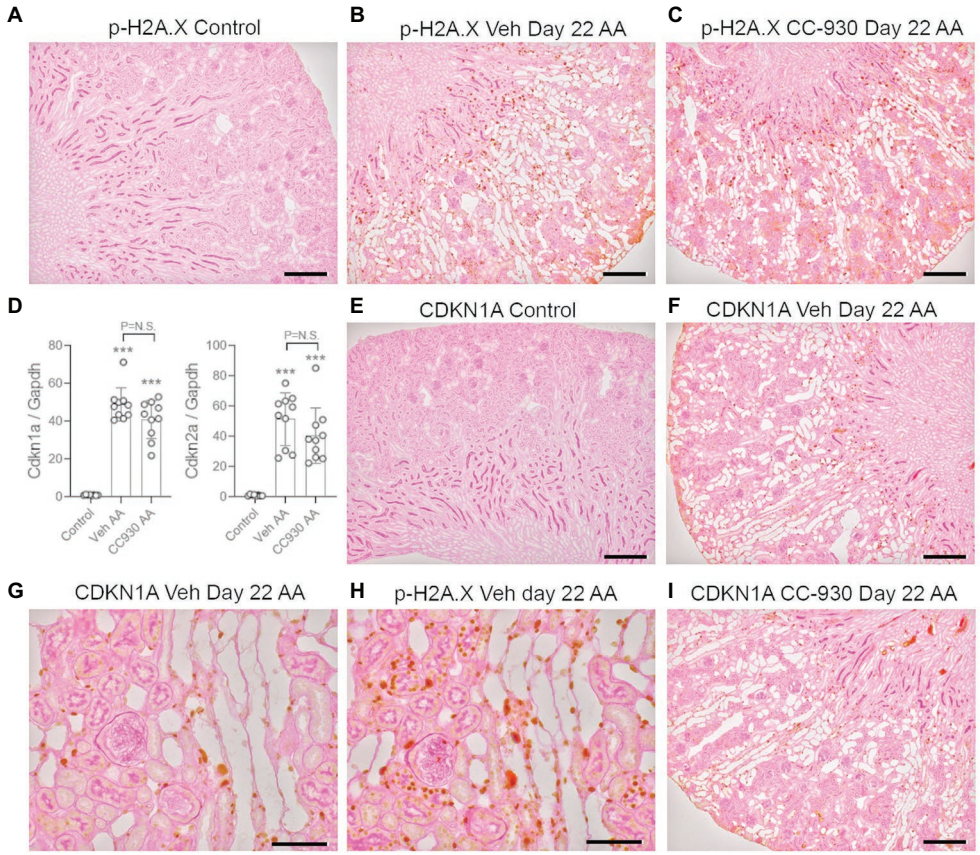

FIGURE 6 | DNA damage (phospho-H2A.X) and senescence markers (CDKN1A) on day 22 of aristolochic acid (AA) induced chronic kidney disease. **(A)** No staining for p-H2A.X is seen the normal control kidney. **(B)** Numerous p-H2A.X stained nuclei are seen in vehicle treated day 22 AA, evident in both atrophied and normal tubules. **(C)** A similar pattern of p-H2A.X nuclear staining is seen in CC-930 treated day 22 AA. **(D)** RT-PCR analysis of kidney tissue for Cdkn1a and Cdkn2a. **(E)** No staining for CDKN1A is seen the normal control kidney. **(F)** Nuclear staining for CDKN1A is evident in cells within atrophied tubules in vehicle treated day 22 AA. **(G)** Higher power view emphasizes that CDKN1A staining is mostly restricted to damaged tubules. **(H)** Serial section to **(F)** showing p-H2A.X staining is also evident damaged tubules, but is more extensive than that of CDKN1A. **(I)** CDKN1a staining in CC-930 treated day 22 AA is similar to that seen in vehicle treated day 22 AA. Bars represent 200 μm **(A–C,E,F,I)**, or 100 μm **(G,H)**. One-way ANOVA with Tukey's multiple comparison test. ***$p < 0.001$ vs. control; N.S., not significant.

KIM-1 and NGAL, while expression of the tubular protective molecule, α-Klotho, was substantially reduced (**Figures 5F–H**).

Chronic administration of low dose AA caused sustained activation of the DNA damage response as shown by immunostaining for p-H2A.X (**Figures 6A,B**), although fewer cells were stained compared to the acute effects of high dose AA on day 3 (**Figure 2B**). Only very low mRNA levels for the senescence markers, Cyclin Dependent Kinase Inhibitor 1A (Cdkn1a/p21^{CIP1}) and Cdkn2a/p16^{INK4A} were evident in control kidney, but both markers were highly upregulated in vehicle treated day 22 AA (**Figure 6D**). No cells were stained for CDKN1A in control kidney, but cell nuclei in atrophic tubules were stained in vehicle treated day 22 AA (**Figures 6E,F**). Analysis of serial sections show CDKN1A stained cells in the same damaged tubules that exhibit p-H2A.X stained cells, although overall more cells were stained for p-H2A.X than for CDKN1A (**Figures 6G,H**).

Chronic administration of the low dose AA also caused sustained activation of the JNK signaling pathway at day 22 in vehicle treated mice. Many p-c-Jun stained cells were evident, particularly in dilated and atrophic tubules, as well as some interstitial p-c-Jun+ cells (**Figures 7A,B**). In addition, a strong band for p-c-Jun was evident Western blots of vehicle treated mice on day 22 of chronic AA administration (**Figures 7D,E**).

Significant interstitial fibrosis was evident on day 22 of chronic AA administration. Increased collagen IV deposition was seen in areas of atrophic tubules (**Figures 8A,B**), which was associated with focal accumulation of α-SMA+ myofibroblasts (not shown). Image analysis showed a 2.5-fold increase in the interstitial deposition of collagen IV in the cortex in the vehicle treated day 22 AA group compared to normal controls (**Figure 8D**). This was accompanied by a significant increase in mRNA levels of collagen III and IV, α-SMA/Acta2, and TGF-β1 (**Figures 8E–H**). There was also a marked macrophage infiltrate as shown by the increase in CD68 mRNA levels (**Figure 8I**), with increases in mRNA levels of macrophage M1-type pro-inflammatory molecules NOS2 and MMP-12 (**Figures 9A,B**). In addition, vehicle

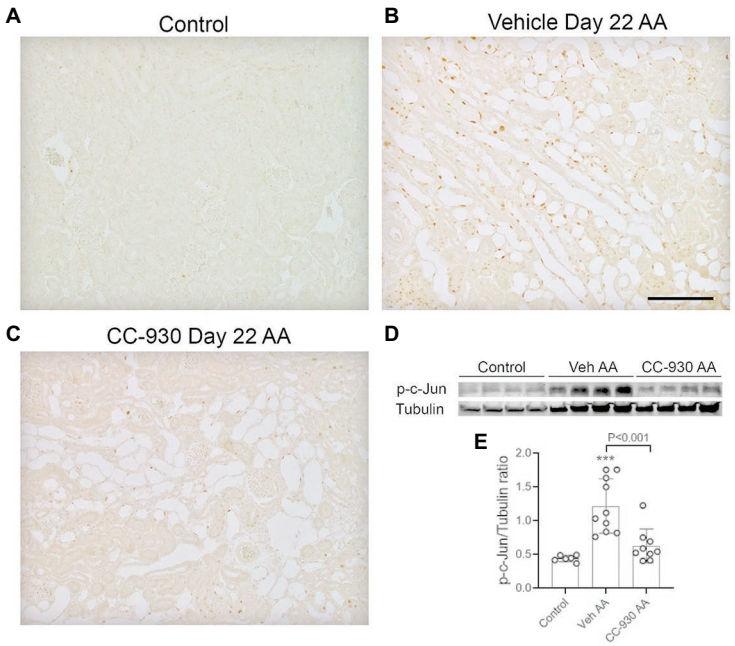

FIGURE 7 | JNK signaling on day 22 of aristolochic acid (AA) induced chronic kidney disease. Vehicle and CC-930 treated AA groups were compared to normal controls. **(A–C)** Immunohistochemistry staining for phospho-c-Jun Ser[63]. **(A)** Normal control kidney shows very little p-c-Jun staining. **(B)** Vehicle treated AA shows tubular cells with nuclear staining for p-c-Jun, being most prominent in dilated and atrophic tubules. **(C)** CC-930 treated AA showing a very substantial reduction in p-c-Jun+ cells. Bar represent 100 μm. **(D)** Western blot of kidney lysates for p-c-Jun and reprobed for α-tubulin. **(E)** Graph showing quantification of Western blots. One-way ANOVA with Tukey's multiple comparison test. ***$p < 0.001$ vs. control.

treated mice on day 22 of AA administration showed increased expression of TNF and IL-36α (**Figures 9E,F**).

Effect of CC-930 Treatment on AA-Induced Chronic Kidney Disease

CC-930 treatment of mice with chronic administration of AA did not affect the loss of kidney function as shown by serum creatinine levels or the development of mild proteinuria (**Figures 5A,B**). Consistent with this, CC-930 treatment did not affect histologic tubular damage or changes in NGAL or α-Klotho mRNA levels (**Figures 5E,G,H**), although the high levels of KIM-1 mRNA were significantly reduced by 18% (**Figure 5F**).

CC-930 treatment did not affect the sustained DNA damage response as shown by immunostaining for p-H2A.X (**Figure 6C**), or the induction of cell senescence as shown by Cdkn1a and Cdkn2a mRNA levels and immunostaining for CDKN1A (**Figures 6D,I**). However, CC-930 treatment did substantially reduce c-Jun phosphorylation as shown by both immunohistochemistry and Western blotting (**Figures 7C–E**).

CC-930 treatment had no significant effect on renal fibrosis on day 22 of AAN in terms of collagen IV deposition and mRNA levels of collagen III and IV, and α-SMA/Acta2 (**Figures 8C–G**), although there was a small reduction in the mRNA levels of TGF-β1 (**Figure 8H**).

CC-930 treatment did not affect the substantial macrophage infiltration on day 22 as shown by CD68 mRNA levels (**Figure 8I**). However, markers of M1-type macrophages, NOS-2 and MMP-12, were substantially reduced by CC-930 treatment and the NOS2:CD68 and MMP-12:CD68 mRNA ratios were also significantly reduced compared to the vehicle treated group (**Figures 9A–D**). Finally, CC-930 treatment significantly reduced TNF, but not IL-36α, mRNA levels (**Figures 9E,F**).

DISCUSSION

This study has shown that inhibition of JNK signaling with CC-930 provided significant protection against high dose AA-induced acute kidney injury, but failed to protect against chronic kidney disease induced by repeated low dose AA administration.

We identified a strong induction of JNK signaling in tubular epithelial cells on day 3 after a single high dose of AA based on amino-terminal phosphorylation of c-Jun at Ser[63], validating previous studies showing that AA induces JNK activation in cultured tubular cells (Yang et al., 2010; Zhou et al., 2010a; Rui et al., 2012). We also demonstrated that chronic administration of low dose AA causes sustained JNK activation in tubular epithelial cells, particularly in atrophic tubular cells. Treatment with CC-930 was highly effective in suppressing JNK signaling.

The protection against acute AA-induced tubular cell damage and renal function impairment is consistent with other studies in which JNK inhibition suppressed acute kidney injury induced

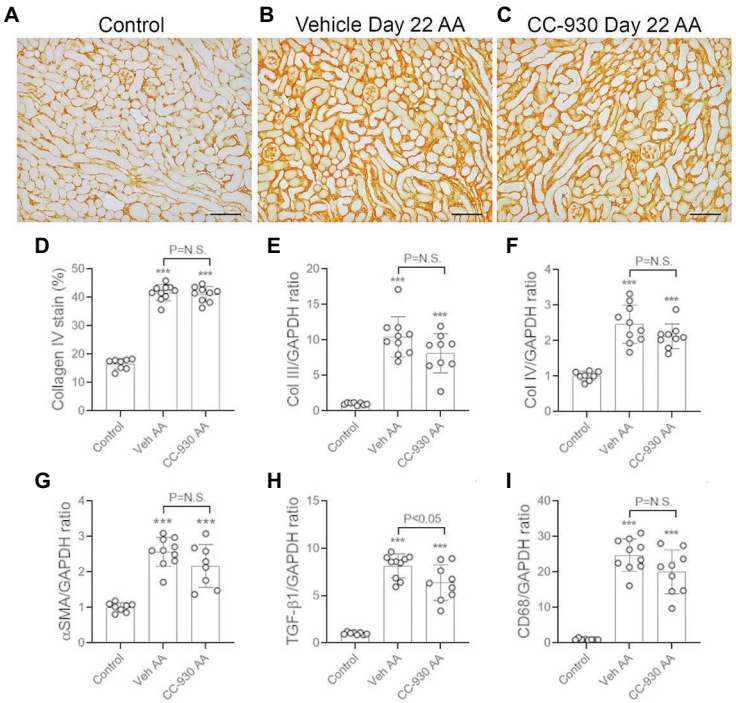

FIGURE 8 | Renal fibrosis on day 22 of AA-induced chronic kidney disease. Vehicle and CC-930 treated AA groups were compared to normal controls. **(A–C)** Immunohistochemistry staining for collagen IV. **(A)** Normal mouse kidney showing collagen IV in the tubular and glomerular basement membranes. **(B)** Vehicle treated day 22 AA shows increased interstitial collagen IV staining, particularly in areas with atrophic tubules. **(C)** CC-930 treated day 22 AA shows a similar increase in interstitial collagen IV deposition as seen with vehicle treatment. Bars represent 100 μm. **(D)** Graph showing quantification of the area of interstitial collagen IV staining. RT-PCR analysis of: **(E)** Collagen III; **(F)** Collagen IV; **(G)** α-SMA/Acta2; **(H)** TGF-β1, and **(I)** CD68. One-way ANOVA with Tukey's multiple comparison test. ***$p < 0.001$ vs. control. N.S., not significant.

by renal ischemia/reperfusion injury (IRI) or cisplatin (Francescato et al., 2007; Wang et al., 2007; Kanellis et al., 2010). The production of ROS is a prominent feature in all three of these models of acute kidney injury, and studies have shown that blockade of JNK signaling prevents ROS-induced death of tubular epithelial cells (Pat et al., 2003; Arany et al., 2004), providing direct evidence that JNK is critical in ROS-induced tubular cell death. While CC-930 treatment did not affect the AA-induced DNA damage response, the protective effect of CC-930 seen in the acute AA model is most likely due to inhibition of tubular damage and cell death caused by the oxidative stress induced by high dose AA. Indeed, this mechanistic link between DNA damage and ROS production is supported by the presence of cells exhibiting JNK signaling and the DNA damage response within damaged tubules.

CC-930 treatment also reduced leukocyte infiltration and the expression of inflammatory cytokines in AA-induced acute kidney injury – a finding consistent with the suppression of inflammation seen with JNK inhibition in the IRI and cisplatin models of acute kidney injury (Francescato et al., 2007; Wang et al., 2007; Kanellis et al., 2010). This may simply reflect the reduction in tubular damage and consequent reduced release of pro-inflammatory and chemotactic danger-associated molecular patterns. Alternatively, this could reflect a specific role for JNK signaling *via* Activator Protein 1 (AP1) in promoting kidney expression of inflammatory molecules since transcription of *Nos2*, *Tnf*, and *Mmp12* genes has been shown to operate *via* AP1 (Newell et al., 1994; Wu et al., 2001; Lin et al., 2007; Grynberg et al., 2017).

Given the significant protective effect of JNK inhibition in AA-induced acute kidney injury, we were surprised by the lack of protection seen with CC-930 in AA-induced chronic kidney disease. In particular, cell culture studies have shown that short-term exposure of tubular epithelial cells to AA induces a strong pro-fibrotic response with increased expression of TGF-β1 and α-SMA, and increased production of collagens (Yang et al., 2010; Zhou et al., 2010a; Rui et al., 2012). In addition, JNK blockade has been shown to reduce inflammation and fibrosis across different models of chronic kidney disease. For example, JNK blockade suppressed tubular cell death, inflammation and the aggressive fibrosis seen in unilateral ureteric obstruction (Ma et al., 2007). JNK blockade prevents the development of crescentic glomerulonephritis in susceptible rats with protection from renal impairment, glomerular damage, and inhibition of the macrophage pro-inflammatory response and renal fibrosis (Flanc et al., 2007). Indeed, intervention in established crescentic disease with a JNK inhibitor still provides significant protection against inflammation and renal fibrosis (Ma et al., 2009). Furthermore, in two other classic models of renal interstitial

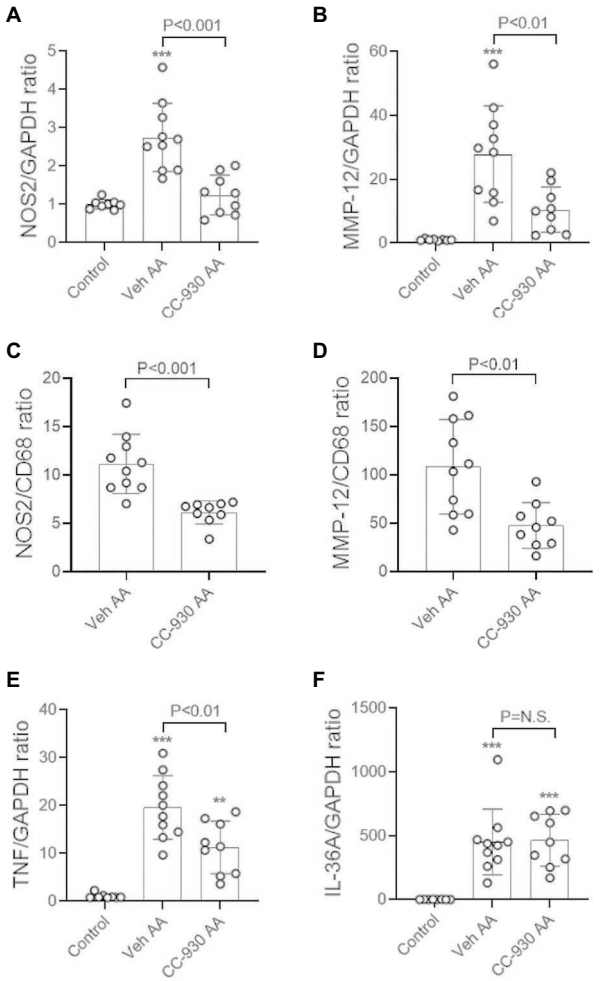

FIGURE 9 | Macrophage activation and inflammation on day 22 of aristolochic acid (AA) induced chronic kidney disease. Vehicle and CC-930 treated AA groups were compared to normal controls. RT-PCR analysis of: **(A)** NOS2, and; **(B)** MMP-12. Graphs show the relative mRNA ratio of: **(C)** NOS2/CD68, and; **(D)** MMP-12/CD68. RT-PCR analysis of: **(E)** TNF, and; **(F)** IL-36A. One-way ANOVA with Tukey's multiple comparison test, or student's *t*-test. $^{**}p < 0.001$ and $^{***}p < 0.001$ vs. control. N.S., not significant.

fibrosis – unilateral IRI and folic acid-induced fibrosis – administration of a JNK inhibitor can suppress both inflammation and fibrosis (de Borst et al., 2009; Jiang et al., 2019).

One possible explanation for the protective effects of JNK inhibition in acute but not chronic AA-induced renal injury is that a different underlying pathogenic mechanism drives chronic injury. Thus, while JNK signaling is important for high dose AA-induced ROS production and cell damage in acute kidney injury, this may have only a minor role in the response to the accumulation of DNA damage occurring in ongoing exposure to low dose AA-induced chronic kidney disease. AA is a potent inducer of the DNA damage response in cultured tubular epithelial cells (Chen et al., 2010a,b). We validated this finding *in vivo* with clear activation of the DNA damage response (phosphorylation of H2A.X) in tubular epithelial cells following acute exposure to high dose AA on day 3, and ongoing activation of the DNA damage response with chronic exposure to low dose AA on day 22. Indeed, DNA damage is potent inducer of cellular senescence (Docherty et al., 2019), and senescent tubular epithelial cells have been implicated in the progression of renal interstitial fibrosis (Kishi et al., 2019). We found the induction of numerous senescent cells in atrophied tubules on the basis of CDKN1A expression and marked upregulation of Cdkn1a and Cdkn2a mRNA levels on day 22 of chronic AA administration. Indeed, the same atrophied tubules contained epithelial cells with activation of the DNA damage response and expression of senescence markers. Many cells in these atrophic tubules also exhibited JNK signaling, but JNK inhibition did not affect either the DNA damage response or the induction of senescence in these damage tubules – providing a rationale for the failure of JNK blockade to suppress renal fibrosis in this setting.

A second possible mechanism driving chronic AA-induced kidney injury is TGF-β/Smad3 signaling (Zhou et al., 2010a). While JNK has been shown to phosphorylate Smad3 to enhance the fibrotic response in short term AA stimulation of tubular epithelial cells (Zhou et al., 2010a), and combined JNK and Smad3 inhibition gives additive benefit in reducing folic acid-induced renal fibrosis (Jiang et al., 2019); it may that chronic administration of AA drives a Smad3-dependent fibrosis that is largely independent of JNK signaling.

Our previous studies found that global macrophage depletion using a c-fms inhibitor suppressed chronic kidney disease in response to repeated AA administration (Dai et al., 2016). This is consistent with other models of kidney disease in which macrophages play an important role in both tubular damage and renal interstitial fibrosis (Han et al., 2013; Meng et al., 2016; Tang et al., 2019). JNK signaling in macrophages has been shown to induce a pro-inflammatory M1 response that induces glomerular and tubulointerstitial renal injury in experimental glomerulonephritis (Ikezumi et al., 2004; Flanc et al., 2007). In the current study, JNK inhibition also suppressed the M1-type pro-inflammatory response in AA-induced chronic kidney disease. However, this did not significantly impact upon disease progression arguing that tubulointerstitial damage by M1-type macrophages is not an important pathogenic mechanism in AA-induced chronic kidney disease.

In conclusion, we have demonstrated that AA administration is a potent inducer of JNK signaling in tubular epithelial cells of the kidney. Blockade of JNK signaling with CC-930 significantly reduced the acute effects of high dose AA on tubular cell damage and renal function impairment, presumably *via* inhibition of ROS-dependent cell damage. However, ongoing JNK blockade was unable to protect against DNA damage-induced tubular cell atrophy and senescence that promote chronic kidney disease caused by ongoing exposure to low dose AA.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was approved (MMCB/2017/21) by the Monash Medical Centre Animal Ethics Committee and performed according to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

AUTHOR CONTRIBUTIONS

FY, DN-P, and FM: conceptualization. FY, EO, KL, FM, and GT: methodology. EO and FM: validation. FY, EO, DN-P, FM, and GT: formal analysis. FY, EO, and FM: investigation. FY and DN-P: data curation. FY and XJ: original draft preparation. XJ and DN-P: final manuscript preparation and supervision. FY, XJ, and DN-P: funding acquisition. All authors contributed to the article and approved the submitted version.

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REFERENCES

Arany, I., Megyesi, J. K., Kaneto, H., Tanaka, S., and Safirstein, R. L. (2004). Activation of ERK or inhibition of JNK ameliorates H_2O_2 cytotoxicity in mouse renal proximal tubule cells. *Kidney Int.* 65, 1231–1239. doi: 10.1111/j.1523-1755.2004.00500.x

Bakhiya, N., Arlt, V. M., Bahn, A., Burckhardt, G., Phillips, D. H., and Glatt, H. (2009). Molecular evidence for an involvement of organic anion transporters (OATs) in aristolochic acid nephropathy. *Toxicology* 264, 74–79. doi: 10.1016/j.tox.2009.07.014

Chang, J. F., Hsieh, C. Y., Lu, K. C., Chen, Y. W., Liang, S. S., Lin, C. C., et al. (2020). Therapeutic targeting of aristolochic acid induced uremic toxin retention, SMAD 2/3 and JNK/ERK pathways in tubulointerstitial fibrosis: nephroprotective role of propolis in chronic kidney disease. *Toxins (Basel)* 12:364. doi: 10.3390/toxins12060364

Chen, Y. Y., Chiang, S. Y., Wu, H. C., Kao, S. T., Hsiang, C. Y., Ho, T. Y., et al. (2010a). Microarray analysis reveals the inhibition of nuclear factor-kappa B signaling by aristolochic acid in normal human kidney (HK-2) cells. *Acta Pharmacol. Sin.* 31, 227–236. doi: 10.1038/aps.2009.197

Chen, Y. Y., Chung, J. G., Wu, H. C., Bau, D. T., Wu, K. Y., Kao, S. T., et al. (2010b). Aristolochic acid suppresses DNA repair and triggers oxidative DNA damage in human kidney proximal tubular cells. *Oncol. Rep.* 24, 141–153. doi: 10.3892/or_00000839

Dai, X. Y., Huang, X. R., Zhou, L., Zhang, L., Fu, P., Manthey, C., et al. (2016). Targeting c-fms kinase attenuates chronic aristolochic acid nephropathy in mice. *Oncotarget* 7, 10841–10856. doi: 10.18632/oncotarget.7460

de Borst, M. H., Prakash, J., Sandovici, M., Klok, P. A., Hamming, I., Kok, R. J., et al. (2009). c-Jun NH2-terminal kinase is crucially involved in renal tubulointerstitial inflammation. *J. Pharmacol. Exp. Ther.* 331, 896–905. doi: 10.1124/jpet.109.154179

Docherty, M. H., O'sullivan, E. D., Bonventre, J. V., and Ferenbach, D. A. (2019). Cellular senescence in the kidney. *J. Am. Soc. Nephrol.* 30, 726–736. doi: 10.1681/ASN.2018121251

Flanc, R. S., Ma, F. Y., Tesch, G. H., Han, Y., Atkins, R. C., Bennett, B. L., et al. (2007). A pathogenic role for JNK signaling in experimental anti-GBM glomerulonephritis. *Kidney Int.* 72, 698–708. doi: 10.1038/sj.ki.5002404

Francescato, H. D., Costa, R. S., Junior, F. B., and Coimbra, T. M. (2007). Effect of JNK inhibition on cisplatin-induced renal damage. *Nephrol. Dial. Transplant.* 22, 2138–2148. doi: 10.1093/ndt/gfm144

Grynberg, K., Ma, F. Y., and Nikolic-Paterson, D. J. (2017). The JNK Signaling pathway in renal fibrosis. *Front. Physiol.* 8:829. doi: 10.3389/fphys.2017.00829

Han, Y., Ma, F. Y., Tesch, G. H., Manthey, C. L., and Nikolic-Paterson, D. J. (2013). Role of macrophages in the fibrotic phase of rat crescentic glomerulonephritis. *Am. J. Physiol. Renal Physiol.* 304, F1043–F1053. doi: 10.1152/ajprenal.00389.2012

Hou, W., Leong, K. G., Ozols, E., Tesch, G. H., Nikolic-Paterson, D. J., and Ma, F. Y. (2018). Cyclophilin D promotes tubular cell damage and the development of interstitial fibrosis in the obstructed kidney. *Clin. Exp. Pharmacol. Physiol.* 45, 250–260. doi: 10.1111/1440-1681.12881

Ikezumi, Y., Hurst, L., Atkins, R. C., and Nikolic-Paterson, D. J. (2004). Macrophage-mediated renal injury is dependent on signaling via the JNK pathway. *J. Am. Soc. Nephrol.* 15, 1775–1784. doi: 10.1097/01.asn.0000131272.06958.de

Jadot, I., Decleves, A. E., Nortier, J., and Caron, N. (2017). An integrated view of Aristolochic acid nephropathy: update of the literature. *Int. J. Mol. Sci.* 18:297. doi: 10.3390/ijms18020297

Jiang, M., Fan, J., Qu, X., Li, S., Nilsson, S. K., Sun, Y. B. Y., et al. (2019). Combined blockade of Smad3 and JNK pathways ameliorates progressive fibrosis in folic acid nephropathy. *Front. Pharmacol.* 10:880. doi: 10.3389/fphar.2019.00880

Kanellis, J., Ma, F. Y., Kandane-Rathnayake, R., Dowling, J. P., Polkinghorne, K. R., Bennett, B. L., et al. (2010). JNK signalling in human and experimental renal ischaemia/reperfusion injury. *Nephrol. Dial. Transplant.* 25, 2898–2908. doi: 10.1093/ndt/gfq147

Kishi, S., Brooks, C. R., Taguchi, K., Ichimura, T., Mori, Y., Akinfolarin, A., et al. (2019). Proximal tubule ATR regulates DNA repair to prevent maladaptive renal injury responses. *J. Clin. Invest.* 129, 4797–4816. doi: 10.1172/JCI122313

Li, Y. C., Tsai, S. H., Chen, S. M., Chang, Y. M., Huang, T. C., Huang, Y. P., et al. (2012). Aristolochic acid-induced accumulation of methylglyoxal and nepsilon-(carboxymethyl)lysine: an important and novel pathway in the pathogenic mechanism for aristolochic acid nephropathy. *Biochem. Biophys. Res. Commun.* 423, 832–837. doi: 10.1016/j.bbrc.2012.06.049

Lin, M. W., Tsao, L. T., Chang, L. C., Chen, Y. L., Huang, L. J., Kuo, S. C., et al. (2007). Inhibition of lipopolysaccharide-stimulated NO production by a novel synthetic compound CYL-4d in RAW 264.7 macrophages involving the blockade of MEK4/JNK/AP-1 pathway. *Biochem. Pharmacol.* 73, 1796–1806. doi: 10.1016/j.bcp.2007.02.009

Ma, F. Y., Flanc, R. S., Tesch, G. H., Bennett, B. L., Friedman, G. C., and Nikolic-Paterson, D. J. (2009). Blockade of the c-Jun amino terminal kinase prevents crescent formation and halts established anti-GBM glomerulonephritis in the rat. *Lab. Investig.* 89, 470–484. doi: 10.1038/labinvest.2009.2

Ma, F. Y., Flanc, R. S., Tesch, G. H., Han, Y., Atkins, R. C., Bennett, B. L., et al. (2007). A pathogenic role for c-Jun amino-terminal kinase signaling in renal fibrosis and tubular cell apoptosis. *J. Am. Soc. Nephrol.* 18, 472–484. doi: 10.1681/ASN.2006060604

Ma, F. Y., Tesch, G. H., and Nikolic-Paterson, D. J. (2014). ASK1/p38 signaling in renal tubular epithelial cells promotes renal fibrosis in the mouse obstructed kidney. *Am. J. Physiol. Renal Physiol.* 307, F1263–F1273. doi: 10.1152/ajprenal.00211.2014

Ma, F. Y., Tesch, G. H., Ozols, E., Xie, M., Schneider, M. D., and Nikolic-Paterson, D. J. (2011). TGF-beta1-activated kinase-1 regulates inflammation and fibrosis in the obstructed kidney. *Am. J. Physiol. Renal Physiol.* 300, F1410–F1421. doi: 10.1152/ajprenal.00018.2011

Meng, X. M., Wang, S., Huang, X. R., Yang, C., Xiao, J., Zhang, Y., et al. (2016). Inflammatory macrophages can transdifferentiate into myofibroblasts during renal fibrosis. *Cell Death Dis.* 7:e2495. doi: 10.1038/cddis.2016.402

Minden, A., Lin, A., Smeal, T., Derijard, B., Cobb, M., Davis, R., et al. (1994). c-Jun N-terminal phosphorylation correlates with activation of the JNK

subgroup but not the ERK subgroup of mitogen-activated protein kinases. *Mol. Cell. Biol.* 14, 6683–6688. doi: 10.1128/MCB.14.10.6683

Newell, C. L., Deisseroth, A. B., and Lopez-Berestein, G. (1994). Interaction of nuclear proteins with an AP-1/CRE-like promoter sequence in the human TNF-alpha gene. *J. Leukoc. Biol.* 56, 27–35. doi: 10.1002/jlb.56.1.27

Pat, B. K., Cuttle, L., Watters, D., Yang, T., Johnson, D. W., and Gobe, G. C. (2003). Fibrogenic stresses activate different mitogen-activated protein kinase pathways in renal epithelial, endothelial or fibroblast cell populations. *Nephrology (Carlton)* 8, 196–204. doi: 10.1046/j.1440-1797.2003.00162.x

Pulverer, B. J., Kyriakis, J. M., Avruch, J., Nikolakaki, E., and Woodgett, J. R. (1991). Phosphorylation of c-Jun mediated by MAP kinases. *Nature* 353, 670–674. doi: 10.1038/353670a0

Reich, N., Tomcik, M., Zerr, P., Lang, V., Dees, C., Avouac, J., et al. (2012). Jun N-terminal kinase as a potential molecular target for prevention and treatment of dermal fibrosis. *Ann. Rheum. Dis.* 71, 737–745. doi: 10.1136/annrheumdis-2011-200412

Rui, H. L., Wang, Y. Y., Cheng, H., and Chen, Y. P. (2012). JNK-dependent AP-1 activation is required for aristolochic acid-induced TGF-beta1 synthesis in human renal proximal epithelial cells. *Am. J. Physiol. Renal Physiol.* 302, F1569–F1575. doi: 10.1152/ajprenal.00560.2011

Tang, P. M., Nikolic-Paterson, D. J., and Lan, H. Y. (2019). Macrophages: versatile players in renal inflammation and fibrosis. *Nat. Rev. Nephrol.* 15, 144–158. doi: 10.1038/s41581-019-0110-2

Vanherweghem, J. L., Depierreux, M., Tielemans, C., Abramowicz, D., Dratwa, M., Jadoul, M., et al. (1993). Rapidly progressive interstitial renal fibrosis in young women: association with slimming regimen including Chinese herbs. *Lancet* 341, 387–391. doi: 10.1016/0140-6736(93)92984-2

Wang, Y., Ji, H. X., Xing, S. H., Pei, D. S., and Guan, Q. H. (2007). SP600125, a selective JNK inhibitor, protects ischemic renal injury via suppressing the extrinsic pathways of apoptosis. *Life Sci.* 80, 2067–2075. doi: 10.1016/j.lfs.2007.03.010

Wu, L., Tanimoto, A., Murata, Y., Fan, J., Sasaguri, Y., and Watanabe, T. (2001). Induction of human matrix metalloproteinase-12 gene transcriptional activity by GM-CSF requires the AP-1 binding site in human U937 monocytic cells. *Biochem. Biophys. Res. Commun.* 285, 300–307. doi: 10.1006/bbrc.2001.5161

Yang, L., Besschetnova, T. Y., Brooks, C. R., Shah, J. V., and Bonventre, J. V. (2010). Epithelial cell cycle arrest in G2/M mediates kidney fibrosis after injury. *Nat. Med.* 16, 535–543. doi: 10.1038/nm.2144

Yang, L., Su, T., Li, X. M., Wang, X., Cai, S. Q., Meng, L. Q., et al. (2012). Aristolochic acid nephropathy: variation in presentation and prognosis. *Nephrol. Dial. Transplant.* 27, 292–298. doi: 10.1093/ndt/gfr291

Yang, B., Xie, Y., Guo, M., Rosner, M. H., Yang, H., and Ronco, C. (2018). Nephrotoxicity and Chinese herbal medicine. *Clin. J. Am. Soc. Nephrol.* 13, 1605–1611. doi: 10.2215/CJN.11571017

Yu, F. Y., Wu, T. S., Chen, T. W., and Liu, B. H. (2011). Aristolochic acid I induced oxidative DNA damage associated with glutathione depletion and ERK1/2 activation in human cells. *Toxicol. In Vitro* 25, 810–816. doi: 10.1016/j.tiv.2011.01.016

Yuan, J., Adamski, R., and Chen, J. (2010). Focus on histone variant H2AX: to be or not to be. *FEBS Lett.* 584, 3717–3724. doi: 10.1016/j.febslet.2010.05.021

Zhou, L., Fu, P., Huang, X. R., Liu, F., Chung, A. C., Lai, K. N., et al. (2010a). Mechanism of chronic aristolochic acid nephropathy: role of Smad3. *Am. J. Physiol. Renal Physiol.* 298, F1006–F1017. doi: 10.1152/ajprenal.00675.2009

Zhou, L., Fu, P., Huang, X. R., Liu, F., Lai, K. N., and Lan, H. Y. (2010b). Activation of p53 promotes renal injury in acute aristolochic acid nephropathy. *J. Am. Soc. Nephrol.* 21, 31–41. doi: 10.1681/ASN.2008111133

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TGF-β1 Signaling: Immune Dynamics of Chronic Kidney Diseases

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Chronic kidney disease (CKD) is a major cause of morbidity and mortality worldwide, imposing a great burden on the healthcare system. Regrettably, effective CKD therapeutic strategies are yet available due to their elusive pathogenic mechanisms. CKD is featured by progressive inflammation and fibrosis associated with immune cell dysfunction, leading to the formation of an inflammatory microenvironment, which ultimately exacerbating renal fibrosis. Transforming growth factor β1 (TGF-β1) is an indispensable immunoregulator promoting CKD progression by controlling the activation, proliferation, and apoptosis of immunocytes via both canonical and non-canonical pathways. More importantly, recent studies have uncovered a new mechanism of TGF-β1 for *de novo* generation of myofibroblast via macrophage-myofibroblast transition (MMT). This review will update the versatile roles of TGF-β signaling in the dynamics of renal immunity, a better understanding may facilitate the discovery of novel therapeutic strategies against CKD.

Keywords: transforming growth factor β, chronic kidney disease, renal inflammation, kidney fibrosis, immunity

INTRODUCTION

Chronic kidney disease (CKD), an increasing contributor to morbidity and mortality, is predicted to become the 5th most common cause of death worldwide in 2040 (1, 2). CKD can be a primary disease or a complication initiated by other disorders, including glomerulonephritis (3), hypertension (4), diabetes (5), infection (6), and genetic causes (7). Its gradual development into end-stage renal disease (ESRD) is featured by the deposition of excessive extracellular matrix (ECM) and loss of kidney function (8). Unfortunately, current treatments are ineffective because of the complicated pathophysiological mechanisms of CKD. Despite there being multiple causes, it is well-accepted that CKD is a consequence of unresolved inflammation and renal fibrosis (9–14). Importantly, increasing evidence suggests the dysregulation of renal immunity is important for CKD development (15–17), e.g., promoting inflammation by their recruitment and adhesion to the renal epithelium (11, 18) and fibrosis by their secretome induced pro-fibrogenic responses respectively (17).

Transforming growth factor-beta (TGF-β) consists of 3 isoforms (TGF-β1, TGF-β2 TGF-β3), TGF-β1 is well-established as an indispensable driver of renal fibrosis in the pathogenesis of CKD, while the role of TGF-β2 and TGF-β3 remains largely undefined (11, 19–21). However, direct targeting of TGF-β1 signaling would affect its physiological functions in the regulation of cell differentiation, apoptosis, and immune homeostasis (22). Consequently, disease-specific pathogenic downstream of TGF-β1 pathway has been proposed to serve as an alternative therapeutic target and prognostic marker for CKD (23, 24). Recently, emerging studies have uncovered the downstream mechanisms of TGF-β1 in both adaptive and innate immunity during CKD. Better understanding of the regulatory mechanisms of TGF-β1 signaling in renal immunity may largely facilitate the therapeutic development of CKD (25).

IMPORTANCE OF TGF-β1 IN CKD PATHOLOGY

TGF-β1 plays an essential role in the pathogenesis of CKD due to its anti-inflammatory and fibrotic actions. TGF-β1 is well-demonstrated as an anti-inflammatory cytokine during the renal repair process at the early stage of kidney injury (26). In a mice model of crescentic glomerulonephritis, TGF-β1 inhibits the release of inflammatory cytokines as well as the infiltration of macrophages and CD3+ T cells for protecting injured kidney (27). TGF-β1 can promote the macrophages transiting from pro-inflammatory M1 into anti-inflammatory M2 phenotype (28). Nevertheless, short-term activation would facilitate the renal repair process, whereas endured activation would lead to renal fibrosis (15). Interestingly, TGF-β1 interrupts NF-κB pathway via Smad7 (29), interacts with β-catenin/Foxo complex (30), or modulates c-Jun N-terminal kinase signaling (31) to exert anti-inflammatory effect. In mice UUO and ischemic/reperfusion models, TGF-β1 also promotes β-catenin/T-cell factor (TCF) interaction, thereby simultaneously driving anti-inflammatory and pro-fibrotic responses via promoting β-catenin binding to Foxo and TCF, respectively (30, 31). Moreover, several studies further demonstrated the pro-fibrotic role of TGF-β1 signaling through mediating the ERK1/2 pathway, P38/MAPK pathway, and Akt/ERKs pathways (32, 33).

CKD would ultimately progress into end-stage renal disease (ESRD) due to the progressive fibrotic processes mediated by TGF-β1 signaling (34). TGF-β1 exerts its pro-fibrotic effects via both canonical (Smads dependent) and non-canonical (Smads independent) pathways. In the canonical pathway, Smad2 and Smad3 are two key downstream mediators of TGF-β receptor that are highly activated in renal fibrosis (35). Subsequently, activated Smad2 and Smad3 first complexed with Smad4 (36), then translocated into the nucleus to transcriptionally regulate pro-fibrotic molecules expression, including collagens, fibronectin, and alpha-smooth muscle actin (37–39), thereby facilitating fibrotic responses. However, each Smads protein is functionally distinct in the pathogenesis of CKD. Smad3 promotes while Smad2 suppresses CKD progression (40–42). Notably, Smad3 and Smad2 bind directly to the target gene, and Smad4 is lack of DNA-binding domains, but Smad4 still serve as regulators of the transcription process (43–47).

In the non-canonical pathways, TGF-β1 directly activates non-Smads signaling pathways, including MAPK pathway (48), PI3K/Akt/mTOR pathway (49), TGF-β1/p38 MAPK pathway (50), ILK (51), EGFR (52), and Wnt/β-catenin pathway (53). These non-canonical pathways largely contribute to the pathogenesis of renal fibrosis, including matrix formation (54), de-differentiation of proximal tubular cells (55), cell proliferation and migration (54), and apoptosis (56).

TGF-β1 signaling is the key mechanism of ECM synthesis by inducing myofibroblasts generation from number of origins, including epithelial cells, endothelial cells, resident fibroblasts, and pericytes. Epithelial to Mesenchymal Transition (EMT) is a well-characterized pathological process of renal fibrosis featured by the conversion of epithelial cells into mesenchymal phenotypes. TGF-β1 signaling drives key events of EMT *in vivo* and *in vitro,* including loss of epithelial adhesion, *de novo* α-SMA expression, and cell migration (57, 58). During EMT, the migratory ability and mesenchymal markers, fibronectin, and α-smooth muscle actin (α-SMA) were acquired, while epithelium adhesion and E-cadherin protein were lost after the transition (59–61). Thus, EMT contributes to the pathogenesis of kidney fibrosis via direct generation of the collagens producing myofibroblasts (62). In the canonical pathway, Smad3 is highly activated in the UUO kidney *in vivo,* and TGF-β1 treated renal tubular epithelial cells *in vitro*, driving EMT for the myofibroblast generation and associated kidney fibrosis, which is blocked by Smad3 deletion and TGF-β1 neutralizing antibody (63–65). Non-canonical pathways, including MAPK, Rho-like GTPase, PI3K/Akt, and Wnt signaling, have been illustrated to have played emerging roles in EMT induction (28, 66, 67). TGF-β1/Smad3 signaling also drives Endothelial to Mesenchymal transitions (EndoMT), where smad3 inhibitor and endothelium-specific TGF-β receptor knockout reduces EndoMT mediated diabetic nephropathy in streptozotocin (STZ)-induced diabetes and tubulointerstitial fibrosis in unilateral ureteral obstruction models *in vivo* (68, 69). Resident fibroblasts and pericytes are rich sources of myofibroblasts, demonstrated by lineage tracing studies with P0-Cre and Foxd1-Cre to label myofibroblasts derived from fibroblasts and pericytes, respectively (70, 71). Resident fibroblasts and pericytes were activated into α-SMA$^+$ myofibroblasts in mice model of obstructive kidney fibrosis via TGF-β1/Smad3 signaling (72–74). Therefore, TGF-β1 activates various cell types via both of the canonical and non-canonical pathways, generating myofibroblast for excess ECM deposition, ultimately contributing to fibrotic responses in CKD.

TGF-β1 IN ADAPTIVE IMMUNITY OF CKD

B Cell

Interestingly, dysregulation of humoral immunity was observed in ESRD patients; only 65% of ESRD patients can produce sufficient titer of antibodies upon vaccination, in contrast to the 95% in healthy control (16, 75). A previous study demonstrated that B1 (CD19+CD5+) and B2 lymphocytes (CD19+CD5–) are negatively associated with the progression of CKD but

positively correlated with the survival of elderly CKD patients, suggesting B cell deficiency could be a prognostic factor of CKD progression (76). Autoantibodies production by B-cells is crucial for the development of IgA nephropathy and lupus nephritis. In the pathogenesis of IgA nephropathy, B-cells produce aberrant galactosylated IgA and its autoantibodies (anti-glycan antibodies) to form immune complexes, which deposition on mesangial cells to initiates glomerulonephritis and subsequent CKD progression (77–79). Similarly, in Lupus nephritis, multiple autoantibodies were involved in the immune complexes formation, including anti-dsDNA (80), anti-C1q (81), and anti-nucleosome (82) autoantibodies. Mechanistically, TGF-β suppresses B-cell maturation into antibody-producing cells, resulting in antibody abnormalities or autoantibodies production (83, 84). TGF-β1 inhibits pre-B cell proliferation via suppressing PI3K/Akt signaling and induces a cell cycle arrest of pre-B cells specifically at the G0/G1 phase (85). TGF-β1 also hinders B cell proliferation and activation indirectly via contacting the regulatory T cells, associated with the upregulation of granzyme A, granzyme B, and perforin (86). TGF-β1 induces B cell-activating factor (BAFF) production from the macrophages via Smad3/4 and PKA/CREB signaling pathways (87). BAFF is a key cytokine regulating B-cells activity, including proliferation, differentiation, apoptosis, and immunoglobulin secretion; excessive BAFF would suppress B-cell development resulting in autoantibodies production in IgA nephropathy and Lupus nephritis (83, 84, 88) Taken together, TGF-β1 suppress B lymphocytes development in the pathogenesis of kidney diseases via both direct and indirect mechanisms.

T Cell

T lymphocyte infiltration has been observed in CKD biopsies (89, 90) and is positively correlated with the deterioration in glomerular filtration rate (91), indicating a pathogenic role of T lymphocytes in the pathogenesis of CKD. Interestingly, CD8+ T cell abundance is significantly associated with the TGF-β1 level in the kidney biopsies of lupus nephritis (92). In a mice model of Crescentic Glomerulonephritis (GN), $CD3^+$ T cell infiltration and associated glomerular and tubulointerstitial injuries were largely suppressed in latent TGF-β1 transgenic mice, compared with wildtype mice (93). TGF-β1 plays a crucial role in the modulation of T cell migration, activation, proliferation, and death. The recruitment and differentiation of $CD4^+$ T cells were regulated by mesenchymal stem cells (MSCs) via TGF-β1 signaling (94) while TGF-β1 enhances $CD8^+$ T-cell activation and proliferation by switching the immune-suppressive myeloid-derived suppressor cells (MDSCs) into immune-stimulating phenotype in a SMAD-2 dependent manner (95). This may explain $CD8^+$ T-cell tubulitis and associated TGF-β1/Smad2/3 signaling activation in a rat model of aristolochic acid nephropathy (AAN) (96). In addition, TGF-β1 induces oxidative stress in injured renal tissue via mitochondrial and NADPH oxidases ROS production and suppression of antioxidant system (97–99). In Mercuric chloride intoxication and Dahl salt-sensitive rat models, elevated ROS level leading to the interstitial CD8+ T cells infiltration and associated tubular damage (100, 101). Adoptive transfer of oxidizing agents treated CD4+ T cells also caused immune complex glomerulonephritis in syngeneic recipient mice (102).

On the other hand, regulatory T cells (Tregs) play a protective role in CKD by suppressing inflammation and immune cell-mediated fibrosis (30, 103–106). Notably, abundance of peripheral Tregs is significantly reduced in CKD patients compared to the healthy controls (107). TGF-β1 is well-characterized as a Tregs inducer (108, 109). TGF-β1 has been demonstrated to increase the proliferation, differentiation, and function of Tregs by not only up-regulating Foxp3 (a master transcription regulator of Tregs) expression via PP2A pathway (110) but also suppressing IL-12R (111). Furthermore, TGF-β1 induces membrane-bound TGF-β1 on the Treg cells to suppress naive CD4+ T cells expansion for immune suppression via activating Smad3 (112). Surprisingly, Tregs are able to convert into TGF-β1-producing cells in the inflammatory environment, which markedly up-regulates the level of TGF-β1 in UUO-obstructed kidney, therefore aggravating chronic inflammation and renal fibrosis (113).

TGF-β1 in Innate Immunity of CKD

Neutrophil

Neutrophils are well-documented because of their aggravating role in inflammation (114), where neutrophil-to-lymphocyte ratio is a popular prognostic marker for estimating the mortality of CKD patients (115). Neutrophils can initiate and amplify inflammatory responses by releasing pro-inflammatory cytokines (114, 116), and serves as a rich source of TGF-β1 in inflamed tissues (117, 118). During inflammation, TGF-β1 facilitates the accumulation of neutrophils (119, 120), therefore inhibiting TGF-β1 effectively alleviates neutrophil infiltration and inflammation (121). Furthermore, TGF-β1 signaling can be blocked by preventing Smad3 activation, which has been proposed as a potential therapeutic strategy for fibrotic diseases driven by neutrophil-mediated inflammation (122, 123).

Dendritic Cell

Dendritic cells (DCs) facilitate renal inflammation via promoting CD8+ T cell proliferation and activation during the development of CKD (124, 125). Mechanistically, TGF-β1 promotes DCs accumulation in fibrotic tissue (126) and modulates DCs-mediated proliferation and activation of T cells (127–130), contributing to the imbalance between Th17 and Treg (131) and the interleukin 17 (IL-17) release from naive $CD4^+$ cells (132). Importantly, TGF-β1 further stimulates TGF-β1 release from DCs in an autocrine manner, serving as a major source of TGF-β1 in the tissue biopsies from stage IV–V CKD patients (133, 134) and suppressing inflammatory cytokines (IL-12, IL-18) production in DCs (135, 136). These findings suggest DCs can regulate the proliferation, activation, differentiation, and function of T cells via TGF-β1 signaling during inflammation. It has been demonstrated that targeting of DCs maybe able to suppress CKD progression by attenuating renal inflammation and fibrosis (94, 137, 138).

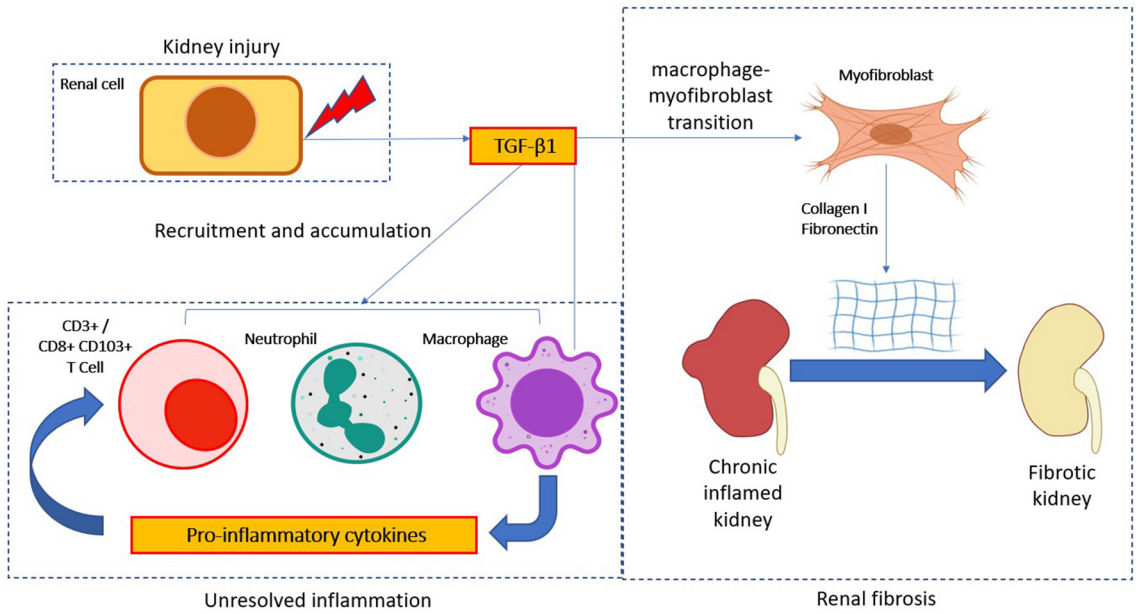

FIGURE 1 | TGF-β1 in immune cell mediate CKD progression. TGF-β1 modulates immune cell activity in the progression of chronic kidney disease (CKD). After kidney injury, TGF-β1 is released by kidney cells to aid the resolution of inflammation. However, persistent TGF-β1 signaling activation would promote a chronic inflammation state via amplification of inflammatory responses. Notably, chronic TGF-β1 signaling activation would further transdifferentiate macrophage into myofibroblast to produce excessive extracellular matrix molecules (Collagen I and fibronectin), thus eventually lead to the pathogenesis of CKD.

Macrophage

Macrophage is a key player in the pathological process of CKD that their infiltration due to their pathogenic actions in both renal inflammation and fibrosis (15, 16, 87, 89, 139, 140). It has been reported that TGF-β1 participated in macrophages-mediated immune dysfunction during the progression of CKD (15, 141, 142). TGF-β1 largely increases macrophages infiltration and accumulation in the injured kidney via stimulating the release of a potent cytokine for macrophages recruitment monocyte chemoattractant protein-1 (MCP-1) from various types of renal cells (143–145). TGF-β1 also regulates macrophage polarization and immunomodulatory cytokines secretion. Upon the kidney injury, TGF-β1 transits M1 macrophage into regulatory M2c phenotype to facilitate kidney repair by producing the immunosuppressive and matrix remodeling activities (146–148). However, the CCL18 secreted from these $CD163^{+}$ macrophages also promotes fibroblast proliferation, leading to the acceleration of kidney fibrosis (149). TGF-β1 also induces the expression of B cell-activating factor (BAFF), a key regulator of B cell activities, in macrophages via Smad3/4 dependent mechanism to influence the macrophages-mediated pathogenic function of B cells (87). The elevated plasma level of BAFF was observed in ESRD patients compared to the control group (150–152). Interestingly, the interaction between macrophages and TGF-β1 is mutual, where macrophage is the effector and a rich source of TGF-β1, actively producing and secreting TGF-β1 in inflamed kidney tissue (153, 154). Thus, blockade of TGF-β1 signaling effectively reduces macrophages infiltration (41, 155, 156) as well as significantly reduces macrophage polarization and extracellular matrix deposition (157, 158).

Novel Fibrotic Mechanism of TGF-β1: Macrophage-Myofibroblast Transition

Myofibroblast is an important effector cell type that contributes to the switching of unresolved inflammation to be renal fibrosis, they featured by a high level of α-SMA expression and excessive extracellular matrix deposition (159). The sources of pathogenic myofibroblasts are highly heterogeneous and still largely unclear and controversial (160, 161). Macrophage-myofibroblast transition (MMT) is a newly-identified phenomenon driven by TGF-β1 signaling as a direct mechanism of macrophage for promoting myofibroblast generation under unresolved renal inflammation (15, 162, 163) (**Figure 1**). Mechanistically, TGF-β1/Smad3 signaling is suggested as the key regulator for initiating MMT during renal fibrosis in a UUO model *in vivo,* where TGF-β1 induces the *de novo* expression of myofibroblast marker α-SMA and effector collagen I in the bone marrow derived macrophages (BMDMs) via a Smad3-dependent mechanism (164). Bioinformatic analysis of TGF-β1/Smad3 dependent transcriptome of MMT *in vitro* further reveals Src and Pou4f1 as the pathogenic mediator in the Smad3 downstream signaling, representing a precise therapeutic target for blocking MMT (24, 165). In brief, TGF-β1/Smad3 directly activates a Src-centric gene network in BMDMs via transcriptional regulation for promoting the MMT process in the fibrosing kidney (15). More importantly, Tang et al. further discovered the importance of a neural-specific homeobox/POU domain protein Pou4f1 in the Smad3

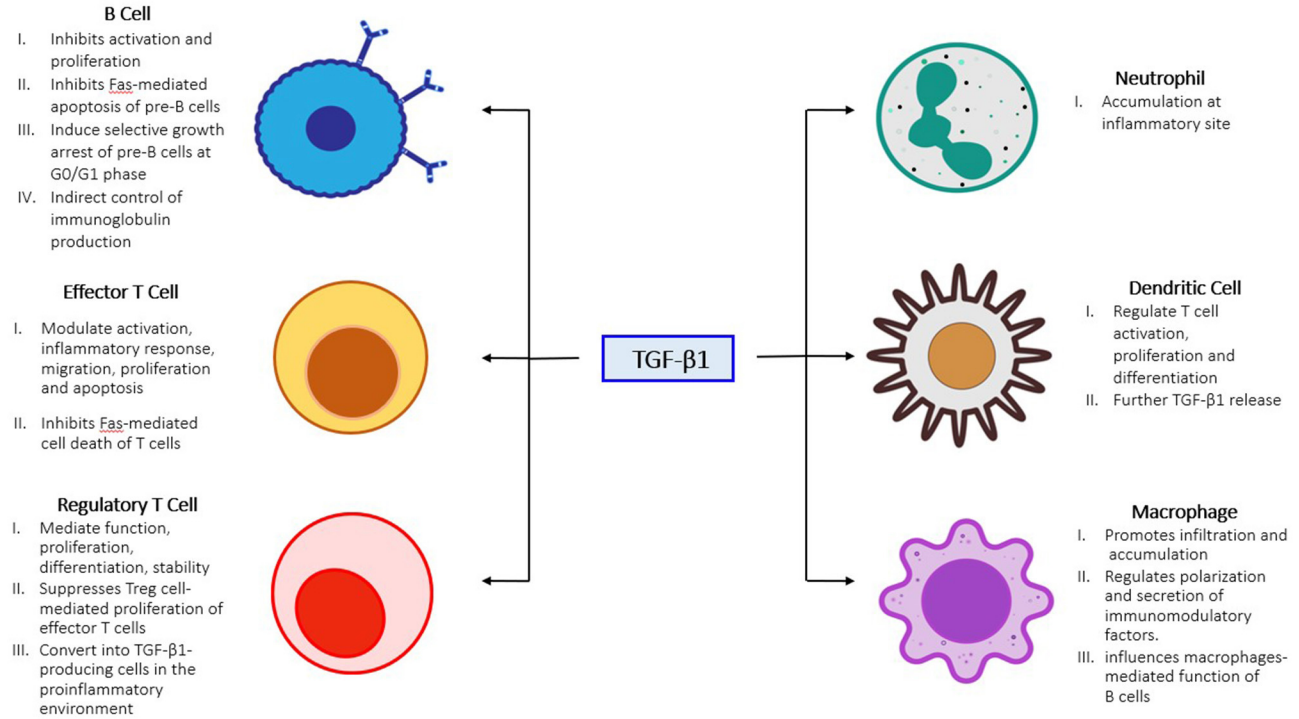

FIGURE 2 | Role of TGF-β1 in unresolved inflammation. TGF-β1 activation promotes innate immune cells accumulation (neutrophil, macrophage, and dendritic cell) at the inflammatory site, which in-turn further activate adaptive immune cells (B and T cells) and dendritic cells to amplify the inflammatory responses in chronic inflamed kidney.

downstream as a specific mediator for regulating MMT (24). Besides, non-canonical TGF-β1 signaling also induces MMT *via* β-catenin/TCF pathway, promoting pro-fibrotic gene expression in the kidney infiltrating macrophages (30, 166). Inhibitor of Src (PP1) and TCF (ICG-001) and BMDM-specific Pou4f1 silencing effectively suppress the MMT process and associated renal fibrosis, suggesting MMT may be therapeutically targeted to restrain CKD progression (24, 165).

THERAPEUTIC STRATEGIES FOR TARGETING THE TGF-β1-MEDIATED CKD

TGF-β1 signaling is essential for the progression of renal fibrosis and has been proposed as a therapeutic target for CKD (**Figure 2**), however systematically targeting TGF-β1 would also suppress its physiological functions and may result in adverse side effects (167, 168). Emerging clinical trials demonstrated that direct targeting TGF-β1 signaling was highly associated with adverse events in 23 to 87% of the kidney patients (167, 169, 170). Nevertheless, alternative approaches that specifically targeting the pathogenic mediators in TGF-β1 downstream may prevent the side effects. The molecular mechanism of Smad3 in renal pathology is intensively elucidated among the other Smads, genetic deletion of Smad3 effectively protected mice against collagen deposition after kidney injury (63, 171, 172). Therefore, several strategies targeting Smad3 have been investigated in a number of pre-clinical studies. Encouragingly, a Smad3 specific inhibitor SIS3 and a natural compound isolated from Poria *cocos* Poricoic acid effectively suppressed renal fibrosis development in experimental models of diabetic nephropathy (68), obstructive nephropathy (173), and ischemia-reperfusion injury (174) *in vivo*. In addition, diterpene and triterpenes (175), 25-O-methylalisol F (176), and IC-2 derivatives (177) are also capable of suppressing Smad3 activation and pro-fibrotic molecules production (Collagen I and fibronectin) in the renal epithelial cells. Importantly, emerging evidence showing macrophages mediate the therapeutic effect of Smad3 inhibition. Smad3 inhibition or genetic deletion suppressed MMT in mouse models of chronic Renal Allograft Injury (178), unilateral ureteric obstruction (164), contributed 50–60% reduction of myofibroblast population, and suppressed macrophage infiltration in type 2 diabetic nephropathy (179), thus contributing to the protective effect of Smad3 targeted therapy. Furthermore, noncoding RNAs including LRNA9884 (180), Erbb4-IR (20, 181), miR-29b (182), anti-miR-433 (183), lnc-TSI (184), and anti-miR-21 (185) were discovered from the TGF-β/Smads signaling for the obstructive and diabetic nephropathy. Among them, RNA therapies targeting LRNA9884 and miR-29b could modulate leukocytes infiltration via inflammatory cytokines expression, thus suppressing renal inflammation in diabetic nephropathy (180, 182, 186, 187). Importantly, these RNA-based therapies effectively restrained CKD progression with minimal side effects thanks to their specificity (188, 189). In addition, targeting the non-canonical

TABLE 1 | Pre-clinical studies for the treatment of CKD by specifically targeting the downstream of TGF-β1.

Drugs	Target	Route and effective dose	Disease model	Results	References
Canonical pathway					
SIS3	Smad3	I.p. 0.2, 2 mg/kg/day	UUO kidneys 1 week BALB/c male mice	↓ Fibrosis ↓ p-Smad3/Fn/Collagen I/III ↓ Myofibroblast (α-SMA^{+} cells)	(173)
SIS3	Smad3	*In vitro* 1 μM I.p. 2.5, 5 μg/g SIS3	TGF-β1/AGEs induced Mouse pancreatic microvascular endothelial cells (MMECs) 5 Days STZ 50 μg/g induced diabetes on Tie2-Cre; Loxp-EGFP mice (C57BL/6J)	↓ p-Smad3 ↓ RAGE-mediated EndoMT ↓ Collagen I/ α-SMA/ Fn	(68)
Poricoic Acid A (PAA)	Smad3	*In vitro* 10 μM 10 mg/kg oral gavage	TGF-β/ hypoxia/reoxygenation treated HK-2 cells Rats IRI model	↓ p-Smad3 ↓ Collagen I/ α-SMA/ Fn	(174)
IC-2 derivatives	Smad3	*In vitro* 10, 20 μM	TGF-β1 induced Tubular epithelial cells HK-2 cells	↓ p-Smad3 ↓ Collagen 1	(177)
25-O-methylalisol F (MAF)	Smad3	*In vitro* 10 μM	TGF-β1/ANG stimulated NRK-52E cells Tubular epithelial cells	↓ p-Smad3 ↓ Wnt/β-catenin ↑ Smad7 expression ↓ Collagen I, Fn, α-SMA	(176)
Diterpene (PZF) and triterpenes (PZH)	Smad3	*In vitro* 10 μM	TGF-β1/ANGII induced Human kidney proximal epithelial cells (HK-2) Immortalized mouse podocytes (MPC5)	↓ p-Smad3 ↓ Collagen I/ α-SMA/ Fn ↓ Wnt/ β-catenin ↓ MMP-7/PAI-1/Fsp-1	(175)
miR-29b	Smad3	Ultrasound microbubble mediated-Mir-29b gene transfer	db/db or db/m mice AGE induced rat MC line and tubular epithelial cell line (NRK52E)	↓ p-Smad3/ Collagen I/III ↓ Microalbuminuria ↓ Mesangial index (histological injury)	(182)
Anti-miR-433	Smad3	Ultrasound-mediated gene transfer of inducible miR-433 shRNA	Obstructive nephropathy mouse model (UUO) Normal rat TEC line, NRK52E	↓ Collagen I/ α-SMA/ Fn ↓ p-Smad3	(183)
lnc-TSI	Smad3	i.v. injection of pcDNA3.1-lnc-TSI	UUO rat model TGF-β1 treated human TECs	↓ Collagen I/ α-SMA/ Fn ↓ Kidney fibrosis (tubular interstitial fibrosis indexes/Serum creatinine)	(184)
Anti-miR-21	Smad3	Ultrasound-mediated gene transfer of inducible miR-21 knockdown	High glucose-induced rat mesangial cell (MC) and tubular epithelial cell (TEC), NRK52E Kidneys of db/db mice	↓ Collagen I/ IV/ Fn ↓ p-Smad3	(185)
Non-canonical pathway					
Trametinib (MEK inhibitor)	ERK1/2, mTORC1	3 mg/kg oral gavage	UUO mouse model	↓ α-SMA/ Vimentin ↓ p-ERK1/2, p-Akt	(191)
Renalase	ERK1/2	Adenovirus renalase gene delivery	UUO mouse model	↓ p-ERK1/2 ↓ Collagen I/ α-SMA/ Fn	(190)
QiShenYiQi (QSYQ) Traditional Chinese Medicines	β-catenin	250, 500 mg/kg/d intra-gastric *In vitro* 5, 10, 20 μg/ml	UUO rat model TGF-β treated Normal kidney proximal tubular (NRK52E) and renal fibroblast cells (NRK49F)	↓ Collagen I/ α-SMA/ Fn ↓ β-catenin	(192)
α1-adrenoceptor inhibitors	p38	Tamsulosin (i.p.) 0.4 mg/kg/day	UUO mouse model	↓ Serum creatinine and urea ↓ KIM-1/NGAL/ PAL-1 ↓ α-SMA/vimentin/Snai1/ Fibronectin	(193)
Aloe-emodin	PI3K/Akt/ mTOR	20 mg/kg/day oral gavage	UUO mouse model	↓ Tubule injury index score. ↓ Masson trichromatic +ve area ↓ Collagen I/Fn ↓ Scr/BUN/urine volume	(49)

UUO, unilateral ureteral obstruction; EMT, epithelial-mesenchymal transition; SIS3, specific Inhibitor of Smad3; CKD, chronic kidney disease; Fn, Fibronectin; Scr, Serum creatinine; BUN, blood urea nitrogen; α-SMA, Alpha-smooth muscle actin; STZ, Streptozotocin; ANG, Angiotensin; KIM-1, Kidney Injury Molecule-1; NGAL-1, neutrophil gelatinase-associated Lipocalin; PAL-1, plasminogen activator inhibitor 1, RAGE MMP-7/PAI-1/Fsp-1.

TGF-β1 signaling including ERK1/2 (190, 191), β-catenin (192), p38 (193), and PI3K/Akt (49) also suppressed the pro-fibrotic actions in obstructive nephropathy, demonstrating the therapeutic potential of targeting the TGF-β1 downstream mediators (**Table 1**).

CONCLUSION AND FUTURE PERSPECTIVES

TGF-β1 exerts its pathogenic roles in the progression of CKD by regulating both of the innate and adaptive immunity in the injured kidney via the canonical and non-canonical pathways including a novel fibrotic mechanism MMT. The TGF-β1 driven development of renal fibrosis from unresolved inflammation is well-observed, but underlying mechanisms remain largely unexplored. Better understanding of the underlying mechanisms of TGF-β1 pathways uncovered a number of novel pathogenic mediators from the downstream signaling, which may represent an effective therapeutic strategy to prevent renal inflammation progress into fibrosis. Moreover, the TGF-β1 regulating immune cells also contribute to other fibrotic diseases. In addition, further studies of TGF-β isoforms (TGF-β2, TGF-β3) on immune cells may reveal their therapeutic potential in renal immunity driven CKD progression. Current clinical trials targeting renal immunity shows promise, further investigation for validating the safety and effectiveness of these therapeutic approaches would discover new hope for patients with fibrotic diseases in the coming future.

AUTHOR CONTRIBUTIONS

PT, AC, C-BZ, CG, and Y-YZ responsible for literature research and writing. K-FT, K-TL, and H-YL reviewed the manuscript and made significant revisions on the drafts. PT supervised and finalized of this work. All authors have read and agreed to the published version of the manuscript.

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REFERENCES

1. Foreman KJ, Marquez N, Dolgert A, Fukutaki K, Fullman N, Mcgaughey M, et al. Forecasting life expectancy, years of life lost, and all-cause and cause-specific mortality for 250 causes of death: reference and alternative scenarios for 2016-40 for 195 countries and territories. *Lancet*. (2018) 392:2052–90. doi: 10.1016/S0140-6736(18)31694-5
2. Peters LJF, Floege J, Biessen EA., Jankowski J, Van Der Vorst EPC. MicroRNAs in chronic kidney disease: four candidates for clinical application. *Int J Mol Sci*. (2020) 21:6547. doi: 10.3390/ijms21186547
3. Han KH, Kim B, Ji SC, Kang HG, Cheong HI, Cho JY, et al. Mechanism of chronic kidney disease progression and novel biomarkers: a metabolomic analysis of experimental glomerulonephritis. *Metabolites*. (2020) 10:169. doi: 10.3390/metabo10040169
4. Ponticelli C, Podestà MA, Moroni G. Hyperuricemia as a trigger of immune response in hypertension and chronic kidney disease. *Kidney Int*. (2020) 98:1149–59. doi: 10.1016/j.kint.2020.05.056
5. Hesp AC, Schaub JA, Prasad PV, Vallon V, Laverman GD, Bjornstad P, et al. The role of renal hypoxia in the pathogenesis of diabetic kidney disease: a promising target for newer renoprotective agents including SGLT2 inhibitors? *Kidney Int*. (2020) 98:579–89. doi: 10.1016/j.kint.2020.02.041
6. Ishigami J, Taliercio JT, Feldman HI, Srivastava A, Townsend RR, Cohen DL, et al. Fibroblast growth factor 23 and risk of hospitalization with infection in chronic kidney disease: the chronic renal insufficiency cohort (CRIC) study. *J Am Soc Nephrol*. (2020) 31:1836–46. doi: 10.1681/ASN.2019101106
7. Groopman EE, Povysil G, Goldstein DB, Gharavi AG. Rare genetic causes of complex kidney and urological diseases. *Nat Rev Nephrol*. (2020) 16:641–56. doi: 10.1038/s41581-020-0325-2
8. Meng XM, Nikolic-Paterson DJ, Lan HY. Inflammatory processes in renal fibrosis. *Nat Rev Nephrol*. (2014) 10:493–503. doi: 10.1038/nrneph.2014.114
9. Ernandez T, Mayadas TN. The changing landscape of renal inflammation. *Trends Mol Med*. (2016) 22:151–63. doi: 10.1016/j.molmed.2015.12.002
10. Hickey FB, Martin F. Role of the immune system in diabetic kidney disease. *Curr Diab Rep*. (2018) 18:20. doi: 10.1007/s11892-018-0984-6
11. Tecklenborg J, Clayton D, Siebert S, Coley SM. The role of the immune system in kidney disease. *Clin Exp Immunol*. (2018) 192:142–50. doi: 10.1111/cei.13119
12. Toba H, Lindsey ML. Extracellular matrix roles in cardiorenal fibrosis: potential therapeutic targets for CVD and CKD in the elderly. *Pharmacol Ther*. (2019) 193:99–120. doi: 10.1016/j.pharmthera.2018.08.014
13. Diaz-Ricart M, Torramade-Moix S, Pascual G, Palomo M, Moreno-Castaño AB, Martinez-Sanchez J, et al. Endothelial damage, inflammation and immunity in chronic kidney disease. *Toxins*. (2020) 12:361. doi: 10.3390/toxins12060361
14. Yang F, Deng L, Li J, Chen M, Liu Y, Hu Y, et al. Emodin retarded renal fibrosis through regulating HGF and TGFβ-Smad signaling pathway. *Drug Des Devel Ther*. (2020) 14:3567–75. doi: 10.2147/DDDT.S245847
15. Tang PM, Nikolic-Paterson DJ, Lan HY. Macrophages: versatile players in renal inflammation and fibrosis. *Nat Rev Nephrol*. (2019) 15:144–58. doi: 10.1038/s41581-019-0110-2
16. Espi M, Koppe L, Fouque D, Thaunat O. Chronic kidney disease-associated immune dysfunctions: impact of protein-bound uremic retention solutes on immune cells. *Toxins*. (2020) 12:300. doi: 10.3390/toxins12050300
17. Huang E, Peng N, Xiao F, Hu D, Wang X, Lu L. The roles of immune cells in the pathogenesis of fibrosis. *Int J Mol Sci*. (2020) 21:5203. doi: 10.3390/ijms21155203
18. Li B, Haridas B, Jackson AR, Cortado H, Mayne N, Kohnken R, et al. Inflammation drives renal scarring in experimental pyelonephritis. *Am J Physiol Renal Physiol*. (2017) 312:F43–53. doi: 10.1152/ajprenal.00471.2016
19. Yu L, Border WA, Huang Y, Noble NA. TGF-beta isoforms in renal fibrogenesis. *Kidney Int*. (2003) 64:844–56. doi: 10.1046/j.1523-1755.2003.00162.x
20. Feng M, Tang PM, Huang XR, Sun SF, You YK, Xiao J, et al. TGF-β mediates renal fibrosis via the Smad3-Erbb4-IR long noncoding RNA Axis. *Mol Ther*. (2018) 26:148–61. doi: 10.1016/j.ymthe.2017.09.024
21. Wei J, Wang Y, Qi X, Fan Z, Wu Y. Melatonin ameliorates hyperglycaemia-induced renal inflammation by inhibiting the activation of TLR4 and TGF-β1/Smad3 signalling pathway. *Am J Transl Res*. (2020) 12:1584–99.
22. Bottinger EP, Bitzer M. TGF-beta signaling in renal disease. *J Am Soc Nephrol*. (2002) 13:2600–10. doi: 10.1097/01.ASN.0000033611.79556.AE
23. Lee S, Kanasaki K, Kalluri R. Circulating TGF-beta1 as a reliable biomarker for chronic kidney disease progression in the African-American population. *Kidney Int*. (2009) 76:10–2. doi: 10.1038/ki.2009.130
24. Tang PM, Zhang YY, Xiao J, Tang PC, Chung JY, Li J, et al. Neural transcription factor Pou4f1 promotes renal fibrosis via

macrophage-myofibroblast transition. *Proc Natl Acad Sci USA*. (2020) 117:20741–52. doi: 10.1073/pnas.1917663117
25. Ponticelli C, Anders H. Thrombospondin immune regulation and the kidney. *Nephrol Dialysis Transplantation*. (2017) 32:1084–9. doi: 10.1093/ndt/gfw431
26. Gu YY, Liu XS, Huang XR, Yu XQ, Lan HY. Diverse role of TGF-β in kidney disease. *Front Cell Dev Biol*. (2020) 8:123. doi: 10.3389/fcell.2020.00123
27. Poveda J, Sanz A, Fernandez-Fernandez B, Carrasco S, Ruiz-Ortega M, Cannata-Ortiz P, et al. MXRA5 is a TGF-β1-regulated human protein with anti-inflammatory and anti-fibrotic properties. *J Cell Mol Med*. (2017) 21:154–64. doi: 10.1111/jcmm.12953
28. Zhang YE. Non-smad signaling pathways of the TGF-β family. *Cold Spring Harb Perspect Biol*. (2017) 9:a022129. doi: 10.1101/cshperspect.a022129
29. Wang W, Huang X, Li A, Liu F, Li J, Truong L, et al. Signaling mechanism of TGF-beta1 in prevention of renal inflammation: role of Smad7. *J Am Soc Nephrol*. (2005) 16:1371–83. doi: 10.1681/ASN.2004121070
30. Qiao X, Rao P, Zhang Y, Liu L, Pang M, Wang H, et al. Redirecting TGF-β signaling through the β-Catenin/Foxo complex prevents kidney fibrosis. *J Am Soc Nephrol*. (2018) 29:557–70. doi: 10.1681/ASN.2016121362
31. Yang Y, Feng X, Liu X, Wang Y, Hu M, Cao Q, et al. Fate alteration of bone marrow-derived macrophages ameliorates kidney fibrosis in murine model of unilateral ureteral obstruction. *Nephrol Dial Transplant*. (2019) 34:1657–68. doi: 10.1093/ndt/gfy381
32. Yi H, Huang C, Shi Y, Cao Q, Zhao Y, Zhang L, et al. Metformin attenuates folic-acid induced renal fibrosis in mice. *J Cell Physiol*. (2018) 233:7045–54. doi: 10.1002/jcp.26505
33. Zhao X, Luo G, Fan Y, Ma X, Zhou J, Jiang H. ILEI is an important intermediate participating in the formation of TGF-β1-induced renal tubular EMT. *Cell Biochem Funct*. (2018) 36:46–55. doi: 10.1002/cbf.3316
34. Carew RM, Wang B, Kantharidis P. The role of EMT in renal fibrosis. *Cell Tissue Res*. (2012) 347:103–16. doi: 10.1007/s00441-011-1227-1
35. Loeffler I, Liebisch M, Allert S, Kunisch E, Kinne RW, Wolf G. FSP1-specific SMAD2 knockout in renal tubular, endothelial, and interstitial cells reduces fibrosis and epithelial-to-mesenchymal transition in murine STZ-induced diabetic nephropathy. *Cell Tissue Res*. (2018) 372:115–33. doi: 10.1007/s00441-017-2754-1
36. Lan HY. Diverse roles of TGF-β/Smads in renal fibrosis and inflammation. *Int J Biol Sci*. 1(2011) 7:1056–67. doi: 10.7150/ijbs.7.1056
37. Wang B, Komers R, Carew R, Winbanks C, Xu B, Herman-Edelstein M, et al. Suppression of microRNA-29 expression by TGF-β1 promotes collagen expression and renal fibrosis. *J Am Soc Nephrol*. (2012) 23:252–65. doi: 10.1681/ASN.2011010055
38. Chakravarthy A, Khan L, Bensler NP, Bose P, De Carvalho DD. TGF-β-associated extracellular matrix genes link cancer-associated fibroblasts to immune evasion and immunotherapy failure. *Nat Commun*. (2018) 9:4692. doi: 10.1038/s41467-018-06654-8
39. Meng P, Zhu M, Ling X, Zhou L. Wnt signaling in kidney: the initiator or terminator? *J Mol Med*. (2020) 98:1511–23. doi: 10.1007/s00109-020-01978-9
40. Meng XM, Huang XR, Chung AC, Qin W, Shao X, Igarashi P, et al. Smad2 protects against TGF-beta/Smad3-mediated renal fibrosis. *J Am Soc Nephrol*. (2010) 21:1477–87. doi: 10.1681/ASN.2009121244
41. Meng XM, Huang XR, Xiao J, Chung AC, Qin W, Chen HY, et al. Disruption of Smad4 impairs TGF-beta/Smad3 and Smad7 transcriptional regulation during renal inflammation and fibrosis *in vivo* and *in vitro*. *Kidney Int*. (2012) 81:266–79. doi: 10.1038/ki.2011.327
42. Duan WJ, Yu X, Huang XR, Yu JW, Lan HY. Opposing roles for Smad2 and Smad3 in peritoneal fibrosis *in vivo* and *in vitro*. *Am J Pathol*. (2014) 184:2275–84. doi: 10.1016/j.ajpath.2014.04.014
43. Dennler S, Itoh S, Vivien D, Ten Dijke P, Huet S, Gauthier JM. Direct binding of Smad3 and Smad4 to critical TGF beta-inducible elements in the promoter of human plasminogen activator inhibitor-type 1 gene. *EMBO J*. (1998) 17:3091–100. doi: 10.1093/emboj/17.11.3091
44. Chen SJ, Yuan W, Mori Y, Levenson A, Trojanowska M, Varga J. Stimulation of type I collagen transcription in human skin fibroblasts by TGF-beta: involvement of Smad 3. *J Invest Dermatol*. (1999) 112:49–57. doi: 10.1046/j.1523-1747.1999.00477.x
45. Piek E, Ju WJ, Heyer J, Escalante-Alcalde D, Stewart CL, Weinstein M, et al. Functional characterization of transforming growth factor beta signaling in Smad2- and Smad3-deficient fibroblasts. *J Biol Chem*. (2001) 276:19945–53. doi: 10.1074/jbc.M102382200
46. Yuan W, Varga J. Transforming growth factor-beta repression of matrix metalloproteinase-1 in dermal fibroblasts involves Smad3. *J Biol Chem*. (2001) 276:38502–10. doi: 10.1074/jbc.M107081200
47. Shi Y, Massague J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell*. (2003) 113:685–700. doi: 10.1016/S0092-8674(03)00432-X
48. Deng B, Yang W, Wang D, Cheng L, Bu L, Rao J, et al. Peptide DR8 suppresses epithelial-to-mesenchymal transition via the TGF-β/MAPK signaling pathway in renal fibrosis. *Life Sci*. (2020) 261:118465. doi: 10.1016/j.lfs.2020.118465
49. Dou F, Liu Y, Liu L, Wang J, Sun T, Mu F, et al. *In Vivo* Aloe-emodin ameliorates renal fibrosis *via* inhibiting PI3K/Akt/mTOR signaling pathway and. *Rejuvenation Res*. (2019) 22:218–29. doi: 10.1089/rej.2018.2104
50. Wang S, Zhou Y, Zhang Y, He X, Zhao X, Zhao H, et al. Roscovitine attenuates renal interstitial fibrosis in diabetic mice through the TGF-β1/p38 MAPK pathway. *Biomed Pharmacother*. (2019) 115:108895. doi: 10.1016/j.biopha.2019.108895
51. Li M, Zhou H, Di J, Yang M, Jia F. ILK participates in renal interstitial fibrosis by altering the phenotype of renal tubular epithelial cells via TGF-β1/smad pathway. *Eur Rev Med PharmacolSci*. (2019) 23:289–96. doi: 10.26355/eurrev_201901_16775
52. Patel S, Tang J, Overstreet J, Anorga S, Lian F, Arnouk A, et al. viaRac-GTPase promotes fibrotic TGF-β1 signaling and chronic kidney disease EGFR, p53, and Hippo/YAP/TAZ pathways. *FASEB J*. (2019) 33:9797–810. doi: 10.1096/fj.201802489RR
53. Yang X, Wang H, Tu Y, Li Y, Zou Y, Li G, et al. WNT1-inducible signaling protein-1 mediates TGF-β1-induced renal fibrosis in tubular epithelial cells and unilateral ureteral obstruction mouse models via autophagy. *J Cell Physiol*. (2020) 235:2009–22. doi: 10.1002/jcp.29187
54. Wang S, Sun Z, Yang S, Chen B, Shi J. CTRP6 inhibits cell proliferation and ECM expression in rat mesangial cells cultured under TGF-β1. *Biomed Pharmacother*. (2018) 97:280–5. doi: 10.1016/j.biopha.2017.10.091
55. Lu Q, Wang WW, Zhang MZ, Ma ZX, Qiu XR, Shen M, et al. ROS induces epithelial-mesenchymal transition via the TGF-β1/PI3K/Akt/mTOR pathway in diabetic nephropathy. *Exp Ther Med*. (2019) 17:835–46. doi: 10.3892/etm.2018.7014
56. Chen J, He Q, Dai M, Kong W. HSP75 inhibits TGF-β1-induced apoptosis by targeting mitochondria in human renal proximal tubular epithelial cells. *Biochem Biophys Res Commun*. (2019) 515:64–71. doi: 10.1016/j.bbrc.2019.05.119
57. Yang J, Liu Y. Dissection of key events in tubular epithelial to myofibroblast transition and its implications in renal interstitial fibrosis. *Am J Pathol*. (2001) 159:1465–75. doi: 10.1016/S0002-9440(10)62533-3
58. Lee M, Kim SH, Jhee JH, Kim TY, Choi HY, Kim HJ, et al. Microparticles derived from human erythropoietin mRNA-transfected mesenchymal stem cells inhibit epithelial-to-mesenchymal transition and ameliorate renal interstitial fibrosis. *Stem Cell Res Ther*. (2020) 11:422. doi: 10.1186/s13287-020-01932-z
59. Strutz F, Okada H, Lo CW, Danoff T, Carone RL, Tomaszewski JE, et al. Identification and characterization of a fibroblast marker: FSP1. *J Cell Biol*. (1995) 130:393–405. doi: 10.1083/jcb.130.2.393
60. Balakumar P, Alqahtani A, Khan NA, Mahadevan N, Dhanaraj SA. Mechanistic insights into hyperuricemia-associated renal abnormalities with special emphasis on epithelial-to-mesenchymal transition: pathologic implications and putative pharmacologic targets. *Pharmacol Res*. (2020) 105209. doi: 10.1016/j.phrs.2020.105209
61. Kanlaya R, Peerapen P, Nilnumkhum A, Plumworasawat S, Sueksakit K, Thongboonkerd V. Epigallocatechin-3-gallate prevents TGF-β1-induced epithelial-mesenchymal transition and fibrotic changes of renal cells via GSK-3β/β-catenin/Snail1 and Nrf2 pathways. *J Nutr Biochem*. (2020) 76:108266. doi: 10.1016/j.jnutbio.2019.108266
62. Liu Y. New insights into epithelial-mesenchymal transition in kidney fibrosis. *J Am Soc Nephrol*. (2010) 21:212–22. doi: 10.1681/ASN.2008121226
63. Sato M, Muragaki Y, Saika S, Roberts AB, Ooshima A. Targeted disruption of TGF-beta1/Smad3 signaling protects against renal tubulointerstitial fibrosis

induced by unilateral ureteral obstruction. *J Clin Invest*. (2003) 112:1486–94. doi: 10.1172/JCI200319270
64. Li A, Zhang X, Shu M, Wu M, Wang J, Zhang J, et al. Arctigenin suppresses renal interstitial fibrosis in a rat model of obstructive nephropathy. *Phytomedicine*. (2017) 30:28–41. doi: 10.1016/j.phymed.2017.03.003
65. Park J, Choi H, Kim D, Kim C, Bae E, Ma S, et al. RON receptor tyrosine kinase regulates epithelial mesenchymal transition and the expression of pro-fibrotic markers via Src/Smad signaling in HK-2 and NRK49F Cells. *Int J Mol Sci.* (2019) 20:5489. doi: 10.3390/ijms20215489
66. Chen KH, Hsu HH, Yang HY, Tian YC, Ko YC, Yang CW, et al. Inhibition of spleen tyrosine kinase (syk) suppresses renal fibrosis through anti-inflammatory effects and down regulation of the MAPK-p38 pathway. *Int J Biochem Cell Biol*. (2016) 74:135–44. doi: 10.1016/j.biocel.2016.03.001
67. Liu Z, Tan R, Liu Y. The many faces of matrix metalloproteinase-7 in kidney diseases. *Biomolecules*. (2020) 10:960. doi: 10.3390/biom10060960
68. Li J, Qu X, Yao J, Caruana G, Ricardo SD, Yamamoto Y, et al. Blockade of endothelial-mesenchymal transition by a Smad3 inhibitor delays the early development of streptozotocin-induced diabetic nephropathy. *Diabetes*. (2010) 59:2612–24. doi: 10.2337/db09-1631
69. Xavier S, Vasko R, Matsumoto K, Zullo JA, Chen R, Maizel J, et al. Curtailing endothelial TGF-beta signaling is sufficient to reduce endothelial-mesenchymal transition and fibrosis in CKD. *J Am Soc Nephrol*. (2015) 26:817–29. doi: 10.1681/ASN.2013101137
70. Humphreys BD, Lin SL, Kobayashi A, Hudson TE, Nowlin BT, Bonventre JV, et al. Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. *Am J Pathol*. (2010) 176:85–97. doi: 10.2353/ajpath.2010.090517
71. Asada N, Takase M, Nakamura J, Oguchi A, Asada M, Suzuki N, et al. Dysfunction of fibroblasts of extrarenal origin underlies renal fibrosis and renal anemia in mice. *J Clin Invest*. (2011) 121:3981–90. doi: 10.1172/JCI57301
72. Wu CF, Chiang WC, Lai CF, Chang FC, Chen YT, Chou YH, et al. Transforming growth factor beta-1 stimulates profibrotic epithelial signaling to activate pericyte-myofibroblast transition in obstructive kidney fibrosis. *Am J Pathol*. (2013) 182:118–31. doi: 10.1016/j.ajpath.2012.09.009
73. Wang N, Deng Y, Liu A, Shen N, Wang W, Du X, et al. Novel mechanism of the pericyte-myofibroblast transition in renal interstitial fibrosis: core fucosylation regulation. *Sci Rep*. (2017) 7:16914. doi: 10.1038/s41598-017-17193-5
74. Zhu Y, Yu C, Zhuang S. Protein arginine methyltransferase 1 mediates renal fibroblast activation and fibrogenesis through activation of Smad3 signaling. *Am J Physiol Renal Physiol*. (2020) 318:F375–87. doi: 10.1152/ajprenal.00487.2019
75. Crosnier J, Jungers P, Courouce AM, Laplanche A, Benhamou E, Degos F, et al. Randomised placebo-controlled trial of hepatitis B surface antigen vaccine in french haemodialysis units: II, Haemodialysis patients. *Lancet*. (1981) 1:797–800. doi: 10.1016/S0140-6736(81)92679-9
76. Lin J, Tang W, Liu W, Yu F, Wu Y, Fang X, et al. Decreased B1 and B2 lymphocytes are associated with mortality in elderly patients with chronic kidney diseases. *Front Med.* (2020) 7:75. doi: 10.3389/fmed.2020.00075
77. Suzuki H, Kiryluk K, Novak J, Moldoveanu Z, Herr AB, Renfrow MB, et al. The pathophysiology of IgA nephropathy. *J Am Soc Nephrol*. (2011) 22:1795–803. doi: 10.1681/ASN.2011050464
78. Wadei HM, Textor SC. The role of the kidney in regulating arterial blood pressure. *Nat Rev Nephrol*. (2012) 8:602–9. doi: 10.1038/nrneph.2012.191
79. Chang S, Li XK. The role of immune modulation in pathogenesis of IgA nephropathy. *Front Med*. (2020) 7:92. doi: 10.3389/fmed.2020.00092
80. Andrejevic S, Jeremic I, Sefik-Bukilica M, Nikolic M, Stojimirovic B, Bonaci-Nikolic B. Immunoserological parameters in SLE: high-avidity anti-dsDNA detected by ELISA are the most closely associated with the disease activity. *Clin Rheumatol*. (2013) 32:1619–26. doi: 10.1007/s10067-013-2330-3
81. Seelen MA, Trouw LA, Daha MR. Diagnostic and prognostic significance of anti-C1q antibodies in systemic lupus erythematosus. *Curr Opin Nephrol Hypertens*. (2003) 12:619–24. doi: 10.1097/00041552-200311000-00008
82. Bigler C, Lopez-Trascasa M, Potlukova E, Moll S, Danner D, Schaller M, et al. Antinucleosome antibodies as a marker of active proliferative lupus nephritis. *Am J Kidney Dis*. (2008) 51:624–9. doi: 10.1053/j.ajkd.2007.10.041
83. Shao J, Peng Y, He L, Liu H, Chen X, Peng X. Capsaicin induces high expression of BAFF and aberrantly glycosylated IgA1 of tonsillar mononuclear cells in IgA nephropathy patients. *Hum Immunol*. (2014) 75:1034–9. doi: 10.1016/j.humimm.2014.08.205
84. Ye M, Peng Y, Liu C, Yan W, Peng X, He L, et al. Vibration induces BAFF overexpression and aberrant O-Glycosylation of IgA1 in cultured human tonsillar mononuclear cells in IgA nephropathy. *Biomed Res Int*. (2016) 2016:9125960. doi: 10.1155/2016/9125960
85. Lanvin O, Guglielmi P, Fuentes V, Gouilleux-Gruart V, Mazière C, Bissac E, et al. TGF-beta1 modulates Fas (APO-1/CD95)-mediated apoptosis of human pre-B cell lines. *Eur J Immunol*. (2003) 33:1372–81. doi: 10.1002/eji.200323761
86. Xu A, Liu Y, Chen W, Wang J, Xue Y, Huang F, et al. TGF-β-induced regulatory T cells directly suppress B cell responses through a noncytotoxic mechanism. *J Immunol*. (2016) 196:3631–41. doi: 10.4049/jimmunol.1501740
87. Kim HA, Jeon SH, Seo GY, Park JB, Kim PH. TGF-beta1 and IFN-gamma stimulate mouse macrophages to express BAFF via different signaling pathways. *J Leukoc Biol*. (2008) 83:1431–9. doi: 10.1189/jlb.1007676
88. Mackay F, Woodcock SA, Lawton P, Ambrose C, Baetscher M, Schneider P, et al. Mice transgenic for BAFF develop lymphocytic disorders along with autoimmune manifestations. *J Exp Med*. (1999) 190:1697–710. doi: 10.1084/jem.190.11.1697
89. Giannopoulou M, Dai C, Tan X, Wen X, Michalopoulos G, Liu Y. Hepatocyte growth factor exerts its anti-inflammatory action by disrupting nuclear factor-kappaB signaling. *Am J Pathol*. (2008) 173:30–41. doi: 10.2353/ajpath.2008.070583
90. Chu C, Hokamp J, Cianciolo R, Dabney A, Brinkmeyer-Langford C, Lees G, et al. RNA-seq of serial kidney biopsies obtained during progression of chronic kidney disease from dogs with X-linked hereditary nephropathy. *Sci Reports*. (2017) 7:16776. doi: 10.1038/s41598-017-16603-y
91. Kuo H, Huang C, Lin T, Lin C. IL-17 and CD40 ligand synergistically stimulate the chronicity of diabetic nephropathy. *Nephrol Dialysis Transplantation.* (2018) 33:248–56. doi: 10.1093/ndt/gfw397
92. Dos Santos M, Bringhenti RN, Rodrigues PG, Do Nascimento JF, Pereira SV, Zancan R, et al. Podocyte-associated mRNA profiles in kidney tissue and in urine of patients with active lupus nephritis. *Int J Clin Exp Pathol*. (2015) 8:4600–13.
93. Huang XR, Chung AC, Zhou L, Wang XJ, Lan HY. Latent TGF-beta1 protects against crescentic glomerulonephritis. *J Am Soc Nephrol*. (2008) 19:233–42. doi: 10.1681/ASN.2007040484
94. Cen S, Wang P, Xie Z, Yang R, Li J, Liu Z, et al. Autophagy enhances mesenchymal stem cell-mediated CD4(+) T cell migration and differentiation through CXCL8 and TGF-β1. *Stem Cell Res Ther*. (2019) 10:265. doi: 10.1186/s13287-019-1380-0
95. Jayaraman P, Parikh F, Newton J, Hanoteau A, Rivas C, Krupar R, et al. TGF-β1 programmed myeloid-derived suppressor cells (MDSC) acquire immune-stimulating and tumor killing activity capable of rejecting established tumors in combination with radiotherapy. *Oncoimmunology*. (2018) 7:e1490853. doi: 10.1080/2162402X.2018.1490853
96. Pozdzik AA, Salmon IJ, Husson CP, Decaestecker C, Rogier E, Bourgeade MF, et al. Patterns of interstitial inflammation during the evolution of renal injury in experimental aristolochic acid nephropathy. *Nephrol Dial Transplant*. (2008) 23:2480–91. doi: 10.1093/ndt/gfn140
97. Locatelli F, Canaud B, Eckardt KU, Stenvinkel P, Wanner C, Zoccali C. Oxidative stress in end-stage renal disease: an emerging threat to patient outcome. *Nephrol Dial Transplant*. (2003) 18:1272–80. doi: 10.1093/ndt/gfg074
98. Dounousi E, Papavasiliou E, Makedou A, Ioannou K, Katopodis KP, Tselepis A, et al. Oxidative stress is progressively enhanced with advancing stages of CKD. *Am J Kidney Dis*. (2006) 48:752–60. doi: 10.1053/j.ajkd.2006.08.015
99. Liu RM, Desai LP. Reciprocal regulation of TGF-beta and reactive oxygen species: a perverse cycle for fibrosis. *Redox Biol*. (2015) 6:565–77. doi: 10.1016/j.redox.2015.09.009
100. De Miguel C, Guo C, Lund H, Feng D, Mattson DL. Infiltrating T lymphocytes in the kidney increase oxidative stress and participate in the

development of hypertension and renal disease. *Am J Physiol Renal Physiol*. (2011) 300:F734–42. doi: 10.1152/ajprenal.00454.2010
101. Pena C, Hernandez-Fonseca JP, Pedreanez A, Viera N, Mosquera J. Renal oxidative stress and renal CD8(+) T-cell infiltration in mercuric chloride-induced nephropathy in rats: role of angiotensin II. *J Immunotoxicol*. (2016) 13:324–34. doi: 10.3109/1547691X.2015.1089960
102. Strickland FM, Li Y, Johnson K, Sun Z, Richardson BC. CD4(+) T cells epigenetically modified by oxidative stress cause lupus-like autoimmunity in mice. *J Autoimmun*. (2015) 62:75–80. doi: 10.1016/j.jaut.2015.06.004
103. Brinkhoff A, Sieberichs A, Engler H, Dolff S, Benson S, Korth J, et al. Pro-inflammatory Th1 and Th17 cells are suppressed during human experimental endotoxemia whereas anti-inflammatory IL-10 producing T-cells are unaffected. *Front Immunol*. (2018) 9:1133. doi: 10.3389/fimmu.2018.01133
104. Sharma R, Kinsey GR. Regulatory T cells in acute and chronic kidney diseases. *Am J Physiol Renal Physiol*. (2018) 314:F679–98. doi: 10.1152/ajprenal.00236.2017
105. Do Valle Duraes F, Lafont A, Beibel M, Martin K, Darribat K, Cuttat R, et al. Immune cell landscaping reveals a protective role for regulatory T cells during kidney injury and fibrosis. *JCI Insight*. (2020) 5:e130651. doi: 10.1172/jci.insight.130651
106. Mu Y, Zhang J, Liu Y, Ma J, Jiang D, Zhang X, et al. CD226 deficiency on regulatory T cells aggravates renal fibrosis via up-regulation of Th2 cytokines through miR-340. *J Leukocyte Biol*. (2020) 107:573–87. doi: 10.1002/JLB.2MA1119-174RR
107. Li Y, Liu X, Wang W, Wang S, Zhang J, Jiang S, et al. Low-dose IL-2 expands CD4(+) regulatory T cells with a suppressive function *in vitro* via the STAT5-dependent pathway in patients with chronic kidney diseases. *Ren Fail*. (2018) 40:280–8. doi: 10.1080/0886022X.2018.1456462
108. Dardalhon V, Awasthi A, Kwon H, Galileos G, Gao W, Sobel RA, et al. IL-4 inhibits TGF-beta-induced Foxp3+ T cells and, together with TGF-beta, generates IL-9+ IL-10+ Foxp3(-) effector T cells. *Nat Immunol*. (2008) 9:1347–55. doi: 10.1038/ni.1677
109. Cai J, Jiao X, Zhao S, Liang Y, Ning Y, Shi Y, et al. Transforming growth factor-β1-overexpressing mesenchymal stromal cells induced local tolerance in rat renal ischemia/reperfusion injury. *Cytotherapy*. (2019) 21:535–45. doi: 10.1016/j.jcyt.2018.12.003
110. Chen X, Feng L, Li S, Long D, Shan J, Li Y. TGF-β1 maintains Foxp3 expression and inhibits glycolysis in natural regulatory T cells via PP2A-mediated suppression of mTOR signaling. *Immunol Lett*. (2020) 226:31–7. doi: 10.1016/j.imlet.2020.06.016
111. Choi G, Na H, Kuen DS, Kim BS, Chung Y. Autocrine TGF-β1 Maintains the Stability of Foxp3(+) Regulatory T Cells via IL-12Rβ2 Downregulation. *Biomolecules*. (2020) 10:819. doi: 10.3390/biom10060819
112. Zhang L, Yi H, Xia XP, Zhao Y. Transforming growth factor-beta: an important role in CD4+CD25+ regulatory T cells and immune tolerance. *Autoimmunity*. (2006) 39:269–76. doi: 10.1080/08916930600753903
113. Wu W, Tsai Y, Lin T, Wu M, Lin C. The attenuation of renal fibrosis by histone deacetylase inhibitors is associated with the plasticity of FOXP3IL-17 T cells. *BMC Nephrol*. (2017) 18:225. doi: 10.1186/s12882-017-0630-6
114. Ai Z, Udalova IA. Transcriptional regulation of neutrophil differentiation and function during inflammation. *J Leukoc Biol*. (2020) 107:419–30. doi: 10.1002/JLB.1RU1219-504RR
115. Woziwodzka K, Dziewierz A, Pawica M, Panek A, Krzanowski M, Gołasa P, et al. Neutrophil-to-lymphocyte ratio predicts long-term all-cause mortality in patients with chronic kidney disease stage 5. *Folia Med Cracov*. (2019) 59:55–70. doi: 10.24425/fmc.2019.131380
116. Chatfield S, Thieblemont N, Witko-Sarsat V. Expanding neutrophil horizons: new concepts in inflammation. *J Innate Immunity*. (2018) 10:422–31. doi: 10.1159/000493101
117. Haddad A, Gaudet M, Plesa M, Allakhverdi Z, Mogas AK, Audusseau S, et al. Neutrophils from severe asthmatic patients induce epithelial to mesenchymal transition in healthy bronchial epithelial cells. *Respir Res*. (2019) 20:234. doi: 10.1186/s12931-019-1186-8
118. Uyama N, Tsutsui H, Wu S, Yasuda K, Hatano E, Qin XY, et al. Anti-interleukin-6 receptor antibody treatment ameliorates postoperative adhesion formation. *Sci Rep*. (2019) 9:17558. doi: 10.1038/s41598-019-54175-1
119. Zhang S. The role of transforming growth factor beta in T helper 17 differentiation. *Immunology*. (2018) 155:24–35. doi: 10.1111/imm.12938
120. Gao P, Tang K, Lu Y, Huang Z, Wang S, Wang M, et al. Pentraxin 3 promotes airway inflammation in experimental asthma. *Respir Res*. (2020) 21:237. doi: 10.1186/s12931-020-01499-6
121. Zhou Y, Wang T, Wang Y, Meng F, Ying M, Han R, et al. Blockade of extracellular high-mobility group box 1 attenuates inflammation-mediated damage and haze grade in mice with corneal wounds. *Int Immunopharmacol*. (2020) 83:106468. doi: 10.1016/j.intimp.2020.106468
122. Sierra-Mondragon E, Rodríguez-Muñoz R, Namorado-Tonix C, Molina-Jijon E, Romero-Trejo D, Pedraza-Chaverri J, et al. All-trans retinoic acid attenuates fibrotic processes by downregulating TGF-β1/Smad3 in early diabetic nephropathy. *Biomolecules*. (2019) 9. doi: 10.3390/biom9100525
123. Silva JD, Lopes-Pacheco M, De Castro LL, Kitoko JZ, Trivelin SA, Amorim NR, et al. Eicosapentaenoic acid potentiates the therapeutic effects of adipose tissue-derived mesenchymal stromal cells on lung and distal organ injury in experimental sepsis. *Stem Cell Res Ther*. (2019) 10:264. doi: 10.1186/s13287-019-1365-z
124. Cao Q, Lu J, Li Q, Wang C, Wang XM, Lee VW, et al. CD103+ dendritic cells elicit CD8+ T cell responses to accelerate kidney injury in adriamycin nephropathy. *J Am Soc Nephrol*. (2016) 27:1344–60. doi: 10.1681/ASN.2015030229
125. Zhang F, Wang C, Wen X, Chen Y, Mao R, Cui D, et al. Mesenchymal stem cells alleviate rat diabetic nephropathy by suppressing CD103(+) DCs-mediated CD8(+) T cell responses. *J Cell Mol Med*. (2020) 24:5817–31. doi: 10.1111/jcmm.15250
126. Chakraborty K, Chatterjee S, Bhattacharyya A. Modulation of CD11c+ lung dendritic cells in respect to TGF-beta in experimental pulmonary fibrosis. *Cell Biol Int*. (2017) 41:991–1000. doi: 10.1002/cbin.10800
127. Lievens D, Habets KL, Robertson AK, Laouar Y, Winkels H, Rademakers T, et al. Abrogated transforming growth factor beta receptor II (TGFbetaRII) signalling in dendritic cells promotes immune reactivity of T cells resulting in enhanced atherosclerosis. *Eur Heart J*. (2013) 34:3717–27. doi: 10.1093/eurheartj/ehs106
128. Esebanmen GE, Langridge WHR. The role of TGF-beta signaling in dendritic cell tolerance. *Immunol Res*. (2017) 65:987–94. doi: 10.1007/s12026-017-8944-9
129. Bourque J, Hawiger D. Immunomodulatory bonds of the partnership between dendritic cells and T cells. *Crit Rev Immunol*. (2018) 38:379–401. doi: 10.1615/CritRevImmunol.2018026790
130. Morris G, Puri BK, Olive L, Carvalho AF, Berk M, Maes M. Emerging role of innate B1 cells in the pathophysiology of autoimmune and neuroimmune diseases: association with inflammation, oxidative and nitrosative stress and autoimmune responses. *Pharmacol Res*. (2019) 148:104408. doi: 10.1016/j.phrs.2019.104408
131. Lu P, Cao Y, Wang M, Zheng P, Hou J, Zhu C, et al. Mature dendritic cells cause Th17/Treg imbalance by secreting TGF-β1 and IL-6 in the pathogenesis of experimental autoimmune encephalomyelitis. *Cent Eur J Immunol*. (2016) 41:143–52. doi: 10.5114/ceji.2016.60987
132. Lyakh L, Trinchieri G, Provezza L, Carra G, Gerosa F. Regulation of interleukin-12/interleukin-23 production and the T-helper 17 response in humans. *Immunol Rev*. (2008) 226:112–31. doi: 10.1111/j.1600-065X.2008.00700.x
133. Kassianos AJ, Wang X, Sampangi S, Muczynski K, Healy H, Wilkinson R. Increased tubulointerstitial recruitment of human CD141(hi) CLEC9A(+) and CD1c(+) myeloid dendritic cell subsets in renal fibrosis and chronic kidney disease. *Am J Physiol Renal Physiol*. (2013) 305:F1391–1401. doi: 10.1152/ajprenal.00318.2013
134. Kassianos AJ, Wang X, Sampangi S, Afrin S, Wilkinson R, Healy H. Fractalkine-CX3CR1-dependent recruitment and retention of human CD1c+ myeloid dendritic cells by *in vitro*-activated proximal tubular epithelial cells. *Kidney Int*. (2015) 87:1153–63. doi: 10.1038/ki.2014.407
135. Fainaru O, Shay T, Hantisteanu S, Goldenberg D, Domany E, Groner Y. TGFbeta-dependent gene expression profile during maturation of dendritic cells. *Genes Immun*. (2007) 8:239–44. doi: 10.1038/sj.gene.6364380
136. Bonnefoy F, Couturier M, Clauzon A, Remy-Martin JP, Gaugler B, Tiberghien P, et al. TGF-beta-exposed plasmacytoid dendritic cells

participate in Th17 commitment. *J Immunol*. (2011) 186:6157–64. doi: 10.4049/jimmunol.1002497
137. Pei G, Yao Y, Yang Q, Wang M, Wang Y, Wu J, et al. Lymphangiogenesis in kidney and lymph node mediates renal inflammation and fibrosis. *Sci Adv*. (2019) 5:eaaw5075. doi: 10.1126/sciadv.aaw5075
138. Wang R, Chen T, Wang C, Zhang Z, Wang XM, Li Q, et al. Flt3 inhibition alleviates chronic kidney disease by suppressing CD103+ dendritic cell-mediated T cell activation. *Nephrol Dial Transplant*. (2019) 34:1853–63. doi: 10.1093/ndt/gfy385
139. Yamate J, Machida Y, Ide M, Kuwamura M, Kotani T, Sawamoto O, et al. Cisplatin-induced renal interstitial fibrosis in neonatal rats, developing as solitary nephron unit lesions. *Toxicol Pathol*. (2005) 33:207–17. doi: 10.1080/01926230490523978
140. Tang PM, Zhang YY, Hung JS, Chung JY, Huang XR, To KF, et al. DPP4/CD32b/NF-kappaB circuit: a novel druggable target for inhibiting CRP-Driven diabetic nephropathy. *Mol Ther*. (2021) 29:365–75. doi: 10.1016/j.ymthe.2020.08.017
141. Meng XM, Nikolic-Paterson DJ, Lan HY. TGF-β: the master regulator of fibrosis. *Nat Rev Nephrol*. (2016) 12:325–38. doi: 10.1038/nrneph.2016.48
142. Narváez A, Guiteras R, Sola A, Manonelles A, Morote J, Torras J, et al. siRNA-silencing of CD40 attenuates unilateral ureteral obstruction-induced kidney injury in mice. *PLoS ONE*. (2019) 14:e0215232. doi: 10.1371/journal.pone.0215232
143. Lecru L, Desterke C, Grassin-Delyle S, Chatziantoniou C, Vandermeersch S, Devocelle A, et al. Cannabinoid receptor 1 is a major mediator of renal fibrosis. *Kidney Int*. (2015) 88:72–84. doi: 10.1038/ki.2015.63
144. Zhang J, Wong MG, Wong M, Gross S, Chen J, Pollock C, et al. A cationic-independent mannose 6-phosphate receptor inhibitor (PXS64) ameliorates kidney fibrosis by inhibiting activation of transforming growth factor-β1. *PLoS ONE*. (2015) 10:e0116888. doi: 10.1371/journal.pone.0116888
145. Zhuang C, Liu G, Barkema HW, Zhou M, Xu S, Ur Rahman S, et al. Selenomethionine suppressed TLR4/NF-κB pathway by activating selenoprotein S to alleviate ESBL *Escherichia coli*-induced inflammation in bovine mammary epithelial cells and macrophages. *Front Microbiol*. (2020) 11:1461. doi: 10.3389/fmicb.2020.01461
146. Nikolic-Paterson DJ, Wang S, Lan HY. Macrophages promote renal fibrosis through direct and indirect mechanisms. *Kidney Int Suppl*. (2014) 4:34–8. doi: 10.1038/kisup.2014.7
147. Ikezumi Y, Suzuki T, Yamada T, Hasegawa H, Kaneko U, Hara M, et al. Alternatively activated macrophages in the pathogenesis of chronic kidney allograft injury. *Pediatr Nephrol*. (2015) 30:1007–17. doi: 10.1007/s00467-014-3023-0
148. Wang L, Ren X, Tian XF, Cheng XL, Zhao YY, Li QY, et al. Protective effects of GPR120 agonist-programmed macrophages on renal interstitial fibrosis in unilateral ureteral obstruction (UUO) rats. *Biomed Pharmacother*. (2019) 117:109172. doi: 10.1016/j.biopha.2019.109172
149. Bellon T, Martinez V, Lucendo B, Del Peso G, Castro MJ, Aroeira LS, et al. Alternative activation of macrophages in human peritoneum: implications for peritoneal fibrosis. *Nephrol Dial Transplant*. (2011) 26:2995–3005. doi: 10.1093/ndt/gfq771
150. Pahl MV, Gollapudi S, Sepassi L, Gollapudi P, Elahimehr R, Vaziri ND. Effect of end-stage renal disease on B-lymphocyte subpopulations, IL-7, BAFF and BAFF receptor expression. *Nephrol Dial Transplant*. (2010) 25:205–12. doi: 10.1093/ndt/gfp397
151. Pieper K, Grimbacher B, Eibel H. B-cell biology and development. *J Allergy Clin Immunol*. (2013) 131:959–71. doi: 10.1016/j.jaci.2013.01.046
152. Fairfax KA, Tsantikos E, Figgett WA, Vincent FB, Quah PS, Lepage M, et al. BAFF-driven autoimmunity requires CD19 expression. *J Autoimmun*. (2015) 62:1–10. doi: 10.1016/j.jaut.2015.06.001
153. Huen SC, Cantley LG. Macrophages in renal injury and repair. *Annu Rev Physiol*. (2017) 79:449–69. doi: 10.1146/annurev-physiol-022516-034219
154. Meng XM, Mak TS, Lan HY. Macrophages in renal fibrosis. *Adv Exp Med Biol*. (2019) 1165:285–303. doi: 10.1007/978-981-13-8871-2_13
155. Tarng DC, Liu IS, Lin LC, Chen NJ. Attenuation of tubular injury and renal fibrosis by TI-HU-YIN via reduction in transforming growth factor-β1 expression in unilateral ureteral obstruction mice. *Chin J Physiol*. (2015) 58:367–76. doi: 10.4077/CJP.2015.BAD326
156. Chung S, Overstreet JM, Li Y, Wang Y, Niu A, Wang S, et al. TGF-β promotes fibrosis after severe acute kidney injury by enhancing renal macrophage infiltration. *JCI Insight*. (2018) 3:e123563. doi: 10.1172/jci.insight.123563
157. Ren J, Li J, Feng Y, Shu B, Gui Y, Wei W, et al. Rictor/mammalian target of rapamycin complex 2 promotes macrophage activation and kidney fibrosis. *J Pathol*. (2017) 242:488–99. doi: 10.1002/path.4921
158. Lu H, Wu L, Liu L, Ruan Q, Zhang X, Hong W, et al. Quercetin ameliorates kidney injury and fibrosis by modulating M1/M2 macrophage polarization. *Biochem Pharmacol*. (2018) 154:203–12. doi: 10.1016/j.bcp.2018.05.007
159. Praga M, Barrio V, Juárez GF, Luño J. Tacrolimus monotherapy in membranous nephropathy: a randomized controlled trial. *Kidney Int*. (2007) 71:924–30. doi: 10.1038/sj.ki.5002215
160. Lebleu VS, Taduri G, O'connell J, Teng Y, Cooke VG, Woda C, et al. Origin and function of myofibroblasts in kidney fibrosis. *Nat Med*. (2013) 19:1047–53. doi: 10.1038/nm.3218
161. Falke LL, Gholizadeh S, Goldschmeding R, Kok RJ, Nguyen TQ. Diverse origins of the myofibroblast-implications for kidney fibrosis. *Nat Rev Nephrol*. (2015) 11:233–44. doi: 10.1038/nrneph.2014.246
162. Meng XM, Wang S, Huang XR, Yang C, Xiao J, Zhang Y, et al. Inflammatory macrophages can transdifferentiate into myofibroblasts during renal fibrosis. *Cell Death Dis*. (2016) 7:e2495. doi: 10.1038/cddis.2016.402
163. Torres Á, Muñoz K, Nahuelpán Y, Ap RS, Mendoza P, Jara C, et al. Intraglomerular monocyte/macrophage infiltration and macrophage-myofibroblast transition during diabetic nephropathy is regulated by the A(2B) adenosine receptor. *Cells*. (2020) 9:1051. doi: 10.3390/cells9041051
164. Wang S, Meng XM, Ng YY, Ma FY, Zhou S, Zhang Y, et al. TGF-β/Smad3 signalling regulates the transition of bone marrow-derived macrophages into myofibroblasts during tissue fibrosis. *Oncotarget*. (2016) 7:8809–22. doi: 10.18632/oncotarget.6604
165. Tang PM, Zhou S, Li CJ, Liao J, Xiao J, Wang QM, et al. The proto-oncogene tyrosine protein kinase Src is essential for macrophage-myofibroblast transition during renal scarring. *Kidney Int*. (2018) 93:173–87. doi: 10.1016/j.kint.2017.07.026
166. Guo Y, Sun L, Xiao L, Gou R, Fang Y, Liang Y, et al. Aberrant Wnt/Beta-catenin pathway activation in dialysate-induced peritoneal fibrosis. *Front Pharmacol*. (2017) 8:774. doi: 10.3389/fphar.2017.00774
167. Voelker J, Berg PH, Sheetz M, Duffin K, Shen T, Moser B, et al. Anti-TGF-β1 Antibody Therapy in Patients with Diabetic Nephropathy. *J Am Soc Nephrol*. (2017) 28:953–62. doi: 10.1681/ASN.2015111230
168. Zhao L, Zou Y, Liu F. Transforming growth factor-beta1 in diabetic kidney disease. *Front Cell Dev Biol*. (2020) 8:187. doi: 10.3389/fcell.2020.00187
169. Vincenti F, Fervenza FC, Campbell KN, Diaz M, Gesualdo L, Nelson P, et al. A phase 2, double-blind, placebo-controlled, randomized study of fresolimumab in patients with steroid-resistant primary focal segmental glomerulosclerosis. *Kidney Int Rep*. (2017) 2:800–10. doi: 10.1016/j.ekir.2017.03.011
170. Lodyga M, Hinz B. TGF-β1 - A truly transforming growth factor in fibrosis and immunity. *Semin Cell Dev Biol*. (2020) 101:123–39. doi: 10.1016/j.semcdb.2019.12.010
171. Fujimoto M, Maezawa Y, Yokote K, Joh K, Kobayashi K, Kawamura H, et al. Mice lacking Smad3 are protected against streptozotocin-induced diabetic glomerulopathy. *Biochem Biophys Res Commun*. (2003) 305:1002–7. doi: 10.1016/S0006-291X(03)00885-4
172. Zhou L, Fu P, Huang XR, Liu F, Chung AC, Lai KN, et al. Mechanism of chronic aristolochic acid nephropathy: role of Smad3. *Am J Physiol Renal Physiol*. (2010) 298:F1006–17. doi: 10.1152/ajprenal.00675.2009
173. Ji X, Wang H, Wu Z, Zhong X, Zhu M, Zhang Y, et al. Specific inhibitor of Smad3 (SIS3) attenuates fibrosis, apoptosis, and inflammation in unilateral ureteral obstruction kidneys by inhibition of transforming growth factor β (TGF-β)/Smad3 signaling. *Med Sci Monit*. (2018) 24:1633–41. doi: 10.12659/MSM.909236
174. Chen DQ, Cao G, Zhao H, Chen L, Yang T, Wang M, et al. Combined melatonin and poricoic acid A inhibits renal fibrosis through modulating the interaction of Smad3 and β-catenin pathway in AKI-to-CKD continuum. *Ther Adv Chronic Dis*. (2019) 10:2040622319869116. doi: 10.1177/2040622319869116

175. Wang M, Chen DQ, Chen L, Liu D, Zhao H, Zhang ZH, et al. Novel RAS Inhibitors Poricoic Acid ZG and Poricoic Acid ZH Attenuate Renal Fibrosis via a Wnt/β-Catenin Pathway and Targeted Phosphorylation of smad3 Signaling. *J Agric Food Chem*. (2018) 66:1828–42. doi: 10.1021/acs.jafc.8b00099
176. Chen H, Yang T, Wang MC, Chen DQ, Yang Y, Zhao YY. Novel RAS inhibitor 25-O-methylalisol F attenuates epithelial-to-mesenchymal transition and tubulo-interstitial fibrosis by selectively inhibiting TGF-β-mediated Smad3 phosphorylation. *Phytomedicine*. (2018) 42:207–18. doi: 10.1016/j.phymed.2018.03.034
177. Hoi S, Tsuchiya H, Itaba N, Suzuki K, Oka H, Morimoto M, et al. WNT/β-catenin signal inhibitor IC-2-derived small-molecule compounds suppress TGF-β1-induced fibrogenic response of renal epithelial cells by inhibiting SMAD2/3 signalling. *Clin Exp Pharmacol Physiol*. (2020) 47:940–6. doi: 10.1111/1440-1681.13270
178. Wang YY, Jiang H, Pan J, Huang XR, Wang YC, Huang HF, et al. Macrophage-to-myofibroblast transition contributes to interstitial fibrosis in chronic renal allograft injury. *J Am Soc Nephrol*. (2017) 28:2053–67. doi: 10.1681/ASN.2016050573
179. Xu BH, Sheng J, You YK, Huang XR, Ma RCW, Wang Q, et al. Deletion of Smad3 prevents renal fibrosis and inflammation in type 2 diabetic nephropathy. *Metabolism*. (2020) 103:154013. doi: 10.1016/j.metabol.2019.154013
180. Zhang YY, Tang PM, Tang PC, Xiao J, Huang XR, Yu C, et al. LRNA9884, a novel Smad3-dependent long noncoding RNA, promotes diabetic kidney injury in db/db Mice via enhancing MCP-1-dependent renal inflammation. *Diabetes*. (2019) 68:1485–98. doi: 10.2337/db18-1075
181. Sun SF, Tang PMK, Feng M, Xiao J, Huang XR, Li P, et al. Novel lncRNA Erbb4-IR Promotes Diabetic Kidney Injury in db/db Mice by Targeting miR-29b. *Diabetes*. (2018) 67:731–44. doi: 10.2337/db17-0816
182. Chen HY, Zhong X, Huang XR, Meng XM, You Y, Chung AC, et al. MicroRNA-29b inhibits diabetic nephropathy in db/db mice. *Mol Ther*. (2014) 22:842–53. doi: 10.1038/mt.2013.235
183. Li R, Chung AC, Dong Y, Yang W, Zhong X, Lan HY. The microRNA miR-433 promotes renal fibrosis by amplifying the TGF-β/Smad3-Azin1 pathway. *Kidney Int*. (2013) 84:1129–44. doi: 10.1038/ki.2013.272
184. Wang P, Luo ML, Song E, Zhou Z, Ma T, Wang J, et al. Long noncoding RNA lnc-TSI inhibits renal fibrogenesis by negatively regulating the TGF-β/Smad3 pathway. *Sci Transl Med*. (2018) 10:eaat2039. doi: 10.3410/f.734198751.793554315
185. Zhong X, Chung AC, Chen HY, Dong Y, Meng XM, Li R, et al. miR-21 is a key therapeutic target for renal injury in a mouse model of type 2 diabetes. *Diabetologia*. (2013) 56:663–74. doi: 10.1007/s00125-012-2804-x
186. Lv W, Booz GW, Wang Y, Fan F, Roman RJ. Inflammation and renal fibrosis: Recent developments on key signaling molecules as potential therapeutic targets. *Eur J Pharmacol*. (2018) 820:65–76. doi: 10.1016/j.ejphar.2017.12.016
187. Zhang Y, Tang PM, Niu Y, Garcia Cordoba CA, Huang XR, Yu C, et al. Long Non-coding RNA LRNA9884 promotes acute kidney injury via regulating NF-kB-mediated transcriptional activation of MIF. *Front Physiol*. (2020) 11:590027. doi: 10.3389/fphys.2020.590027
188. Tang PM, Zhang YY, Lan HY. LncRNAs in TGF-beta-driven tissue fibrosis. *Noncoding RNA*. (2018) 4:26. doi: 10.3390/ncrna4040026
189. Tang PM, Zhang YY, Mak TS, Tang PC, Huang XR, Lan HY. Transforming growth factor-beta signalling in renal fibrosis: from Smads to non-coding RNAs. *J Physiol*. (2018) 596:3493–503. doi: 10.1113/JP274492
190. Wu Y, Wang L, Deng D, Zhang Q, Liu W. Renalase protects against renal fibrosis by inhibiting the activation of the ERK signaling pathways. *Int J Mol Sci*. (2017) 18:855. doi: 10.3390/ijms18050855
191. Andrikopoulos P, Kieswich J, Pacheco S, Nadarajah L, Harwood SM, O'riordan CE, et al. The MEK inhibitor trametinib ameliorates kidney fibrosis by suppressing ERK1/2 and mTORC1 signaling. *J Am Soc Nephrol*. (2019) 30:33–49. doi: 10.1681/ASN.2018020209
192. Zhou Z, Hu Z, Li M, Zhu F, Zhang H, Nie J, et al. QiShenYiQi attenuates renal interstitial fibrosis by blocking the activation of β-Catenin. *PLoS ONE*. (2016) 11:e0162873. doi: 10.1371/journal.pone.0162873
193. Ren H, Zuo S, Hou Y, Shang W, Liu N, Yin Z. Inhibition of α1-adrenoceptor reduces TGF-β1-induced epithelial-to-mesenchymal transition and attenuates UUO-induced renal fibrosis in mice. *FASEB J*. (2020) 34:14892–904. doi: 10.1096/fj.202000737RRR

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Spleen Tyrosine Kinase Inhibition Ameliorates Tubular Inflammation in IgA Nephropathy

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Spleen tyrosine kinase (Syk) is a non-receptor tyrosine kinase involved in signal transduction in a variety of immune responses. It has been demonstrated that Syk plays a pathogenic role in orchestrating inflammatory responses and cell proliferation in human mesangial cells (HMC) in IgA nephropathy (IgAN). However, whether Syk is involved in tubular damage in IgAN remains unknown. Using human kidney biopsy specimens, we found that Syk was activated in renal tubules of biopsy-proven IgAN patients with an increase in total and phosphorylated levels compared to that from healthy control subjects. *In vitro*, cultured proximal tubular epithelial cells (PTECs) were stimulated with conditioned medium prepared from human mesangial cells incubated with polymeric IgA (IgA-HMC) from patients with IgAN or healthy control. Induction of IL-6, IL-8, and ICAM-1 synthesis from cultured PTECs incubated with IgA-HMC conditioned medium was significantly suppressed by treatment with the Syk inhibitor R406 compared to that from healthy control. Furthermore, R406 downregulated expression of phosphorylated p65 NF-κB and p-42/p-44 MAPK, and attenuated TNF-α-induced cytokine production in PTECs. Taken together, our findings suggest that Syk mediates IgA-HMC conditioned medium-induced inflammation in tubular cells *via* activation of NF-κB and p-42/p-44 MAPK signaling. Inhibition of Syk may be a potential therapeutic approach for tubulointerstitial injury in IgAN.

Keywords: IgA nephropathy, spleen tyrosine kinase, inflammation, NF-κB, MAPK

INTRODUCTION

IgA nephropathy (IgAN) was first described over 50 years ago (Tang, 2018) and remains the most common form of primary glomerulonephritis worldwide with a hallmark feature of IgA1 deposits in the glomerular mesangium (Wyatt and Julian, 2013). Most patients with IgAN present a slowly progressive clinical course and about 30–40% of patients will develop

kidney failure within 20–30 years of diagnosis (Lai et al., 2016). Apart from a prediction tool that takes into account clinicopathologic features at the time of kidney biopsy (Barbour et al., 2019), there are no other means that take into account pathogenetic processes that accurately predict who will progress to kidney failure. Studies over the past decades have indicated that circulating glycosylated IgA1 binds to autoantibodies and forms immune complexes that deposit in the mesangium, resulting in activated resident cells and local inflammation (Novak et al., 2018). More evidence have shown that mesangial-derived mediators contribute to the pathogenesis of tubulointerstitial damage and podocyte injury *via* mesangial–podocytic–tubular crosstalk (Leung et al., 2018). Genome-wide association studies have also demonstrated that genetic components are implicated in disease pathogenesis and identified several susceptibility genes and loci associated with immune regulation (Zhang et al., 2017). Although important progress has been made in understanding the pathogenic mechanism of IgAN since the disease was first discovered, an effective and specific therapy for IgAN is still lacking (Barratt and Tang, 2018). Tampering the immune system stems from partial treatment efficacy using various forms of immunosuppression such as corticosteroid (Lv et al., 2017), mycophenolate (Tang et al., 2010), and more recently hydroxychloroquine (Liu et al., 2019).

Spleen tyrosine kinase (Syk) is a cytoplasmic tyrosine kinase highly expressed in most immune cells, where Syk plays a critical role in cell signaling during hematopoietic cell activation and differentiation (Mocsai et al., 2010). Syk is activated by stimulation of immunoreceptor expressed on immune cells, the two SH2 domains of Syk specifically bind to the dual phosphorylated immunoreceptor tyrosine-based activation motifs (ITAMS), triggering kinase activation and multiple downstream signaling pathways (Kaur et al., 2013). Syk is also expressed in various non-hematopoietic cells including epithelial cells, endothelial cells, fibroblasts, and neuronal cells. Numerous studies have revealed a diverse biological role of Syk in cell adhesion, platelet activation, vascular development, and cancer growth (Yanagi et al., 2001; Bartaula-Brevik et al., 2018). Given that Syk is an upstream mediator of multiple signaling pathways in the immune responses, it has been used as a potential therapeutic target for autoimmune diseases and immune-mediated disorders (Ghosh and Tsokos, 2010; Lucas and Tan, 2014; Szilveszter et al., 2019).

Syk is also expressed in human and murine mesangial cells and plays a pathogenetic role in IgAN. Syk expression is required for the IgA-induced production of inflammatory cytokines including MCP-1, IL-6, IL-8, and RANTES in human mesangial cells (Kim et al., 2012). IgA may bind to a novel Fcα receptor that mediates phosphorylation of Syk and MCP-1 synthesis in IgA-activated mesangial cells (Barratt et al., 2000; Tsuge et al., 2003), though the expression of Fcα receptor on human mesangial cells is controversial (Leung et al., 2000). Other potential IgA receptor present on mesangial cells such as transferrin receptor (CD71) and galactosyltransferase 1 have been identified to recruit Syk for activation (Moura et al., 2001; Molyneux et al., 2017). *In vivo*, treatment with fostamatinib, the selective Syk inhibitor and a prodrug of R406, significantly reduces proteinuria, glomerular macrophage infiltration, and tissue damage in an animal model of antibody-mediated glomerulonephritis, suggesting a potential protective effect of Syk inhibition on IgAN (Smith et al., 2010).

In this study, we report that Syk is an important mediator of tubular activation in IgAN. Both expression and phosphorylation of Syk were upregulated in renal tubules of kidney biopsies from patients with IgAN. We also found that inhibition of Syk could attenuate the inflammatory changes in PTECs triggered by glomerulotubular crosstalk, *via* downregulation of NF-κB and p-42/p-44 MAPK signaling.

MATERIALS AND METHODS

Sample Collection

Serum samples were collected from Chinese patients (age 49 ± 12 years) with clinical (eGFR 60.52 ± 25.04 ml/min per 1.73 m^2 and serum creatinine 169 ± 130 μmol/l) and renal immunopathological diagnosis of primary IgAN (n = 20) and healthy subjects (n = 20) with no microscopic hematuria or proteinuria as normal controls. Renal tissue biopsy were obtained from patients with IgA nephropathy (n = 5). Normal portions of renal tissues removed from nephrectomy specimens for the treatment of solitary renal carcinoma in the opposite pole were used as control (n = 5). **Table 1** provides the clinical characteristics of the five patients with IgAN at the time of biopsy. This study was conducted in accordance with the principles of the Declaration of Helsinki. The use of serum and tissue specimens for this study was approved by the Research Ethics Committee/Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster. Written informed consent was obtained from all subjects before sample collection.

Isolation of IgA1

Polymeric IgA1 was isolated and purified from sera of IgAN patients and healthy subjects as described previously (Leung et al., 2001). Briefly, IgA1 was purified using a jacalin-agarose affinity column from Pierce (Rockford, IL, United States), and the purity was confirmed by SDS-PAGE. Five groups of pooled IgA, each from four different IgAN patients or healthy control subjects, were used in the subsequent experiment.

Immunohistochemical Staining

Kidney biopsies were fixed in 10% neutral-buffered formalin and paraffin-embedded. Immunohistochemical staining was performed on tissue sections (4 μm) by incubation with primary antibody against total Syk and phosphor-Syk (Cell Signaling Technology, Beverly, MA), followed by peroxidase conjugated secondary antibodies. All sections were counterstained with hematoxylin. The percentage of stained area was quantified by Image J software (NIH).

TABLE 1 | Clinical characteristics of patients with IgAN at the time of biopsy.

Pt ID	Sex	Age (years)	sCr (μmol/L)	eGFR (ml/min per 1.73 m^2)	UPCR (mg/mmol Cr)	Hypertension (Y/N)	On RAAS blocker (Y/N)	On Corticosteroid (Y/N)
1	M	71	92	70	77	Y	Y	N
2	F	58	81	63	189	Y	Y	N
3	F	28	202	26	113	N	Y	N
4	M	60	82	83	540	N	N	N
5	F	54	73	72	99	N	Y	N

eGFR, estimated glomerular filtration rate; IgAN, IgA nephropathy; Pt, patient; RAAS, Renin-angiotensin-aldosterone system; sCr, serum creatinine; UPCR, spot urine protein-to-creatinine ratio.

Cell Culture

Normal human mesangial cells (HMCs) were obtained from Lonza (Walkersville, MD, United States) and grown in RPMI 1640 medium supplemented with 5% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, United States) at 37°C in 5% CO_2 atmosphere. Human primary renal proximal tubular epithelial cells (PTECs) were obtained from Lonza and cultured in renal epithelial cell growth basal medium (REBM) with growth supplements at 37°C in 5% CO_2 atmosphere. In all experiments, cells grown between passages 3–6 were used and serum starved for 24 h before stimulation.

Preparation of IgA HMC Conditioned Media and Stimulation of PTECs

Conditioned media (IgA HMC medium) were prepared by incubating HMCs (1×10^6 cells in a six-well culture plate) with pooled IgA (50 μg/ml) from IgAN patients or healthy control in RPMI 1640 medium containing 0.5% FBS for 48 h. Cells incubated with RPMI medium alone were used as the medium control. The supernatant of cell culture was collected and stored at −70°C until use. Confluent, growth-arrested PTECs were incubated in RPMI 1640 medium containing 0.5% FBS and 20-fold diluted conditioned medium for 4 h (gene analysis) and 48 h (protein analysis) with or without pre-treatment of Syk inhibitor R406 (2 μM) for 1 h. PTECs were incubated with TNF-α (10 ng/ml) for 48 h.

RNA Extraction and Real-Time PCR Analysis

Total RNAs were isolated from PTECs using Trizol reagent (Invitrogen). One micrograms of total RNAs were reverse transcribed to cDNA and mRNA expression were detected by ABI Real-time PCR System using SYBR Green Master Mix (Applied Biosystems, Carlsbad, CA, United States). Primer sequences were IL-6, forward 5'-ATGAACTCCTTCTCCACAAG-3' and reverse 5'-TGTCAATTCGTTCTGAAGAG-3'; IL-8, forward 5'-GTGCAGTTTTGCCAAGGAGT-3' and reverse 5'-TAATTTCTGTGTTGGCGCAG-3'; ICAM-1, 5'-GGCCTCAGTCAGTGTGA-3' and reverse 5'-AACCCCATTCAGCGTCA-3'; β-actin, forward 5'-TGACGTGGACATCCGCAAAG-3' and reverse 5'-CTGGAAGGTGGACAGCGAGG-3'. Relative gene expression was obtained after normalization with β-actin, and followed by comparison to control group using SDS software (Applied Biosystems).

Cytokine Detection by ELISA

Culture media were collected after 48 h incubation. IL-6, IL-8, and ICAM-1 protein levels were quantified using commercial kit (PeproTech, Rocky Hill, NJ) according to manufacturer's instructions. The detection sensitivity range is 24–1,500 pg/ml for IL-6, 8–1,000 pg/ml for IL-8, and 23–3,000 pg/ml for ICAM-1.

Western Blot Analysis

Cells were lysed with lysis buffer containing protease inhibitor cocktails (Sigma-Aldrich, St. Louis, MO). Equal amounts of protein were resolved in 12% SDS-PAGE gel (Invitrogen) and transferred to PVDF membrane (Millipore, Bedford, MA, United States). After blocking, the membranes were incubated with antibodies against phosphor-NF-κB (p-p65), NF-κB (p65), phosphor-p42/p-p44 MAPK (p-p42/p-p44), and total p42/p44 MAPK (Cell Signaling Technology), and subsequently incubated with peroxidase-conjugated secondary antibody (Dako, Carpinteria, CA, United States). The immunocomplex was visualized with ECL prime chemiluminescence (GE Healthcare, Buckinghamshire, United Kingdom) using the ChemiDoc XRS+ system (Bio-Rad, Hercules, CA, United States). Quantification of protein bands was performed by the ImageJ program (NIH, Bethesda, MD, United States).

Statistical Analysis

All the data were obtained from at least three independent experiments and expressed as mean ± SEM. Differences between multiple groups were evaluated with one-way analysis of variance followed by Bonferroni's comparison using GraphPad Prism, version 4 (GraphPad Software, San Diego, CA, United States). Data were considered statistically significant at $p < 0.05$ ($^{*}p < 0.05$; $^{**}p < 0.01$, and $^{***}p < 0.001$).

RESULTS

Increased Expression of Total and Phosphorylated Syk in Renal Biopsies From Patients With IgAN

To confirm the activation of Syk signaling in IgAN, expression of total and phosphor Syk was evaluated in human renal

biopsies by immunohistochemistry. Syk (total) was detected on renal tubules, but not in glomeruli of normal kidney tissue from patient with renal carcinoma. The expression level of total Syk was markedly increased in renal tubular cells and slightly increased in glomeruli from patients with IgAN compared to normal control. Similarly, the expression level of phosphorylated Syk (phosphor) was significantly upregulated in both renal tubules and glomeruli. Phosphorylated Syk was not detectable in both compartments of normal kidney tissue (**Figure 1**).

Syk Inhibitor Suppressed the Activation of PTECs Cultured With IgA-HMC Medium

Following the immunohistochemical findings of increased Syk expression levels in renal tubules of IgAN, we next determined whether tubulointerstitial inflammation in IgAN was mediated *via* Syk activation. Cultured PTECs were pretreated with or without Syk inhibitor R406 before incubation with IgA-HMC medium. The expression of IL-6, IL-8, and ICAM-1 mRNA were significantly upregulated in PTECs incubated with conditioned medium from patients with IgAN compared with conditioned medium from healthy control and medium control. Pretreatment with R406 significantly suppressed IgA-HMC medium-induced cytokine expressions from PTECs. Similar inhibitory effect of R406 was observed when PTECs were incubated with medium alone (**Figure 2A**). Likewise, IL-6, IL-8, and ICAM-1 synthesis was upregulated when PTECs were incubated with IgA-HMC medium and the production of these mediators were attenuated in the presence of R406 (**Figure 2B**).

Syk Inhibitor Relieved the Activation of NF-κB and MAPK Signaling PTECs Cultured With IgA-HMC Medium

Activation of NF-κB and MAPK signal pathway has been implicated in the pro-inflammatory responses of tubular epithelial cells. We then investigated the effect of Syk inhibition on phosphorylation of p65 NF-κB and p42/p44 MAPK by Western blot analysis. Incubation with IgA-HMC medium from patients with IgAN significantly activated the expression level of phosphorylated p65 (p-p65) and p42/p44 (p-p42/p-p44) in PTECs compared to cells incubated with IgA HMC medium from healthy control. The activation of these signal pathways were markedly suppressed by treatment with Syk inhibitor R406 (**Figure 3**).

Syk Activation Was Involved in TNF-α-Induced Pro-inflammatory Cytokine Production in PTECs

Our previous study shows that TNF-α derived from HMC stimulated with IgA from IgAN patients activates tubular cells (Chan et al., 2005b), and it is a potent mediator for NF-κB and p42/p44 MAPK signal transduction. We tested whether Syk activation was involved in TNF-α-induced pro-inflammatory cytokine production in PTECs. In response to TNF-α, synthesis of IL-6, IL-8, and ICAM-1 in supernatant of PTECs was significantly increased as determined by ELISA. Treatment with

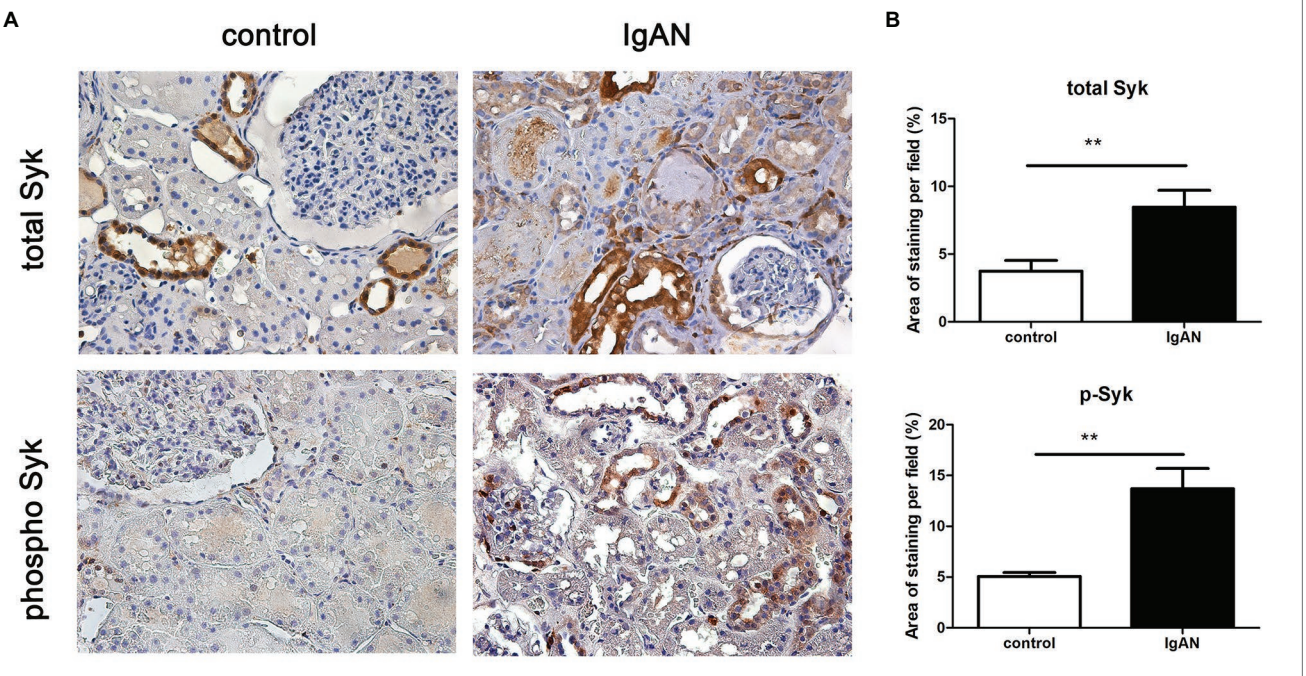

FIGURE 1 | Spleen tyrosine kinase (Syk) expression in human kidney tissues. **(A)** Total and phosphor Syk protein were detected renal biopsies from normal control and patients with IgA nephropathy (IgAN) using immunohistochemical staining. **(B)** Quantitative analysis of different groups. $^{**}p < 0.01$ between groups as indicated. 400X magnification.

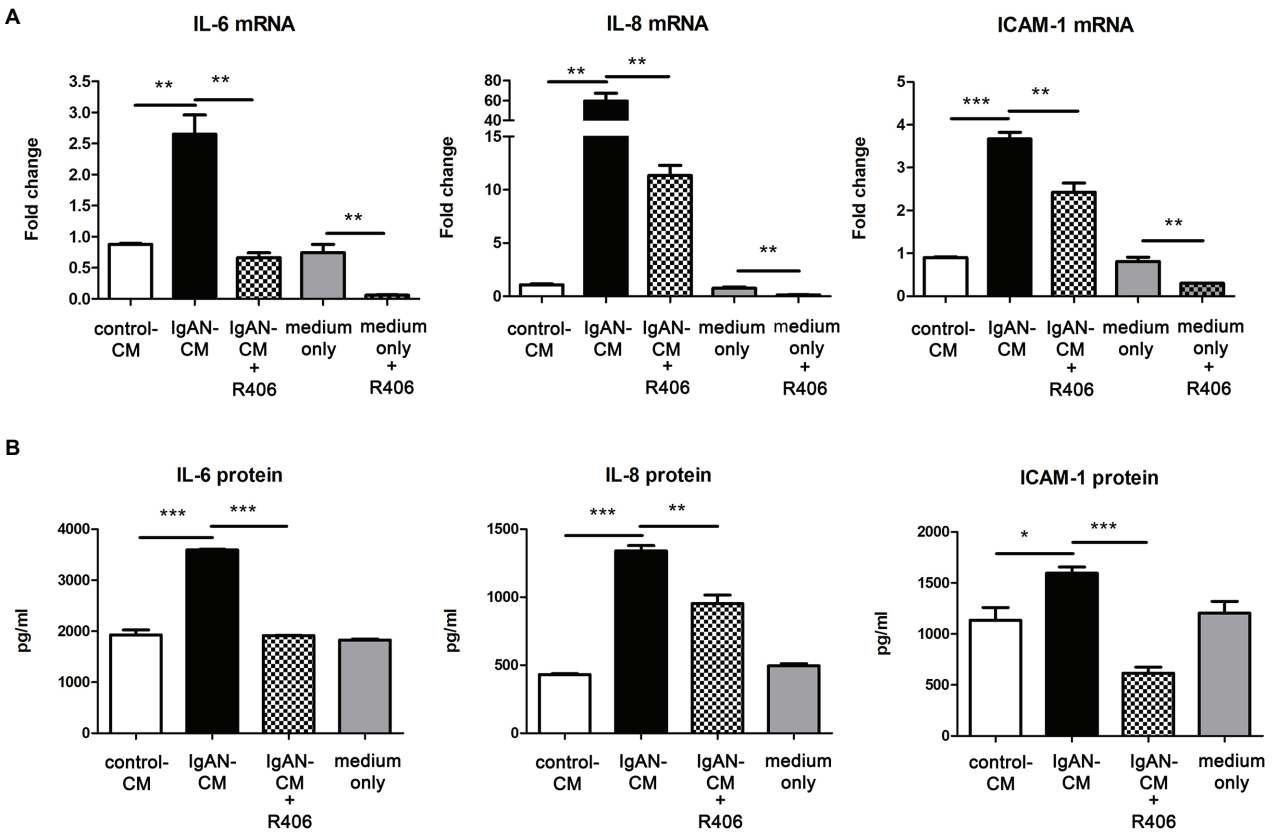

FIGURE 2 | Inhibition of Syk by R406 suppresses inflammatory cytokine expression in proximal tubular epithelial cells (PTECs). **(A)** qPCR and **(B)** ELISA with quantitative analysis on IL-6, IL-8, and ICAM-1 expression in PTECs incubated with conditioned medium from patients with IgAN (IgAN-CM), conditioned medium from healthy control subjects (control-CM) and medium control. *$P < 0.05$; **$P < 0.01$ and ***$P < 0.001$ between groups as indicated.

Syk inhibitor R406 downregulated cytokine synthesis of all three kinds whereas PTECs treated with R406 only had no effect on cytokine synthesis (**Figure 4**).

DISCUSSION

This study demonstrates that the expression level of Syk is significantly higher in kidney biopsies from patients with IgAN compared to that from normal control. Moreover, the expression level of phosphorylated Syk is also markedly increased in renal tubules, indicating kinase activation of Syk in tubular cells from patients with IgAN. Of note, increased Syk expression and phosphorylation is observed in the glomeruli from IgAN, which is consistent to the previous study showing the pathogenic role of Syk in IgAN (Kim et al., 2012). Syk is an immunoreceptor-associated signaling protein, and therefore upregulation of phosphorylated Syk confirms the inflammatory events in the tubulointerstitium or activation of tubular epithelial cells in IgAN. Our findings demonstrate that Syk activation not only occurs in glomerular lesions as reported in the previous study, but it also plays a significant role in tubulointerstitial injury.

To confirm our hypothesis that Syk mediates inflammatory responses of activated tubular cells in IgAN, we conducted a pharmacological inhibition study with Syk inhibitor on cultured PTECs. Based on our previous data that tubulointerstitial damage in IgAN is mediated by glomerulotubular communication, *via* humoral factors, rather than direct binding of IgA to PTECs (Chan et al., 2004), cultured PTECs was incubated with conditioned medium from IgA-stimulated HMCs. Syk inhibition by R406 blocks *in vitro* tubular activation by suppressing NF-κB and p42/p44 MAPK signaling pathway and cytokine production in PTECs. R406, the active metabolite of fostamatinib, is a potent Syk inhibitor for blocking of its kinase activity. Numerous studies have demonstrated that R406 reduces immunoreceptor-mediated leukocyte activation and inflammation in the animal model of antibody-induced arthritis (Braselmann et al., 2006), ANCA-associated glomerulonephritis (Mcadoo et al., 2020) and lupus nephritis (Kitai et al., 2017). Syk is important for downstream signal transduction from cell surface immunoreceptor. Our finding shows that IgA-HMC medium prepared from IgAN patients induces activation of NF-κB and p42/p44 MAPK signaling in PTECs, which can be suppressed by Syk inhibitor. Taken together, our data suggest that Syk is required for

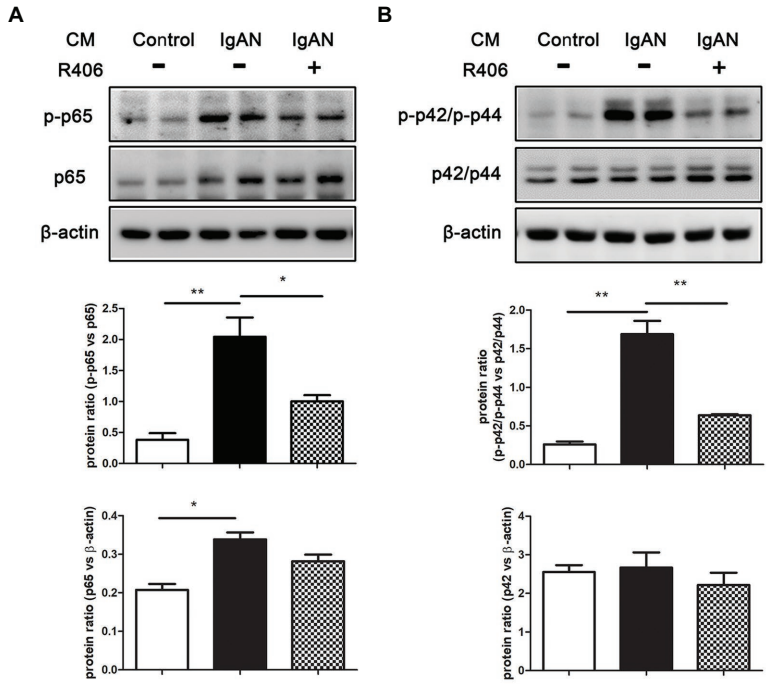

FIGURE 3 | Inhibition of Syk by R406 attenuates activation of NF-κB and MAPK signaling pathway in PTECs. Western blot analysis with quantitative analysis on expression of **(A)** p-p65 and total p65 of NF-κB and **(B)** p-p42/p-p44 and total p42/p44 of MAPK in PTECs incubated with conditioned medium from patients with IgAN (IgAN-CM) with and without R406, and conditioned medium from healthy control subjects (control-CM). β-actin was used as loading control. $^{*}p < 0.05$ and $^{**}p < 0.01$ between groups as indicated.

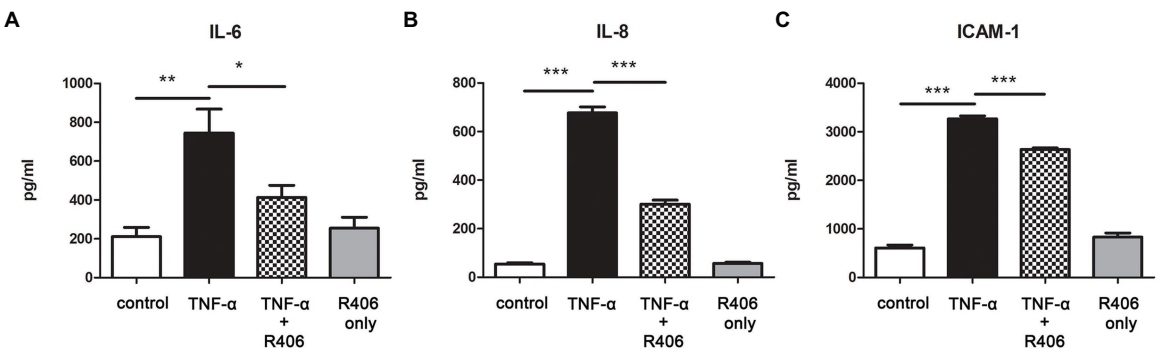

FIGURE 4 | Inhibition of Syk by R406 reduces TNF-α-induced inflammatory cytokine production in PTECs. ELISA with quantitative analysis on protein expression of **(A)** IL-6, **(B)** IL-8 and **(C)** ICAM-1 in culture supernatant from PTECs incubated with control medium, TNF-α and TNF-α pretreated with R406. $^{*}p < 0.05$; $^{**}p < 0.01$ and $^{***}p < 0.001$ between groups as indicated.

triggering activation of downstream NF-κB and p42/p44 MAPK pathway in tubular inflammation in IgAN.

Activation of Syk is crucial for regulating intracellular signal transduction in innate immune cells (Lowell, 2011). For example, in human monocytes, tyrosine kinase inhibitor blocks intergrin-mediated Syk phosphorylation and NF-κB-driven IL-β expression (Lin et al., 1995). Other studies have revealed that ligation of C-type lectin receptors in myeloid cells, *via* Syk activation, is coupled to aggregation of caspase-recruitment domain-containing 9 (CARD9) or CARD-containing MAGUK protein 1 (CARMA1) with mucosa-associated lymphoid tissue 1 (Malt1) and B-cell lymphoma 10 (Bcl10), which in turn leads to the activation of downstream NF-κB pathway (Klemm et al., 2006; Hara et al., 2007; Hara and Saito, 2009). Tubular NF-κB expression is correlated with the degree of macrophage infiltration, tubulointerstitial fibrosis and renal survival in renal tissue from patients with primary IgAN (Silva et al., 2011). Thus, activation of tubular epithelial

cells is closely associated with renal deterioration. Previous data have found that upregulation of p42/p44 MAPK signaling is detected in activated PTECs *via* glomerulotubular communication. Release of Angiotensin II (Ang II) from IgA-stimulated HMCs binds to Ang II type 1 receptor (AT1R) on PTECs, triggering the phosphorylation of p-42/p44 MAPK and subsequent inflammatory responses (Chan et al., 2005a). Our finding indicates that Syk activation plays a role in p42/p44 MAPK signaling transduction in tubular inflammation in IgAN. However, it is unclear how Syk activation mediates the activation of p42/p44 MAPK signaling pathway. Phosphoinositide 3-kinases (PI3K) have been shown to be a direct binding partner of activated Syk. The Syk–PI3K interaction triggers Akt and PKC signaling pathway, which mediates the activation of p42/p44 MAPK signal transduction (Mocsai et al., 2010). Thus, it is possible that Syk activation mediates downstream p42/p44 MAPK signaling *via* PI3K-Akt-PKC pathway.

In hematopoietic cells, the engagement of immunoreceptor with ITAM triggers Syk activation and the subsequent downstream signal pathways (Turner et al., 2000). In mesangial cells, several potential IgA receptors have been identified for IgA-mediated immune responses. For example, blockade of galactosyltransferase 1 inhibits IgA-induced phosphorylation of Syk and synthesis of IL-6 in HMCs, suggesting IgA binds to galactosyltransferase 1 and leads to Syk activation (Molyneux et al., 2017). However, IgA1 deposits are rarely detected in tubulointerstitium in IgAN and there is no binding of IgA to tubular epithelial cells (Frasca et al., 1982), it remains unclear how Syk is activated in PTECS. Our data show that inhibition of Syk suppresses TNF-α-induced cytokine production in PTECs, suggesting that TNF-α may activate Syk, which in turn induce NF-κB signal transduction. Our previous study demonstrates that TNF-α is a mesangial-derived cytokine that has been implicated in the glomerulotubular crosstalk (Chan et al., 2005b). Indeed, immunoprecipitation study have shown that TNF-α induces the binding of Syk to two TNF-α receptors, TNFR1 and TNFR2, in Jurkat cells and recruitment of Syk modulates TNF-α-induced activation of NF-κB (Takada and Aggarwal, 2004). Taken together, Syk in tubular epithelial cells may be activated by mesangial-derived TNF-α, leading to activation of NF-κB and p42/p44 MAPK signaling pathway.

The importance of Syk activation in the innate immunity is well recognized for decades. Several Syk inhibitors including fostamatinib (R788), entospletinib (GS-9973), cerdulatinib (PRT062070) and TAK-659 are being evaluated under clinical trials (Liu and Mamorska-Dyga, 2017). Accumulating evidence shows that Syk is a crucial player in the pathogenesis of immune-mediated glomerulonephritis such as IgAN, anti-GBM glomerulonephritis and lupus nephritis (Ma et al., 2017). Syk targeting therapy in mouse model of UUO demonstrates a beneficial effect on renal fibrosis (Chen et al., 2016). Treatment with fostamatinib reduces proteinuria and renal inflammation in rats with anti-GBM glomerulonephritis (Chen et al., 2016). Although, we and others have demonstrated the pathogenic role of Syk in mediating inflammatory events in both mesangial cells and tubular epithelial cells, the renoprotective effect of Syk inhibitor has not been investigated in animal model of IgAN due to the lack of a murine line that consistently developing IgAN and the difference in IgA1 between human and rodents. Recent advance in the development of grouped ddY mouse model of IgAN may be a useful tool for future investigation (Suzuki et al., 2014). Furthermore, a phase II randomized controlled trial has been conducted to evaluate the efficacy and safety of fostamatinib in treatment of patients with IgAN (Ma et al., 2016).

In this study, we have demonstrated that Syk is activated in renal tubular cells from patients with IgAN. Inhibition of Syk can suppress inflammatory responses in activated tubular epithelial cells evoked by glomerulotubular crosstalk, *via* downregulation of NF-κB and TNF-α signal transduction. Our findings are in line with previous data on the beneficial effect of Syk inhibition in renal diseases and provide further evidence on the important role of Syk in tubulointerstitial injury and Syk inhibition is a potential therapeutics for IgAN in the future.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Research Ethics Committee/Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

WY designed and performed the experiment, and wrote the manuscript. KC acquired the clinical specimens and data. LC and JL analyzed and interpreted the results. KL reviewed and edited the manuscript. ST conceived and supervised the study. All authors contributed to the article and approved the submitted version.

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REFERENCES

Barbour, S. J., Coppo, R., Zhang, H., Liu, Z. H., Suzuki, Y., Matsuzaki, K., et al. (2019). Evaluating a new international risk-prediction tool in IgA nephropathy. *JAMA Intern. Med.* 179, 942–952. doi: 10.1001/jamainternmed.2019.0600

Barratt, J., Greer, M. R., Pawluczyk, I. Z., Allen, A. C., Bailey, E. M., Buck, K. S., et al. (2000). Identification of a novel Fcalpha receptor expressed by human mesangial cells. *Kidney Int.* 57, 1936–1948. doi: 10.1046/j.1523-1755.2000.00043.x

Barratt, J., and Tang, S. C. W. (2018). Treatment of IgA nephropathy: evolution over half a century. *Semin. Nephrol.* 38, 531–540. doi: 10.1016/j.semnephrol.2018.05.023

Bartaula-Brevik, S., Lindstad Brattas, M. K., Tvedt, T. H. A., Reikvam, H., and Bruserud, O. (2018). Splenic tyrosine kinase (SYK) inhibitors and their possible use in acute myeloid leukemia. *Expert Opin. Investig. Drugs* 27, 377–387. doi: 10.1080/13543784.2018.1459562

Braselmann, S., Taylor, V., Zhao, H., Wang, S., Sylvain, C., Baluom, M., et al. (2006). R406, an orally available spleen tyrosine kinase inhibitor blocks fc receptor signaling and reduces immune complex-mediated inflammation. *J. Pharmacol. Exp. Ther.* 319, 998–1008. doi: 10.1124/jpet.106.109058

Chan, L. Y., Leung, J. C., and Lai, K. N. (2004). Novel mechanisms of tubulointerstitial injury in IgA nephropathy: a new therapeutic paradigm in the prevention of progressive renal failure. *Clin. Exp. Nephrol.* 8, 297–303. doi: 10.1007/s10157-004-0324-9

Chan, L. Y., Leung, J. C., Tang, S. C., Choy, C. B., and Lai, K. N. (2005a). Tubular expression of angiotensin II receptors and their regulation in IgA nephropathy. *J. Am. Soc. Nephrol.* 16, 2306–2317. doi: 10.1681/ASN.2004121117

Chan, L. Y., Leung, J. C., Tsang, A. W., Tang, S. C., and Lai, K. N. (2005b). Activation of tubular epithelial cells by mesangial-derived TNF-alpha: glomerulotubular communication in IgA nephropathy. *Kidney Int.* 67, 602–612. doi: 10.1111/j.1523-1755.2005.67116.x

Chen, K. H., Hsu, H. H., Yang, H. Y., Tian, Y. C., Ko, Y. C., Yang, C. W., et al. (2016). Inhibition of spleen tyrosine kinase (syk) suppresses renal fibrosis through anti-inflammatory effects and down regulation of the MAPK-p38 pathway. *Int. J. Biochem. Cell Biol.* 74, 135–144. doi: 10.1016/j.biocel.2016.03.001

Frasca, G. M., Vangelista, A., Biagini, G., and Bonomini, V. (1982). Immunological tubulo-interstitial deposits in IgA nephropathy. *Kidney Int.* 22, 184–191. doi: 10.1038/ki.1982.151

Ghosh, D., and Tsokos, G. C. (2010). Spleen tyrosine kinase: an Src family of non-receptor kinase has multiple functions and represents a valuable therapeutic target in the treatment of autoimmune and inflammatory diseases. *Autoimmunity* 43, 48–55. doi: 10.3109/08916930903374717

Hara, H., Ishihara, C., Takeuchi, A., Imanishi, T., Xue, L., Morris, S. W., et al. (2007). The adaptor protein CARD9 is essential for the activation of myeloid cells through ITAM-associated and toll-like receptors. *Nat. Immunol.* 8, 619–629. doi: 10.1038/ni1466

Hara, H., and Saito, T. (2009). CARD9 versus CARMA1 in innate and adaptive immunity. *Trends Immunol.* 30, 234–242. doi: 10.1016/j.it.2009.03.002

Kaur, M., Singh, M., and Silakari, O. (2013). Inhibitors of switch kinase 'spleen tyrosine kinase' in inflammation and immune-mediated disorders: a review. *Eur. J. Med. Chem.* 67, 434–446. doi: 10.1016/j.ejmech.2013.04.070

Kim, M. J., McDaid, J. P., McAdoo, S. P., Barratt, J., Molyneux, K., Masuda, E. S., et al. (2012). Spleen tyrosine kinase is important in the production of proinflammatory cytokines and cell proliferation in human mesangial cells following stimulation with IgA1 isolated from IgA nephropathy patients. *J. Immunol.* 189, 3751–3758. doi: 10.4049/jimmunol.1102603

Kitai, M., Fukuda, N., Ueno, T., Endo, M., Maruyama, T., Abe, M., et al. (2017). Effects of a spleen tyrosine kinase inhibitor on progression of the lupus nephritis in mice. *J. Pharmacol. Sci.* 134, 29–36. doi: 10.1016/j.jphs.2017.02.015

Klemm, S., Gutermuth, J., Hultner, L., Sparwasser, T., Behrendt, H., Peschel, C., et al. (2006). The Bcl10-Malt1 complex segregates Fc epsilon RI-mediated nuclear factor kappa B activation and cytokine production from mast cell degranulation. *J. Exp. Med.* 203, 337–347. doi: 10.1084/jem.20051982

Lai, K. N., Tang, S. C., Schena, F. P., Novak, J., Tomino, Y., Fogo, A. B., et al. (2016). IgA nephropathy. *Nat. Rev. Dis. Primers.* 2:16001. doi: 10.1038/nrdp.2016.1

Leung, J. C. K., Lai, K. N., and Tang, S. C. W. (2018). Role of Mesangial-Podocytic-tubular cross-talk in IgA nephropathy. *Semin. Nephrol.* 38, 485–495. doi: 10.1016/j.semnephrol.2018.05.018

Leung, J. C., Tang, S. C., Lam, M. F., Chan, T. M., and Lai, K. N. (2001). Charge-dependent binding of polymeric IgA1 to human mesangial cells in IgA nephropathy. *Kidney Int.* 59, 277–285. doi: 10.1046/j.1523-1755.2001.00489.x

Leung, J. C., Tsang, A. W., Chan, D. T., and Lai, K. N. (2000). Absence of CD89, polymeric immunoglobulin receptor, and asialoglycoprotein receptor on human mesangial cells. *J. Am. Soc. Nephrol.* 11, 241–249.

Lin, T. H., Rosales, C., Mondal, K., Bolen, J. B., Haskill, S., and Juliano, R. L. (1995). Integrin-mediated tyrosine phosphorylation and cytokine message induction in monocytic cells. A possible signaling role for the Syk tyrosine kinase. *J. Biol. Chem.* 270, 16189–16197. doi: 10.1074/jbc.270.27.16189

Liu, D., and Mamorska-Dyga, A. (2017). Syk inhibitors in clinical development for hematological malignancies. *J. Hematol. Oncol.* 10:145. doi: 10.1186/s13045-017-0512-1

Liu, L. J., Yang, Y. Z., Shi, S. F., Bao, Y. F., Yang, C., Zhu, S. N., et al. (2019). Effects of hydroxychloroquine on proteinuria in IgA nephropathy: a randomized controlled trial. *Am. J. Kidney Dis.* 74, 15–22. doi: 10.1053/j.ajkd.2019.01.026

Lowell, C. A. (2011). Src-family and Syk kinases in activating and inhibitory pathways in innate immune cells: signaling cross talk. *Cold Spring Harb. Perspect. Biol.* 3:a002352. doi: 10.1101/cshperspect.a002352

Lucas, M. C., and Tan, S. L. (2014). Small-molecule inhibitors of spleen tyrosine kinase as therapeutic agents for immune disorders: will promise meet expectations? *Future Med. Chem.* 6, 1811–1827. doi: 10.4155/fmc.14.126

Lv, J., Zhang, H., Wong, M. G., Jardine, M. J., Hladunewich, M., Jha, V., et al. (2017). Effect of Oral methylprednisolone on clinical outcomes in patients with IgA nephropathy: the TESTING randomized clinical trial. *JAMA* 318, 432–442. doi: 10.1001/jama.2017.9362

Ma, T. K., McAdoo, S. P., and Tam, F. W. (2016). Spleen tyrosine kinase: a crucial player and potential therapeutic target in renal disease. *Nephron* 133, 261–269. doi: 10.1159/000446879

Ma, T. K., McAdoo, S. P., and Tam, F. W. (2017). Targeting the tyrosine kinase signaling pathways for treatment of immune-mediated glomerulonephritis: from bench to bedside and beyond. *Nephrol. Dial. Transplant.* 32, i129–i138. doi: 10.1093/ndt/gfw336

McAdoo, S. P., Prendecki, M., Tanna, A., Bhatt, T., Bhangal, G., McDaid, J., et al. (2020). Spleen tyrosine kinase inhibition is an effective treatment for established vasculitis in a pre-clinical model. *Kidney Int.* 97, 1196–1207. doi: 10.1016/j.kint.2019.12.014

Mocsai, A., Ruland, J., and Tybulewicz, V. L. (2010). The SYK tyrosine kinase: a crucial player in diverse biological functions. *Nat. Rev. Immunol.* 10, 387–402. doi: 10.1038/nri2765

Molyneux, K., Wimbury, D., Pawluczyk, I., Muto, M., Bhachu, J., Mertens, P. R., et al. (2017). beta1,4-galactosyltransferase 1 is a novel receptor for IgA in human mesangial cells. *Kidney Int.* 92, 1458–1468. doi: 10.1016/j.kint.2017.05.002

Moura, I. C., Centelles, M. N., Arcos-Fajardo, M., Malheiros, D. M., Collawn, J. F., Cooper, M. D., et al. (2001). Identification of the transferrin receptor as a novel immunoglobulin (Ig)A1 receptor and its enhanced expression on mesangial cells in IgA nephropathy. *J. Exp. Med.* 194, 417–425. doi: 10.1084/jem.194.4.417

Novak, J., Barratt, J., Julian, B. A., and Renfrow, M. B. (2018). Aberrant glycosylation of the IgA1 molecule in IgA nephropathy. *Semin. Nephrol.* 38, 461–476. doi: 10.1016/j.semnephrol.2018.05.016

Silva, G. E., Costa, R. S., Ravinal, R. C., Ramalho, L. Z., Dos Reis, M. A., Coimbra, T. M., et al. (2011). NF-kB expression in IgA nephropathy outcome. *Dis. Markers* 31, 9–15. doi: 10.1155/2011/940827

Smith, J., McDaid, J. P., Bhangal, G., Chawanasuntorapoj, R., Masuda, E. S., Cook, H. T., et al. (2010). A spleen tyrosine kinase inhibitor reduces the severity of established glomerulonephritis. *J. Am. Soc. Nephrol.* 21, 231–236. doi: 10.1681/ASN.2009030263

Suzuki, H., Suzuki, Y., Novak, J., and Tomino, Y. (2014). Development of animal models of human IgA nephropathy. *Drug Discov. Today Dis. Model.* 11, 5–11. doi: 10.1016/j.ddmod.2014.07.002

Szilveszter, K. P., Nemeth, T., and Mocsai, A. (2019). Tyrosine kinases in autoimmune and inflammatory skin diseases. *Front. Immunol.* 10:1862. doi: 10.3389/fimmu.2019.01862

Takada, Y., and Aggarwal, B. B. (2004). TNF activates Syk protein tyrosine kinase leading to TNF-induced MAPK activation, NF-kappaB activation, and apoptosis. *J. Immunol.* 173, 1066–1077. doi: 10.4049/jimmunol.173.2.1066

Tang, S. C. W. (2018). An overview of IgA nephropathy: 50 years on. *Semin. Nephrol.* 38, 433–434. doi: 10.1016/j.semnephrol.2018.05.024

Tang, S. C., Tang, A. W., Wong, S. S., Leung, J. C., Ho, Y. W., and Lai, K. N. (2010). Long-term study of mycophenolate mofetil treatment in IgA nephropathy. *Kidney Int.* 77, 543–549. doi: 10.1038/ki.2009.499

Tsuge, T., Suzuki, Y., Shimokawa, T., Horikoshi, S., Okumura, K., Ra, C., et al. (2003). Monocyte chemoattractant protein (MCP)-1 production via functionally reconstituted Fcalpha receptor (CD89) on glomerular mesangial cells. *Inflamm. Res.* 52, 428–432. doi: 10.1007/s00011-003-1200-x

Turner, M., Schweighoffer, E., Colucci, F., Di Santo, J. P., and Tybulewicz, V. L. (2000). Tyrosine kinase SYK: essential functions for immunoreceptor signaling. *Immunol. Today* 21, 148–154. doi: 10.1016/S0167-5699(99)01574-1

Wyatt, R. J., and Julian, B. A. (2013). IgA nephropathy. *N. Engl. J. Med.* 368, 2402–2414. doi: 10.1056/NEJMra1206793

Yanagi, S., Inatome, R., Takano, T., and Yamamura, H. (2001). Syk expression and novel function in a wide variety of tissues. *Biochem. Biophys. Res. Commun.* 288, 495–498. doi: 10.1006/bbrc.2001.5788

Zhang, Y. M., Zhou, X. J., and Zhang, H. (2017). What genetics tells us about the pathogenesis of IgA nephropathy: the role of immune factors and infection. *Kidney Int. Rep.* 2, 318–331. doi: 10.1016/j.ekir.2017.02.005

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A Metabolic Reprogramming of Glycolysis and Glutamine Metabolism Is a Requisite for Renal Fibrogenesis—Why and How?

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Chronic Kidney Disease (CKD) is characterized by organ remodeling and fibrosis due to failed wound repair after on-going or severe injury. Key to this process is the continued activation and presence of matrix-producing renal fibroblasts. In cancer, metabolic alterations help cells to acquire and maintain a malignant phenotype. More recent evidence suggests that something similar occurs in the fibroblast during activation. To support these functions, pro-fibrotic signals released in response to injury induce metabolic reprograming to meet the high bioenergetic and biosynthetic demands of the (myo)fibroblastic phenotype. Fibrogenic signals such as TGF-β1 trigger a rewiring of cellular metabolism with a shift toward glycolysis, uncoupling from mitochondrial oxidative phosphorylation, and enhanced glutamine metabolism. These adaptations may also have more widespread implications with redirection of acetyl-CoA directly linking changes in cellular metabolism and regulatory protein acetylation. Evidence also suggests that injury primes cells to these metabolic responses. In this review we discuss the key metabolic events that have led to a reappraisal of the regulation of fibroblast differentiation and function in CKD.

Keywords: fibroblast, fibrosis, glycolysis, glutaminolysis, metabolic, metabolism, priming, TGF-β1

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INTRODUCTION

While the kidney can recover from acute injury, persistent and/or severe injury results in the chronic accumulation of scar tissue (fibrosis) and progressive renal failure. Understanding the mechanisms that regulate the transition from acute kidney injury to chronic kidney disease (CKD) is important, because once fibrosis is initiated it can be extremely difficult to switch off or reverse (Hewitson, 2009; Hewitson et al., 2017a). A defining characteristic of this transition is a maladaptive repair and a persistent activation of fibroblast-like cells (Darby and Hewitson, 2007). These cells are the major source of the excess extracellular matrix (ECM) proteins in a process that is inherently similar in different organ pathologies.

WHAT IS A FIBROBLAST?

Single cell sequencing has highlighted the considerable heterogeneity of the fibroblast population in the kidney (Wu et al., 2019). One of the most vexed questions in fibrosis research is therefore—how

do we define a fibroblast? Potentially fibroblasts include any ECM-producing cell in the connective tissue stroma. Consistent with their diversity, multiple cellular origins for the fibroblast have been suggested including epithelial- and endothelial-to-mesenchymal transition, infiltration of bone marrow precursors and macrophages (Duffield et al., 2013), along with a resident quiescent mesenchymal stem cell precursor, often simply referred to as a pericyte (Chou et al., 2020).

ROLE OF THE MYOFIBROBLAST

It has repeatedly been shown that a population of activated fibroblasts can be identified by *de novo* expression of α-smooth muscle actin (αSMA), a protein normally associated with smooth muscle cells (Darby and Hewitson, 2007). These cells display properties of both fibroblasts and smooth muscle cells including prodigious synthesis of ECM and contractile functions through the formation of actin-myosin fibers (Tomasek et al., 2002). Interstitial myofibroblasts are a feature of both primary and secondary tubulointerstitial pathologies in the kidney.

The myofibroblast was first described in skin wound healing where its transient presence in granulation tissue is responsible for repair and contraction of the wound area to facilitate healing. Based on this, it is often assumed that they have a similar acute repair role in the kidney in response to injury. Accordingly, some have postulated that these cells are initially recruited to synthesize ECM in order to stabilize injured tubules (Kaissling et al., 2013). Regardless, their ongoing presence in the kidney, as elsewhere, results in scar tissue formation and contracture.

ACTIVATION OF FIBROBLASTS

The initial differentiation of fibroblasts to so-called myofibroblasts is a process driven by cytokines/growth factors released by injured parenchyma and infiltrating inflammatory cells (Darby and Hewitson, 2007). While a number of pro-fibrotic influences exist, transforming growth factor-β1 (TGF-β1) has consistently been shown to be the pre-eminent fibrogenic signal (Meng et al., 2016).

Fibroblasts Can Act Autonomously

During fibrosis, fibroblasts begin to act autonomously and independently of tubular and leukocyte inflammation (Leaf and Duffield, 2017). Indeed, recently Buhl et al. have elegantly shown that activation of fibroblasts *per se* is sufficient to drive progressive fibrosis and systemic features of CKD (Buhl et al., 2020), independent of any surrounding tubular and interstitial pathologies.

This process seems particularly pertinent to TGF-β1, which is secreted as a latent protein bound to adjacent ECM and with activation on release from the latency complex. Thus, TGF-β1 synthesized by tubules can't simply diffuse across basement membranes to activate interstitial fibroblasts (Venkatachalam and Weinberg, 2015). This key difference between TGF-β1 and many other growth factors and cytokines, highlights an important autocrine regulatory mechanism. Myofibroblasts isolated from hypertrophic scars exhibit a stably differentiated and contractile phenotype *via* a TGF-β1-dependent autocrine loop involving focal adhesion proteins (Dabiri et al., 2008). Here TGF-β1 is activated by myofibroblasts "pulling" on the TGF-β1 complex to release the active form. This dependence on generating tissue tension demonstrates that feed-forward loops can drive myofibroblast differentiation (Dabiri et al., 2008). TGF-β1 can also operate as a feed forward loop to amplify the effects of other novel pro-fibrotic growth factors such as FGF23 (Smith et al., 2017a,b).

METABOLIC REGULATION OF FIBROBLAST FUNCTION

While a major focus of fibrosis research has been on the transcriptional regulation of collagen synthesis, we now also appreciate that TGF-β1 is a trigger for a metabolic reprogramming that is needed for fibroblast synthesis and contraction.

Physiological cellular metabolism consists of specific metabolic reactions involving conversion of a carbon source into the building blocks needed for macromolecule biosynthesis, energetics and cellular homeostasis (see Altman et al., 2016; O'Neill et al., 2016). Alterations in cellular metabolism, including the so-called "Warburg effect," are thought to play an essential role in the acquisition and maintenance of a malignant phenotype in tumor cells (Chen et al., 2018). Fibroblasts in a number of organs undergo similar dramatic metabolic changes during activation that are necessary to meet the increased bioenergetic and biosynthetic demands of mitogenesis and ECM synthesis (fibrogenesis) [reviewed in (Xie et al., 2015; Para et al., 2019; Zhao et al., 2020)] (**Table 1**). Because TGF-β1 has been identified as the specific orchestrator in many cases (Negmadjanov et al., 2015; Ding et al., 2017; Si et al., 2019; Barcena-Varela et al., 2020; Bates et al., 2020; Henderson et al., 2020; Smith and Hewitson, 2020), the molecular events underlying these global adaptations are of particular mechanistic interest.

Rewiring Glycolysis

Principal amongst the metabolic changes induced by TGF-β1 is an increase in glycolytic flux, despite the availability of oxygen (aerobic glycolysis), and uncoupling from oxidative phosphorylation in the mitochondria (**Figure 1A**) (Ding et al., 2017; Smith et al., 2019; Smith and Hewitson, 2020). This metabolic shift is also supported clinically by a metabolomic sub-analysis of the Chronic Renal Insufficiency Cohort (CRIC) study showing that a decrease in the tricarboxylic acid (TCA) cycle intermediates, citrate and aconitate, correlate with declining eGFR in diabetic kidney disease (DKD) (Kwan et al., 2020).

There are several reasons why this redirection of carbon may be functionally significant in fibrosis. Although glycolysis is less efficient at producing adenosine triphosphate (ATP) than oxidative phosphorylation coupled with the electron transport

Abbreviations: Gls, glutaminase; OxPhos, oxidative phosphorylation; IPF: Idiopathic pulmonary fibrosis; αSMA, α-smooth muscle actin; TGF-β1, transforming growth factor-β1.

TABLE 1 | Representative *in vitro* examples of metabolic adaptations in fibroblast-like cells.

Organ	Cell	Metabolic adaptations	References
Skin	Normal human skin and keloid fibroblasts	↑glycolysis in keloid fibroblasts vs. normal fibroblasts	Li et al., 2018
Liver	Human hepatic stellate cell line	TGF-β1 ↑glycolysis ↓OxPhos	Bates et al., 2020
Liver	Hepatic stellate cell line (LS2)	TGF-β1 ↑glycolysis ↓OxPhos	Barcena-Varela et al., 2020
Lung	Normal human and IPF lung Fibroblasts	↑glycolysis in IPF	Xie et al., 2015
Peritoneum	Human mesothelial cell line (MCT-5A)	TGF-β1 ↑glycolysis ↓OxPhos	Si et al., 2019
Kidney	Rat interstitial fibroblast	TGF-β1 ↑glycolysis ↓OxPhos ↑Glutaminolysis	Smith and Hewitson, 2020
Lung	Normal human and IPF lung fibroblasts	↑Gls mRNA in IPF vs. normal	Choudhury et al., 2020
Lung	Human Lung fibroblasts	Absence of glutamine ↓TGF-β1 stimulated collagen and αSMA	Hamanaka et al., 2019
Lung	Human Lung fibroblasts	TGF-β1 ↑Gls and glutamine consumption	Ge et al., 2018

chain, glycolysis produces ATP faster (Para et al., 2019). Additionally, this means that carbon can be redirected to other biosynthetic pathways (e.g., pentose phosphate pathway and nucleotide synthesis etc.), and critically with respect to collagen production, enhances generation of non-essential amino acids, such as glycine, which constitutes 35% of all amino acids in collagen (Nigdelioglu et al., 2016). The ultimate conversion of pyruvate to lactate, and its export, results in acid-induced TGF-β1 activation *in vitro* (Kottmann et al., 2012, 2015). Indeed, genetic or pharmacologic approaches that block glycolysis decrease contraction and reduce TGF-β1-induced αSMA and collagen expression in the fibroblastioc IMR-90 cell line (Bernard et al., 2015). In the mouse, blockade of glycolytic flux with shikonin, an inhibitor of pyruvate kinase M2, ameliorates fibrosis after unilateral ureteric obstruction (Wei et al., 2019).

Metabolic Switches

A more in depth analysis of the metabolic reprogramming in renal fibroblasts suggests that inactivation of the pyruvate dehydrogenase complex (PDC) is an important metabolic switch in maintaining a chronically activated fibrogenic state (Smith and Hewitson, 2020). The PDC catalyzes a series of rate-limiting reactions involved in the oxidative decarboxylation of pyruvate to acetyl-CoA (**Figure 1**). Analysis of the canonical pathways differentially regulated by TGF-β1 identified inhibition of acetyl-CoA biosynthesis *via* inactivation of PDC as a potential metabolic regulator of fibroblast activation in cells derived from fibrotic kidneys (Smith and Hewitson, 2020). Accordingly, TGF-β1 induces a profound reduction in cellular acetyl-CoA stores. Inhibition of PDC through increased activity of pyruvate dehydrogenase kinases (PDK) also leads to a fibrogenic Warburg-like phenotype in cardiac fibroblasts (Tian et al., 2020). Other direct targets of TGF-β1 include the glycolytic enzymes phosphofructokinase (PFK) (Calvier et al., 2017) and hexokinase (Yin et al., 2019) which catalyze key regulatory steps in the conversion of glucose to pyruvate.

Metabolic Regulation of Protein Acetylation

Augmented glycolysis also has more widespread implications (Ghosh-Choudhary et al., 2020). Acetyl-CoA is not only a substrate for the TCA cycle and other biosynthetic pathways (e.g., fatty acid synthesis), but it is also the obligatory acetyl donor for regulatory protein lysine acetylation (Sivanand et al., 2018), thus providing a potential direct link between changes in cell metabolism and protein function (Kori et al., 2017; Weinert et al., 2018).

Acetylation of amino acid residues has regulatory functions at various steps between transcription and protein degradation (Spange et al., 2009). These include changes in both the size and electrostatic charge of amino acid side chains, altered enzyme activity through changing binding preference, competitive interaction with other modifications and finally, the creation of new protein docking sites (Spange et al., 2009).

Under physiological conditions, glucose accounts for up to 90% of the acetyl-CoA pool (Kamphorst et al., 2014), with the majority being generated in the mitochondria through the decarboxylation of pyruvate *via* PDC. Mitochondrial acetyl-CoA is exported as citrate, and converted back into acetyl-CoA, and used for acetylation within both the cytoplasm and nucleus. Nuclear acetyl-CoA may also be supplemented *in situ* by nuclear PDC (Sutendra et al., 2014).

To date, most interest in acetylation has focused on the role of nuclear histone acetylation in epigenetic regulation of transcription. However, the significance of protein acetylation extends well beyond histones (Choudhary et al., 2009, 2014) with proteomic analysis revealing that in excess of 2,000 proteins can be acetylated in the kidney (Lundby et al., 2012). Consistent with a reduction in global acetyl-CoA levels, it has been shown that TGF-β1 produces a corresponding reduction in lysine acetylation of many, as yet unidentified, proteins in rat kidney fibroblasts (Smith and Hewitson, 2020), thus extending our earlier finding that TGF-β1 regulates histone H3 acetylation (Hewitson et al., 2017b; Smith et al., 2019). These effects were ameliorated when PDC was maintained in an activated state with dichloroacetate, a reversible inhibitor of inactivating PDKs (Smith and Hewitson, 2020).

Kinetic studies of acetylation show that it is rapidly reversible (Kori et al., 2017; Weinert et al., 2018) suggesting that it can act as a modification-based switch, analogous to that seen with phosphorylation. A noteworthy example of this exists in the regulation of fibrosis. Canonical TGF-β1

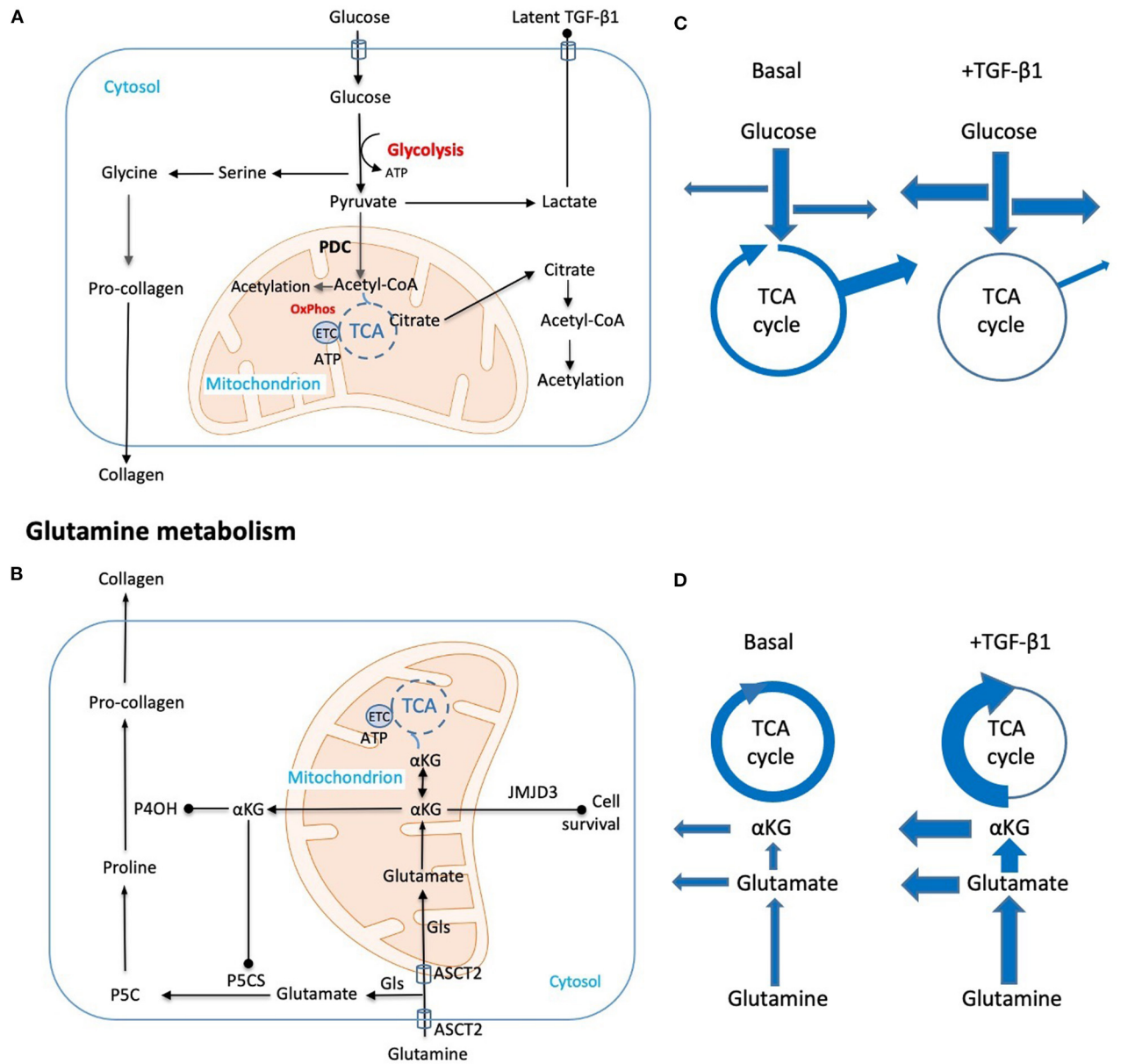

FIGURE 1 | Schematic representation of key metabolic adaptations in fibroblasts to support collagen synthesis. Metabolic and biosynthetic fates of **(A)** glucose and **(B)** glutamine highlight potential synthetic (arrow head) and regulatory (solid dot) functions in fibrogenesis. **(C,D)** Putative changes in metabolic flux caused by TGF-β1 are diagrammatically indicated by changes in arrow thickness. These include **(C)** a shift from oxidative phosphorylation to glycolysis with commensurate increases in amino acid and nucleotide synthesis and a reduction in Acetyl-CoA generation. Export of the end product lactate may also be pro-fibrotic as local changes to pH contribute to activation of latent TGF-β. **(D)** Parallel hypothesized TGF-β1 induced changes in glutamine metabolism both supplement amino acid synthesis and TCA intermediates lost through metabolic shifts in glucose metabolism. Key: Acetyl-CoA, Acetyl coenzyme-A; ASCT2, alanine-serine-cysteine transporter 2; αKG, α-ketoglutarate; ATP, adenosine triphosphate; ETC, electron transport chain; Gls, glutaminase; JMJD3, Jumonji domain-containing protein D3; OxPhos, oxidative phosphorylation; P4OH, prolyl-4-hydroxylase; P5CS, pyrroline-5-carboxylate synthase; P5C, pyrroline-5-carboxylate; PDC, pyruvate dehydrogenase complex; TCA, tricarboxylic acid cycle; TGF-β1, transforming growth factor-β1.

signaling is *via* the transcription factors Smad 2, 3, and 4, in turn counter balanced by actions of the inhibitory Smad 7 (Meng et al., 2016). Acetylation protects Smad 7 from degradation by ubiquitination. Conversely, deacetylation increases ubiquitination and degradation, thus removing a well-described anti-fibrotic brake. In this context, findings that

TGF-β1 is responsible for the deacetylation of Smad 7 (Fukasawa et al., 2004) establish a functional significance.

Glutamine Metabolism

Enhanced glycolytic flux alone cannot meet the high metabolic demands of fibroblasts and increased carbon supply through alternate pathways is needed to support biosynthetic requirements. The maintenance of high levels of glutamine in the blood through diet and synthesis by muscle and other organs provides a ready source of carbon and nitrogen to support cell growth. Physiologically glutamine in the kidney is metabolized most in tubules. As in tumor cells, along with an increase in glycolysis, glutamine metabolism is also increased *in vitro* by TGF-β1 in lung (Bernard et al., 2018; Ge et al., 2018; Hamanaka et al., 2019) and dermal (Henderson et al., 2020) fibroblast-like cells. Clinically, epidemiological analyses have shown that a decrease in urine glutamine is predictive of progression from moderate to severe albuminuria in DKD (Pena et al., 2014), consistent with increased use in biosynthetic pathways.

Glutamine is transported into the cell by the solute carrier Slc1A5 (ASCT2), where it is converted into glutamate by glutaminase (Gls 1 or Gls 2 depending on the tissue). Augmented glutamine consumption in lung myofibroblasts appears largely driven by elevated levels of Gls1 (Bernard et al., 2018; Ge et al., 2018). Proline, which together with glycine accounts for 57% of amino acid residues in collagen (Li and Wu, 2018), is generated directly from glutamate *via* pyrroline-5-carboxylate (P5C) (Li and Wu, 2018) in the cytosol, while in the mitochondrion glutamine can be converted to α-ketoglutarate (αKG), an intermediate of the TCA cycle (**Figure 1B**). Thus, increased glutamine metabolism acts to replenish the TCA cycle through anaplerotic reactions, but also provides proline for collagen synthesis.

Glutamine has been shown to increase mRNA transcripts for pro-collagen I (Bellon et al., 1995), but this is not a universal finding (Ge et al., 2018). In the latter case, glutamine-derived αKG increases in pro-collagen I protein levels were contingent on activation of mTOR and consequent phosphorylation of p70-S6K and 4E-BP1 rather than changes in gene transcription (Ge et al., 2018). The formation of αKG from glutamate is reversible, meaning that αKG can also serve as a precursor in the conversion of glutamate to proline. Additionally, αKG also stabilizes intracellular collagen by promoting proline hydroxylation *via* activation of the enzyme prolyl hydroxylase (Ge et al., 2018). The relationship between αKG and proline hydroxylation is particularly interesting. Hydroxylation of prolines in hypoxia inducible factor-1 (HIF-1α) is necessary for ubiquitin-mediated proteasome degradation of this enigmatic protein. Conversely, the same post-translational modification protects intracellular collagen from being degraded in lung fibroblasts (Li and Wu, 2018), suggesting that proline hydroxylation in collagens does not create a docking site for ubiquitin binding. Finally, by acting as a co-factor for a Jumonji family histone demethylase (JMJD3), αKG promotes histone methylation at the cell survival genes X-linked IAP (XIAP) and survivin (Bai et al., 2019), thereby providing a mechanistic link between metabolism, epigenetic regulation of cell survival and resistance of fibroblasts to apoptosis.

The relative importance of changes in glycolysis and glutaminolysis is unclear. Recent data highlights that amino acids, rather than glucose, might be responsible for most of the cell mass in proliferation (Du et al., 2018), Replenishment of the TCA cycle with αKG due to a TGF-β1-induced shift toward glycolysis is logical and such a change in flux has been demonstrated in lung fibroblasts using carbon tracing (Bernard et al., 2018). Functional measures suggest that the main function of glutamine metabolism in fibroblasts is to feed biosynthetic pathways rather than ATP generation (Hamanaka et al., 2019).

METABOLIC REGULATION OF FIBROBLAST DIFFERENTIATION

An interesting question has been whether metabolic reprogramming is a signal for fibroblast differentiation as well as increased activity *per se*. Not surprisingly these roles are difficult to isolate. Nevertheless, triggering of development signals like the hedgehog-Yap axis in both glycolysis (Chen et al., 2012) and glutaminolysis (Du et al., 2018) have suggested a more direct role in differentiation. Indeed, subsequent studies showed that both glutamine-depleted media and Gls inhibition prevent the differentiation of quiescent hepatic stellate cells into myofibroblasts, an effect that was not seen with glucose deprivation (Du et al., 2018).

FIBROBLAST PRIMING

Although we have long known that fibroblasts from human fibrotic kidneys are inherently more proliferative and synthesize more collagen than their counterparts from normal kidneys (Rodemann and Muller, 1990), only recently have we started to understand this at the molecular level.

In normal cutaneous granulation tissue, completion of physiological healing is accompanied by a loss of myofibroblasts due to apoptosis (Darby et al., 1990). In pathological conditions, such as experimental pulmonary fibrosis, the persistence of fibroblasts has been shown to be due to an inherent resistance of fibroblasts to apoptosis (Huang et al., 2013). Likewise dermal fibroblasts from skin lesions in systemic scleroderma show concordant and stable gene expression differences when compared to fibroblasts isolated from healthy donors (Shin et al., 2019). In these cells, a signature of aberrant TGF-β1 signaling was sustained in isolated dermal fibroblasts maintained in culture (Shin et al., 2019). Similarly, fibrotic renal fibroblasts have upregulated responses to cytokine stimulation compared to their counterparts derived from uninjured tissue, in part due to increased cell-surface TGF-β receptor expression (Smith et al., 2017a,b).

This predisposition also extends to metabolic adaptations. Under basal conditions, skin fibroblasts derived from keloids have a higher rate of ATP synthesis than their normal skin counterparts (Vincent et al., 2008); glycolysis is the primary energy source in these cells, while normal skin fibroblasts derive

their ATP mainly from oxidative phosphorylation (Vincent et al., 2008). Fibroblasts from lungs of patients with idiopathic pulmonary fibrosis (IPF) have augmented glycolysis relative to cells from normal lungs (Xie et al., 2015). In renal studies, the effect of TGF-β1 on PDC regulation was observed in fibroblasts grown from kidneys after unilateral ureteric obstruction, and not in cells from unobstructed kidneys (Smith and Hewitson, 2020), while Tian et al. have recently described a metabolic memory over multiple cell passages in left ventricular fibroblasts derived from patients with pulmonary arterial hypertension, implying an epigenetic basis (Tian et al., 2020). Finally, fibroblasts derived from patients with IPF express more Gls mRNA than their normal lung counterparts (Choudhury et al., 2020). There is however a clear heterogeneity, with some fibroblasts derived from patients with IPF (Bai et al., 2019) and systemic scleroderma (Henderson et al., 2020) more sensitive (primed) than others.

CELLULAR METABOLISM AS A THERAPEUTIC TARGET

Metabolic pathways that support persistent activation and ECM synthesis in fibroblasts may offer the opportunity for therapeutic interventions to block fibrogenesis. Such novel therapeutic strategies for fibrosis have been proposed based on proof-of-principle studies in a variety of experimental models and organs that have targeted different steps in central carbon and glutamine metabolism; 6-phosphofructo-2-kinase/fructose-2, 6-biphosphatase 3 (PFKFB3) (Xie et al., 2015) and PDC (Wei et al., 2019; Tian et al., 2020), serine-glycine synthesis (Hamanaka et al., 2018) and Gls (Cui et al., 2019), amongst others. However, although there is much *in vitro* and pre-clinical evidence, human data remains limited and indirect. Unsurprisingly, we again take our lead from cancer, where multiple small molecule inhibitors targeting cellular metabolism are under investigation in phase1/2 clinical trials [reviewed in (Akins et al., 2018)].

CONCLUSIONS

In cancer cells, metabolic adaptations appear as prerequisites for the acquisition and maintenance of a malignant phenotype. Rapidly accumulating evidence now suggests that renal fibroblasts may likewise be metabolically reprogrammed, with glucose and glutamine consumption linked to several possible mechanisms in (myo)fibroblast activation and fibrogenesis (**Figure 1**). Despite the similarities with malignant cells, differences do exist, highlighting the need to define this process at an organ and cell-specific level, and to confirm both changes in metabolic flux and synthetic fate over time. Likewise, while non–renal (myo)fibroblasts offer an exciting glimpse into the metabolic adaptions in fibrosis, we eagerly await further confirmation of such adaptations in human kidney disease.

AUTHOR CONTRIBUTIONS

TH and ES jointly wrote and edited the manuscript. Both authors approved the manuscript for submission and agree to be accountable for its contents.

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REFERENCES

Akins, N. S., Nielson, T. C., and Le, H. V. (2018). Inhibition of glycolysis and glutaminolysis: an emerging drug discovery approach to combat cancer. *Curr. Top Med. Chem.* 18, 494–504. doi: 10.2174/1568026618666180523111351

Altman, B. J., Stine, Z. E., and Dang, C. V. (2016). From Krebs to clinic: glutamine metabolism to cancer therapy. *Nat. Rev. Cancer* 16, 619–634. doi: 10.1038/nrc.2016.71

Bai, L., Bernard, K., Tang, X., Hu, M., Horowitz, J. C., Thannickal, V. J., et al. (2019). Glutaminolysis epigenetically regulates antiapoptotic gene expression in idiopathic pulmonary fibrosis fibroblasts. *Am. J. Respir. Cell Mol. Biol.* 60, 49–57. doi: 10.1165/rcmb.2018-0180OC

Barcena-Varela, M., Paish, H., Alvarez, L., Uriarte, I., Latasa, M. U., Santamaria, E., et al. (2020). Epigenetic mechanisms and metabolic reprogramming in fibrogenesis: dual targeting of G9a and DNMT1 for the inhibition of liver fibrosis. *Gut* 70, 388–400. doi: 10.1136/gutjnl-2019-320205

Bates, J., Vijayakumar, A., Ghoshal, S., Marchand, B., Yi, S., Kornyeyev, D., et al. (2020). Acetyl-CoA carboxylase inhibition disrupts metabolic reprogramming during hepatic stellate cell activation. *J. Hepatol.* 73, 896–905. doi: 10.1016/j.jhep.2020.04.037

Bellon, G., Chaqour, B., Wegrowski, Y., Monboisse, J. C., and Borel, J. P. (1995). Glutamine increases collagen gene transcription in cultured human fibroblasts. *Biochim. Biophys. Acta* 1268, 311–323. doi: 10.1016/0167-4889(95)00093-8

Bernard, K., Logsdon, N. J., Benavides, G. A., Sanders, Y., Zhang, J., Darley-Usmar, V. M., et al. (2018). Glutaminolysis is required for transforming growth factor-β1-induced myofibroblast differentiation and activation. *J. Biol. Chem.* 293, 1218–1228. doi: 10.1074/jbc.RA117.000444

Bernard, K., Logsdon, N. J., Ravi, S., Xie, N., Persons, B. P., Rangarajan, S., et al. (2015). Metabolic reprogramming is required for myofibroblast contractility and differentiation. *J. Biol. Chem.* 290, 25427–25438. doi: 10.1074/jbc.M115.646984

Buhl, E. M., Djudjaj, S., Klinkhammer, B. M., Ermert, K., Puelles, V. G., Lindenmeyer, M. T., et al. (2020). Dysregulated mesenchymal PDGFR-β drives kidney fibrosis. *EMBO Mol. Med.* 12:e11021. doi: 10.15252/emmm.201911021

Calvier, L., Chouvarine, P., Legchenko, E., Hoffmann, N., Geldner, J., Borchert, P., et al. (2017). PPARγ links BMP2 and TGFβ1 pathways in vascular smooth muscle cells, regulating cell proliferation and glucose metabolism. *Cell Metab.* 25, 1118–1134.e1117. doi: 10.1016/j.cmet.2017.03.011

Chen, Y., Choi, S. S., Michelotti, G. A., Chan, I. S., Swiderska-Syn, M., Karaca, G. F., et al. (2012). Hedgehog controls hepatic stellate cell fate by regulating metabolism. *Gastroenterology* 143, 1319–1329.e1311. doi: 10.1053/j.gastro.2012.07.115

Chen, Z., Liu, M., Li, L., and Chen, L. (2018). Involvement of the Warburg effect in non-tumor diseases processes. *J. Cell Physiol.* 233, 2839–2849. doi: 10.1002/jcp.25998

Chou, Y. H., Pan, S. Y., Shao, Y. H., Shih, H. M., Wei, S. Y., Lai, C. F., et al. (2020). Methylation in pericytes after acute injury promotes chronic kidney disease. *J. Clin. Invest.* 130, 4845–4857. doi: 10.1172/JCI135773

Choudhary, C., Kumar, C., Gnad, F., Nielsen, M. L., Rehman, M., Walther, T. C., et al. (2009). Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science* 325, 834–840. doi: 10.1126/science.1175371

Choudhary, C., Weinert, B. T., Nishida, Y., Verdin, E., and Mann, M. (2014). The growing landscape of lysine acetylation links metabolism and cell signalling. *Nat. Rev. Mol. Cell Biol.* 15, 536–550. doi: 10.1038/nrm3841

Choudhury, M., Yin, X., Schaefbauer, K. J., Kang, J. H., Roy, B., Kottom, T. J., et al. (2020). SIRT7-mediated modulation of glutaminase 1 regulates TGF-β-induced pulmonary fibrosis. *FASEB J.* 34, 8920–8940. doi: 10.1096/fj.202000564R

Cui, H., Xie, N., Jiang, D., Banerjee, S., Ge, J., Sanders, Y. Y., et al. (2019). Inhibition of Glutaminase 1 attenuates experimental pulmonary fibrosis. *Am. J. Respir. Cell Mol. Biol.* 61, 492–500. doi: 10.1165/rcmb.2019-0051OC

Dabiri, G., Tumbarello, D. A., Turner, C. E., and Van de Water, L. (2008). Hic-5 promotes the hypertrophic scar myofibroblast phenotype by regulating the TGF-beta1 autocrine loop. *J. Invest. Dermatol.* 128, 2518–2525. doi: 10.1038/jid.2008.90

Darby, I., Skalli, O., and Gabbiani, G. (1990). Alpha-smooth muscle actin is transiently expressed by myofibroblasts during experimental wound healing. *Lab. Invest.* 63, 21–29.

Darby, I. A., and Hewitson, T. D. (2007). Fibroblast differentiation in wound healing and fibrosis. *Int. Rev. Cytol.* 257, 143–179. doi: 10.1016/S0074-7696(07)57004-X

Ding, H., Jiang, L., Xu, J., Bai, F., Zhou, Y., Yuan, Q., et al. (2017). Inhibiting aerobic glycolysis suppresses renal interstitial fibroblast activation and renal fibrosis. *Am. J. Physiol. Renal. Physiol.* 313, F561–f575. doi: 10.1152/ajprenal.00036.2017

Du, K., Hyun, J., Premont, R. T., Choi, S. S., Michelotti, G. A., Swiderska-Syn, M., et al. (2018). Hedgehog-YAP signaling pathway regulates glutaminolysis to control activation of hepatic stellate cells. *Gastroenterology* 154, 1465–1479.e1413. doi: 10.1053/j.gastro.2017.12.022

Duffield, J. S., Lupher, M., Thannickal, V. J., and Wynn, T. A. (2013). Host responses in tissue repair and fibrosis. *Annu. Rev. Pathol.* 8, 241–276. doi: 10.1146/annurev-pathol-020712-163930

Fukasawa, H., Yamamoto, T., Togawa, A., Ohashi, N., Fujigaki, Y., Oda, T., et al. (2004). Down-regulation of Smad7 expression by ubiquitin-dependent degradation contributes to renal fibrosis in obstructive nephropathy in mice. *Proc. Natl. Acad. Sci. U.S.A.* 101, 8687–8692. doi: 10.1073/pnas.0400035101

Ge, J., Cui, H., Xie, N., Banerjee, S., Guo, S., Dubey, S., et al. (2018). Glutaminolysis promotes collagen translation and stability via α-Ketoglutarate-mediated mTOR activation and proline hydroxylation. *Am. J. Respir. Cell Mol. Biol.* 58, 378–390. doi: 10.1165/rcmb.2017-0238OC

Ghosh-Choudhary, S., Liu, J., and Finkel, T. (2020). Metabolic regulation of cell fate and function. *Trends Cell Biol.* 30, 201–212. doi: 10.1016/j.tcb.2019.12.005

Hamanaka, R. B., Nigdelioglu, R., Meliton, A. Y., Tian, Y., Witt, L. J., O'Leary, E., et al. (2018). Inhibition of phosphoglycerate dehydrogenase attenuates bleomycin-induced pulmonary fibrosis. *Am. J. Respir. Cell Mol. Biol.* 58, 585–593. doi: 10.1165/rcmb.2017-0186OC

Hamanaka, R. B., O'Leary, E. M., Witt, L. J., Tian, Y., Gokalp, G. A., Meliton, A. Y., et al. (2019). Glutamine metabolism is required for collagen protein synthesis in lung fibroblasts. *Am. J. Respir. Cell Mol. Biol.* 61, 597–606. doi: 10.1165/rcmb.2019-0008OC

Henderson, J., Duffy, L., Stratton, R., Ford, D., and O'Reilly, S. (2020). Metabolic reprogramming of glycolysis and glutamine metabolism are key events in myofibroblast transition in systemic sclerosis pathogenesis. *J. Cell Mol. Med.* 24, 14026–14038. doi: 10.1111/jcmm.16013

Hewitson, T. D. (2009). Renal tubulointerstitial fibrosis: common but never simple. *Am. J. Physiol. Renal. Physiol.* 296, F1239–F1244. doi: 10.1152/ajprenal.90521.2008

Hewitson, T. D., Holt, S. G., and Smith, E. R. (2017a). Progression of tubulointerstitial fibrosis and the chronic kidney disease phenotype—role of risk factors and epigenetics. *Front. Pharmacol.* 8:520. doi: 10.3389/fphar.2017.00520

Hewitson, T. D., Holt, S. G., Tan, S. J., Wigg, B., Samuel, C. S., and Smith, E. R. (2017b). Epigenetic modifications to H3K9 in renal tubulointerstitial cells after unilateral ureteric obstruction and TGF-beta1 stimulation. *Front. Pharmacol.* 8:307. doi: 10.3389/fphar.2017.00307

Huang, S. K., Scruggs, A. M., Donaghy, J., Horowitz, J. C., Zaslona, Z., Przybranowski, S., et al. (2013). Histone modifications are responsible for decreased Fas expression and apoptosis resistance in fibrotic lung fibroblasts. *Cell Death Dis.* 4, e621–e621. doi: 10.1038/cddis.2013.146

Kaissling, B., Lehir, M., and Kriz, W. (2013). Renal epithelial injury and fibrosis. *Biochim. Biophys. Acta* 1832, 931–939. doi: 10.1016/j.bbadis.2013.02.010

Kamphorst, J. J., Chung, M. K., Fan, J., and Rabinowitz, J. D. (2014). Quantitative analysis of acetyl-CoA production in hypoxic cancer cells reveals substantial contribution from acetate. *Cancer Metab.* 2:23. doi: 10.1186/2049-3002-2-23

Kori, Y., Sidoli, S., Yuan, Z. F., Lund, P. J., Zhao, X., and Garcia, B. A. (2017). Proteome-wide acetylation dynamics in human cells. *Sci. Rep.* 7:10296. doi: 10.1038/s41598-017-09918-3

Kottmann, R. M., Kulkarni, A. A., Smolnycki, K. A., Lyda, E., Dahanayake, T., Salibi, R., et al. (2012). Lactic acid is elevated in idiopathic pulmonary fibrosis and induces myofibroblast differentiation via pH-dependent activation of transforming growth factor-β. *Am. J. Respir. Crit. Care Med.* 186, 740–751. doi: 10.1164/rccm.201201-0084OC

Kottmann, R. M., Trawick, E., Judge, J. L., Wahl, L. A., Epa, A. P., Owens, K. M., et al. (2015). Pharmacologic inhibition of lactate production prevents myofibroblast differentiation. *Am. J. Physiol. Lung Cell Mol. Physiol.* 309, L1305–1312. doi: 10.1152/ajplung.00058.2015

Kwan, B., Fuhrer, T., Zhang, J., Darshi, M., Van Espen, B., Montemayor, D., et al. (2020). Metabolomic markers of kidney function decline in patients with diabetes: evidence from the Chronic Renal Insufficiency Cohort (CRIC) study. *Am. J. Kidney Dis.* 76, 511–520. doi: 10.1053/j.ajkd.2020.01.019

Leaf, I. A., and Duffield, J. S. (2017). What can target kidney fibrosis? *Nephrol. Dial. Transplant.* 32, i89–i97. doi: 10.1093/ndt/gfw388

Li, P., and Wu, G. (2018). Roles of dietary glycine, proline, and hydroxyproline in collagen synthesis and animal growth. *Amino Acids* 50, 29–38. doi: 10.1007/s00726-017-2490-6

Li, Q., Qin, Z., Nie, F., Bi, H., Zhao, R., Pan, B., et al. (2018). Metabolic reprogramming in keloid fibroblasts: aerobic glycolysis and a novel therapeutic strategy. *Biochem. Biophys. Res. Commun.* 496, 641–647. doi: 10.1016/j.bbrc.2018.01.068

Lundby, A., Lage, K., Weinert, B. T., Bekker-Jensen, D. B., Secher, A., Skovgaard, T., et al. (2012). Proteomic analysis of lysine acetylation sites in rat tissues reveals organ specificity and subcellular patterns. *Cell Rep.* 2, 419–431. doi: 10.1016/j.celrep.2012.07.006

Meng, X. M., Nikolic-Paterson, D. J., and Lan, H. Y. (2016). TGF-beta: the master regulator of fibrosis. *Nat. Rev. Nephrol.* 12, 325–338. doi: 10.1038/nrneph.2016.48

Negmadjanov, U., Godic, Z., Rizvi, F., Emelyanova, L., Ross, G., Richards, J., et al. (2015). TGF-beta1-mediated differentiation of fibroblasts is associated with increased mitochondrial content and cellular respiration. *PLoS ONE* 10:e0123046. doi: 10.1371/journal.pone.0123046

Nigdelioglu, R., Hamanaka, R. B., Meliton, A. Y., O'Leary, E., Witt, L. J., Cho, T., et al. (2016). Transforming growth factor (TGF)-beta promotes *de novo* serine synthesis for collagen production. *J. Biol. Chem.* 291, 27239–27251. doi: 10.1074/jbc.M116.756247

O'Neill, L. A., Kishton, R. J., and Rathmell, J. (2016). A guide to immunometabolism for immunologists. *Nat. Rev. Immunol.* 16, 553–565. doi: 10.1038/nri.2016.70

Para, R., Romero, F., George, G., and Summer, R. (2019). Metabolic reprogramming as a driver of fibroblast activation in pulmonary fibrosis. *Am. J. Med. Sci.* 357, 394–398. doi: 10.1016/j.amjms.2019.02.003

Pena, M. J., Lambers Heerspink, H. J., Hellemons, M. E., Friedrich, T., Dallmann, G., Lajer, M., et al. (2014). Urine and plasma metabolites predict the development of diabetic nephropathy in individuals with Type 2 diabetes mellitus. *Diabet. Med.* 31, 1138–1147. doi: 10.1111/dme.12447

Rodemann, H. P., and Muller, G. A. (1990). Abnormal growth and clonal proliferation of fibroblasts derived from kidneys with interstitial fibrosis. *Proc. Soc. Exp. Biol. Med.* 195, 57–63. doi: 10.3181/00379727-195-43118

Shin, J. Y., Beckett, J. D., Bagirzadeh, R., Creamer, T. J., Shah, A. A., McMahan, Z., et al. (2019). Epigenetic activation and memory at a TGFB2 enhancer in systemic sclerosis. *Sci. Transl. Med.* 11:eaaw0790. doi: 10.1126/scitranslmed.aaw0790

Si, M., Wang, Q., Li, Y., Lin, H., Luo, D., Zhao, W., et al. (2019). Inhibition of hyperglycolysis in mesothelial cells prevents peritoneal fibrosis. *Sci. Transl. Med.* 11:aav5341. doi: 10.1126/scitranslmed.aav5341

Sivanand, S., Viney, I., and Wellen, K. E. (2018). Spatiotemporal control of Acetyl-CoA metabolism in chromatin regulation. *Trends Biochem. Sci.* 43, 61–74. doi: 10.1016/j.tibs.2017.11.004

Smith, E. R., and Hewitson, T. D. (2020). TGF-beta1 is a regulator of the pyruvate dehydrogenase complex in fibroblasts. *Sci. Rep.* 10:17914. doi: 10.1038/s41598-020-74919-8

Smith, E. R., Holt, S. G., and Hewitson, T. D. (2017a). FGF23 activates injury-primed renal fibroblasts via FGFR4-dependent signalling and enhancement of TGF-beta autoinduction. *Int. J. Biochem. Cell Biol.* 92, 63–78. doi: 10.1016/j.biocel.2017.09.009

Smith, E. R., Tan, S. J., Holt, S. G., and Hewitson, T. D. (2017b). FGF23 is synthesised locally by renal tubules and activates injury-primed fibroblasts. *Sci. Rep.* 7:3345. doi: 10.1038/s41598-017-02709-w

Smith, E. R., Wigg, B., Holt, S., and Hewitson, T. D. (2019). TGF-beta1 modifies histone acetylation and acetyl-coenzyme A metabolism in renal myofibroblasts. *Am. J. Physiol. Renal. Physiol.* 316, F517–F529. doi: 10.1152/ajprenal.00513.2018

Spange, S., Wagner, T., Heinzel, T., and Kramer, O. H. (2009). Acetylation of non-histone proteins modulates cellular signalling at multiple levels. *Int. J. Biochem. Cell. Biol.* 41, 185–198. doi: 10.1016/j.biocel.2008.08.027

Sutendra, G., Kinnaird, A., Dromparis, P., Paulin, R., Stenson, T. H., Haromy, A., et al. (2014). A nuclear pyruvate dehydrogenase complex is important for the generation of acetyl-CoA and histone acetylation. *Cell* 158, 84–97. doi: 10.1016/j.cell.2014.04.046

Tian, L., Wu, D., Dasgupta, A., Chen, K. H., Mewburn, J., Potus, F., et al. (2020). Epigenetic metabolic reprogramming of right ventricular fibroblasts in pulmonary arterial hypertension: a pyruvate dehydrogenase kinase-dependent shift in mitochondrial metabolism promotes right ventricular fibrosis. *Circ. Res.* 126, 1723–1745. doi: 10.1161/CIRCRESAHA.120.316443

Tomasek, J. J., Gabbiani, G., Hinz, B., Chaponnier, C., and Brown, R. A. (2002). Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat. Rev. Mol. Cell. Biol.* 3, 349–363. doi: 10.1038/nrm809

Venkatachalam, M. A., and Weinberg, J. M. (2015). Fibrosis without fibroblast TGF-beta receptors? *Kidney Int.* 88, 434–437. doi: 10.1038/ki.2015.170

Vincent, A. S., Phan, T. T., Mukhopadhyay, A., Lim, H. Y., Halliwell, B., and Wong, K. P. (2008). Human skin keloid fibroblasts display bioenergetics of cancer cells. *J. Invest. Dermatol.* 128, 702–709. doi: 10.1038/sj.jid.5701107

Wei, Q., Su, J., Dong, G., Zhang, M., Huo, Y., and Dong, Z. (2019). Glycolysis inhibitors suppress renal interstitial fibrosis via divergent effects on fibroblasts and tubular cells. *Am. J. Physiol. Renal. Physiol.* 316, F1162–f1172. doi: 10.1152/ajprenal.00422.2018

Weinert, B. T., Narita, T., Satpathy, S., Srinivasan, B., Hansen, B. K., Schölz, C., et al. (2018). Time-resolved analysis reveals rapid dynamics and broad scope of the CBP/p300 acetylome. *Cell* 174, 231–f 244.e212. doi: 10.1016/j.cell.2018.04.033

Wu, H., Kirita, Y., Donnelly, E. L., and Humphreys, B. D. (2019). Advantages of single-nucleus over single-cell RNA sequencing of adult kidney: rare cell types and novel cell states revealed in fibrosis. *J. Am. Soc. Nephrol.* 30, 23–32. doi: 10.1681/ASN.2018090912

Xie, N., Tan, Z., Banerjee, S., Cui, H., Ge, J., Liu, R. M., et al. (2015). Glycolytic reprogramming in myofibroblast differentiation and lung fibrosis. *Am. J. Respir. Crit. Care Med.* 192, 1462–1474. doi: 10.1164/rccm.201504-0780OC

Yin, X., Choudhury, M., Kang, J. H., Schaefbauer, K. J., Jung, M. Y., Andrianifahanana, M., et al. (2019). Hexokinase 2 couples glycolysis with the profibrotic actions of TGF-β. *Sci. Signal.* 12:eaax4067. doi: 10.1126/scisignal.aax4067

Zhao, X., Kwan, J. Y. Y., Yip, K., Liu, P. P., and Liu, F. F. (2020). Targeting metabolic dysregulation for fibrosis therapy. *Nat. Rev. Drug Discov.* 19, 57–75. doi: 10.1038/s41573-019-0040-5

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frontiers in Medicine

ORIGINAL RESEARCH
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Significance of Glomerular Immune Reactivity in Time Zero Biopsies for Allograft Survival Beyond IgA

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The quality of a renal transplant can influence the clinical course after transplantation. Glomerular immune reactivity in renal transplants has previously been described, focusing particularly on IgA, and has been shown to disappear in most cases without affecting the outcome. Here, we describe a cohort of time zero biopsies with regard to glomerular immune reactivity and implications for histomorphology and follow-up. 204 Time zero biopsies were analyzed by immunohistochemistry for glomerular immune reactivity. Time zero and 1-year biopsies were evaluated for histomorphological changes, which, together with clinical and follow-up data, were assessed for associations with glomerular immune profiles. Nearly half of the analyzed time zero biopsies showed glomerular immune reactivity with mesangial C3 being the most common (32.9%), followed by IgA (13.7%) and fullhouse patterns (6.9%). Strong C3 deposits (C3high) were only observed in deceased transplants. In the majority of cases immune reactivity was undetectable in follow-up biopsies and had no adverse effect on transplant function in follow-up of 5 years. In kidney pairs transplanted to different recipients a strong concordance of immune profiles in both kidneys was observed. Moreover, an association of male donor sex and deceased donor transplantation with the presence of immune reactivity was observed. In conclusion, glomerular immune reactivity is a very frequent finding in time zero biopsies, which seems to be determined by donor parameters including male sex and deceased donor transplants. It had no adverse impact on transplant function in 5-year follow-up. Glomerular immune reactivity in time zero biopsies, therefore, does not appear to indicate an inferior quality of the transplant.

Keywords: complement, renal tranplantation, time zero biopsy, transplant quality, immune reactivity

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INTRODUCTION

Time zero biopsies offer the possibility to assess donor kidneys at the time of transplantation, which can help to interpret findings in later biopsies. In order to interpret the significance of time zero biopsy findings for the fate of the transplant it is important to correlate these findings with donor parameters and future transplant function.

Besides structural changes, a variety of glomerular immune reactivities have been described in time zero biopsies of donor kidneys in the past. In several series of time zero biopsies the incidence of mesangial IgA deposits was reported to be between 6.9 and 32.1% (1–8). This IgA reactivity gradually disappeared in most cases (4, 6–8) without influencing graft survival (4, 8) and function (6, 8). Glomerular IgM and C3 (7, 8) and less frequently IgG (3, 8) were also reported to vanish in the majority of cases in follow-up biopsies without adverse effects (7). Whereas, the vast majority of previous reports have been focusing on glomerular IgA (1–6, 8), in the present report we analyzed and compared different immune profiles in time zero biopsies including immunohistochemical fullhouse patterns and C3 reactivity as potential markers of complement activation in the transplanted organs.

Here, we analyzed a European cohort of time zero biopsies for the presence of glomerular immune reactivity and subdivided staining patterns into subgroups, including IgA, fullhouse, C3high, C3low and cases without significant immune reactivity. We compared morphologic, clinical and follow-up data between the groups.

MATERIALS AND METHODS

Patients

Consecutive and extended cohort: A total of 204 time zero specimens (203 kidney biopsies and one small kidney resection) of 191 donors, performed at the University Hospital of Erlangen and submitted to the Dept. of Nephropathology, were included. To assess the prevalence of glomerular immune reactivity we considered a consecutive cohort of 163 time zero biopsies performed between 06/2011 and 05/2013. Two cases were excluded for the lack of glomeruli, so that 161 cases were analyzed (**Figure 1**). As numbers of cases with immune reactivity were low in the consecutive cohort, for further analyses we randomly selected 43 additional time zero biopsies from 2010 to 2016, taken before or after the above-mentioned time span. These showed one of the below-defined glomerular immune patterns, in order to improve representativeness and comparability, for a total of 204 analyzed biopsies. The use of archived renal specimens was approved by the local Ethics committee (reference number 4415). Donor parameters included: sex, age, living/deceased donor, creatinine (mg/dl) and glomerular filtration rate (GFR; ml/min) before transplantation, body-mass index (BMI), cold and warm ischemia time (minutes), presence/absence of proteinuria, of a history of smoking, of diabetes mellitus, and arterial hypertension. Recipient parameters included sex, age, BMI; native kidney disease, number of hemodialyses required after transplantation, renal transplant (Rtx) and patient survival (yes/no), post-transplant renal function (primary/delayed/no function, organ loss or death), follow-up creatinine and GFR at 1 to 5 years post transplantation. GFR was calculated according to the CKD-EPI formula as described by Levely et al. (9) using patient serum creatinine and age under specification of race, sex and serum creatinine level. To achieve better comparability children <16 years were excluded from statistical analyses of creatinine and GFR.

Immunohistochemistry

Immunohistochemical stainings with antibodies specific for IgA, IgG, IgM, C1q, C3c (all polyclonal: Cat. No. IgA A0262, IgG A0423, RRID:AB_2335700; IgM A04202, RRID:AB_578520; C1q A013602, RRID:AB_578496; C3c A006202, RRID:AB_578477, Agilent, Santa Clara, CA, USA) were performed on formalin fixed and paraffin-embedded (FFPE, 1μm sections) material with current standard methods after digestion with protease from Streptomyces griseus (Sigma-Aldrich, Munich, Germany, P5147) on a Ventana Benchmark stainer (Roche, Basel, Switzerland) or manually before 2011. Limited staining in the glomerular vascular pole was scored as negative. The intensity of staining was categorized into 4 grades: grade 0 (none), grade 1 (mild), grade 2 (moderate), grade 3 (strong).

In case of IgA reactivity in the time zero biopsy additional stainings for C4d (polyclonal, rabbit anti-C4d, 1:500, Cat. No. RBK061, RRID:AB_2864450, Zytomed Systems GmbH, Bargteheide, Germany; antigen-retrieval with ULTRA CC1 buffer, Roche) and galactose-deficient IgA (rat anti-Gd-IgA1, clone KM55, 1:100, Cat. No. 10777, Immuno-Biological Laboratories, Minneapolis, MA, USA; antigen-retrieval with protease digestion) were performed manually.

Study Groups

According to the immunohistochemical findings biopsies were divided into the following subgroups: (i) negative: no glomerular immune reactivity except IgM and/or C1q, (ii) IgA: IgA deposits without fullhouse pattern, (iii) FH: fullhouse pattern with positivity of IgA, IgG, IgM, C1q and C3, (iv) C3high: C3 2+/3+ without IgA, IgG or C1q, (v) C3low: C3 1+ without IgA, IgG or C1q, (vi) MN: membranous pattern, (vii) NOS (not otherwise specified): mesangial immune reactivity exceeding the definition of negative, not meeting criteria of any other pattern.

Follow-Up Biopsies

A total of 176 follow-up biopsies were evaluated, which were taken 4-1227 days post transplantation. These included 1-year biopsies ($n = 111$) and follow-up biopsies at other points in time ($n = 65$). Clinical indications for renal biopsy as retrieved from the accompanying files in the total of 176 follow-up biopsies were protocol biopsy (3 months or 1-year) in 96, rise in creatinine in 45 (one with simultaneous proteinuria), delayed graft function in 15, positive polyomavirus serology in seven, proteinuria in four, donor-specific antibodies in six, suspicion of rejection in two and not reported in one. Main diagnoses in the original reports included no rejection/significant pathology in 57, at least moderate acute tubular injury in 33, Borderline changes in 24, T-cell mediated rejection (TCMR) in 20, antibody-mediated rejection (ABMR) in 4 and suspicion of ABMR in 2, combined TCMR/ABMR or Borderline/ABMR in 2, polyomavirus-nephropathy in 10 (one accompanied by TCMR), interstitial fibrosis and tubular atrophy of ≥ 20% as main finding in 12, glomerulonephritis in eight deriving from 5 patients and interpreted as recurrence in three patients (glomerulonephritis was accompanied by acute tubular injury in three, by Borderline changes in two and by vascular hyaline microthrombi in one biopsy), cholesterol embolism in one,

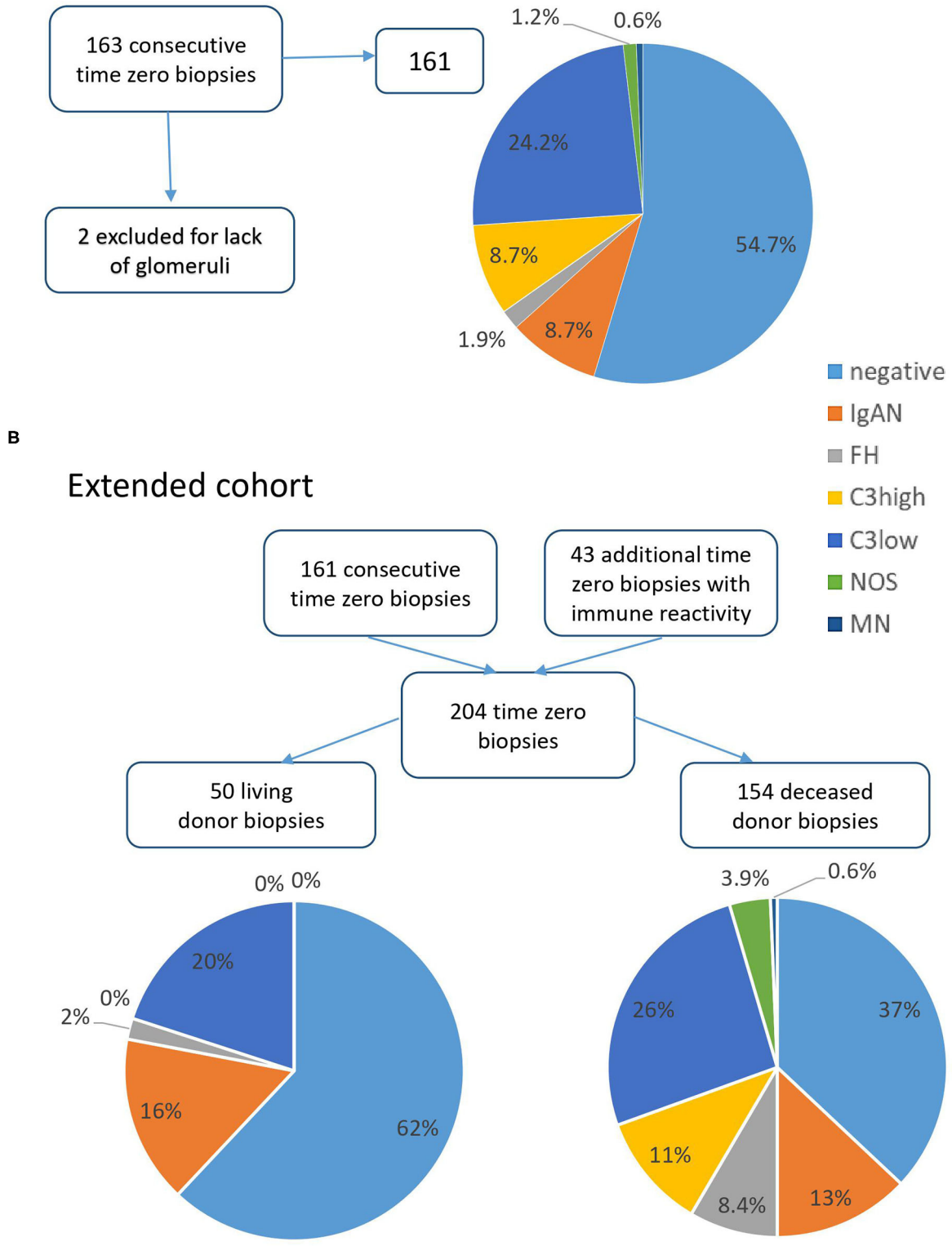

FIGURE 1 | Relative distribution of immune pattern in time zero biopsies. **(A)** In the consecutive cohort 161 time zero biopsies were analyzed after exclusion of two cases with insufficient material. Over half of the cases showed no significant immune reactivity, about a quarter of cases mild C3 deposits (C3low) and the remainder different immune patterns including IgA, fullhouse (FH), and C3high. **(B)** Comparing living and deceased donor biopsies in the total cohort of 204 time zero specimens, negative cases were more prevalent in living donor organs and C3high staining was only observed in deceased donor transplants.

thrombotic microangiopathy in one, ischemic infarction in one and findings were insufficient for a diagnosis in one.

For all cases included in the cohort of time zero biopsies the respective 1-year biopsies were evaluated whenever available ($n = 111$) including all 12-months protocol biopsies and, in cases without protocol biopsy, any other biopsy taken between 9 and 15 months after transplantation, whenever available. Additionally, in IgA (22/28), FH (12/14), C3high (16/17), C3low (24/50), NOS (4/6) and MN (1/1) first follow-up biopsies after transplantation (irrespective of the time of biopsy) were assessed by immunohistochemistry (markers positive in the time zero biopsy were re-analyzed) to evaluate for persistence of glomerular immune reactivity. Immunohistochemistry was performed in 79 first follow-up biopsies, of which 16 were 1-year biopsies. In cases with persistence of glomerular immune reactivity in 1st follow-up 1-year biopsies or other available biopsies were analyzed by immunohistochemistry until negative or no further biopsy was available. A 2nd follow-up biopsy after persistence was evaluated in seven cases (6 1-year biopsies) and a 3rd biopsy in one case.

Histological Evaluation of Time Zero Biopsies and 1-Year Biopsies

In time zero and 1-year biopsies total and globally sclerosed glomeruli were counted, interstitial fibrosis and tubular atrophy (IFTA) was estimated in steps of 5%, arteriosclerosis was scored as described previously (10). Matrix expansion (mesangial matrix >2 mesangial cell nuclei) was assessed as present or absent. In time zero biopsies, additionally, the degree of acute tubular injury (0: no/minimal, 1 <25%, 2:25–49%, 3:50–74%, 4 ≥ 75% of tubules involved) was scored.

Statistical Analyses

Statistical analyses were performed using IBM SPSS Version 24. For the comparison of ordinal and numerical variables between either two or more independent groups Mann-Whitney and Kruskal-Wallis tests were used, respectively. Bonferroni correction was applied after *post-hoc* testing for Kruskal-Wallis. To test the association between nominal variables Pearson's chi-square test was used or alternatively Fisher's exact test when expected values were <5. Results with p-values <0.05 were considered statistically significant. *Post-hoc* analysis for Pearson's chi-square test was performed using the standardized residuals (11) and correcting p-values using Bonferroni.

RESULTS

Recipient and Donor Characteristics

Recipients were 50.1 years old (mean, standard deviation (SD) 16) including 131 men and 73 women. Native kidney disease in the clinical files included IgA in 18 cases, other glomerulonephritis (GN) in 31, hypertensive nephropathy (NP) in 25, autosomal dominant polycystic kidney disease in 28, diabetic NP in 12, congenital renal dysplasia in 10, vesico-ureteral reflux in five, amyloidosis and focal-segmental glomerulosclerosis each in three, interstitial nephritis in two and hemolytic uremic syndrome, nephronophthisis, calcineurin-inhibitor toxicity and familial mediteranean fever in one each and was unknown in 62 patients.

The mean age of the donors was 51.1 years (SD 15.4) including 88 men and 103 women. 67/184 donors were smokers, 11/177 were diabetic, 56/178 had a history of arterial hypertension and 43/182 were proteinuric. 154 (75.5%) transplant kidneys derived from deceased (mean age 50.5 years, SD 17.5; 69 men/72 women; 57/136 smokers; 11/128 diabetic; 49/129 hypertensive, 43/135 proteinuric) and 50 (24.5%) from living donors (mean age 52.7 years, SD 6.5; 19 men/31 women; 10/48 smokers; 0/49 diabetic; 7/49 hypertensive, 0/47 proteinuric).

Composition of the Consecutive Cohort

Of 163 consecutive time zero biopsies performed between 06/2011 and 05/2013 161 were included for further analyses to estimate the prevalence of different immune patterns (**Figure 1A**). In this cohort, 88 biopsies (54.7%) showed no immune reactivity (negative), in 14 biopsies (8.7%) IgA was found, in 3 a fullhouse pattern (FH, 1.9%), in 14 (8.7%) moderate to strong C3 reactivity (C3high) and in 39 (24.2%) mild C3 positivity (C3low). In two (1.2%) biopsies a mesangial immune pattern was present not fitting one of the before described patterns (NOS, not otherwise specified) and in 1 biopsy (0.6%) positivity in a membranous pattern (MN) was present.

Extended Cohort and Persistence

In order to increase group sizes for further statistical analyses and for better comparability 43 additional cases with glomerular immune reactivity in the time zero biopsy were added, making a total of 204 cases (**Figure 1B**). Comparing the distribution of immune patterns between living and deceased donor specimens (**Figure 1B**), in 50 transplants from living donors 31 (62%) showed negative immunohistochemistry and none of the cases showed C3high reactivity as opposed to transplants of deceased donors, which showed negative immunohistochemistry in only 57/154 specimens (37%, $p = 0.002$) and C3high in 17 cases (11%, $p = 0.015$). In the other groups no significant differences between living and deceased donors were observed (all $p > 0.05$).

In total, in the extended cohort 88 negative cases, 28 IgA, 14 FH, 17 C3high, 50 C3low, six immune reactivity NOS and 1 MN were included (**Figure 2**). Immunohistochemical findings are shown in **Table 1**. Isolated mesangial IgM reactivity (sometimes accompanied by C1q) was present in the vast majority of biopsies (79.4%) and was interpreted as unspecific deposition. IgM was associated with higher glomerular filtration rates and lower creatinine values of the donors prior to explantation compared to cases without glomerular IgM ($p = 0.017$ and 0.035 in all biopsies and $p = 0.028$ and 0.036 assessing the negative group only, data not shown).

The cohort included 13 kidney pairs, which were transplanted to different recipients. In 12 of them the immune profile in the time zero biopsy was the same in both organs including one case with fullhouse, 3 with IgA, 3 with C3high, 2 with C3low reactivity and three negative for glomerular immune reactivity. In one case mild mesangial C3 reactivity (C3low) was seen in

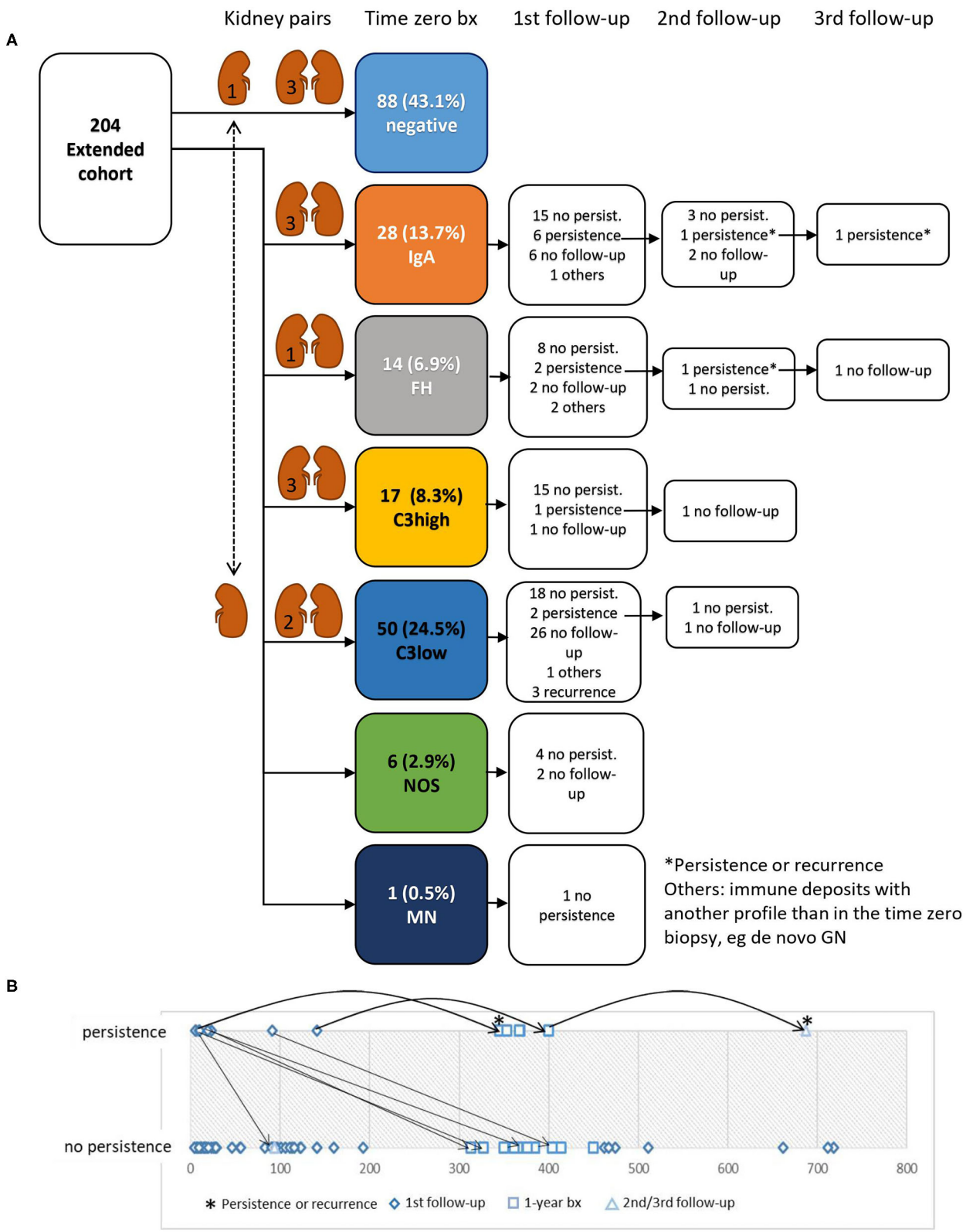

FIGURE 2 | Disappearance and persistence of immune reactivity in follow-up biopsies. **(A)** Of 204 specimens included in the analysis 116 showed glomerular immune reactivity exceeding the definition of negative. In 79 cases immunohistochemical follow-up analyses were performed. In the majority of cases immune reactivity had

(Continued)

FIGURE 2 | already vanished in the 1st follow-up biopsy. In two cases with persistence (asterisks) it was unclear whether immunoreactivity was due to persistence or a recurrence of the native kidney disease. The cohort included 13 kidney pairs transplanted to different recipients, which in 12 cases showed the same immune profile (indicated by pictograms of kidney pairs and the number of cases in the left kidney). In one kidney pair one biopsy showed a C3low pattern and the corresponding kidney biopsy was negative for immune reactivity. **(B)** Time course of persistence of glomerular immune reactivity. In the majority of 1st follow-up biopsies (diamond) and 1-year biopsies (square) no persistence was observed from the beginning. In four of the biopsies with persistence and first follow-up shortly after transplantation (<100 days) immune reactivity vanished in the 2nd or 1-year follow-up biopsies. The two cases with persistence even in the 2nd or 3rd follow-up (asterisks) were possible recurrences of the native kidney disease.

TABLE 1 | Immunohistochemical findings in time zero biopsies.

	All	Negative	IgA	FH	C3high	C3low	NOS	MN
IgA	42/204, 20.6%	0/88	28/28	14/14	0/17	0/50	0/6	0/1
IgG	23/204, 11.3%	0/88	5/28	14/14	0/17	0/50	3/6	1/1
IgM	162/204, 79.4%	66/88	25/28	14/14	12/17	39/50	5/6	1/1
C1q	30/204, 14.7%	8/88	2/28	14/14	0/17	0/50	6/6	0/1
C3	112/204, 54.9%	0/88	25/28	14/14	17/17	50/50	6/6	0/1
C4d	n.d.	n.d.	2/24	8/12	n.d.	n.d.	n.d.	n.d.
KM55	n.d.	n.d.	1/27	0/12	n.d.	n.d.	n.d.	n.d.

Data are shown as number of positive cases/number of total cases, percentage. n.d., not done.

one kidney, but not in the other organ, which was negative in immunohistochemistry (**Figure 2A**).

Immunohistochemical follow-up was performed in 79 cases (**Figure 2A**) with immune reactivity in time zero biopsies. Immunohistochemistry turned negative in 65 cases (**Figure 3**), in four cases another immune profile than observed in the time zero biopsy developed and in three cases recurrence of the native kidney disease, as documented in the clinical files, was observed. In seven cases the findings persisted without further follow-up available. One of these cases showed again IgA reactivity and had IgA nephropathy as native kidney disease and one showed a fullhouse pattern and had a diagnosis of systemic lupus erythematosus. Hence, in these two cases a recurrence of the native kidney disease was a possible differential diagnosis of persistence of the initial findings observed in the time zero biopsy.

Having a glance at the time-course of persistence of immune deposits one can appreciate that persistence was much more frequent in the period of time shortly after transplantation and became more infrequent at later points in time (**Figure 2B**).

In 6 follow-up biopsies with persistent immune reactivity electron microscopy was performed, to evaluate for the ultrastructural correlate of immunohistochemical findings. In two biopsies definite osmiophilic deposits were observed (**Supplementary Figure 1**), in two questionable traces of osmiophilic deposits and in two no deposits could be appreciated.

Clinical and Morphological Findings in Time Zero Biopsies With Glomerular Immune Reactivity

When comparing controls without immune reactivity with cases with IgA, FH, C3high or C3low no significant differences with regard to histomorphological parameters in time zero and 1-year biopsies were observed (**Supplementary Table 1**). Female donors were more common in the negative group ($p = 0.002$), and proteinuria in the donor was significantly more common in the C3high group ($p = 0.002$) compared to the remainder of analyzed groups. Donor age, creatinine, GFR and BMI were not associated with any one of the groups (**Table 2**). Moreover, no significant differences were seen with regard to adverse biopsy proven events in the 1st year (**Supplementary Table 1**) or creatinine and GFR in the first 5 years after transplantation (**Supplementary Table 2**, **Figure 4**).

C3high and C3low groups were separately analyzed as donor proteinuria was more frequent in donors with C3high deposits than in C3low cases ($p = 0.003$, data not shown).

When comparing all cases with glomerular immune reactivity to the control group, presence of glomerular immune reactivity was significantly associated with deceased and male donors ($p = 0.003$ and 0.001, respectively). Cold ischemia was significantly longer ($p = 0.024$) and donor creatinine higher ($p = 0.024$) in transplants with immune reactivity than in controls (**Table 2**). No differences were observed with regard to histological findings in time zero and 1-year biopsies, biopsy-proven adverse events, creatinine and GFR in the 5 years after transplantation or other donor parameters (**Tables 1**, **2**, **Supplementary Tables 1**, **2** and **Figure 4**).

DISCUSSION

In our experience, glomerular immune reactivity in time zero biopsies is a frequent finding. To substantiate this observation and to better understand its relevance for future organ function we analyzed a total of 204 time zero biopsies including 161 consecutive cases by immunohistochemistry.

Earlier studies mainly focusing on IgA in time zero biopsies reported frequencies of glomerular IgA-reactivity ranging from 6.9 to 32.1% (1–8). Our findings with 8.7% IgA-reactivity were in the lower range of these earlier reports. This may in part be a consequence of differences in the ethnicities in different cohorts

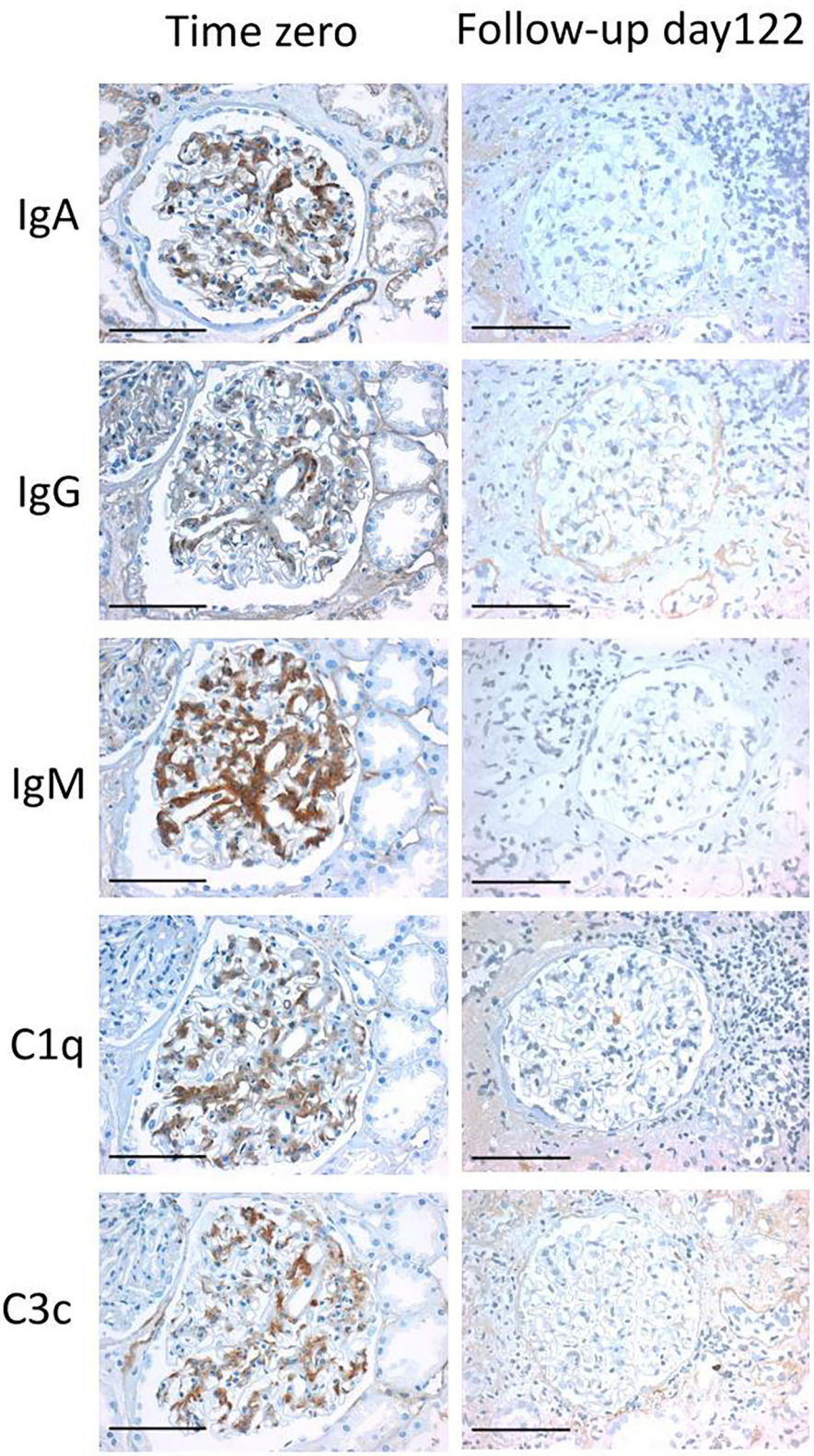

FIGURE 3 | Fullhouse pattern disappearing in the follow-up biopsy. An example of an immunohistochemical fullhouse pattern is depicted in a time zero biopsy (left column), which has completely disappeared in the follow-up biopsy at day 122 (right column). All light microscopic pictures were taken with an AxioCam MRc and an Imager.A1 Axio microscope (Zeiss, Germany) at an original magnification of 400x. Scale bars indicate 100 μm.

TABLE 2 | Comparison of donor parameters and ischemia time in immunohistochemical subgroups of time zero biopsies.

		Positive	Negative	IgA	FH	C3high	C3low	*p*-value association with positive/ negative	*p*-value association with negative/ IgA/FH/ C3high/ C3low
Donor parameters	Donor male/female	60/47	28/57	14/11	10/3	7/7	24/24	**0.001**	**0.016, negative 0.027**
	Living/deceased	19/88	31/54	8/17	1/12	0/14	10/38	**0.003**	0.013, p.h. n.s.
	Donor age	51 (6/82), 107	54 (1/86), 85	57 (28/82), 25	48.0 (8/66), 13	54.5 (22/79), 14	50 (14/75), 48	0.053	0.08
	Donor creatinine* (mg/dl)	0.89 (0.32/5.63), 98	0.8 (0.28-4.1), 79	0.9 (0.58/1.86), 25	0.96 (0.32/3.92), 9	0.99 (0.50/5.63), 14	0.8 (0.44/4.12), 44	**0.024**	0.059
	Donor GFR (ml/min)*	90.2 (13.16/135.13), 98	88.2 (17.51/158.79), 79	88.55 (36.48/119.87), 25	85.56 (19.97/134.86), 9	58.27 (13.16/135.13), 14	92.98 (17.53/118.96), 44	0.465	0.305
	Donor smoker (y/n)	40/62	27/56	6/18	6/6	3/11	22/23	0.347	0.123
	Donor proteinuria (y/n)	29/73	14/67	5/17	4/9	9/5	10/36	0.077	**0.005, C3 high 0.002**
	Donor BMI	26.12 (9.8/65.3), 99	26.3 (15.7/48.4), 81	27.0 (22/44), 25	26.01 (9.8/34.0), 10	25.68 (20.2/63.4), 14	26,2 (20.2/65.3), 44	0.437	0.809
	Donor DM (y/n)	8/89	3/78	1/20	1/11	2/12	2/42	0.21	0.561
	Donor aHT (y/n)	30/68	27/54	5/16	4/8	6/8	12/32	0.697	0.750
Ischemia	Cold (minutes)	664.5 (30/1438), 110	540 (26/1148), 86	492 (44/1438), 28	689 (368/860), 11	700.5 (345/1056), 16	664.5 (30/1192), 48	**0.024**	0.134
	Warm (minutes)	35 (16/121), 111	37 (23/90), 87	36.5 (16/91), 28	30 (19/121), 11	33 (24/55), 16	38 (16/92), 49	0.406	0.693

*Parameters are shown as either median (min/max), total number of analyzed cases or number of yes/no (y/n) cases, if not indicated otherwise. *before organ explantation. For the kidney pairs with the same immune profile only one was included into the statistical analysis of donor parameters. DM, diabetes mellitus; aHT, arterial hypertension; p.h.n.s., post hoc not significant. Data with a gray background show groups with significant differences (indicated in bold) after post-hoc testing.*

(8). Besides IgA-reactivity we frequently found glomerular C3 (32.9%), fullhouse patterns (1.9%) and mesangial immune-reactivity NOS (1.2%) as well as one case of membranous pattern (0.6%). We decided to separately assess cases with little mesangial C3 reactivity (C3low, 24.2%) and moderate to strong C3 reactivity (C3high, 8.7%), as in the latter donor proteinuria was significantly more common than in the C3low group, which could indicate a different significance of both findings. Interestingly, high levels of C3 (C3high) were only detected in time zero biopsies of deceased donors, whereas mild C3 reactivity was found with similar frequencies in kidneys from living and deceased donors. Proteinuria is an exclusion criterion for living donors, which could explain this finding. Additionally, the alternative complement pathway is activated by spontaneous hydrolysis (12). One could postulate that this mechanism might be augmented during the perimortal phase and transplantation procedure in deceased donors, which might trigger the activation of the complement pathway. The significance of glomerular C3 reactivity remains, however, unclear. It might indicate genuine and relevant complement activation in the donor or just an epiphenomenon of perimortal processes without pathophysiological relevance.

Mesangial reactivity for C3 and also IgM has been observed previously in time zero biopsies. Curschellas et al. reported glomerular C3 and IgM in 18.6 and 65.7%, respectively, which was not interpreted as glomerulonephritis and had no impact on serum creatinine 1 year after transplantation (7). Others reported C3 and IgM in 6.1 and 31.4% of time zero biopsies (3) and 39.4 and 52.6% of donor kidneys (8). In contrast, in a large study of necropsies including subjects, which had committed suicide or died of a violent death, mesangial IgM was present in only 2.5 and C3b in 0.2% (13). The reason why in our time zero biopsies the frequency of C3 deposits was very high may have something to do with the fact that C3 is of limited stability (14) and might get lost especially in the case of necropsies. Moreover, in contrast to the previous studies we applied immunohistochemistry on FFPE-material instead of immunofluorescence on frozen sections, frequently used before (3, 13). Isolated deposits of IgM and/or C1q (found in 79.4 and 14.7%) were not interpreted as a significant finding by us, as some degree of mesangial IgM is observed in the vast majority of renal biopsies [personal observation, (7)]. The molecular sizes of IgM-pentamers and C1q-protein complexes are very large (15, 16), so that both proteins might be prone to trapping in the mesangium. Our observation that mesangial IgM was associated with higher glomerular filtration rates in the donors might support this notion, meaning that with stronger filtration and increased transglomerular flow more protein can be trapped.

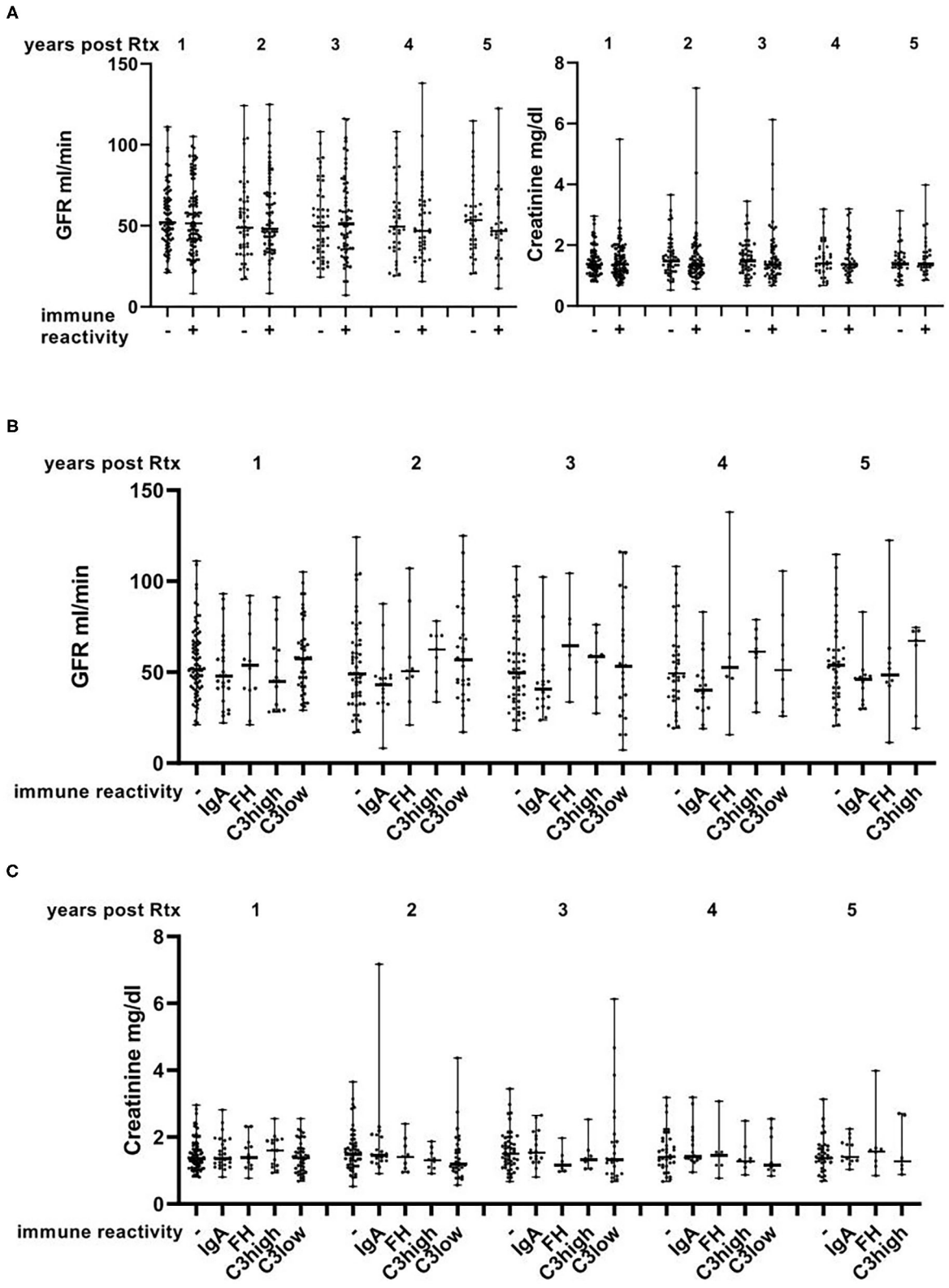

FIGURE 4 | Course of GFR and creatinine in the first 5 years after transplantation. No differences with regard to GFR and creatinine were observed between cases that were positive and negative for immune reactivity **(A)** or across the different assessed subgroups **(B,C)** and no apparent overall deterioration of the renal function. Data are shown with median and range.

In our cohort 13 kidney pairs were included, which were transplanted to different recipients. Interestingly, all but one pair showed the same immune profile in both time zero biopsies. In only one case one kidney showed negative findings whereas the other showed mild mesangial C3 deposits. This concordance in the vast majority of time zero biopsies implicates that the immune-reactivity observed is largely determined by donor parameters rather than by peri-transplantation circumstances. Accordingly, IgA deposits were observed in 4–10.8% (13, 17–19) of unpreselected renal specimens, which also argues that immune reactivity can be found in randomly selected native kidneys and is not a mere epiphenomenon of the transplantation procedure. Moreover, presence of immune reactivity was associated with deceased donor transplants and male gender of the donors, further substantiating the idea that donor parameters are relevant for the observed immune reactivity. The association of immune reactivity with longer cold ischemia may be a consequence of the increased numbers of deceased donors in this group and does not necessarily indicate that cold ischemia is causative for glomerular immune reactivity. Accordingly, in the group of deceased donors the presence or absence of immune reactivity was not significantly associated with cold ischemia ($p = 0.95$, data not shown).

Looking at the time-course of persistence of immune reactivity in follow-up biopsies, a positive result of immunohistochemistry was particularly common early after transplantation and in most cases disappeared in further follow-up biopsies, when available. This disappearance of glomerular immune reactivity over time goes well in line with earlier reports (4, 6–8) and might indicate a wash-out mechanism being operational. In two cases, persistence in more than one biopsy was observed. Intriguingly, in both cases the immune reactivity had an immune profile compatible with a recurrence of the native kidney disease, as reported in the clinical files, so that it was not possible to decide whether a true persistence or a recurrence was observed in the transplant. In fact, it was proposed that latent IgA deposits in time zero biopsies might predispose to IgA-recurrence in the transplant (5).

Immune reactivity in time zero biopsies neither influenced the histomorphological parameter in time zero and 1-year biopsies nor the number of biopsy-proven adverse events in the 1st year after renal transplantation. Moreover, no significant impact on 1 to 5-year follow-up with regard to graft function and survival was observed. Accordingly, no influence on 1-year creatinine was observed by Curschellas et al. (7) and also Sofue et al. did not observe a difference in renal function between cases with and without IgA deposits in time zero biopsies at 1 year (6). Another report stated that cases with mesangial IgA deposition were prone to delayed graft function and development of borderline changes when compared to controls, whereas graft survival at 1 and 3 years was similar in both groups (4). In this cohort, however, only cases with mesangial proliferation and marked IgA deposition where included, mitigating the comparability with our cohort (4).

Limitations of our study include the retrospective nature of the analyses as well as the relatively low numbers of cases included in the different subgroups, which we tried to compensate by extending the cohort. Time zero biopsies were collected before transplantation, therefore, prognostically adverse deposition of complement and immunoglobulins at a later point in time due to ischemia/reperfusion injury cannot be ruled out, as this would have been missed in our analyses. Native kidney disease in most cases with immune reactivity in time zero biopsies was usually not diagnosed in-house, so that one had to rely on the clinically reported diagnoses when evaluating for recurrence of native kidney disease. Moreover, we did not perform light chain immunohistochemistry, so that subtypes of glomerulonephritis with monoclonal immunoglobulin deposition might have been overlooked.

Taken together, we found glomerular immune reactivity to be a very frequent finding in time zero biopsies, which largely seems to be determined by donor parameters, maybe with some enhancement of the complement pathway in deceased donors. In the vast majority of cases this reactivity disappeared after transplantation. It had no impact on graft function or survival, not only in the 1st year as previously reported, but also in the long-run up to 5 years post renal transplantation and did not promote increased scarring of the transplant in 1 year biopsies. Therefore, it appears very justifiable to proceed with the current routine to transplant organs without testing for incidental glomerular immune reactivity before transplantation, as no increased risk of inferior outcome or raised need for closer follow-up or increased immunosuppression appears to be warranted.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the Friedrich-Alexander-University (Re.-No. 4415). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

EV collected and analyzed data, performed experiments, and edited the manuscript. SS collected analyzed data and performed experiments. CD collected and analyzed data and edited the manuscript. KA collected data and edited the manuscript. JS and BB collected clinical data and edited the manuscript. FF contributed to statistical analyses and edited the manuscript. KH and HA collected clinical data. MB-H initiated the study

and collected and analyzed data and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.656840/full#supplementary-material

Supplementary Figure 1 | Ultrastructural findings in persisting immune reactivity in follow-up biopsies. In the left column examples of persisting immune reactivity in follow-up biopsies are shown as indicated. The right column shows the corresponding ultrastructural findings with osmiophilic deposits indicated by arrows, which in the lower panel were very small. All light microscopic pictures were taken at an original magnification of 400x. Scale bars indicate 100 μm. Ultrastructural micrographs all in 5,000x original magnification.

Supplementary Table 1 | Comparison of biopsy findings in immunohistochemical subgroups of time zero biopsies.

Supplementary Table 2 | Comparison of follow-up parameters in immunohistochemical subgroups of time zero biopsies.

REFERENCES

1. Rosenberg H, Martinez P, Vaccarezza A, MartinezL. [A morphologic study of 103 kidneys donated for renal transplantation. *Rev Med Chil.* (1989) 117:1344–50.
2. Rosenberg HG, Martinez PS, Vaccarezza AS, MartinezLV. Morphological findings in 70 kidneys of living donors for renal transplant. *Pathol Res Pract.* (1990) 186:619–24. doi: 10.1016/S0344-0338(11)80225-6
3. Suzuki K, Honda K, Tanabe K, Toma K, Nihei H, YamaguchiY. Incidence of latent mesangial IgA deposition in renal allograft donors in Japan. *Kidney Int.* (2003) 63:2286–94. doi: 10.1046/j.1523-1755.63.6s.2.x
4. Ji S, Liu M, Chen J, Yin L, Sha G, Chen H, et al. The fate of glomerular mesangial IgA deposition in the donated kidney after allograft transplantation. *Clin Transplant.* (2004) 18:536–40. doi: 10.1111/j.1399-0012.2004.00206.x
5. Moriyama T, Nitta K, Suzuki K, Honda K, Horita S, Uchida K, et al. Latent IgA deposition from donor kidney is the major risk factor for recurrent IgA nephropathy in renal transplantation. *Clin Transplant.* (2005) 19(Suppl. 14):41–8. doi: 10.1111/j.1399-0012.2005.00403.x
6. Sofue T, Inui M, Hara T, Moritoki M, Nishioka S, Nishijima Y, et al. Latent IgA deposition from donor kidneys does not affect transplant prognosis, irrespective of mesangial expansion. *Clin Transplant.* (2013) 27 (Suppl. 26):14–21. doi: 10.1111/ctr.12158
7. Curschellas E, Landmann J, Durig M, Huser B, Kyo M, Basler V, et al. Morphologic findings in "zero-hour" biopsies of renal transplants. *Clin Nephrol.* (1991) 36:215–22.
8. Gaber LW, Khan FN, Graviss EA, Nguyen DT, Moore LW, Truong LD, et al. Prevalence, characteristics, and outcomes of incidental IgA glomerular deposits in donor kidneys. *Kidney Int Rep.* (2020) 5:1914–24. doi: 10.1016/j.ekir.2020.08.018
9. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Ann Int Med.* (2009) 150:604–12. doi: 10.7326/0003-4819-150-9-200905050-00006
10. Roufosse C, Simmonds N, Clahsen-van Groningen M, Haas M, Henriksen KJ, Horsfield C, et al. A 2018 reference guide to the banff classification of renal allograft pathology. *Transplantation.* (2018) 102:1795–14. doi: 10.1097/TP.0000000000002366
11. Beasley TM, Schumacker RE. Multiple regression approach to analyzing contingency tables: post hoc and planned comparison procedures. *J Exp Educ.* (1995) 64:79–93. doi: 10.1080/00220973.1995.9943797
12. Thurman JM, Nester CM. All things complement. *Clin J Am Soc Nephrol.* (2016) 11:1856–66. doi: 10.2215/CJN.01710216
13. Varis J, Rantala I, Pasternack A, Oksa H, Jantti M, Paunu ES, et al. Immunoglobulin and complement deposition in glomeruli of 756 subjects who had committed suicide or met with a violent death. *J Clin Pathol.* (1993) 46:607–10. doi: 10.1136/jcp.46.7.607
14. Larsen S. Glomerular immune deposits in kidneys from patients with no clinical or light microscopic evidence of glomerulonephritis. Assessment of the influence of autolysis on identification of immunoglobulins and complement. *Acta Pathol Microbiol Scand A.* (1979) 87:313–9. doi: 10.1111/j.1699-0463.1979.tb00058.x
15. Roberts-Thomson PJ, Shepherd K. Molecular size heterogeneity of immunoglobulins in health and disease. *Clin Exp Immunol.* (1990) 79:328–34. doi: 10.1111/j.1365-2249.1990.tb08091.x
16. Mii A, Shimizu A, Masuda Y, Fujita E, Aki K, Ishizaki M, et al. Current status and issues of C1q nephropathy. *Clin Exp Nephrol.* (2009) 13:263–74. doi: 10.1007/s10157-009-0159-5
17. Waldherr R, Rambausek M, Duncker WD, Ritz E. Frequency of mesangial IgA deposits in a non-selected autopsy series. *Nephrol Dial Transplant.* (1989) 4:943–6. doi: 10.1093/ndt/4.11.943
18. Sinniah R. Occurrence of mesangial IgA and IgM deposits in a control necropsy population. *J Clin Pathol.* (1983) 36:276–9. doi: 10.1136/jcp.36.3.276
19. Varis J, Rantala I, Pasternack A. Immunofluorescence of immunoglobulins and complement in kidneys taken at necropsy. *J Clin Pathol.* (1989) 42:1211–4. doi: 10.1136/jcp.42.11.1211

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REVIEW
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Epigenetics and Inflammation in Diabetic Nephropathy

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Diabetic nephropathy (DN) leads to high morbidity and disability. Inflammation plays a critical role in the pathogenesis of DN, which involves renal cells and immune cells, the microenvironment, as well as extrinsic factors, such as hyperglycemia, chemokines, cytokines, and growth factors. Epigenetic modifications usually regulate gene expression via DNA methylation, histone modification, and non-coding RNAs without altering the DNA sequence. During the past years, numerous studies have been published to reveal the mechanisms of epigenetic modifications that regulate inflammation in DN. This review aimed to summarize the latest evidence on the interplay of epigenetics and inflammation in DN, and highlight the potential targets for treatment and diagnosis of DN.

Keywords: diabetic nephropathy, epigenetics, DNA methylation, histone modifications, non-coding RNAs, inflammation

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INTRODUCTION

The latest Diabetes Atlas by the International Diabetes Federation indicates that the current number of patients with diabetes mellitus (DM) is 463 million in 2019, which is estimated to increase to 578 million by 2030 and to 700 million by 2045 (International Diabetes Federation, 2019). DM and its complications seriously affect patients' quality of life and result in tremendous socioeconomic burdens (GBD, 2017 Disease and injury incidence and prevalence collaborators, 2018; Lin et al., 2020b). Diabetic nephropathy (DN), one of the most common microvascular complications of DM, is the major contributor to chronic kidney disease (CKD) and end-stage renal disease (Ruiz-Ortega et al., 2020). Approximately 30–40% of DM patients gradually develop DN (Lim, 2014). Current therapies, including intensive glucose control and the treatment of hypertension through renin-angiotensin-aldosterone system (RAAS) blockers, only slow down the progression of DN and fail to reverse or stop it (Sanz et al., 2019; Ruiz-Ortega et al., 2020). Therefore, early diagnosis and novel treatment for DN are of great significance while recognizing its etiology remains urgent.

The biologist Conrad Waddington firstly introduced 'epigenetics' which describes a phenomenon of inheritance that is independent of DNA sequence (Russo et al., 1996; Goldberg et al., 2007). This concept has become one of the frontiers of genetic research over the years. Epigenetic modifications modulate gene expressions through DNA methylation, histone modification, and non-coding RNAs involving in the pathogenesis of DN (Keating and El-Osta, 2013; Reddy et al., 2015). Studies have also shown that the modifications are reversible indicating potential therapeutic value for DN (Hotamisligil, 2017; Kato and Natarajan, 2019). Low grade chronic inflammation is a major characteristic in the pathogenesis of DN, but the pathophysiological relevance between epigenetics and inflammation has not been

fully summarized. In this review, we highlighted recent epigenetic modifications relevant to inflammation and its signaling pathways in DN. The prespecified search strategies were shown in the **Supplementary Material 1**.

INFLAMMATION IN THE PROGRESSION OF DN

The Role of Renal Resident and Immune Cells in the Inflammatory Response

Hyperglycemia and glucose metabolites such as advanced glycation end products (AGEs) have long been regarded as initial factors of DN which promote the loss of podocytes, the hyperfiltration of endothelial cells, the expansion of mesangial cells and the thickening of glomerular basement membrane, and finally result in the deposition of extracellular matrix in the glomerulus (Schena and Gesualdo, 2005; Grabias and Konstantopoulos, 2014). The injured resident cells in kidney release chemokines and cytokines to attract the infiltration of immune cells (e.g., monocytes, macrophages, dendritic cells, and lymphocytes) (Tang and Yiu, 2020). Macrophages/monocytes are found to be the most predominant immune cells through both clinical and experimental studies. Previous study shows that macrophages are positively associated with pathological lesions in DN (Chow et al., 2004). A recent study of single cell RNA sequencing (scRNA-seq) indicates proportions of endothelial cells and immune cells are significantly increased while mesangial cells and podocytes are decreased in the glomerular cells in diabetic mouse kidney (Fu et al., 2019a). Among of immune cells in this study, macrophages are predominant, particularly M1 phenotype macrophages (Fu et al., 2019a). It has been also demonstrated that infiltration of macrophages in the glomeruli and tubulointerstitial tissues was increased in renal biopsies of patients (Klessens et al., 2017). In addition, the depletion of macrophages significantly reduces proteinuria and glomerular pathological changes in diabetic mice (You et al., 2013). The scRNA-seq analysis of kidney cortex from diabetic (n = 3) and non-diabetic patients (n = 3) shows patients in early diabetic nephropathy have 78 folds of leukocytes, including T cells, B cells, monocytes and plasma cells, compared to non-diabetic patients (Wilson et al., 2019). Few macrophages are observed in early diabetic kidneys (Wilson et al., 2019). Proportions of kidney cells and immune cells, and their roles at different stages of DN needed to be further studies. Epigenetic modifications in diabetic kidneys are shown in **Figure 1**.

The infiltration of macrophages is promoted by chemokines and adhesion molecules which are released from resident cells under the stimulation of high glucose and AGEs (Hickey and Martin, 2013). Notably, MCP-1 is an important mediator in the infiltration of macrophages and the progression of inflammation (Chow et al., 2006). The deletion of MCP-1 in mice and inhibition of MCP-1 in type 2 diabetic patients have been shown to improve renal function (Chow et al., 2006). Previous studies have shown that an increase in M1 macrophages is negatively associated with renal function (Wang et al., 2017), while the induction of M2 macrophages has been shown to attenuate renal damage in DN mouse model (Sun et al., 2015). High glucose and AGEs promote macrophages to M1 polarization and the release of inflammatory cytokines, such as tumor necrosis factor (TNF), contributing to pathogenesis in the early stage of diabetes (Webster et al., 1997). Additionally, macrophages can also act as myofibroblasts through the process of macrophage-myofibroblast transition (MMT) to deteriorate renal fibrosis, replace parenchyma tissue with (Tang et al., 2020b) extracellular matrix (ECM), and also contribute to the production of reactive oxygen species (ROS) and proteases (Meng et al., 2014; Torres et al., 2020). Orchestrated by TGF-β/Smad signaling pathway, MMT is a newly known fibrosis process which has been rarely found neither in acute inflammation, nor in normal kidney, indicating that chronic inflammation was the principle contributor to fibrosis (Meng et al., 2016; Tang et al., 2019). A recent study has found that brain-specific transcription factor POU4F1 is the only transcription factor taking part in the TGF-β1/Smad3-driven MMT and thus could be a new therapeutic target in chronic inflammation induced MMT fibrosis (Tang et al., 2020b). The proto-oncogene tyrosine protein kinase *SRC* presents as a direct SMAD3 target gene and is also essential for MMT in macrophages (Tang et al., 2018b). In general, the accumulation of macrophage are not only related to the degree of inflammation and kidney function, but also correlated to glomerulosclersis and the degree of interstitial fibrosis (Tang et al., 2019). Studies have shown that aberrant intrarenal infiltration and activation of T cells are involved in the pathogenesis of DN in both clinical samples and streptozotocin (STZ)-induced diabetes mice (Moon et al., 2012). Clinical findings show that T cell immunity and TNF-α signaling pathway are activated during the early development of DN in patients (Moon et al., 2012; Lampropoulou et al., 2020). The proportions of T helper cells (Th1, Th2, Th17 and regulatory T (Treg) cells) in DN are altered with the increased levels of Th1 and Th17, and the decreased level of Treg (Zhang et al., 2014). Adoptive transfer of CD4 + Foxp3 + Treg cells in mice have been found to ameliorate diabetic kidney injuries and insulin resistance by inhibiting inflammation (Eller et al., 2011).

The Role of Inflammatory Mediators and Signaling Pathways in DN

Several signaling pathways contribute to the inflammation and the release of inflammatory cytokines (**Figure 2**; Newton and Dixit, 2012). Interleukins (ILs) play critical roles in the regulation of the immune system. Studies have shown that the circulating level of IL-6 is positively correlated with the progression of DN in patients (Saraheimo et al., 2003), and IL-1β, IL-18, and IL-17A are associated with the occurrence and development of DN (Cortvrindt et al., 2017; Lemos et al., 2018; Lin et al., 2020a). TNF-α is involved in the development of various diseases, such as psoriasis, rheumatoid arthritis, and CKD (Elliott et al., 1994; Pina et al., 2016). Studies have demonstrated that macrophages are the main source of renal TNF-α (Awad et al., 2015). In diabetic mice, the inhibition of TNF-α leads to decreased urinary albumin excretion, and in a clinical trial where DN patients were treated with pentoxifylline,

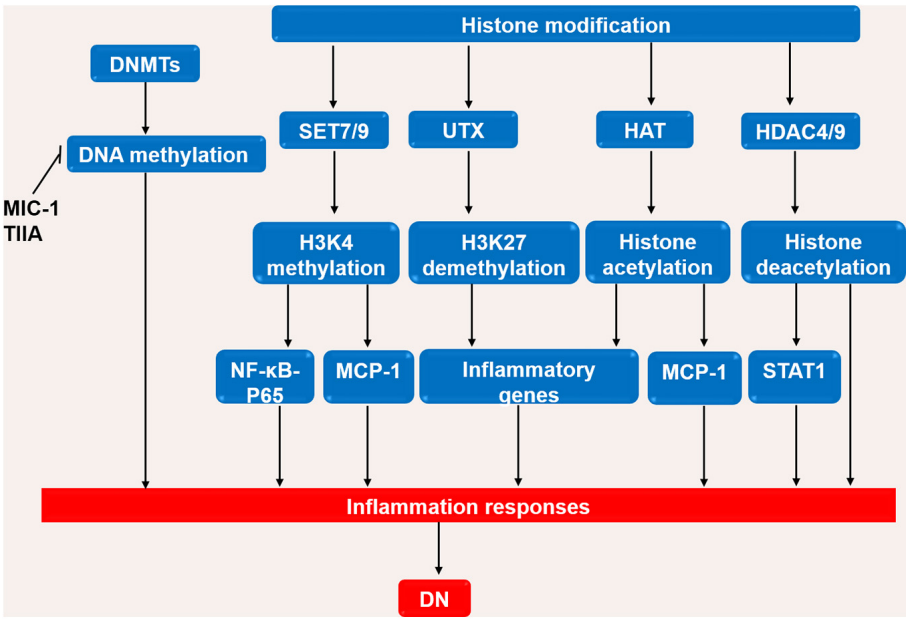

FIGURE 1 | Interaction of immune cells, kidney intrinsic cells and epigenetic modifications. DNA methylation, histone modifications, and non-coding RNA modifications activate inflammatory pathways by interactions of immune cells and kidney intrinsic cells. OS, oxidative stress; ROS, reactive oxygen species; HG, high glucose; Ang II, angiotensin II; AGE-RAGE, advanced glycation end-products-receptor for advanced glycation end products; IL, interleukin; TNF, tumor necrosis factor; MCP-1, monocyte chemoattractant protein 1; NF-κB, nuclear factor-κB; JAK-STAT, Janus kinase/signal transducer and activator of transcription; NRF2, Nuclear Factor-2 Erythroid Related Factor; NLRP, NOD-like receptor pyrin domain-containing protein.

a methylxanthine derivative with anti-inflammatory function, the reduction in urinary TNF-α concentration was directly correlated with the change in albuminuria, suggesting the role of TNF-α in the pathogenesis of DN (Moriwaki et al., 2007; Navarro-González et al., 2015).

Nuclear factor-κB (NF-κB) is the basic transcription factor that plays a pivotal role in inflammation in DN patients. Activated by upstream signals such as AGEs, angiotensin II, and oxidative stress (OS), NF-κB dissociates from its inhibitor IκB proteins and is transferred into the nucleus to regulate the expression of inflammatory gene including cytokines, chemokines, and adhesion molecules such as IL-6, TNF-α, and MCP-1 (Wada and Makino, 2016; Mikuda et al., 2018). One of the upstream signal pathways stimulated by AGEs is called the p38 mitogen-activated protein kinase (MAPK) pathway (Wu et al., 2002). The p38 MAPK pathway induces the activation of NF-κB in the infiltrating macrophages of DN (Adhikary et al., 2004). In turn, in renal parenchymal cells, elevated IL-1 and TNF-α have been shown to promote the phosphorylation of p38 MAPK, demonstrating their inflammatory roles in DN (Adhikary et al., 2004). Similarly, PI3K/AKT/mTOR is a widely studied signaling pathway that mediates the phenotype and injury of podocytes in DN. Stimulated by AGEs, PI3K/AKT can also promote NF-κB and aggravate inflammation (Ahmad et al., 2013; Hong et al., 2017). Recently, C-reactive protein (CRP) has been found to trigger a novel NF-κB-involved signaling pathway in the progression of DN, more narrowly, in human CRP transfected-db/db mice and cultured renal tubular epithelial cells, CRP is proved to promote inflammation via the evoking and dimerization of dipeptidyl peptidase-4 (DPP4) through DPP4/CD32b/NF-kB signaling circuit. The blockage of the circuit by the DPP4 inhibitor, linagliptin, attenuates DN, suggesting the potential therapeutic effect for DN (Tang et al., 2021).

TGF-β/SMAD signaling pathway plays a criticial role in diabetic kidney injuries (Chen et al., 2011, 2014a; Liu et al., 2011; Lan, 2012; Zhong et al., 2013; Li et al., 2014; Zhang et al., 2019b; Xu et al., 2020a; Yang et al., 2020). In the diabetic kidney, high glucose and AGEs enhance the phosphorylation of SMAD3 and decrease the phosphorylation SMAD7. SMAD3 deficiency prevents renal inflammation and fibrosis in SMAD3-db/db mice via regulations of lncRNA Erbb4-IR.transcription and miR-29b (Xu et al., 2020a). SMAD3 deficiency protects against diabetes-associated beta cell dysfunction and loss in DN mice (Sheng et al., 2021). SMAD3 also promotes autophagy dysregulation and kidney injury (Yang et al., 2020). SMAD7 inhibits IκBα, an NF-κB inhibitor, suppressing the activation of NF-κB pathway (Chung et al., 2009). The deletion of SMAD7 significantly aggravates renal inflammation as evidenced by the upregulation of IL-1β, TNF-α, and MCP-1 in diabetic mice by crosstalk with NF-κB pathway, and the addition of SMAD7 attenuates the kidney injuries (Chen et al., 2011). Thus, TGF-β/SMAD and NF-κB crosstalk pathway may act as a novel prevention and therapeutic targets for diabetic nephropathy.

Activation of OS signaling pathways contributes to renal inflammation in DN. Nuclear factor-2 erythroid related factor (NRF2) is a protein that has the ability to alleviate inflammation and act as an antioxidant mediator in the process

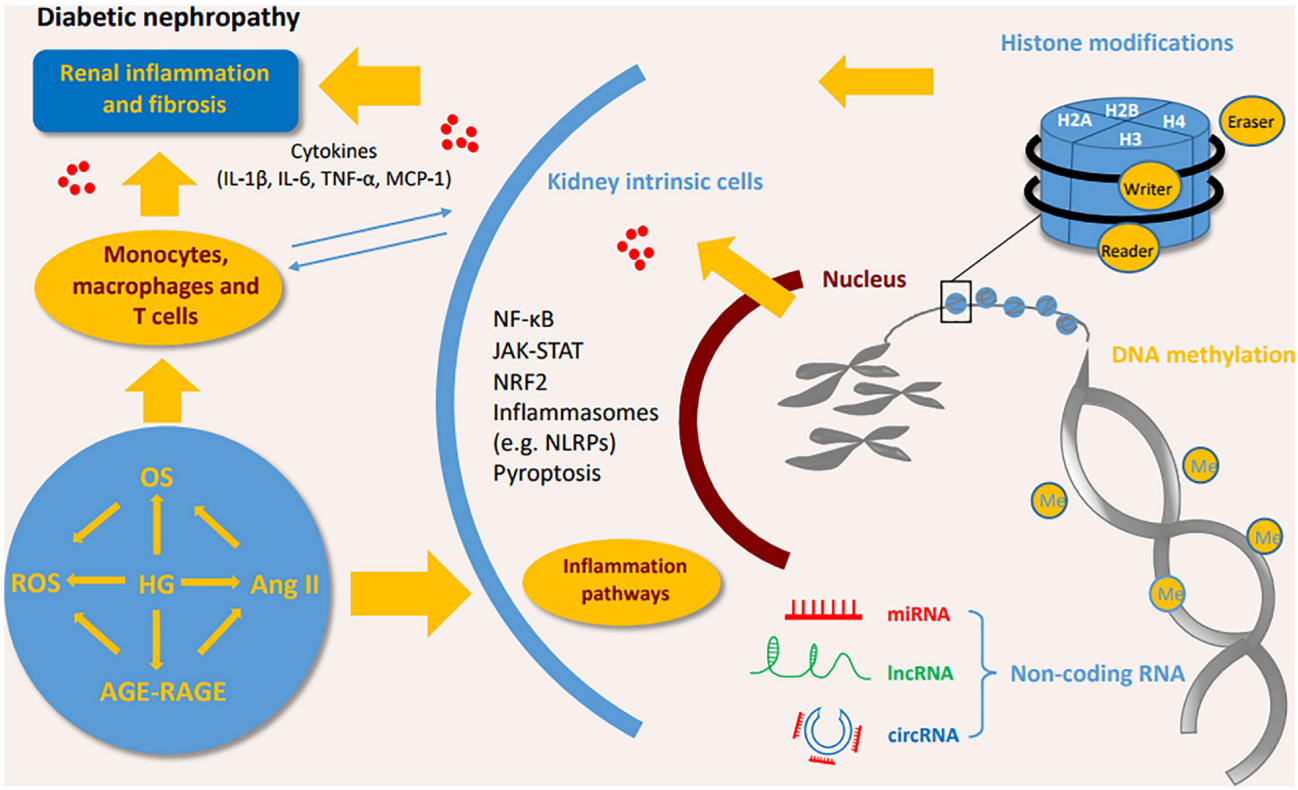

FIGURE 2 | The inflammatory pathways involved in the DN process. High glucose or stimulating factors cause the activation of multiple pathways, including P38/MAPK, PI3K/AKT, TLR4/NLRP3 and the novel CRP/DPP4/CD32b, which all further activate NF-κB pathway, and transcriptionally promote the expression of multiple inflammatory cytokines to enhance inflammation. SMAD7 can inhibit NF-κB activity. NRF2 has anti-inflammatory and antioxidant functions in DN. In addition, JAK/STAT pathway induces IL-6 to promote the inflammatory response in DN. DN, diabetic nephropathy; IL, interleukin; TNF, tumor necrosis factor; NF-κB, Nuclear factor-κB; MCP-1, monocyte chemoattractant protein-1; STAT, Signal transducer and activator of transcription; NRF2, Nuclear factor-2 erythroid related factor; NLRP3, NOD-like receptor protein 3; ROS, reactive oxygen species; DPP4, dipeptidyl peptidase-4; MAPK, mitogen-activated protein kinase; JAK2, Janus kinase; TLR4, Toll-like receptors 4; CRP, C-reactive protein; AGE, advanced glycation end product; RAGE, Receptor for advanced glycosylation end products; Ang II, angiotensin II; TGF, Transforming growth factor.

of OS. NRF2 reduces the infiltration of macrophages and proinflammatory cytokines by ameliorating oxidative overload. Clinical studies have demonstrated that the NRF2 activator, such as bardoxolone methyl, improves kidney function in diabetic patients (Pergola et al., 2011).

The nucleotide-binding oligomerization domain (NOD) family and NOD-like receptor pyrin domain-containing protein (NLRP) family are involved in DN. NOD2 promotes the endothelial-to-mesenchymal transition in DN (Shang et al., 2017). NLRP3 promotes the generation of IL-1β and IL-18 by activating the NLRP3-Caspase-1-IL-1β pathway in diabetic kidneys (Chi et al., 2020; Wang et al., 2020). NLRP3 has interactions with Toll-like receptors, ROS and NF-κB pathway to promote inflammation in DN (Shahzad et al., 2015; Wang et al., 2020).

The Janus kinase/signal transducer and activator of transcription (JAK-STAT) pathway is involved in processing extracellular signals (cytokines and chemokines) to the cell nucleus, resulting in gene expression (O'shea et al., 2013). The clinical findings show that JAK2 is increased in the podocytes of patients with early DN (Berthier et al., 2009). Podocyte-specific JAK2 overexpression in diabetic mice aggravates glomerulopathy while the inhibition of JAK1/2 attenuates the phenotypic changes of diabetic kidney (Zhang et al., 2017).

DN AND EPIGENETIC MODIFICATIONS INVOLVED IN INFLAMMATION

DNA Methylation Involved in Inflammation of DN

DNA methylation promotes inflammatory activation of immune cells in diabetic kidney disease and demethylating agents prevent the progressive kidney disease (Larkin et al., 2018; Chen et al., 2019b). DNA methylation is a process in which the methyl group of S-adenosylmethionine is transferred to the cytosine of DNA under the catalysis of DNA methyltransferases (DNMTs), resulting in down-regulation of the gene expression (Yagi et al., 2012). DNMTs mainly include DNMT1, DNMT3a, and DNMT3b. DNMT1 has been found to contribute to the maintenance of methylation and the other DNMTs are related

to *de novo* methylation (Hsieh, 1999). DNA methylation occurs specifically at the 5' site of the CpG dinucleotide cytosine residue, hindering the binding of transcription factors and promoters, subsequently inhibiting transcription (Yagi et al., 2012). The genome-wide DNA methylation analysis shows that DNA methylation is associated the kidney injuries and kidney inflammation in DN patients (VanderJagt et al., 2015; Park et al., 2019). *In vivo* study also indicates high-glucose induced high levels of methylation in kidney cells. It is found that there are 173 differentially methylated regions (DMRs) in high glucose (HG)-treated mesangial cells compared to the low-glucose (LG) treatment (Li et al., 2020d). Suppression of methylation by bioactive constituent extracted from plants, e.g., moringa isothiocyanate (MIC-1), potentially down-regulates expression of *TGF-β1*, and changes the *Nrf2, Col4a2, Tceal3, Ret, and Agt* expressions (Li et al., 2020d; Cheng et al., 2019).

Aberrant cytosine methylation of the upstream regulators of the mammalian target of rapamycin (mTOR) promotes inflammation by the upregulation of DNMT1 in DN (Chen et al., 2019b). Notably, DNA methylation is dynamic and can be altered by environmental factors. Studies have found that hyperglycemia in T2DM patients triggers a self-regulatory mechanism leading to the reduction of 5mC levels in the peripheral blood, which indicates that the DNA might undergo demethylation via the upregulation of ten-eleven-translocation 2 (TET2), a DNA demethylation enzyme (Yuan et al., 2019).

Histone Modifications Involved in Inflammation of DN

The nucleosome is the basic unit of chromatin consisting of DNA and wrapped histone proteins. The post-translational modifications (PTMs) on chromatin histone include acetylation, ubiquitination, phosphorylation, and methylation. Recently, the genome-wide analysis of chromatin binding proteins and histone modifications has been conducted through chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq) or by microarrays. The modifications are mainly mediated by three types of enzymes: writer, eraser, and reader. Writers/erasers carry on the modifications by adding/removing methyl or acetyl groups at amino acid residues in histone, such as histone acetyltransferase, histone methyltransferase (HMT), histone deacetylase (HDAC) and histone demethylase (HDM) (Bhatt et al.). Readers are the effectors that can identify and interpret post-translational modifications. Histone acetylation promotes gene transcription, while histone methylation promotes or inhibits gene transcription (Kouzarides, 2007). Specifically, the methylation of histone mostly happens on the residues of lysine and arginine. There are three types of methylation in lysine, namely monomethylation, dimethylation and trimethylation, and all three types of methylation of H3 at lysine 4 (H3K4me1, H3K4me2 and H3K4me3, respectively) exert an active effect (Kato and Natarajan, 2019). Similarly, H3K36me2 and H3K36me3 are enriched at transcriptional activation genome regions (Kato and Natarajan, 2019). Conversely, the methylation of H3K9me3, H3K27me3 and H4K20me3 are associated with gene repression (Kato and Natarajan, 2019). These modifications usually happen at promoters, insulators, enhancers, and other cis-regulatory regions, and finally lead to aberrant gene expression (Barski et al., 2007; Heintzman et al., 2009; Pradeepa et al., 2016).

Histone PTMs are involved in the pathogenesis of DN (Kato and Natarajan, 2019). HG and other danger signals increase the expression of pro-inflammatory genes by histone PTMs (Kato and Natarajan, 2019). *TXNIP*, pro-inflammatory gene, has been demonstrated to play an important role in the development of DN (Chen et al., 2008). In hyperglycemia-induced DN mice, HG-induced *Txnip* expression is associated with the enrichment of activated histone marks H3K9ac, H3K4me3, H3K4me1, and the repressive histone mark H3K27me3 at the promoter region of the gene, which has also been proved in human mesanginal cells (De Marinis et al., 2016). Furthermore, histone methylation take part in the process of inflammation via the secretion of inflammatory cytokines in diabetes. Specifically, H3K4 methylation could be mediated by HMT SET7 (Cheng et al., 2005). It is reported that transient HG causes the recruitment of HMT SET7 and increases H3K4 methylation at the NF-κB -P65 promoter, which promotes the expression of P65, MCP-1 and VCAM-1 in endothelial cells (El-Osta et al., 2008). Meanwhile, in endoplasmic reticulum (ER) stress induced kidney model of db/db mice, the increased expression of *Mcp-1* is associated with the enrichment of H3K4me1 at *Mcp-1* promoters, and could be significantly attenuated by the methyltransferase SET7/9 gene silencing (Chen et al., 2014c). The other study indicates that SET7/9 modifies chromatin histone lysine at promoters of *MCP-1*and *TNF-α* which promotes the inflammation in THP-1 monocytes (Li et al., 2008). In contrast, UTX (ubiquitously transcribed tetratricopeptide repeat, X chromosome) is a histone demethylase that can remove di- and tri-methyl groups from H3K27 (Choi et al., 2015). Studies have reported that the expression of UTX is upregulated in podocytes, tubular and mesangial cells of DN patients *in vitro* and *in vivo* (Majumder et al., 2018). Moreover, the knockout of UTX or the treatment of UTX inhibitor, GSK-J4, can reduce palmitic acid-induced increase of inflammation and DNA damage (Chen et al., 2019c). Furthermore, one study demonstrated that the inhibition of UTX could inhibit hypertrophy, a key event in glomerular dysfunction (Jia et al., 2019). In parallel, TGF-β down-regulates Enhancer of Zeste homolog 2 (EZH2), a H3K27me3 methyltransferase, by inducing miR-101b, which targets the 3'-untranslated region (3'-UTR) of *EZH2*. Meanwhile, TGF-β up-regulates UTX, a key role for H3K27me3 demethylases in renal mesangial cells. TGF-β-induced the inhibition of H3K27me3 augments pathological genes via dysregulation of associated histone-modifying enzymes and miR-101b in DN (Jia et al., 2019). Another H3K27me3 demethylase JMJD3 regulates inflammatory genes in macrophages (De Santa et al., 2007). To conclude, these studies suggest that the inhibition of H3K27me3 augments the expression of inflammation genes and the progression of DN.

Similarly, acetylation and deacetylation of histones via histone acetyltransferases (HATs) and histone deacetylase (HDACs) contribute to the pathogenesis of DN. Hyperglycemia promotes chromatin histone acetylation at inflammatory genes promoter regions and enhances inflammatory gene expressions *in vivo*

(Miao et al., 2004). Levels of H3K9ac, H3K9ac/S10p, H3K18ac, H3K23ac and H3K56ac are increased in the kidneys of db/db mice (Huang et al., 2015). Furthermore, the expression of *Sirt6* (a histone deacetylase) is reduced in podocytes of STZ-induced mice which results high levels of H3K9ac at promoters of *Notch1* and *Notch4*, and exacerbates the inflammation in kidney (Liu et al., 2017). The silencing of HDAC9 attenuates renal injuries as demonstrated by the decrease in glomerulosclerosis, inflammatory cytokines, and alteration of podocyte apoptosis (Liu et al., 2016). The elevated HDAC4 in diabetic kidney exacerbates inflammation via suppressing STAT1 signaling and the silencing of HDAC4 is associated with the decreases of cytokines (TNF-α, TGF-β, IL-8, MCP-1) (Wang et al., 2014). The roles of DNA methylation and histone modification in the DN process are briefly shown in **Figure 3**.

Non-coding RNAs Involved in Inflammation of DN

Non-coding RNAs (ncRNAs) commonly include transfer RNA, ribosomal RNA, long ncRNA (lncRNA), small ncRNA (e.g., microRNA, piRNAs, snoRNA, snRNA, exRNA) and circular RNA (circRNA) (Storz, 2002; Yang, 2015). Roles of microRNA (miRNA), lncRNA and circRNA in DN have been recently studied (Loganathan et al., 2020; Zhou et al., 2021). MiRNA is the best characterized non-coding RNA for transcriptional gene regulation by targeting the 3′-UTR of a specific mRNA. Typically, miRNAs exert their inhibitory actions on the gene via RNA silencing and translational repression (Wilczynska and Bushell, 2015).

MiRNAs play significant roles in regulating inflammation in DN (Zhou et al., 2021). Recent studies involving models of DN podocytes have found that downregulation of the miR-17∼92 cluster ameliorates inflammation and podocyte injury by targeting ABCA1 (ATP-binding cassette transporter A1) (Fan et al., 2020). Similarly, the inhibition of miR-21-5p in a macrophage-derived extracellular vesicle model could also exert podocyte protective effect by the restraint of inflammasome activation (Ding et al., 2020). Moreover, miRNAs are also found to regulate inflammation in renal tubular epithelial cells. The overexpression of miR-199a-3p improves the injury in high glucose induced HK-2 cell damage model, following with decreased IL-1, IL-6 and TNF-α level, which is also consistent with the clinical finding that miR-199a-3p is negatively correlated with the progression of DN (Zhang et al., 2020b). The protective effects of miR-199a-3p is via suppressing miR-199a-3p mediated IKKβ/NF-κB pathway (Zhang et al., 2020b). *In vitro* experiments, the overexpression of miR-26a-5p significantly inhibits the bovine serum albumin (BSA)-induced IL-6 and TNF-α expression in HK2 cells while the inhibition of miR-26a-5p promotes the expression of inflammatory cytokines (Li et al., 2020c). MiR-26a-5p is also found to activate NF-κB pathway by targeting on *CHAC1* and *TLR4* genes (Zhong et al., 2018;

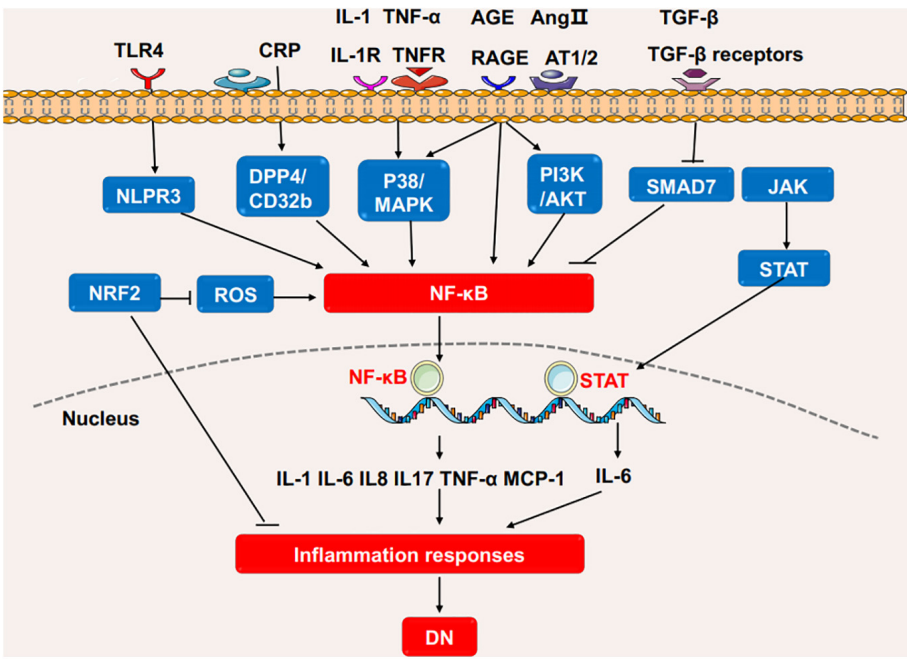

FIGURE 3 | The roles of DNA methylation and histone modification in the DN process. High glucose or stimulating factors cause DNA methylation and histone modification. DNA methylation is mainly regulated by DNMTs. MIC-1 and TIIA inhibits DNA methylation and reduces inflammation in DN. In the processes of histone modification, SET7/9 regulates H3K4 methylation, UTX regulates H3K27 demethylation, HAT promotes histone acetylation, and HDAC4/9 promotes histone deacetylation. The above processes regulate inflammatory genes, in turn affects the inflammatory response in DN. DN, diabetic nephropathy; DNMTs, DNA methyltransferases; UTX, ubiquitously transcribed tetratricopeptide repeat, X chromosome; HAT, histone acetyltransferases; HDAC, histone deacetylase; NF-κB, Nuclear factor-κB; MCP-1, monocyte chemoattractant protein-1; STAT1, Signal transducer and activator of transcription 1.

TABLE 1 | The target genes and potential mechanisms of miRNAs associated with inflammation in DN.

miR	Targeted Genes/Pathway	Inflammation Pathway/Related mediator	Sample	Model	Effect on Inflammation	References
miR-15b-5p	*Sema3A*	IL-1β, TNF-α, and IL-6.	Cells	Mouse podocytes	Alleviate	Fu et al., 2019b
miR-21	*Timp3*	Podocyte apoptosis	Rats	STZ-induced DN rats	Promote	Chen et al., 2018
			Cells	HG-treated podocytes		
miR-21	N/D	NF-κB	Mice	Db/db mice	Promote	Zhong et al., 2013
miR-29	*KEAP1*	SIRT1/NF-κB/microR-29/Keap1	Rats	STZ-induced DN rats	Alleviat	Zhou et al., 2015
			Cells	HG-induced injury in HK-2 cells		
miR-29b	*Sp1*	TNF-α, MCP-1/NF-κB	Mice	Db/db mice	Alleviate	Chen et al., 2014b
miR-31	N/D	The recruitment of leukocytes to vascular walls	Serum	DN patients	Alleviate	Rovira-Llopis et al., 2018
miR-126	*Vegf*	PI3K/AKT/mTOR IL-1β, IL-6, IL-18 and TNF-α	Rats	STZ-induced DN rats	Alleviate	Lou et al., 2020
			Cells	NRK52E		
miR-133	N/D	MAPK/ERK	Rats Cells	STZ-induced DN rats HG-induced injury in HK-2 cells	Promote	Shao et al., 2019
miR-140-5p	N/D	TLR4, NF-κB	Tissue	Kidney tissues from DN patients	Alleviate	Lou et al., 2020
			Cells	HG-induced injury in HK-2 cells		
miR-217	N/D	SIRT1/VEGF/HIF-1α	Serum	DN patients	Promote	Shao et al., 2017
miR-217	N/D	N/A	Cells	Rat glomerular mesangial cells	Promote	Shao et al., 2016
miR-218	N/D	NF-κB	Rats	Rat streptozotocin-induced model of DN	Alleviate	Li et al., 2020b
miR-218	*DACH1*	TNF-α and IL-1β	Cells	HG-induced injury in HK-2 cells	Peomote	Zhang et al., 2020c
miR-218	*GPRC5A*	N/A	Cells	HG-induced injury in HK-2 cells	Promote	Su et al., 2020
miR-325-3p	*CCL19*	N/A	Cells	HK-2 and human MC cells	Promote	Sun et al., 2020a
miR-328-3p	*Tlr4*	TLR4, NF-κB	Cells	MP5 cells	Alleviate	Duan et al., 2020
miR-34b	N/D	IL-6R/JAK2 /STAT3	Cells	HG-induced HK-2 cells	Promote	Lv et al., 2019
miR-451	*Lmp7*	NF-κB	Mice	Db/db mice	Alleviate	Sun et al., 2016
			Cells	HG-induced MCs		
miR-485	*NOX5*	N/A	Cells	Human MCs	Alleviate	Wu et al., 2020
miR-544	*Fasn*	NF-κB	Mice	Db/db mice	Alleviate	Sun et al., 2020b
miR-770-5p	*Timp3*	IL-1β, TNF-α	Cells	HG-induced mouse podocytes	Promote	Wang and Li, 2020
miR-874	N/D	TLR4	Rats	STZ-induced DN rats	Alleviate	Yao et al., 2019
			Cells	HG-induced podocytes		

N/D, not determined; N/A, not available; IL, interleukin; TNF, tumor necrosis factor; DN, diabetic nephropathy; STZ, streptozotocin; HG, High glucose; NF-κB, Nuclear factor-κB; SIRT1, Silent information regulator 1; KEAP1, Kelch-like ECH-associated protein 1; HK-2, human kidney 2; Sp1, specificity protein 1; MCP-1, monocyte chemoattractant protein-1; VEGF, vascular endothelial growth factor; HIF-1α, hypoxia-inducible factor-1α; NRK52E, rat kidney tubular epithelial cells; MCs, mesangial cells; TLR4, Toll-like receptors 4; MAPK/ERK, mitogen-activated protein kinase/extracellular signal-regulated kinase; JAK2, Janus kinase 2; STAT3, Signal transducer and activator of transcription 3.

Li et al., 2020c). MiR-155 and miR-146a have also been found to be correlated with renal damage, possibly due to the increased expression of TNF-α, TGF-β1, and NF-κB, and their roles in inflammation-mediated glomerular endothelial damage (Huang et al., 2014). Moreover, miRNAs regulate inflammation by modulating macrophage polarization. As mentioned before, macrophage M1 polarization act as an inflammation driver. In miR-146a deficiency diabetic mice, the expression of M1 markers is increased while the M2 response is diminished which is in accordance with the upregulated pro-inflammatory cytokines, suggesting the anti-inflammatory proporities of miR-146a (Bhatt et al., 2016). M2 macrophages ameliorate podocyte injury is related to miR-25-3p (Huang et al., 2020). It is found that autophagy deficiency in diabetic mice increases macrophage infiltration in proximal tubules (Ma et al., 2020), and the induction of miR-214 enhances the autophagy impairment, thus aggravating renal inflammation (Li et al., 2011). MiR-214 in monocytes is upregulated by AGEs, which in turn impairs the expression of the phosphatase and tensin homolog (PTEN) and delays spontaneous apoptosis of monocytes (Li et al., 2011). Additionally, miR-27a is downregulated by an adipokine, omentin-1, which alleviates inflammation and OS by directly targeting the 3′-UTR of *Nrf2* (Song et al., 2018). MiR-29b attenuates podocyte injury by targeting the 3′-UTR of HADC4 in DN (Gondaliya et al., 2020). MiR-125b has been found to inhibit the chromatin histone H3K9

TABLE 2 | The target genes and potential mechanisms of lncRNAs associated with inflammation in DN.

lncRNA	Targeted Axis/ Inflammation Pathway	Sample	Model	Effect on Inflammation	References
XIST	miR-485/*PSMB8*	Human	DN patients	Promote	Wang, 2020
		Cells	Human MCs		
RPPH1	*Gal-3/Mek/Erk*	Mice	Db/db mice	Promote	Zhang et al., 2019a
		Cells	HG-induced MCs		
NEAT1	miR-34c/NLRP3- CASPASE-1-IL-1β	Rats	STZ-induced DN rats	Alleviate	Zhan et al., 2020
		Cells	HBZY-1		
MEG3	miR-181a/*Egr-1*/TLR4 pathway	Rats	DN rat models	Promote	Zha et al., 2019
KCNQ1OT1	miR-506-3p/NLRP3-CASPASE-1-IL-1β	Cells	HG-induced HK-2 cells	Promote	Li et al., 2017
MALAT1	miR-23c/NLRP3-CASPASE-1-IL-1β	Rats Cells	STZ-induced DN rats HG-induced HK-2 cells	Promote	Li et al., 2017
Gm4419	NF-κB/NLRP3 inflammasome	Cells	HG-induced MCs	Promote	Yi et al., 2017
NON-HSAG053901	*Egr-1/TGF*-β	Mice Cells	STZ-induced mice Mesangial cells	Promote	Peng et al., 2019
HOTTIP	miR-455-3p/*WNT-2B*	Cells	HG-inducedSV40-MES13 cells and HK-2 cells	Promote	Zhu et al., 2019
GAS5	miR-452-5p/NLRP3-CASPASE-1-IL-1β	Cells	HK-2 cells	Alleviate	Xie et al., 2019
UCA1	miRNA-206	Rats	DN rat models	Alleviate	Yu et al., 2019
		Cells	HK-2 cells		
LRNA9884	*Mcp-1/Smad3*	Mice	Db/db	Promote	Zhang et al., 2019b
		Cells	Mouse tubular epithelial cells		

PSMB8, proteasome subunit beta type-8; DN, diabetic nephropathy; MCs, mesangial cells; HG, high glucose; STZ, streptozotocin; NLRP3, NOD-like receptor protein 3; IL, interleukin; STZ, streptozotocin; HBZY-1, Rat glomerular mesangial cell line; TLR4, Toll-like receptors 4; Egr-1, Early growth response protein 1; HK-2, human kidney tubular epithelial cell 2; WNT-2B, a protein of the Wnt signaling pathway.

methyltransferase to regulate inflammatory genes in diabetic mice (Villeneuve et al., 2010). Hyperglycemia induces miR-101b, which targets the EZH2, leading to mesangial dysfunction in DN (Jia et al., 2019).

Moreover, accumulating evidence shows that a lot of miRNAs are involved in the regulation of inflammation in DN as shown in **Table 1**.

LncRNAs also contribute to the development and progression of DN. LncRNA myocardial infarction associated transcript (MIAT) promotes hyperglycemia-induced podocyte inflammation by sponging miR-130a-3p and the regulation of TLR4 (Zhang et al., 2020a). LncRNA 4930556M19Rik has been found to protect against HG-induced podocyte damage by downregulation miR-27a-3p (Fan and Zhang, 2020). Macrophage-specific lncRNA_7949 mediates macrophage-induced kidney inflammation by the controlling of MCP-1 transcription through TLR4/NF-κB pathway (Lv et al., 2015). TGF-β/Smad3 transits the miRNA profile and promotes renal diseases via regulating transcriptional levels of non-coding RNAs. SMAD3-dependent lncRNAs have been recently uncovered in kidney diseases (Tang et al., 2018a, 2020a). LncRNA Erbb4-IR is responsible for TGF-β/Smad3-regulated renal fibrosis by inhibiting SMAD7 (Feng et al., 2018). It has been reported that lncRNA Erbb4-IR enhances diabetic kidney injury by mediating miR-29b in db/db Mice. Deletion of SMAD3 could down-regulate the lncRNA Erbb4-IR transcription, and therefore protect against renal injury in db/db mice (Sun et al., 2018). LRNA9884, a novel SMAD3-dependent lncRNA, is not only involved into NF-κB-mediated inflammatory responses by activation of macrophage migration inhibitory factor (MIF) in AKI, but also enhances diabetic renal injury via promoting MCP-1-dependent renal inflammation in db/db mice (Zhang et al., 2019b, 2020d; Xu et al., 2020a). The lncRNAs involved in the inflammation of DN are shown in **Table 2**.

CircRNAs regulate gene expressions by acting as sponges of miRNA (Kristensen et al., 2019), and play an important role in renal diseases (Jin et al., 2020). As a sponge of miR-135a, circRNA_010383 is markedly decreased in the kidney of db/db mice and HG-induced kidney resident cells, and overexpression of circRNA_010383 in kidney protects kidney from proteinuria and fibrosis in DN (Peng et al., 2021). CircLRP6, as a sponge of miR-205, activates TLR4/NF-κB pathway and induces inflammation in high glucose treated mesangial cells (Chen et al., 2019a). CircACTR2 induces inflammation and pyroptosis in high glucose treated renal tubular cells (Wen et al., 2020). Circ_0003928 attenuates the high glucose-induced inflammation in HK-2 cells by targeting miR-151-3p/Anxa2 (An et al., 2020). CircWBSCR17 aggravates inflammation and fibrosis in high glucose-induced HK-2 cells via miR-185-5p/SOX6 axis (Li et al., 2020a). Circ0000285 enhances inflammation via sponging miR-654-3p in high glucose treated podocytes and diabetic mouse kidney (Yao et al., 2020).

DISCUSSION

The current evidence reveals epigenetics (methylation, acetylation, and non-coding RNA modification) modulate inflammation via intrinsic cells, immune cells, and numerous inflammatory pathways in the development of DN. Persistent inflammation in DN promotes the renal fibrosis, thus resulting in CKD and even end-stage renal disease (Tang et al., 2020a). Anti-inflammatory therapy has long been considered to have enormous benefits for either the alleviation or the prevention of DN (Barutta et al., 2015). In this review, we summarized the evidence linking epigenetic modifications and inflammation in DN. Thus, it may be an effective approach to target these modifications for DN treatment. As for histone modification, the inhibition of HATs/HDACs provides as a class of new agents or therapeutic targets for the treatment of DN. Most of agents are non-selective inhibitors hindering the clinical application (Wang et al., 2014). Valproic acid is a specific HDAC1 inhibitor, which attenuates proteinuria, fibrosis, and inflammatory effects and even acute pancreatitis (Van Beneden et al., 2011; Jain et al., 2019). However, effects of specific HDAC inhibitors for DN remain largely unexplored.

LncRNAs have been considered the novel markers as well as the potential therapeutic targets, and novel drug delivery vehicles (e.g., exosome-ncRNAs). Metformin has been found to protect against inflammation and ECM accumulation in mesangial cells via the H19/miR-143-3p/TGF-β1 axis, suggesting that the H19/miR-143-3p/TGF-β1 axis could be a potential therapeutic target for the management of DN (Xu et al., 2020b). The competing endogenous RNA (ceRNA) network analysis on human miRNA indicates that RP11-363E7.4/TTN-AS1/HOTAIRM1-hsa-miR-106b-5p-PTGER3 and LINC00960-hsa-miR-1237-3p-MMP-2 interaction pairs are significant in diabetic kidney (Yu et al., 2021). Drugs such as iloprost, treprostinil, and captopril that target PTGER3 and MMP-2 might benefit patients with DN (Yu et al., 2021).

Intriguingly, several studies show miRNA-192 is upregulated in diabetic patients with microalbuminuria, but downregulated in macroalbumnuria compared to normalbuminuria (Krupa et al., 2010; Jia et al., 2016). However, another study shows that miR-192 is increased in DN patients with over proteinuria (ACT >300 mg/g) compared to microalbuminuria (Chien et al., 2016). These studies indicate miRNA-mediated epigenetic modifications may have various roles in different stages of a disease.

Besides DNA methylation, histone modification and non-coding RNA, RNA methylation plays an important role in the mRNA post-translational modification. For example, N6-methyladenosine (m^6A) methylation is the most chemically modified form of eukaryotic messenger RNA (mRNA) which modifies the adenosine at the 3'-UTR and the stop codon of a mRNA (Fu et al., 2014; Roundtree et al., 2017). Roles of epigenetic modifications are not fully elucidated. Recently, single nucleus ATCT-seq integrated with snRNA-seq has been used to detect the cell-type-specific chromatin accessibility which enable to deep understanding of cell heterogeneity in kidney (Bansal et al., 2020; Muto et al., 2021). It may provide a new approach to understand the epigentic modifications in DN.

Collectively, further studies are warranted to reveal the precise regulatory mechanisms in the different stages of DN as well as potential therapeutic targets and diagnostic biomarkers for DN.

AUTHOR CONTRIBUTIONS

H-YC and X-MM conceived, revised and edited the manuscript. B-YS and S-FZ collected studies and drafted the manuscript. H-DL assisted in data extraction and revised the manuscript. All authors approved the final version of the manuscript for publication.

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SUPPLEMENTARY MATERIAL

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REFERENCES

Adhikary, L., Chow, F., Nikolic-Paterson, D. J., Stambe, C., Dowling, J., Atkins, R. C., et al. (2004). Abnormal p38 mitogen-activated protein kinase signalling in human and experimental diabetic nephropathy. *Diabetologia* 47, 1210–1222. doi: 10.1007/s00125-004-1437-0

Ahmad, A., Biersack, B., Li, Y., Kong, D., Bao, B., Schobert, R., et al. (2013). Targeted regulation of PI3K/Akt/mTOR/NF-κB signaling by indole compounds and their derivatives: mechanistic details and biological implications for cancer therapy. *Anti Cancer Agents Med. Chem.* 13, 1002–1013. doi: 10.2174/18715206113139990078

An, L., Ji, D., Hu, W., Wang, J., Jin, X., Qu, Y., et al. (2020). Interference of Hsa_circ_0003928 alleviates high glucose-induced cell apoptosis and inflammation in HK-2 cells via miR-151-3p/Anxa2. *Diabetes Metab Syndr Obes* 13, 3157–3168. doi: 10.2147/dmso.s265543

Awad, A. S., You, H., Gao, T., Cooper, T. K., Nedospasov, S. A., Vacher, J., et al. (2015). Macrophage-derived tumor necrosis factor-α mediates diabetic renal injury. *Kidney Int.* 88, 722–733. doi: 10.1038/ki.2015.162

Bansal, A., Balasubramanian, S., Dhawan, S., Leung, A., Chen, Z., and Natarajan, R. (2020). Integrative omics analyses reveal epigenetic memory in diabetic renal cells regulating genes associated with kidney dysfunction. *Diabetes* 69, 2490–2502. doi: 10.2337/db20-0382

Barski, A., Cuddapah, S., Cui, K., Roh, T.-Y., Schones, D. E., Wang, Z., et al. (2007). High-resolution profiling of histone methylations in the human genome. *Cell* 129, 823–837. doi: 10.1016/j.cell.2007.05.009

Barutta, F., Bruno, G., Grimaldi, S., and Gruden, G. (2015). Inflammation in diabetic nephropathy: moving toward clinical biomarkers and targets for treatment. *Endocrine* 48, 730–742. doi: 10.1007/s12020-014-0437-1

Berthier, C. C., Zhang, H., Schin, M., Henger, A., Nelson, R. G., Yee, B., et al. (2009). Enhanced expression of a janus kinase–signal transducer and activator

of transcription pathway members in human diabetic nephropathy. *Diabetes* 58, 469–477. doi: 10.2337/db08-1328

Bhatt, K., Lanting, L. L., Jia, Y., Yadav, S., Reddy, M. A., Magilnick, N., et al. (2016). Anti-inflammatory role of microRNA-146a in the pathogenesis of diabetic nephropathy. *J. Am. Soc. Nephrol.* 27, 2277–2288. doi: 10.1681/asn.2015010111

Chen, B., Li, Y., Liu, Y., and Xu, Z. (2019a). circLRP6 regulates high glucose-induced proliferation, oxidative stress, ECM accumulation, and inflammation in mesangial cells. *J. Cell. Physiol.* 234, 21249–21259. doi: 10.1002/jcp.28730

Chen, G., Chen, H., Ren, S., Xia, M., Zhu, J., Liu, Y., et al. (2019b). Aberrant DNA methylation of mTOR pathway genes promotes inflammatory activation of immune cells in diabetic kidney disease. *Kidney Int.* 96, 409–420. doi: 10.1016/j.kint.2019.02.020

Chen, H., Huang, Y., Zhu, X., Liu, C., Yuan, Y., Su, H., et al. (2019c). Histone demethylase UTX is a therapeutic target for diabetic kidney disease. *J. Physiol.* 597, 1643–1660. doi: 10.1113/jp277367

Chen, H., Zhong, X., Huang, X. R., Meng, X. M., You, Y., Chung, A. C., et al. (2014a). MicroRNA-29b inhibits diabetic nephropathy in db/db mice. *Mol. Ther.* 22, 842–853. doi: 10.1038/mt.2013.235

Chen, H. Y., Huang, X. R., Wang, W., Li, J. H., Heuchel, R. L., Chung, A. C., et al. (2011). The protective role of Smad7 in diabetic kidney disease: mechanism and therapeutic potential. *Diabetes* 60, 590–601. doi: 10.2337/db10-0403

Chen, H. Y., Zhong, X., Huang, X. R., Meng, X. M., You, Y., Chung, A. C., et al. (2014b). MicroRNA-29b inhibits diabetic nephropathy in db/db mice. *Mol. Ther.* 22, 842–853.

Chen, J., Guo, Y., Zeng, W., Huang, L., Pang, Q., Nie, L., et al. (2014c). ER stress triggers MCP-1 expression through SET7/9-induced histone methylation in the kidneys of db/db mice. *Am. J. Physiol. R. Physiol.* 306, F916–F925.

Chen, J., Saxena, G., Mungrue, I. N., Lusis, A. J., and Shalev, A. (2008). Thioredoxin-interacting protein: a critical link between glucose toxicity and β-cell apoptosis. *Diabetes* 57, 938–944. doi: 10.2337/db07-0715

Chen, X., Zhao, L., Xing, Y., and Lin, B. (2018). Down-regulation of microRNA-21 reduces inflammation and podocyte apoptosis in diabetic nephropathy by relieving the repression of TIMP3 expression. *Biomed. Pharmacother.* 108, 7–14. doi: 10.1016/j.biopha.2018.09.007

Cheng, D., Gao, L., Su, S., Sargsyan, D., Wu, R., Raskin, I., et al. (2019). Moringa isothiocyanate activates Nrf2: potential role in diabetic nephropathy. *AAPS J.* 21:31.

Cheng, X., Collins, R. E., and Zhang, X. (2005). Structural and sequence motifs of protein (histone) methylation enzymes. *Annu. Rev. Biophys. Biomol. Struct.* 34, 267–294. doi: 10.1146/annurev.biophys.34.040204.144452

Chi, K., Geng, X., Liu, C., Cai, G., and Hong, Q. (2020). Research progress on the role of inflammasomes in kidney disease. *Med. Inf.* 2020:8032797.

Chien, H. Y., Chen, C. Y., Chiu, Y. H., Lin, Y. C., and Li, W. C. (2016). Differential microRNA profiles predict diabetic nephropathy progression in taiwan. *Int. J. Med. Sci.* 13, 457–465. doi: 10.7150/ijms.15548

Choi, H. J., Park, J. H., Park, M., Won, H. Y., Joo, H. S., Lee, C. H., et al. (2015). UTX inhibits EMT-induced breast CSC properties by epigenetic repression of EMT genes in cooperation with LSD 1 and HDAC 1. *EMBO Rep.* 16, 1288–1298. doi: 10.15252/embr.201540244

Chow, F., Nikolic-Paterson, D. J., Ozols, E., Atkins, R. C., Rollin, B., and Tesch, G. H. (2006). Monocyte chemoattractant protein-1 promotes the development of diabetic renal injury in streptozotocin-treated mice. *Kidney Int.* 69, 73–80. doi: 10.1038/sj.ki.5000014

Chow, F., Ozols, E., Nikolic-Paterson, D. J., Atkins, R. C., and Tesch, G. H. (2004). Macrophages in mouse type 2 diabetic nephropathy: correlation with diabetic state and progressive renal injury. *Kidney Int.* 65, 116–128. doi: 10.1111/j.1523-1755.2004.00367.x

Chung, A. C., Huang, X. R., Zhou, L., Heuchel, R., Lai, K. N., and Lan, H. Y. (2009). Disruption of the Smad7 gene promotes renal fibrosis and inflammation in unilateral ureteral obstruction (UUO) in mice. *Nephrol. Dialysis Transplant.* 24, 1443–1454. doi: 10.1093/ndt/gfn699

Cortvrindt, C., Speeckaert, R., Moerman, A., Delanghe, J. R., and Speeckaert, M. M. (2017). The role of interleukin-17A in the pathogenesis of kidney diseases. *Pathology* 49, 247–258. doi: 10.1016/j.pathol.2017.01.003

De Marinis, Y., Cai, M., Bompada, P., Atac, D., Kotova, O., Johansson, M. E., et al. (2016). Epigenetic regulation of the thioredoxin-interacting protein (TXNIP) gene by hyperglycemia in kidney. *Kidney Int.* 89, 342–353. doi: 10.1016/j.kint.2015.12.018

De Santa, F., Totaro, M. G., Prosperini, E., Notarbartolo, S., Testa, G., and Natoli, G. (2007). The histone H3 lysine-27 demethylase Jmjd3 links inflammation to inhibition of polycomb-mediated gene silencing. *Cell* 130, 1083–1094. doi: 10.1016/j.cell.2007.08.019

Ding, X., Jing, N., Shen, A., Guo, F., Song, Y., Pan, M., et al. (2020). MiR-21-5p in macrophage-derived extracellular vesicles affects podocyte pyroptosis in diabetic nephropathy by regulating A20. *J. Endocrinol. Invest.* Epub ahead of print,

Duan, Y., Luo, Q., Wang, Y., Ma, Y., Chen, F., Zhu, X., et al. (2020). Adipose mesenchymal stem cell-derived extracellular vesicles containing microRNA-26a-5p target TLR4 and protect against diabetic nephropathy. *J. Biol. Chem.* 295, 12868–12884. doi: 10.1074/jbc.ra120.012522

Eller, K., Kirsch, A., Wolf, A. M., Sopper, S., Tagwerker, A., Stanzl, U., et al. (2011). Potential role of regulatory T cells in reversing obesity-linked insulin resistance and diabetic nephropathy. *Diabetes* 60, 2954–2962. doi: 10.2337/db11-0358

Elliott, M. J., Maini, R. N., Feldmann, M., Kalden, J. R., Antoni, C., Smolen, J. S., et al. (1994). Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor α (cA2) versus placebo in rheumatoid arthritis. *Lancet* 344, 1105–1110. doi: 10.1016/s0140-6736(94)90628-9

El-Osta, A., Brasacchio, D., Yao, D., Pocai, A., Jones, P. L., Roeder, R. G., et al. (2008). Transient high glucose causes persistent epigenetic changes and altered gene expression during subsequent normoglycemia. *J. Exp. Med.* 205, 2409–2417. doi: 10.1084/jem.20081188

Fan, H., and Zhang, W. (2020). Overexpression of Linc 4930556M19Rik Suppresses High Glucose-Triggered Podocyte Apoptosis, Fibrosis and Inflammation via the miR-27a-3p/Metalloproteinase 3 (TIMP3) Axis in Diabetic Nephropathy. *Med. Sci. Monit. Int. Med. J. Exp. Clin. Res.* 26:e925361.

Fan, X., Hao, Z., Li, Z., Wang, X., and Wang, J. (2020). Inhibition of miR-17˜92 cluster ameliorates high glucose-induced podocyte damage. *Med. Inf.* 2020:6126490.

Feng, M., Tang, P. M.-K., Huang, X.-R., Sun, S.-F., You, Y.-K., Xiao, J., et al. (2018). TGF-β mediates renal fibrosis via the Smad3-Erbb4-IR long noncoding RNA axis. *Mol. Ther.* 26, 148–161. doi: 10.1016/j.ymthe.2017.09.024

Fu, J., Akat, K. M., Sun, Z. G., Zhang, W. J., Schlondorff, D., Liu, Z. H., et al. (2019a). Single-cell RNA profiling of glomerular cells shows dynamic changes in experimental diabetic kidney disease. *J. Am. Soc. Nephrol.* 30, 533–545. doi: 10.1681/asn.2018090896

Fu, Y., Dominissini, D., Rechavi, G., and He, C. (2014). Gene expression regulation mediated through reversible m 6 A RNA methylation. *Nat. Rev. Gene.* 15, 293–306. doi: 10.1038/nrg3724

Fu, Y., Wang, C., Zhang, D., Chu, X., Zhang, Y., and Li, J. (2019b). miR-15b-5p ameliorated high glucose-induced podocyte injury through repressing apoptosis, oxidative stress, and inflammatory responses by targeting Sema3A. *J. Cell. Physiol.* 234, 20869–20878. doi: 10.1002/jcp.28691

GBD. (2018). global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the global burden of disease study 2017. *Lancet* 392, 1789–1858.

Goldberg, A. D., Allis, C. D., and Bernstein, E. (2007). Epigenetics: a landscape takes shape. *Cell* 128, 635–638. doi: 10.1016/j.cell.2007.02.006

Gondaliya, P. A., Jash, K., Tekade, R. K., Srivastava, A., and Kalia, K. (2020). miR-29b attenuates histone deacetylase-4 mediated podocyte dysfunction and renal fibrosis in diabetic nephropathy. *J. Diabetes Metab Dis.*19, 13–27. doi: 10.1007/s40200-019-00469-0

Grabias, B. M., and Konstantopoulos, K. (2014). The physical basis of renal fibrosis: effects of altered hydrodynamic forces on kidney homeostasis. *Am. J. Physiol. R. Physiol.* 306, F473–F485.

Heintzman, N. D., Hon, G. C., Hawkins, R. D., Kheradpour, P., Stark, A., Harp, L. F., et al. (2009). Histone modifications at human enhancers reflect global cell-type-specific gene expression. *Nature* 459, 108–112. doi: 10.1038/nature07829

Hickey, F. B., and Martin, F. (2013). Diabetic kidney disease and immune modulation. *Curr. Opin. Pharmacol.* 13, 602–612. doi: 10.1016/j.coph.2013.05.002

Hong, J. N., Li, W. W., Wang, L. L., Guo, H., Jiang, Y., Gao, Y. J., et al. (2017). Jiangtang decoction ameliorate diabetic nephropathy through the regulation of PI3K/Akt-mediated NF-κB pathways in KK-Ay mice. *Chin. Med.* 12, 1–16.

Hotamisligil, G. S. (2017). Inflammation, metaflammation and immunometabolic disorders. *Nature* 542, 177–185. doi: 10.1038/nature21363

Hsieh, C. L. (1999). In vivo activity of murine de novo methyltransferases, Dnmt3a and Dnmt3b. *Mol. Cell. Biol.* 19, 8211–8218. doi: 10.1128/mcb.19.12.8211

Huang, H., Liu, H., Tang, J., Xu, W., Gan, H., Fan, Q., et al. (2020). M2 macrophage-derived exosomal miR-25-3p improves high glucose-induced podocytes injury through activation autophagy via inhibiting DUSP1 expression. *IUBMB Life* 72, 2651–2662. doi: 10.1002/iub.2393

Huang, J., Wan, D., Li, J., Chen, H., Huang, K., and Zheng, L. (2015). Histone acetyltransferase PCAF regulates inflammatory molecules in the development of renal injury. *Epigenetics* 10, 62–72. doi: 10.4161/15592294.2014.990780

Huang, Y., Liu, Y., Li, L., Su, B., Yang, L., Fan, W., et al. (2014). Involvement of inflammation-related miR-155 and miR-146a in diabetic nephropathy: implications for glomerular endothelial injury. *BMC Nephrol.* 15:142.

International Diabetes Federation (2019). *IDF DIABETES ATLAS*, 9th Edn. Available online at: https://diabetesatlas.org/en/ (accessed October 10, 2020).

Jain, A., Haque, I., Tayal, V., and Roy, V. (2019). Valproic acid-induced acute pancreatitis. *Indian J. Psychiatry* 61, 421–422. doi: 10.4103/psychiatry.indianjpsychiatry_383_18

Jia, Y., Guan, M., Zheng, Z., Zhang, Q., Tang, C., Xu, W., et al. (2016). miRNAs in urine extracellular vesicles as predictors of early-stage diabetic nephropathy. *J. Diabetes Res.* 2016:7932765. doi: 10.1155/2016/7932765

Jia, Y., Reddy, M. A., Das, S., Oh, H. J., Abdollahi, M., Yuan, H., et al. (2019). Dysregulation of histone H3 lysine 27 trimethylation in transforming growth factor-β1–induced gene expression in mesangial cells and diabetic kidney. *J. Biol. Chem.* 294, 12695–12707. doi: 10.1074/jbc.ra119.007575

Jin, J., Sun, H., Shi, C., Yang, H., Wu, Y., Li, W., et al. (2020). Circular RNA in renal diseases. *J. Cell. Mol. Med.* 24, 6523–6533.

Kato, M., and Natarajan, R. (2019). Epigenetics and epigenomics in diabetic kidney disease and metabolic memory. *Nat. Rev. Nephrol.* 15, 327–345. doi: 10.1038/s41581-019-0135-6

Keating, S. T., and El-Osta, A. (2013). Glycemic memories and the epigenetic component of diabetic nephropathy. *Curr. Diabetes Rep.* 13, 574–581. doi: 10.1007/s11892-013-0383-y

Klessens, C. Q., Zandbergen, M., Wolterbeek, R., Bruijn, J. A., Rabelink, T. J., Bajema, I. M., et al. (2017). Macrophages in diabetic nephropathy in patients with type 2 diabetes. *Nephrol. Dialysis Trans.* 32, 1322–1329.

Kouzarides, T. (2007). Chromatin modifications and their function. *Cell* 128, 693–705. doi: 10.1016/j.cell.2007.02.005

Kristensen, L. S., Andersen, M. S., Stagsted, L. V. W., Ebbesen, K. K., Hansen, T. B., and Kjems, J. (2019). The biogenesis, biology and characterization of circular RNAs. *Nat. Rev. Gene.* 20, 675–691.

Krupa, A., Jenkins, R., Luo, D. D., Lewis, A., Phillips, A., and Fraser, D. (2010). Loss of MicroRNA-192 promotes fibrogenesis in diabetic nephropathy. *J. Am. Soc. Nephrol.* 21, 438–447. doi: 10.1681/asn.2009050530

Lampropoulou, I. T., Stangou, M, Sarafidis, P., Gouliovaki, A., Giamalis, P., Tsouchnikas, I., et al. (2020). TNF-α pathway and T-cell immunity are activated early during the development of diabetic nephropathy in type II diabetes mellitus. *Clin. Immunol.* 215:108423. doi: 10.1016/j.clim.2020.108423

Lan, H. Y. (2012). Transforming growth factor-beta/smad signalling in diabetic nephropathy. *Clin. Exp. Pharmacol. Physiol.* 39, 731–738. doi: 10.1111/j.1440-1681.2011.05663.x

Larkin, B. P., Glastras, S. J., Chen, H., Pollock, C. A., and Saad, S. (2018). DNA methylation and the potential role of demethylating agents in prevention of progressive chronic kidney disease. *FASEB J.* 32, 5215–5226. doi: 10.1096/fj.201800205r

Lemos, D. R., Mcmurdo, M., Karaca, G., Wilflingseder, J., Leaf, I. A., Gupta, N., et al. (2018). Interleukin-1β activates a MYC-dependent metabolic switch in kidney stromal cells necessary for progressive tubulointerstitial fibrosis. *J. Am. Soc. Nephrol.* 29, 1690–1705. doi: 10.1681/asn.2017121283

Li, G., Qin, Y., Qin, S., Zhou, X., Zhao, W., and Zhang, D. (2020a). Circ_WBSCR17 aggravates inflammatory responses and fibrosis by targeting miR-185-5p/SOX6 regulatory axis in high glucose-induced human kidney tubular cells. *Life Sci.* 259:118269. doi: 10.1016/j.lfs.2020.118269

Li, L. M., Hou, D. X., Guo, Y. L., Yang, J. W., Liu, Y., Zhang, C. Y., et al. (2011). Role of microRNA-214–targeting phosphatase and tensin homolog in advanced glycation end product-induced apoptosis delay in monocytes. *J. Immunol.* 186, 2552–2560. doi: 10.4049/jimmunol.1001633

Li, M., Guo, Q., Cai, H., Wang, H., Ma, Z., and Zhang, X. (2020b). miR-218 regulates diabetic nephropathy via targeting IKK−β and modulating NK−κB-mediated inflammation. *J. Cell. Physiol.* 235, 3362–3371. doi: 10.1002/jcp.29224

Li, R., Chung, A. C., Yu, X., and Lan, H. Y. (2014). MicroRNAs in diabetic kidney disease. *Int. J. Endocrinol.* 2014:593956.

Li, S., Jia, Y., Xue, M., Hu, F., Zheng, Z., Zhang, S., et al. (2020c). Inhibiting Rab27a in renal tubular epithelial cells attenuates the inflammation of diabetic kidney disease through the miR-26a-5p/CHAC1/NF-kB pathway. *Life Sci.* 261:118347. doi: 10.1016/j.lfs.2020.118347

Li, S., Li, W., Wu, R., Yin, R., Sargsyan, D., Raskin, I., et al. (2020d). Epigenome and transcriptome study of moringa isothiocyanate in mouse kidney mesangial cells induced by high glucose, a potential model for diabetic-induced nephropathy. *AAPS J.* 22:8.

Li, X., Zeng, L., Cao, C., Lu, C., Lian, W., Han, J., et al. (2017). Long noncoding RNA MALAT1 regulates renal tubular epithelial pyroptosis by modulated miR-23c targeting of ELAVL1 in diabetic nephropathy. *Exp. Cell Res.* 350, 327–335. doi: 10.1016/j.yexcr.2016.12.006

Li, Y., Reddy, M. A., Miao, F., Shanmugam, N., Yee, J.-K., Hawkins, D., et al. (2008). Role of the histone H3 lysine 4 methyltransferase, SET7/9, in the regulation of NF-κB-dependent inflammatory genes: relevance to diabetes and inflammation. *J. Biol. Chem.* 283, 26771–26781. doi: 10.1074/jbc.m802800200

Lim, A. K. (2014). Diabetic nephropathy–complications and treatment. *Int. J. Nephrol. Renovascular Dis.* 7:361. doi: 10.2147/ijnrd.s40172

Lin, J., Cheng, A., Cheng, K., Deng, Q., Zhang, S., Lan, Z., et al. (2020a). New insights into the mechanisms of pyroptosis and implications for diabetic kidney disease. *Int. J. Mol. Sci.* 21:7057. doi: 10.3390/ijms21197057

Lin, X., Xu, Y., Pan, X., Xu, J., Ding, Y., Sun, X., et al. (2020b). Global, regional, and national burden and trend of diabetes in 195 countries and territories: an analysis from 1990 to 2025. *Sci. Rep.* 10:14790.

Liu, F., Chen, H. Y., Huang, X. R., Chung, A. C., Zhou, L., Fu, P., et al. (2011). C-reactive protein promotes diabetic kidney disease in a mouse model of type 1 diabetes. *Diabetologia* 54, 2713–2723. doi: 10.1007/s00125-011-2237-y

Liu, F., Zong, M., Wen, X., Li, X., Wang, J., Wang, Y., et al. (2016). Silencing of histone deacetylase 9 expression in podocytes attenuates kidney injury in diabetic nephropathy. *Sci. Rep.* 6:33676.

Liu, M., Liang, K., Zhen, J., Zhou, M., Wang, X., Wang, Z., et al. (2017). Sirt6 deficiency exacerbates podocyte injury and proteinuria through targeting Notch signaling. *Nat. Commun.* 8, 1–15. doi: 10.1016/j.biochi.2014.12.015

Loganathan, T. S., Sulaiman, S. A., Abdul Murad, N. A., Shah, S. A., Abdul Gafor, A. H., Jamal, R., et al. (2020). Interactions among non-coding RNAs in diabetic nephropathy. *Front. Pharmacol.* 11:191. doi: 10.3389/fphar.2020.0019

Lou, Z., Li, Q., Wang, C., and Li, Y. (2020). The effects of microRNA-126 reduced inflammation and apoptosis of diabetic nephropathy through PI3K/AKT signalling pathway by VEGF. *Arch. Physiol. Biochem.* 25, 1–10. doi: 10.1080/13813455.2020.1767146

Lv, L., Tang, P., You, Y. K., Huang, X., Liu, B.-C., and Lan, H.-Y. (2015). Long noncoding RNA-7949 regulates macrophage activation in renal inflammation via the TLR4/NF-KB pathway. *Hong Kong J. Nephrol.* 2:S76.

Lv, N., Li, C., Liu, X., Qi, C., and Wang, Z. (2019). miR-34b alleviates high glucose-induced inflammation and apoptosis in human HK-2 cells via IL-6R/JAK2/STAT3 signaling pathway. *Med. Sci. Monitor Int. Med. J. Exp. Clin. Res.* 25:8142. doi: 10.12659/msm.917128

Ma, Z., Li, L., Livingston, M. J., Zhang, D., Mi, Q., Zhang, M., et al. (2020). p53/microRNA-214/ULK1 axis impairs renal tubular autophagy in diabetic kidney disease. *J. Clin. Invest.* 130, 5011–5026. doi: 10.1172/jci135536

Majumder, S., Thieme, K., Batchu, S. N., Alghamdi, T. A., Bowskill, B. B., Kabir, M. G., et al. (2018). Shifts in podocyte histone H3K27me3 regulate mouse and human glomerular disease. *J. Clin. Invest.* 128, 483–499. doi: 10.1172/jci95946

Meng, X. M., Nikolic-Paterson, D. J., and Lan, H. Y. (2014). Inflammatory processes in renal fibrosis. *Nat. Rev. Nephrol.* 10, 493–503. doi: 10.1038/nrneph.2014.114

Meng, X. M., Wang, S., Huang, X. R., Yang, C., Xiao, J., Zhang, Y., et al. (2016). Inflammatory macrophages can transdifferentiate into myofibroblasts during renal fibrosis. *Cell Death Dis.* 7:e2495. doi: 10.1038/cddis.2016.402

Miao, F., Gonzalo, I. G., Lanting, L., and Natarajan, R. (2004). In vivo chromatin remodeling events leading to inflammatory gene transcription under diabetic conditions. *J. Biol. Chem.* 279, 18091–18097. doi: 10.1074/jbc.m311786200

Mikuda, N., Kolesnichenko, M., Beaudette, P., Popp, O., Uyar, B., Sun, W., et al. (2018). The IκB kinase complex is a regulator of mRNA stability. *EMBO J.* 37:e98658.

Moon, J. Y., Jeong, K. H., Lee, T. W., Ihm, C. G., Lim, S. J., and Lee, S. H. (2012). Aberrant recruitment and activation of T cells in diabetic nephropathy. *Am. J. Nephrol.* 35, 164–174. doi: 10.1159/000334928

Moriwaki, Y., Inokuchi, T., Yamamoto, A., Ka, T., Tsutsumi, Z., Takahashi, S., et al. (2007). Effect of TNF-α inhibition on urinary albumin excretion in experimental diabetic rats. *Acta Diabetologica* 44, 215–218. doi: 10.1007/s00592-007-0007-6

Muto, Y., Wilson, P., Wu, H., Waikar, S., and Humphreys, B. (2021). Single cell transcriptional and chromatin accessibility profiling redefine cellular heterogeneity in the adult human kidney. *Nat. Commun.* 12:2190. doi: 10.1038/s41467-021-22368-w

Navarro-González, J. F., Mora-Fernández, C., De Fuentes, M. M., Chahin, J., Méndez, M. L., Gallego, E., et al. (2015). Effect of pentoxifylline on renal function and urinary albumin excretion in patients with diabetic kidney disease: the PREDIAN trial. *J. Am. Soc. Nephrol.* 26, 220–229. doi: 10.1681/asn.2014010012

Newton, K., and Dixit, V. M. (2012). Signaling in innate immunity and inflammation. *Cold Spring Harbor Perspect. Biol.* 4:a006049.

O'shea, J. J., Kontzias, A., Yamaoka, K., Tanaka, Y., and Laurence, A. (2013). Janus kinase inhibitors in autoimmune diseases. *Ann. Rheumatic Dis.* 72:ii111. doi: 10.1136/annrheumdis-2012-202576

Park, J., Guan, Y., Sheng, X., Gluck, C., Seasock, M. J., Hakimi, A. A., et al. (2019). Functional methylome analysis of human diabetic kidney disease. *JCI Insight* 4:e128886.

Peng, F., Gong, W., Li, S., Yin, B., Zhao, C., Liu, W., et al. (2021). circRNA_010383 acts as a sponge for miR-135a, and its downregulated expression contributes to renal fibrosis in diabetic nephropathy. *Diabetes* 70, 603–615. doi: 10.2337/db20-0203

Peng, W. F., Huang, S., Shen, L. S., Tang, Y. B., Li, H. H., and Shi, Y. Q. (2019). Long noncoding RNA NONHSAG053901 promotes diabetic nephropathy via stimulating Egr-1/TGF-beta-mediated renal inflammation. *J. Cell. Physiol.* 234, 18492–18503. doi: 10.1002/jcp.28485

Pergola, P. E., Raskin, P., Toto, R. D., Meyer, C. J., Huff, J. W., Grossman, E. B., et al. (2011). Bardoxolone methyl and kidney function in CKD with type 2 diabetes. *New Engl. J. Med.* 365, 327–336.

Pina, T., Corrales, A., Lopez-Mejias, R., Armesto, S., Gonzalez-Lopez, M. A., Gómez-Acebo, I., et al. (2016). Anti-tumor necrosis factor-alpha therapy improves endothelial function and arterial stiffness in patients with moderate to severe psoriasis: a 6-month prospective study. *J. Dermatol.* 43, 1267–1272. doi: 10.1111/1346-8138.13398

Pradeepa, M. M., Grimes, G. R., Kumar, Y., Olley, G., Taylor, G. C., Schneider, R., et al. (2016). Histone H3 globular domain acetylation identifies a new class of enhancers. *Nat. Genet.* 48:681. doi: 10.1038/ng.3550

Reddy, M. A., Zhang, E., and Natarajan, R. (2015). Epigenetic mechanisms in diabetic complications and metabolic memory. *Diabetologia* 58, 443–455. doi: 10.1007/s00125-014-3462-y

Roundtree, I. A., Evans, M. E., Pan, T., and He, C. (2017). Dynamic RNA modifications in gene expression regulation. *Cell* 169, 1187–1200. doi: 10.1016/j.cell.2017.05.045

Rovira-Llopis, S., Escribano-Lopez, I., Diaz-Morales, N., Iannantuoni, F., Lopez-Domenech, S., Andújar, I., et al. (2018). Downregulation of miR-31 in diabetic nephropathy and its relationship with inflammation. *Cell. Physiol. Biochem.* 50, 1005–1014. doi: 10.1159/000494485

Ruiz-Ortega, M., Rayego-Mateos, S., Lamas, S., Ortiz, A., and Rodrigues-Diez, R. R. (2020). Targeting the progression of chronic kidney disease. *Nat. Rev. Nephrol.* 16, 269–288.

Russo, V. E., Martienssen, R. A., and Riggs, A. D. (1996). *Epigenetic Mechanisms of Gene Regulation*. New York, NY: Cold Spring Harbor Laboratory Press.

Sanz, A. B., Ramos, A. M., Soler, M. J., Sanchez-Niño, M. D., Fernandez-Fernandez, B., Perez-Gomez, M. V., et al. (2019). Advances in understanding the role of angiotensin-regulated proteins in kidney diseases. *Exp. Rev. Proteomics* 16, 77–92. doi: 10.1080/14789450.2018.1545577

Saraheimo, M., Teppo, A.-M., Forsblom, C., Fagerudd, J., Groop, P.-H., and Group, F. S. (2003). Diabetic nephropathy is associated with low-grade inflammation in type 1 diabetic patients. *Diabetologia* 46, 1402–1407. doi: 10.1007/s00125-003-1194-5

Schena, F. P., and Gesualdo, L. (2005). Pathogenetic mechanisms of diabetic nephropathy. *J. Am. Soc. Nephrol.* 16, S30–S33.

Shahzad, K., Bock, F., Dong, W., Wang, H., Kopf, S., Kohli, S., et al. (2015). Nlrp3-inflammasome activation in non-myeloid-derived cells aggravates diabetic nephropathy. *Kidney Int.* 87, 74–84. doi: 10.1038/ki.2014.271

Shang, J., Zhang, Y., Jiang, Y., Li, Z., Duan, Y., Wang, L., et al. (2017). NOD2 promotes endothelial-to-mesenchymal transition of glomerular endothelial cells via MEK/ERK signaling pathway in diabetic nephropathy. *Biochem. Biophys. Res. Commun.* 484, 435–441. doi: 10.1016/j.bbrc.2017.01.155

Shao, X., Kong, W. X., and Li, Y. T. (2019). MiR-133 inhibits kidney injury in rats with diabetic nephropathy via MAPK/ERK pathway. *Eur. Rev. Med. Pharmacol. Sci.* 23, 10957–10963.

Shao, Y., Lv, C., Wu, C., Zhou, Y., and Wang, Q. (2016). Mir-217 promotes inflammation and fibrosis in high glucose cultured rat glomerular mesangial cells via Sirt1/HIF-1α signaling pathway. *Diabetes Metab. Res. Rev.* 32, 534–543. doi: 10.1002/dmrr.2788

Shao, Y., Ren, H., Lv, C., Ma, X., Wu, C., and Wang, Q. (2017). Changes of serum Mir-217 and the correlation with the severity in type 2 diabetes patients with different stages of diabetic kidney disease. *Endocrine* 55, 130–138. doi: 10.1007/s12020-016-1069-4

Sheng, J., Wang, L., Tang, P. M., Wang, H. L., Li, J. C., Xu, B. H., et al. (2021). Smad3 deficiency promotes beta cell proliferation and function in db/db mice via restoring Pax6 expression. *Theranostics* 11, 2845–2859. doi: 10.7150/thno.51857

Song, J., Zhang, H., Sun, Y., Guo, R., Zhong, D., Xu, R., et al. (2018). Omentin-1 protects renal function of mice with type 2 diabetic nephropathy via regulating miR-27a-Nrf2/Keap1 axis. *Biomed. Pharmacother.* 107, 440–446. doi: 10.1016/j.biopha.2018.08.002

Storz, G. (2002). An expanding universe of noncoding RNAs. *Science* 296, 1260–1263. doi: 10.1126/science.1072249

Su, S.-S., Li, B.-P., Li, C.-L., Xiu, F.-R., Wang, D.-Y., and Zhang, F.-R. (2020). Downregulation of MiR-218 can alleviate high-glucose-induced renal proximal tubule injury by targeting GPRC5A. *Biosci. Biotechnol. Biochem.* 84, 1123–1130. doi: 10.1080/09168451.2020.1717330

Sun, H., Tian, J., Xian, W., Xie, T., and Yang, X. (2015). Pentraxin-3 attenuates renal damage in diabetic nephropathy by promoting M2 macrophage differentiation. *Inflammation* 38, 1739–1747. doi: 10.1007/s10753-015-0151-z

Sun, J., Wang, J., Lu, W., Xie, L., Lv, J., Li, H., et al. (2020a). MiR-325-3p inhibits renal inflammation and fibrosis by targeting CCL19 in diabetic nephropathy. *Clin. Exp. Pharmacol. Physiol.* 47, 1850–1860.

Sun, S. F., Tang, P. M., Feng, M., Xiao, J., Huang, X. R., Li, P., et al. (2018). Novel lncRNA Erbb4-IR promotes diabetic kidney injury in db/db mice by targeting miR-29b. *Diabetes* 67, 731–744. doi: 10.2337/db17-0816

Sun, T., Liu, Y., Liu, L., and Ma, F. (2020b). MicroRNA-544 attenuates diabetic renal injury via suppressing glomerulosclerosis and inflammation by targeting FASN. *Gene* 723:143986. doi: 10.1016/j.gene.2019.143986

Sun, Y., Peng, R., Peng, H., Liu, H., Wen, L., Wu, T., et al. (2016). miR-451 suppresses the NF-kappaB-mediated proinflammatory molecules expression through inhibiting LMP7 in diabetic nephropathy. *Mol. Cell. Endocrinol.* 433, 75–86. doi: 10.1016/j.mce.2016.06.004

Tang, P. C.-T., Zhang, Y.-Y., Chan, M. K.-K., Lam, W. W.-Y., Chung, J. Y.-F., Kang, W., et al. (2020a). The emerging role of innate immunity in chronic kidney diseases. *Int. J. Mol. Sci.* 21:4018. doi: 10.3390/ijms21114018

Tang, P. M. K., Nikolic-Paterson, D. J., and Lan, H. Y. (2019). Macrophages: versatile players in renal inflammation and fibrosis. *Nat. Rev. Nephrol.* 15, 144–158. doi: 10.1038/s41581-019-0110-2

Tang, P. M. K., Zhang, Y. Y., Hung, J. S. C., Chung, J. Y. F., Huang, X. R., To, K. F., et al. (2021). DPP4/CD32b/NF-κB Circuit: a novel druggable target for inhibiting CRP-driven diabetic nephropathy. *Mol. Ther.* 29, 365–375. doi: 10.1016/j.ymthe.2020.08.017

Tang, P. M. K., Zhang, Y. Y., Mak, T. S. K., Tang, P. C. T., Huang, X. R., and Lan, H. Y. (2018a). Transforming growth factor−β signalling in renal fibrosis: from Smads to non-coding RNAs. *J. Physiol.* 596, 3493–3503. doi: 10.1113/jp274492

Tang, P. M. K., Zhang, Y. Y., Xiao, J., Tang, P. C. T., Chung, J. Y. F., Li, J., et al. (2020b). Neural transcription factor Pou4f1 promotes renal fibrosis via macrophage–myofibroblast transition. *Proc. Natl. Acad. Sci.* 117, 20741–20752. doi: 10.1073/pnas.1917663117

Tang, P. M. K., Zhou, S., Li, C. J., Liao, J., Xiao, J., Wang, Q. M., et al. (2018b). The proto-oncogene tyrosine protein kinase Src is essential for macrophage-myofibroblast transition during renal scarring. *Kidney Int.* 93, 173–187. doi: 10.1016/j.kint.2017.07.026

Tang, S. C. W., and Yiu, W. H. (2020). Innate immunity in diabetic kidney disease. *Nat. Rev. Nephrol.* 16, 206–222. doi: 10.1038/s41581-019-0234-4

Torres, Á, Muñoz, K., Nahuelpán, Y., R Saez, A.-P., Mendoza, P., Jara, C., et al. (2020). Intraglomerular monocyte/macrophage infiltration and macrophage–myofibroblast transition during diabetic nephropathy is regulated by the A2B adenosine receptor. *Cells* 9:1051. doi: 10.3390/cells9041051

Van Beneden, K., Geers, C., Pauwels, M., Mannaerts, I., Verbeelen, D., Van Grunsven, L. A., et al. (2011). Valproic acid attenuates proteinuria and kidney injury. *J. Am. Soc. Nephrol.* 22, 1863–1875. doi: 10.1681/asn.2010111196

VanderJagt, T. A., Neugebauer, M. H., Morgan, M., Bowden, D. W., and Shah, V. O. (2015). Epigenetic profiles of pre-diabetes transitioning to type 2 diabetes and nephropathy. *World J. Diabetes* 6:1113. doi: 10.4239/wjd.v6.i9.1113

Villeneuve, L. M., Kato, M., Reddy, M. A., Wang, M., Lanting, L., and Natarajan, R. (2010). Enhanced levels of microRNA-125b in vascular smooth muscle cells of diabetic db/db mice lead to increased inflammatory gene expression by targeting the histone methyltransferase Suv39h1. *Diabetes* 59, 2904–2915. doi: 10.2337/db10-0208

Wada, J., and Makino, H. (2016). Innate immunity in diabetes and diabetic nephropathy. *Nat. Rev. Nephrol.* 12:13. doi: 10.1038/nrneph.2015.175

Wang, J., Shen, X., Liu, J., Chen, W., Wu, F., Wu, W., et al. (2020). High glucose mediates NLRP3 inflammasome activation via upregulation of ELF3 expression. *Cell Death Dis.* 11, 1–14.

Wang, L., and Li, H. (2020). MiR-770-5p facilitates podocyte apoptosis and inflammation in diabetic nephropathy by targeting TIMP3. *Biosci. Rep.* 40:BSR20193653.

Wang, Q. (2020). XIST silencing alleviated inflammation and mesangial cells proliferation in diabetic nephropathy by sponging miR-485. *Arch. Physiol. Biochem.* 15, 1–7. doi: 10.1080/13813455.2020.1789880

Wang, X., Liu, J., Zhen, J., Zhang, C., Wan, Q., Liu, G., et al. (2014). Histone deacetylase 4 selectively contributes to podocyte injury in diabetic nephropathy. *Kidney Int.* 86, 712–725. doi: 10.1038/ki.2014.111

Wang, X., Yao, B., Wang, Y., Fan, X., Wang, S., Niu, A., et al. (2017). Macrophage cyclooxygenase-2 protects against development of diabetic nephropathy. *Diabetes* 66, 494–504. doi: 10.2337/db16-0773

Webster, L., Abordo, E. A., Thornalley, P. J., and Limb, G. A. (1997). Induction of TNFα and IL-1β mRNA in monocytes by methylglyoxal-and advanced glycated endproduct-modified human serum albumin. *Biochem. Soc. Trans.* 25:250S. doi: 10.1042/bst025250s

Wen, S., Li, S., Li, L., and Fan, Q. (2020). circACTR2: a novel mechanism regulating high glucose-induced fibrosis in renal tubular cells via pyroptosis. *Biol. Pharm. Bull.* 43, 558–564. doi: 10.1248/bpb.b19-00901

Wilczynska, A., and Bushell, M. (2015). The complexity of miRNA-mediated repression. *Cell Death Differ.* 22, 22–33. doi: 10.1038/cdd.2014.112

Wilson, P. C., Wu, H. J., Kirita, Y., Uchimura, K., Ledru, N., Rennke, H. G., et al. (2019). The single-cell transcriptomic landscape of early human diabetic nephropathy. *Proc. Natl. Acad. Sci. U.S.A.* 116, 19619–19625. doi: 10.1073/pnas.1908706116

Wu, C. H., Huang, C. M., Lin, C. H., Ho, Y. S., Chen, C. M., and Lee, H. M. (2002). Advanced glycosylation end products induce NF-κB dependent iNOS expression in RAW 264.7 cells. *Mol. Cell. Endocrinol.* 194, 9–17. doi: 10.1016/s0303-7207(02)00212-5

Wu, J., Lu, K., Zhu, M., Xie, X., Ding, Y., Shao, X., et al. (2020). miR-485 suppresses inflammation and proliferation of mesangial cells in an in vitro model of diabetic nephropathy by targeting NOX5. *Biochem. Biophys. Res. Commun.* 521, 984–990. doi: 10.1016/j.bbrc.2019.11.020

Xie, C., Wu, W., Tang, A., Luo, N., and Tan, Y. (2019). lncRNA GAS5/miR-452-5p reduces oxidative stress and pyroptosis of high-glucose-stimulated renal tubular cells. *Diabetes Metab. Syndrome Obes. Targets Ther.* 12:2609. doi: 10.2147/dmso.s228654

Xu, B. H., Sheng, J., You, Y. K., Huang, X. R., Ma, R. C. W., Wang, Q., et al. (2020a). Deletion of Smad3 prevents renal fibrosis and inflammation in type 2 diabetic nephropathy. *Metabolism* 103:154013. doi: 10.1016/j.metabol.2019.154013

Xu, J., Xiang, P., Liu, L., Sun, J., and Ye, S. (2020b). Metformin inhibits extracellular matrix accumulation, inflammation and proliferation of mesangial cells in diabetic nephropathy by regulating H19/miR-143-3p/TGF−β1 axis. *J. Pharmacy Pharmacol.* 72, 1101–1109. doi: 10.1111/jphp.13280

Yagi, S., Hirosawa, M., and Shiota, K. (2012). DNA methylation profile: a composer-, conductor-, and player-orchestrated mammalian genome consisting of genes and transposable genetic elements. *J. Reproduct. Deve.* 58, 265–273. doi: 10.1262/jrd.2011-030

Yang, C., Chen, X. C., Li, Z. H., Wu, H. L., Jing, K. P., Huang, X. R., et al. (2020). SMAD3 promotes autophagy dysregulation by triggering lysosome depletion in tubular epithelial cells in diabetic nephropathy. *Autophagy* Epub ahead of print,

Yang, L. (2015). Splicing noncoding RNAs from the inside out. *Wiley Interdisciplinary Rev. RNA* 6, 651–660. doi: 10.1002/wrna.1307

Yao, T., Zha, D., Gao, P., Shui, H., and Wu, X. (2019). MiR-874 alleviates renal injury and inflammatory response in diabetic nephropathy through targeting toll-like receptor-4. *J. Cell. Physiol.* 234, 871–879. doi: 10.1002/jcp.26908

Yao, T., Zha, D., Hu, C., and Wu, X. (2020). Circ_0000285 promotes podocyte injury through sponging miR-654-3p and activating MAPK6 in diabetic nephropathy. *Gene* 747:144661. doi: 10.1016/j.gene.2020.144661

Yi, H., Peng, R., Zhang, L. Y., Sun, Y., Peng, H. M., Liu, H. D., et al. (2017). LincRNA-Gm4419 knockdown ameliorates NF-kappa B/NLRP3 inflammasome-mediated inflammation in diabetic nephropathy. *Cell Death Dis.* 8:e2583. doi: 10.1038/cddis.2016.451

You, H., Gao, T., Cooper, T. K., Brian Reeves, W., and Awad, A. S. (2013). Macrophages directly mediate diabetic renal injury. *Am. J. Physiol. R. Physiol.* 305, F1719–F1727.

Yu, R., Zhang, Y., Lu, Z., Li, J., Shi, P., and Li, J. (2019). Long-chain non-coding RNA UCA1 inhibits renal tubular epithelial cell apoptosis by targeting microRNA-206 in diabetic nephropathy. *Arch. Physiol. Biochem.* Epub ahead of print,

Yu, Y., Jia, Y. Y., Wang, M., Mu, L., and Li, H. J. (2021). PTGER3 and MMP-2 play potential roles in diabetic nephropathy via competing endogenous RNA mechanisms. *BMC Nephrol.* 22:27.

Yuan, E. F., Yang, Y., Cheng, L., Deng, X., Chen, S. M., Zhou, X., et al. (2019). Hyperglycemia affects global 5-methylcytosine and 5-hydroxymethylcytosine in blood genomic DNA through upregulation of SIRT6 and TETs. *Clin. Epigenet.* 11, 1–9.

Zha, F., Qu, X., Tang, B., Li, J., Wang, Y., Zheng, P., et al. (2019). Long non-coding RNA MEG3 promotes fibrosis and inflammatory response in diabetic nephropathy via miR-181a/Egr-1/TLR4 axis. *Aging (Albany NY)* 11:3716. doi: 10.18632/aging.102011

Zhan, J. F., Huang, H. W., Huang, C., Hu, L. L., and Xu, W. W. (2020). Long non-coding RNA NEAT1 regulates pyroptosis in diabetic nephropathy via mediating the miR-34c/NLRP3 Axis. *Kidney Blood Pres. Res.* 45, 589–602. doi: 10.1159/000508372

Zhang, C., Xiao, C., Wang, P., Xu, W., Zhang, A., Li, Q., et al. (2014). The alteration of Th1/Th2/Th17/Treg paradigm in patients with type 2 diabetes mellitus: Relationship with diabetic nephropathy. *Hum. Immunol.* 75, 289–296. doi: 10.1016/j.humimm.2014.02.007

Zhang, H., Nair, V., Saha, J., Atkins, K. B., Hodgin, J. B., Saunders, T. L., et al. (2017). Podocyte-specific JAK2 overexpression worsens diabetic kidney disease in mice. *Kidney Int.* 92, 909–921. doi: 10.1016/j.kint.2017.03.027

Zhang, M., Zhao, S., Xu, C., Shen, Y., Huang, J., Shen, S., et al. (2020a). Ablation of lncRNA MIAT mitigates high glucose-stimulated inflammation and apoptosis of podocyte via miR-130a-3p/TLR4 signaling axis. *Biochem. Biophys. Res. Commun.* 533, 429–436. doi: 10.1016/j.bbrc.2020.09.034

Zhang, P., Sun, Y., Peng, R., Chen, W., Fu, X., Zhang, L., et al. (2019a). Long non-coding RNA Rpph1 promotes inflammation and proliferation of mesangial cells in diabetic nephropathy via an interaction with Gal-3. *Cell Death Dis.* 10, 1–16.

Zhang, R., Qin, L., and Shi, J. (2020b). MicroRNA-199a-3p suppresses high glucose-induced apoptosis and inflammation by regulating the IKKβ/NF−κB signaling

pathway in renal tubular epithelial cells. *Int. J. Mol. Med.* 46, 2161–2171. doi: 10.3892/ijmm.2020.4751

Zhang, Y. L., Wang, J. M., Yin, H., Wang, S. B., He, C. L., and Liu, J. (2020c). DACH1, a novel target of miR-218, participates in the regulation of cell viability, apoptosis, inflammatory response, and epithelial-mesenchymal transition process in renal tubule cells treated by high-glucose. *REN Fail* 42, 463–473. doi: 10.1080/0886022x.2020.1762647

Zhang, Y. Y., Tang, P. M., Niu, Y., Córdoba, G. A., Alexandra, C., Huang, X. R., et al. (2020d). Long non-coding RNA LRNA9884 promotes acute kidney injury via regulating NF-kB-mediated transcriptional activation of MIF. *Front. Physiol.* 11:1399. doi: 10.3389/fphys.2020.590027

Zhang, Y. Y., Tang, P. M. K., Tang, P. C. T., Xiao, J., Huang, X. R., Yu, C., et al. (2019b). LRNA9884, a novel smad3-dependent long noncoding RNA, promotes diabetic kidney injury in db/db mice via enhancing MCP-1–dependent renal inflammation. *Diabetes* 68, 1485–1498. doi: 10.2337/db18-1075

Zhong, X., Chung, A. C. K., Chen, H., Dong, Y., Meng, X., Li, R., et al. (2013). miR-21 is a key therapeutic target for renal injury in a mouse model of type 2 diabetes. *Diabetologia* 56, 663–674. doi: 10.1007/s00125-012-2804-x

Zhong, X., Zhang, L., Li, Y., Li, P., Li, J., and Cheng, G. (2018). Kaempferol alleviates ox-LDL-induced apoptosis by up-regulation of miR-26a-5p via inhibiting TLR4/NF-κB pathway in human endothelial cells. *Biomedicine Pharmacother.* 108, 1783–1789. doi: 10.1016/j.biopha.2018.09.175

Zhou, H., Ni, W.-J., Meng, X.-M., and Tang, L.-Q. (2021). MicroRNAs as regulators of immune and inflammatory responses: potential therapeutic targets in diabetic nephropathy. *Front. Cell Dev. Biol.* 8:1837. doi: 10.3389/fcell.2020.618536

Zhou, L., Xu, D. Y., Sha, W. G., Shen, L., Lu, G. Y., Yin, X., et al. (2015). High glucose induces renal tubular epithelial injury via Sirt1/NF-kappaB/microR-29/Keap1 signal pathway. *J. Trans. Med.* 13, 1–12.

Zhu, X. J., Gong, Z., Li, S. J., Jia, H. P., and Li, D. L. (2019). Long non-coding RNA Hottip modulates high-glucose-induced inflammation and ECM accumulation through miR-455-3p/WNT2B in mouse mesangial cells. *Int. J. Clin. Exp. Pathol.* 12:2435.

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REVIEW
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Transforming Growth Factor-β and Long Non-coding RNA in Renal Inflammation and Fibrosis

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Renal fibrosis is one of the most characterized pathological features in chronic kidney disease (CKD). Progressive fibrosis eventually leads to renal failure, leaving dialysis or allograft transplantation the only clinical option for CKD patients. Transforming growth factor-β (TGF-β) is the key mediator in renal fibrosis and is an essential regulator for renal inflammation. Therefore, the general blockade of the pro-fibrotic TGF-β may reduce fibrosis but may risk promoting renal inflammation and other side effects due to the diverse role of TGF-β in kidney diseases. Long non-coding RNAs (lncRNAs) are RNA transcripts with more than 200 nucleotides and have been regarded as promising therapeutic targets for many diseases. This review focuses on the importance of TGF-β and lncRNAs in renal inflammation, fibrogenesis, and the potential applications of TGF-β and lncRNAs as the therapeutic targets and biomarkers in renal fibrosis and CKD are highlighted.

Keywords: long non-coding RNA, renal fibrosis, inflammation, TGF-β, SMADs, molecular therapy

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INTRODUCTION

Chronic kidney disease (CKD) has become a significant public health problem with the rising mortality and morbidity over the past three decades (Provenzano et al., 2019). Renal fibrosis is one of the most prominent pathogenic features and the best predictor for CKD progression (Majo et al., 2019). Triggered by the initial renal insults, the fibrotic process evokes to establish repairs. However, as severe or persistent injuries prolong, renal resident cells, together with infiltrating cells, may contribute to the initiation and progression of fibrosis with excessive deposition of extracellular matrix (ECM) in the glomerulus, tubulointerstitium, and vasculature (Glassock et al., 2017). Moreover, unresolved renal inflammation could also trigger the fibrotic process by releasing pro-fibrotic growth factors, cytokines, and chemokines (Chung and Lan, 2011; Meng et al., 2014). Injuries from mesangial cells, endothelial cells (ECs), podocytes, tubular epithelial cells (TECs), and inflammatory cells could also lead to renal glomerular and interstitial fibrosis (**Figure 1**). Progressive renal fibrosis and inflammation can then impair the function of nephrons and results in albuminuria and the reduction of eGFR. Renal fibrosis culminates in renal failure, well known as end-stage renal disease (ESRD) (Liu, 2011).

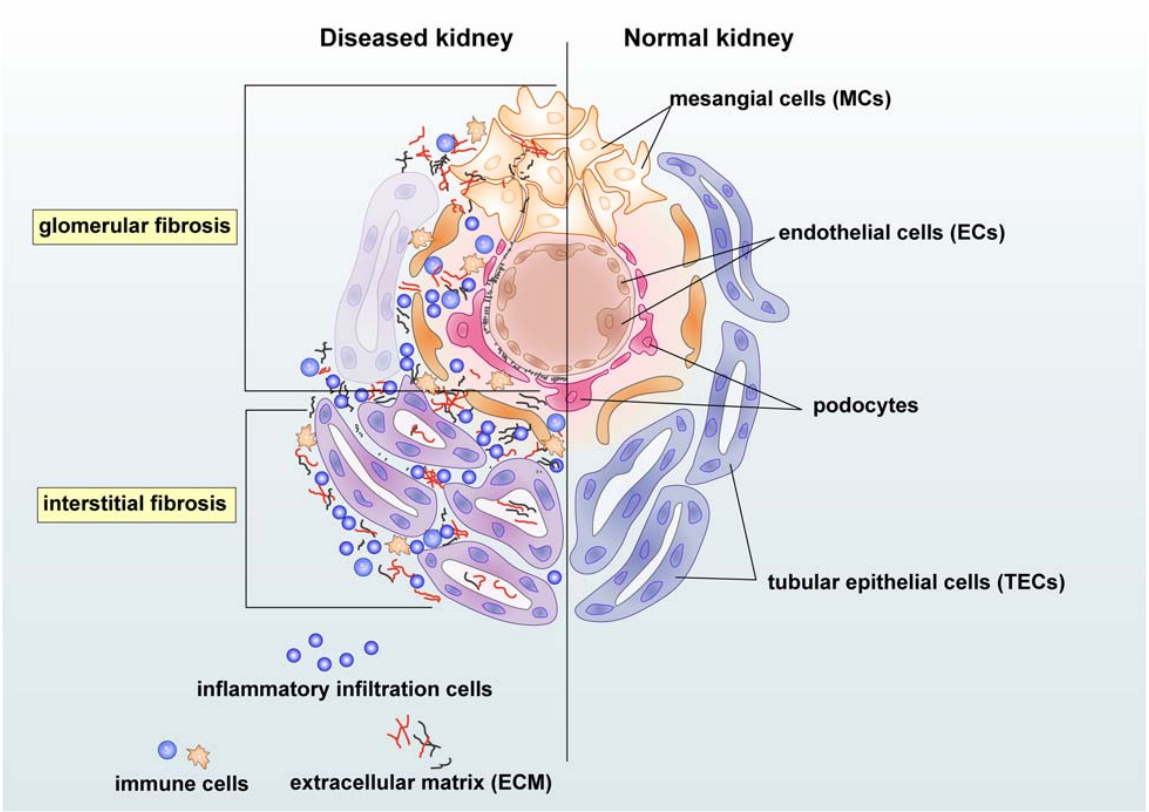

FIGURE 1 | Renal intrinsic and inflammatory cells in glomerular and interstitial fibrosis during kidney injury. Damaged mesangial cells (MCs), endothelial cells (ECs), and podocytes are essential in the glomerular fibrosis. The mesangial cell may produce pro-fibrotic and pro-inflammatory growth factors and cytokines and enhance proliferation to cause deposition of the mesangial matrix. Damages on endothelial cells and podocytes may lead to albuminuria and endothelial dysfunction in chronic kidney disease (CKD). Injured tubular epithelial cells (TECs) may produce pro-fibrotic and pro-inflammatory factors, resulting in the accumulation of extracellular matrix (ECM) and inflammatory cells in the tubulointerstitium area. Immune cells, including macrophages and T cells, may participate in renal fibrosis by producing growth factors and becoming collagen-producing myofibroblasts under the regulation of transforming growth factor-β (TGF-β)/Smad3 signaling.

Transforming growth factor-β (TGF-β) is a primary pathophysiologic cytokine that instigates the process of fibrosis (Meng et al., 2016a). TGF-β can induce transcription of fibrotic products such as α-SMA and collagens by canonical and non-canonical signaling pathways. Fibrotic mediators include angiotensin II (Ang II), reactive oxygen species (ROS), as well as advanced glycation end products (AGEs) that may activate individual pathways to crosstalk with TGF-β/Smad signaling to regulate renal fibrosis and inflammation (Chung et al., 2010; Lan, 2011). However, current anti-fibrotic therapies by targeting TGF-β are ineffective with unexpected side effects, underscoring the complexities of the TGF-β signaling pathway (Yoshimura and Muto, 2011; Gu et al., 2020a).

With the new technologies of high-throughput assays, we can now update our understanding of the genomes. The transcriptomic studies have demonstrated that the vast majority of the genomes in mammals produce large numbers of non-protein-coding RNAs (ncRNAs) (Quinn and Chang, 2016). These ncRNAs are classified into long non-coding RNAs (lncRNAs), microRNAs (miRNAs), small interfering RNAs (siRNAs), small nuclear RNAs (snRNAs), small nucleolar RNAs (snoRNAs), and PIWI-interacting RNAs (piRNAs) (Van der Hauwaert et al., 2019). Of these ncRNAs, lncRNAs are characterized as RNAs being transcribed over 200 nucleotides in length. They have been considered the major players in fibrotic diseases's pathogenesis due to their tissue and cell-type specificity and the regulations on DNAs, RNAs, and proteins (Jiang and Zhang, 2017). Of note, many TGF-β/Smad3-regulated lncRNAs have been reported as essential mediators in the process of renal fibrosis and inflammation (Tang et al., 2017, 2018a,b).

In this review, the underlying mechanistic signaling pathways by which TGF-β and lncRNAs drive renal fibrosis are to be discussed. The developments of biomarkers and therapeutic potential for renal inflammation and fibrosis by targeting TGF-β/Smad signaling and lncRNAs are also described.

DIVERSE ROLES OF TGF-β/SMAD SIGNALING PATHWAY IN RENAL INFLAMMATION AND FIBROSIS

Transforming growth factor-β is a pleiotropic cytokine that plays diverse roles in a wide range of biological and pathological processes. Indeed, TGF-β acts as either deleterious or protective

functions in kidney diseases (Lopez-Hernandez and Lopez-Novoa, 2012). TGF-β may induce renal fibrosis by canonical and non-canonical signaling pathways (Lan and Chung, 2012; Isaka, 2018). Besides, TGF-β promotes renal fibrosis by stimulating ECM accumulation and alternatively activating the pro-fibrotic immune cells, facilitating the transitions from various cell types into pro-fibrotic cells (Gu et al., 2020b). Therefore, understanding the diverse roles of TGF-β is of utmost importance in the development of anti-fibrotic therapies.

Transforming growth factor-β is a well-characterized member that belongs to the TGF-β superfamily. Among three isoforms of TGF-β, TGF-β1 is considered the pro-fibrotic molecule that drives the fibrotic process via canonical and non-canonical signaling pathways (Lodyga and Hinz, 2019). In particular, high expression of TGF-β1 is observed in most, not all progressive forms of human and rodent kidney diseases (Kopp et al., 1996; Fan et al., 1999; Lan, 2012b; Lan and Chung, 2012), demonstrating the pathogenic role for TGF-β1 in CKD. To induce transcriptions of target genes, likely as α-SMA and collagens, the latent TGF-β1 becomes active and binds to TGF-β receptors, promoting the transduction of a series of Smad proteins to regulate fibrogenesis (Derynck and Zhang, 2003). Regarding the downstream TGF-β/Smad signaling, although the functions of Smad2 and Smad4 have been well studied (Tsuchida et al., 2003; Ju et al., 2006; Meng et al., 2012; Morishita et al., 2014; Loeffler et al., 2018), their mechanistic roles are diverse and unclear due to the limited availability of animal models, which still warranted for further exploration.

It is widely acknowledged that Smad3 is pro-fibrotic, while Smad2 and Smad7 are anti-fibrotic. Smad3 is highly activated in a wide range of renal disease; evidence on animal models suggest that the inhibition or blockade of Smad3 may reduce the fibrotic response (Wang et al., 2006; Yang et al., 2009, 2010; Li et al., 2010; Zhou et al., 2010; Liu et al., 2012; Zhang et al., 2018). By contrast, the function of Smad2 and Smad7 is protective, which negatively regulates the TGF-β/Smad3 signaling in renal fibrosis and inflammation (Lan, 2008, 2012a; Chen et al., 2011). Many studies support this finding, showing that overexpression of Smad7 improves renal fibrogenesis in obstructive, diabetic, hypertensive, toxin-induced nephropathy and autoimmune crescentic glomerulonephritis (Li et al., 2002; Lan et al., 2003; Hou et al., 2005; Ng et al., 2005; Ka et al., 2007; Chung et al., 2009; Liu et al., 2013, 2014; Dai et al., 2015) by inhibiting the TGF-β/Smad3 and NF-κB signaling pathways. However, the contradictory findings have also reported that the overexpression of Smad7 could promote TGF-β-driven apoptosis in podocytes (Schiffer et al., 2001, 2002). Collectively, although restoring the imbalance between Smad3 and Smad7 may serve as an ideal therapy to halt the fibrotic process (Nie et al., 2014; Zhao et al., 2014, 2016; Meng et al., 2015; Du et al., 2018b), Smad3 and Smad7 also serve as the vital downstream molecules in other signaling pathways. Therefore, new specific targets should be sought.

Transforming growth factor-β may also be produced by damaged renal intrinsic cells or immune cells in acute and CKDs, thus promoting the transition of tubular cells into myofibroblasts (Mack and Yanagita, 2015). Myofibroblasts produce fibronectin and collagens and contribute to ECM accumulation (Yuan et al., 2019). Based on current studies, the sources of myofibroblast origins include pericytes (Wu et al., 2013), renal resident fibroblasts, tubular epithelial cell-myofibroblast transition (EMT) (Iwano et al., 2002), endothelial cell-myofibroblast transition (EndoMT) (Zeisberg et al., 2008) and bone marrow-derived macrophage-myofibroblast transition (MMT) (Fan et al., 1999; Meng et al., 2016b; Wang et al., 2017). TGF-β/Smad signaling pathway tightly regulates these transitions.

To halt the fibrotic process, strategies to inhibit the function of TGF-β include the utilization of neutralizing antibodies (Border et al., 1990), small molecule inhibitors against TGF-β receptors (Bonafoux and Lee, 2009), latent form of TGF-β (Huang et al., 2008a,b) and antisense oligonucleotides to TGF-β1 (March et al., 2018). These findings have conferred a vital pathological role of TGF-β in renal inflammation and fibrosis, implying the urgent need for anti-TGF-β therapy.

THERAPEUTIC EFFECT OF ANTI-TGF-β TREATMENT ON KIDNEY DISEASES

Anti-TGF-β therapy is an issue of considerable debate. On the one hand, TGF-β is the crucial mediator that regulates fibrosis in all organs, especially in kidneys (Györfi et al., 2018). On the other hand, TGF-β regulates a wide range of biological and pathological processes and acts as essential roles in the immune cells, such as macrophages, conventional and unconventional T cells (Meng, 2019; Gu et al., 2020a). Over the past decades, a number of therapeutic drugs and clinical trials for the treatment of CKD targeting TGF-β have further revealed the underlying mechanisms and renewed our understanding of TGF-β signaling (Ruiz-Ortega et al., 2020).

Targeting on the TGF-β family, LY2382770 and fresolimumab have proven no efficacy on improvements in neither proteinuria, eGFR, nor serum creatinine in focal and segmental glomerulosclerosis (FSGS) and diabetic nephropathy (DN) (Trachtman et al., 2011; Vincenti et al., 2017; Voelker et al., 2017). Besides, various side effects induced by blocking TGF-β, including herpes zoster, skin lesions, pustular rash, bleeding events, and cancers, have demonstrated the awkward situation of the anti-TGF-β therapies. Hopefully, with the rapid development of pharmacology, a promising synthetic anti-TGF-β agent, pirfenidone, is proven to improve the eGFR decline in patients with DN and FSGS (Cho et al., 2007; Sharma et al., 2011). Further studies and clinical trials on pirfenidone's renal protective effects are still ongoing (NCT02689778, NCT02408744, and NCT00001959).

Nevertheless, the by-effects such as gastrointestinal disorders and photosensitive dermatitis of pirfenidone are inevitable, raising safety concerns to the clinical application of anti-TGF-β therapies. Current anti-TGF-β therapies have limited effectiveness, underscoring the urgent need to develop specific therapeutic targets to halt the progression of renal fibrosis.

THE EMERGING ROLE OF LONG NON-CODING RNAs IN RENAL INFLAMMATION AND FIBROSIS

The genomic and transcriptional landscape is far more complicated than we previously appreciated. With the development of large-scale transcriptome analyses, we have now acknowledged that the vast majority of genomic sequence is transcribed into a group of lncRNAs (Hangauer et al., 2013). However, these lncRNAs were initially ignored as "transcriptional noise" or "evolutionary debris," dating from the 1970s (Ohno, 1972). In the 1990s, the functions of some classically defined lncRNAs are discovered, such as X inactive specific transcript (XIST) in X chromosome inactive specific, raising the possibility that lncRNAs may play an essential role in cellular biology and disease (Brockdorff et al., 1991; Brown et al., 1991). Of note, the number of identified lncRNAs is rapidly rising to date. Based on the GENCODE[1] (version 33), 17952 lncRNA and 19957 protein-coding genes have been identified in the human genome, but the functions of lncRNAs in renal development and diseases remain largely unknown. In the context of lncRNA function in kidney diseases, lncRNAs may act as scaffolds, decoys, or guides to control the recruitment or dismissal of chromatin-modifying complexes.

Although lncRNAs produce in deficient amounts, their expression patterns are highly restricted to specific cell types, tissue, developmental stage, or disease state, suggesting the distinctive roles of lncRNAs in different physiological or pathological contexts (Batista and Chang, 2013; Flynn and Chang, 2014). Pathologically, the fibrotic and inflammatory processes in the kidneys may be triggered by a wide range of renal injuries in the attempt to establish tissue repair. Pathological hallmarks include TGF-β activation, myofibroblast differentiation and transition, ECM deposition, and inflammatory responses. Of note, TGF-β is a master regulator of immune cell trades that it correlates closely with the development, homeostasis, and differentiation of immune cells such as T cells (Li and Flavell, 2008). T cells are the predominant players in TGF-β-driven renal fibrosis and inflammation (Kinsey and Okusa, 2014; Ludwig-Portugall and Kurts, 2014; Hu et al., 2016). The hematopoietic-specific TGF-β and cytokines produced by inflammatory immune cells may activate innate and acquired immune response (Gu et al., 2020b). These may well be associated with the functions of lncRNAs in renal inflammation. For instance, lncRNAs may act as mediators in lupus nephritis pathogenesis to regulate inflammation and apoptosis of renal cells (Xue et al., 2017; Liao et al., 2019; Chen et al., 2020).

Nevertheless, lncRNAs take part in the fibrotic or inflammatory transcriptional regulation by direct interactions with RNA polymerase II (Pol II), transcription factors (TFs), and other regulators. Furthermore, some lncRNAs may act as competing endogenous RNAs (ceRNAs), which play the competitive role as the sponges to bind with miRNAs and reduce the concentration of fibrotic or inflammatory miRNAs, therefore competing with these miRNAs in binding to their target mRNA transcripts.

As previously mentioned, the group of lncRNAs identified in kidneys is highly specific to cell type or disease state. Studies carried out over these years have identified a group of anti- or pro-fibrotic and inflammatory lncRNAs in diabetic, acute and chronic renal diseases (Tang et al., 2017, 2018a; Ren et al., 2019; Gu et al., 2020c) (**Table 1**).

For example, hyperglycemia is one of the most driving forces in renal fibrosis. Zhang et al. has revealed the anti-fibrotic effect of lncRNA growth arrest-specific transcript (GAS5) in the progression of DN. lncRNA GAS5 may downregulate the expression of pro-inflammatory MMP9 by recruiting EZH2 to the MMP9 promoter region, therefore inhibiting renal interstitial fibrosis and inflammatory (Zhang et al., 2020a). lncRNA CRNDE also interacts with miR-181a-5p to to protect sepsis-induced AKI from apoptosis (Wang et al., 2020a). Moreover, overexpression of lncRNA CCAT1 may down-regulate miR-155, thus inhibiting inflammation and promoting proliferation (Lu et al., 2020). LncRNA zinc finger E-box binding homeobox1-antisense RNA 1 (ZEB1-AS1) provides a binding site in its promoter region for p53. It may promote H3K4me3 histone modification on ZEB1 promoter to exhibit anti-fibrotic effect (Wang et al., 2018a). In the context of fibrosis, the function of lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) has been well-studied in cardiac and in hepatic fibrosis (Jiang et al., 2019; Riaz and Li, 2019; Che et al., 2020). MALAT1 has caught much attention in renal diseases for its anti-inflammatory effect in AKI. The expression of LncRNA 1700020I14Rik tends to decrease under high glucose conditions, but the overexpression of LncRNA 1700020I14Rik exerts an anti-fibrotic effects by inhibiting cell proliferation and regulating the miR-34a-5p/Sirt1/HIF-1α pathway (Li et al., 2018). Moreover, lncRNA CYP4B1-PS1-001 significantly reduces in the early stage of DN; the proliferation and fibrosis of mesangial cells are reversed as the overexpression of CYP4B1-PS1-001 regulates the ubiquitination and degradation of Nucleolin (Wang et al., 2016a, 2018c). ENSMUST00000147869 is significantly downregulated in the DN model. Overexpression of ENSMUST00000147869 may inhibit fibrosis and proliferation of mesangial cells by the possible regulation of the Cyp4a12a gene (Wang et al., 2016b). To interact with miRNAs and proteins in podocytes, pericytes, or TECs, lncRNAs such as taurine upregulated gene 1 (TUG1) (Zhao et al., 2019; Cao et al., 2020a,b), Rian (Bijkerk et al., 2019), 3110045C21Rik (Arvaniti et al., 2016) also function as anti-fibrotic lncRNA to participate in the pathogenesis of systematic erythematosus lupus (SLE), ischemia-reperfusion injury and obstructive nephropathy, respectively.

Studies on pro-fibrotic lncRNAs are shown in **Table 2**. LncRNA myocardial infarction-associated transcript (Miat) has been identified to function as miRNA sponges in TECs and pericytes, thus regulating their transitions into myofibroblast (Bijkerk et al., 2019; Wang et al., 2020b). In diabetes-induced renal injury, lncRNA nuclear enriched abundant transcript 1 (NEAT1) is found to be increased in the serum of DN patients. A further mechanistic study has revealed that lncRNA NEAT1 may progress the development of DN by sponging miR-23c.

[1] http://www.gencodegenes.org

TABLE 1 | Anti-fibrotic or anti-inflammatory long non-coding RNAs (lncRNAs) in renal diseases.

lncRNA	Model	Mechanism/target	Pathological output(s)	Year	References
GAS5	STZ-induced DN and rat	Recruits EZH2 to the promoter region of MMP9	Anti-fibrotic; anti-inflammatory	2020	Zhang et al., 2020a
CRNDE	Sepsis-induced AKI, rat, and TECs	Regulation of miR-181a-5p/PPARα pathway	Anti-inflammatory	2020	Wang et al., 2020a
CCAT1	LPS-induced AKI mice and TECs	Overexpression of CCAT1 sequesters miR-155 and leads to upregulation of SIRT1 and TECs damage	Anti-inflammatory	2020	Lu et al., 2020
TUG1	SLE patient serum and SLE mouse	/	Anti-fibrotic; anti-inflammatory	2020	Cao et al., 2020a,b
	LPS-induced podocyte injury	Targets miR-197/MAPK1	Anti-inflammatory	2019	Zhao et al., 2019
Rian/RIAN	UUO mouse, AKI mouse, and pericytes	Possible interactions with 14q32 miRNA cluster	Anti-fibrotic	2019	Bijkerk et al., 2019
Malat1/MALAT1	AKI; mice; and TECs	Regulates HIF-1α expression through NF-κB signaling	Anti-inflammatory	2018	Tian et al., 2018
ZEB1-AS1	DN mouse and DN patient	Binds to H3K4 methyltransferase myeloid and MLL1 to promote ZEB1 expression Provides a binding site for p53	Anti-fibrotic	2018	Wang et al., 2018a
1700020I14Rik	DN mouse and MCs	Interacts with miR-34a-5p, Sirt1/HIF-1α	Anti-fibrotic	2018	Li et al., 2018
CYP4B1-PS1-001	DN mouse and MCs	Regulates Nucleolin to inhibit proliferation and fibrosis of MCs	Anti-fibrotic	2018 2016	Wang et al., 2016a, 2018c
3110045C21Rik	UUO mouse and TECs	Contains binding sites for Pol II and H3K4m3	Anti-fibrotic	2016	Arvaniti et al., 2016
ENSMUST00000147869	DN mouse and MCs	Possibly targets on *Cyp4a12a* gene	Anti-fibrotic	2016	Wang et al., 2016b

Moreover, studies from Yang et al. and Huang et al. have drawn similar conclusions. At the same time, they further demonstrated that lncRNA NEAT1 might promote fibrosis in TECs and MCs by regulating the ERK1/2 or Akt/mTOR signaling pathways (Huang et al., 2019; Yang et al., 2020). It is reported that NEAT1 may also promote renal inflammation in lupus nephritis by upregulating the expression of TRAF6 and activating the NF-κB signaling in lupus nephritis (Zhang et al., 2020b). lncRNA LOC105375913 is upregulated in the TECs of FSGS patients and functions to promote tubulointerstitial fibrosis by regulating C3a/p38/XBP signaling pathway and by increasing the expression of Snail and binding to miR-27b (Han et al., 2019). lncRNA LINC00667 is also upregulated in kidney tissues related to the proliferation of TECs. A further mechanistic study has revealed that LINC00667 promotes renal fibrosis by regulating the miR-19b-3p/LINC00667/CTGF signaling pathway (Chen et al., 2019). lncRNA TapSAKI is reported as a biomarker with pro-inflammation and pro-apoptosis in injured TECs and can predict mortality in AKI patients (Lorenzen et al., 2015a).

In diabetic kidney disease, ribonuclease P RNA component H1 (Rpph1) (Zhang et al., 2019b)and Blnc1 are marked as pro-inflammatory lncNRAs (Feng et al., 2019). Moreover, studies on DN have observed the upregulation of lncRNA MALAT1 in TECs and podocytes under high glucose-induced conditions. Induced by TGF-β1, MALAT1 facilitates EMT and promotes fibrosis by acting as a sponge for miR-145 or as a feedback regulator of the Wnt/β-catenin signaling pathway (Hu et al., 2017; Liu et al., 2019a; Zhang et al., 2019a). However, the pathogenic role of lncRNA MALAT1 in hypoxia-induced AKI remains unclear. Kölling et al. have identified an increased level of lncRNA MALAT1 in renal biopsies and plasma of AKI patients; *in vitro* study has also shown a decreased number and proliferation in MALAT1-inhibited ECs. The mechanistic study has discovered that it is transcriptionally activated by hypoxia-inducible factor 1α (HIF-1α). However, no significant differences in inflammation and fibrosis were shown on MALAT1 knockout and wild-type mice in hypoxia-induced AKI (Kölling et al., 2018).

Also, microarray data have shown a pro-fibrotic role of lncRNA LINC00963 by targeting on FoxO3 gene to regulate the FoxO signaling pathway (Chen et al., 2018). Pro-inflammatory cytokines, together with NLRP3 inflammasome, may also drive the progression of fibrosis under diabetic conditions. Furthermore, lncRNA Gm4419 is increased in DN and promotes renal fibrosis and inflammation by activating the NF-κB/NLRP3 inflammasome signaling pathway in MCs (Yi et al., 2017). However, the functional roles of lncRNA ENSRNOG00000037522 and CHCHD4P4 are remained to be further investigated (Zhang et al., 2017a; Ling et al., 2018).

TRANSFORMING GROWTH FACTOR-β/SMAD3-DEPENDENT LNCRNA IN RENAL INFLAMMATION AND FIBROSIS

Fibrotic responses triggered by TGF-β/Smad3 signaling are of importance in renal fibrogenesis. However, generally blocking the

TABLE 2 | Pro-fibrotic or pro-inflammatory lncRNAs in renal diseases.

lncRNA	Model	Mechanism/function	Pathological output(s)	Year	References
Miat/MIAT	UUO mouse and TECs	Sponge for miR-145	Pro-fibrotic	2020	Wang et al., 2020b
	UUO mouse, IRI mouse, and pericytes	Possible interactions with miR-150	Pro-fibrotic	2019	Bijkerk et al., 2019
Neat1/NEAT1	DN mouse and TECs	Regulates the Klotho/ERK1/2 signaling	Pro-fibrotic	2020	Yang et al., 2020
	Plasma from DN patient, DN mouse, and MCs	Sponge for miR-23c	Pro-fibrotic	2020	Li et al., 2020
	DN rat and MCs	Possible regulation of Akt/mTOR	Pro-fibrotic	2019	Huang et al., 2019
	MCs	Targets miR-146b to promote TRAF6 expression	Pro-inflammatory	2019	Zhang et al., 2020b
LOC105375913	FSGS patient and TECs	Regulated by C3a/p38/XBP-1s signaling and binds to miR-27b	Pro-fibrotic	2019	Han et al., 2019
LINC00667	CKD patient, CKD rat, and TECs	Promotes fibrosis via miR-19b-3p/LINC00667/CTGF signaling	Pro-fibrotic	2019	Chen et al., 2019
TapSAKI	Sepsis-induced AKI; rats; and TECs	Promotes apoptosis and inflammation of TECs via TaqSAKI/miR-22/TLR4/NF-κB signaling pathway	Pro-inflammatory	2019	Shen et al., 2019
Rpph1	db/db mice and MCs	Promotes inflammation and MCs proliferation through Gal-3/Mek/Erk signaling	Pro-inflammatory	2019	Zhang et al., 2019b
Blnc1	DN patient, STZ-induced DN, and TECs	Interaction with NRF2/HO-1 and NF-κB signaling	Pro-fibrotic; Pro-inflammatory	2019	Feng et al., 2019
Malat1/MALAT1	DN and TECs	Regulation of Wnt/β-catenin signaling	Pro-fibrotic	2019	Zhang et al., 2019a
	DN mouse and TECs	Sponge for miR-145	Pro-fibrotic	2019	Liu et al., 2019a
	Plasma, renal biopsies from AKI patients, IRI mouse, TECs, and ECs	Regulated by HIF-1α	No significant effect	2018	Kölling et al., 2018
	DN mouse and podocytes	Binds to SRSF1, interacts with β-catenin	Pro-fibrotic	2017	Hu et al., 2017
	STZ-induced mice and ECs	Upregulated IL-6, TNF-α by activating SAA3	Pro-inflammatory	2015	Puthanveetil et al., 2015
ENSRNOG00000037522	DN rat and podocytes	/	Pro-fibrotic	2018	Ling et al., 2018
NR_033515	Serum from DN patient and MCs	Negatively regulates miR-743b-5p	Pro-fibrotic	2018	Gao et al., 2018
LINC00963	5/6 nephrectomy and rat	Activates the FoxO signaling	Pro-fibrotic	2018	Chen et al., 2018
CHCHD4P4	Kidney stone, mouse and TECs	/	Pro-fibrotic	2017	Zhang et al., 2017a
ASncmtRNA-2	DN mouse and MCs	Upregulated by ROS	Pro-fibrotic	2017	Gao et al., 2017
Gm4419	DN mouse and MCs	Activates NF-κB/NLRP3-mediated inflammation and interacts with p50	Pro-fibrotic; pro-inflammatory	2017	Yi et al., 2017
PVT1	AKI; and LPS-induced TECs	Binds to TNF-α and inhibits JNK/NF-κB signaling pathway	Pro-inflammatory	2017	Huang et al., 2017
RP23-45G16.5	UUO mouse and TECs	Shows positive correlation with *cdkn1b* gene	Pro-fibrotic	2016	Arvaniti et al., 2016

upstream TGF-β signaling may risk promoting inflammation and other side effects. We are beginning to learn that the involvement of TGF-β in many other biological processes has been the main obstacle for anti-TGF-β therapy. Nevertheless, the majority of studies continue to seek therapeutic targets for anti-fibrotic treatments. miRNA targeting downstream TGF-β signaling has been one of the optimal options.

However, the off-target effects and cytotoxicity of miRNA therapies have caught the attention of their specificity and safety. Encouragingly, it has been reported that a group of characterized lncRNAs is involved in TGF-β/Smad3-mediated renal fibrosis and inflammation (Zhou et al., 2014, 2015b) (**Table 3**). These emerging studies should provide possibilities for lncRNA treatment in the future. Ptprd-IR is a novel lncRNA that promotes inflammatory response on TECs in the UUO model. It contains a binding site for Smad3 in its promoter region and is downregulated by deleting Smad3. In contrast, the overexpression of Ptprd-IR enhances inflammatory response by upregulating TGF-β1-, interleukin-1β (IL-1β)-induced NF-κB-driven production of pro-inflammatory cytokines but shows no effect on the TGF-β1-induced renal fibrosis (Pu et al., 2020). Other novel lncRNA, lncRNA Erbb4-IR, of which expression is induced by TGF-β1 via Smad3-dependent mechanism, is significantly increased in the fibrotic UUO model (Feng et al., 2018). Erbb4-IR binds to the inhibitory Smad7 and blocks TGF-β/Smad3-induced renal fibrosis, while overexpression of Erbb4-IR may promote fibrosis by downregulating the expression of Smad7. Of note, Erbb4-IR may also be induced by advanced

glycosylation end products (AGEs) in DN. It promotes the expression of collagens by binding to miR-29b and hence transcriptionally suppresses miR-29b. Silencing renal Erbb4-IR leads to the upregulation of protective miR-29b and prevents fibrosis (Sun et al., 2018; Xu et al., 2020). Besides, lncRNA AT-rich interactive domain 2-IR (Arid2-IR) also contains a Smad3 binding site in the promoter region. Further *in vivo* study has shown that deletion of Smad3 may abolish upregulation of Arid2-IR in the diseased kidney. Arid2-IR shares a similar mechanism with Ptprd-IR that overexpression of Arid2-IR may promote TGF-β1-, IL-1β-induced NF-κB-driven inflammation without affecting TGF-β/Smad3-mediated renal fibrosis (Zhou et al., 2015a). Nevertheless, the study from Yang et al. (2019) has demonstrated the upregulation and pro-fibrotic effect of Arid2-IR on MCs in DN, that Arid2-IR may be positively regulated by the early growth response protein-1 (Egr1) and promote ECM production.

A novel Smad3-dependent lncRNA, LRNA9884, is induced by AGEs and tightly regulated by Smad3 in the development and progression of DN. Mechanistically, LRNA9884 directly binds to MCP-1 and enhances the promoter activity of MCP-1 at the transcriptional level, thus aggravating the renal injury driven by progressive inflammation (Zhang et al., 2019c). The kidney-enriched TGF-β/Smad3-interacting lncRNA, term as Inc-TSI, is another novel lncRNA that serves as a potential target for renal fibrosis (Wang et al., 2018b). lnc-TSI inhibits renal fibrosis by binding to the MH2 domain of Smad3, therefore blocking the interaction of Smad3 and TβRI and inhibiting the phosphorylation of Smad3. Meanwhile, the overexpression of lnc-TSI prevents the nuclear translocation of Smad2/3/4, resulting in the decreased expression of fibrotic proteins. The anti-fibrotic role of lnc-TSI has further confirmed that the fibrosis index of IgAN patients is negatively correlated with the expression of lnc-TSI.

Collectively, the TGF-β/Smad3-mediated lncRNAs may act as anti-fibrotic or pro-fibrotic mediators in the fibrotic process by binding to Smad3, Smad7, or inflammatory molecules to inhibit or enhance renal fibrosis and inflammation. It

TABLE 3 | TGF-β/Smad3-dependent lncRNAs in renal fibrosis and inflammation.

ncRNA	Model	Mechanism/function	Pathological output(s)	Year	References
Ptprd-IR (np_4334)	UUO mouse and TECs	Contains a binding site for Smad3 and promotes NF-κB-driven inflammation	Pro-inflammatory	2020	Pu et al., 2020
Erbb4-IR (np_5318)	DN mouse, TECs, and MCs	Binds to miR-29b to downregulate miR-29b expression	Pro-fibrotic	2020 2018	Sun et al., 2018; Xu et al., 2020
	UUO mouse and TECs	Binds to Smad7 to downregulate Smad7 expression	Pro-fibrotic	2018	Feng et al., 2018
Arid2-IR (np_28496)	DN mouse and MCs	Upregulated by Egr-1-induced ECM production	Pro-fibrotic	2019	Yang et al., 2019
	UUO mouse, anti-GBM mouse, and TECs	Contains a binding site for Smad3 and promotes NF-κB-driven inflammation	Pro-inflammatory	2015	Zhou et al., 2015a
LRNA9884	DN mouse, TECs, and MCs	Directly triggers the MCP-1 production	Pro-inflammatory	2019	Zhang et al., 2019c
NONHSAG053901	DN mouse and MCs	Directly binds to Egr-1	Pro-fibrotic; pro-inflammatory	2019	Peng et al., 2019
HOTAIR	UUO rat and TECs	Regulation of miR-124 /Notch1	Pro-fibrotic	2019	Zhou et al., 2019
lncRNA-ATB	UUO rat and TECs	Regulated by Livin to promote EMT	Pro-fibrotic	2019	Zhou and Jiang, 2019
MEG3	TECs	Regulated by miR-185/DNMT1 axis to inhibit fibrosis	Anti-fibrotic	2019	Xue et al., 2019
	TECs; acute renal allograft; and mice	Function as target of miR-181b-5p to regulate the expression of TNF-α	Pro-inflammatory	2019	Pang et al., 2019
ENST00000453774.1	Human renal fibrotic tissue, UUO mouse, and TECs	Activates autophagy by promoting ROS defense activates Nrf2/HO-1 signaling	Anti-fibrotic	2019	Xiao et al., 2019
lnc-TSI	IgAN patient and UUO mouse	Binds with Smad3 to block the interaction between Smad3 and TβR1	Anti-fibrotic	2018	Wang et al., 2018b
TCONS_00088786	UUO mouse and TECs	Possible regulation of miR-132	Pro-fibrotic	2018	Zhou et al., 2018
	UUO rat and TECs	/	Pro-fibrotic	2017	Sun et al., 2017
TCONS_01496394	UUO rat and TECs	/	Pro-fibrotic	2017	Sun et al., 2017
H19	UUO mouse, DN mouse, and TECs	Stimulated by TGF-β2 and serves as a sponge for miR-17	Pro-fibrotic	2016	Xie et al., 2016

CKD, chronic kidney disease; FSGS, focal and segmental glomerulosclerosis; STZ, streptozotocin; DN, diabetic nephropathy; SLE, systematic erythematosus lupus; IgAN, IgA nephropathy; anti-GBM, anti-glomerular basement membrane; LPS, lipopolysaccharides; UUO, unilateral ureteral obstruction; AKI, acute kidney injury; IRI, ischemia-reperfusion injury; MCs, mesangial cells; TECs, tubular epithelial cells; ECs, endothelial cells; SAA3, serum amyloid antigen 3.

has been demonstrated by a large number of studies that lncRNAs act like an endogenous RNA to compete for miRNA to regulate the target transcripts at the transcriptional or post-transcriptional level during renal fibrosis. In the early stage of DN, the expression of lncRNA NONHSAG053901 is highly increased in DN mice and MCs. The functional study has revealed that the overexpression of NONHSAG053901 promotes fibrosis, inflammation, and proliferation in MCs. Mechanistically, NONHSAG053901 directly binds to Egr-1, which later interacts with TGF-β to upregulate the release of pro-inflammatory cytokines to promote Egr-1/ TGF-β mediated renal inflammation (Peng et al., 2019). In addition, the pro-fibrotic lncRNA HOTAIR is significantly upregulated in TGF-β1-induced TECs and UUO rat kidney. Depletion on HOTAIR upregulates miR-124 to block the Notch1 signal pathway, therefore improving the EMT and reducing the accumulation of fibrotic proteins such as fibronectin and α-SMA (Zhou et al., 2019).

lnc-ATB has also been proven to be the critical regulator stimulated by TGF-β that mediates the EMT process. The expression of lncRNA-ATB is significantly increased in TECs and the UUO kidney under TGF-β and Livin regulation (Zhou and Jiang, 2019). Another lncRNA regulated by TGF-β is maternally expressed gene 3 (MEG3), inhibited in TGF-β-stimulated TECs. DNA methyltransferases 1 (DNMT1), regulated by miR-185, can positively modulate the methylation state of CpG islands in the promoter region of MEG3. Overexpression of lncRNA MEG3 reverses TGF-β-induced fibrosis in TECs. Thus, lncRNA MEG3 exerts an anti-fibrotic effect in TGF-β-promoted EMT and is regulated by the miR-185/DNMT1 signaling pathway (Xue et al., 2019). However, one study had investigated the pro-inflammatory effect of MEG3 in the acute renal allograft model (Pang et al., 2019). The anti-fibrotic lncRNA, ENST00000453774.1, is also downregulated in TGF-β-induced TECs and UUO model, especially in the fibrotic renal biopsies from patients. ENST00000453774.1 may regulate the Nrf2-keap1/Nrf2 nuclei translocation/HO-1 and NQO-1 signaling to activate the pro-survival autophagy of TECs, therefore promoting ROS defense and reducing the production of ECM markers such as fibronectin and collagen I (Xiao et al., 2019).

Nevertheless, the mechanism of some pro-fibrotic lncRNAs is still obscure. Based on the transcriptome sequencing study, a group of lncRNAs that contain Smad3 binding motifs in the promoter region has been identified. Among these lncRNAs, TCONS_00088786 and TCONS_01496394 are confirmed to be regulated by TGF-β in a time and dose-dependent manner. Knockdown of TCONS_00088786 may inhibit the mRNA expression profile of the gene Acta1, Col1a1, and Col3a1, while knockdown of TCONS_01496394 decreases the mRNA expression of Ctgf and Fn1, suggesting their potential in promoting renal fibrosis (Sun et al., 2017). Although a functional study has shown a positive regulation of TCONS_00088786 on miR-132, the underlying mechanism is unclear (Zhou et al., 2018). Interestingly, the expression of lncRNA H19 is also increased in TECs and the UUO model. lncRNA H19 is activated in embryonic cells, but its expression is significantly decreased after birth. Under the renal fibrotic condition, H19 is upregulated by TGF-β2 to promote the production of ECM-related proteins. Knockdown of H19 restores the renal functions and inhibits TGF-β2-induced fibrosis. It is demonstrated that H19 serves as a sponge for miR-17 and negatively regulates miR-17 in the process of fibrogenesis (Xie et al., 2016). However, further evidence on how H19 and miR-17 contribute to the network of renal fibrosis remains unclear.

FUTURE PERSPECTIVES: LNCRNA AS A NOVEL THERAPEUTIC TARGET FOR KIDNEY DISEASE

The activation of TGF-β/Smad signaling is one of the most characterized features in fibrosis. Although TGF-β is the crucial driver of fibrotic response, it also acts as an anti-inflammatory cytokine and essential mediator that regulates a wide range of biological processes in different cell types and disease conditions. Numerous studies reveal that lncRNAs participate in the emergence and progression of kidney diseases. An outline is becoming manifest in the contribution of TGF-β/Smad-mediated lncRNAs in renal fibrogenesis.

We are now getting better closer to understand how these lncRNAs regulate fibrosis. They can bind to the Smads proteins to exert either anti- or pro-fibrotic effects. They can also serve as miRNA sponges and interact with other signaling pathways to regulate ECM accumulation, EMT, MMT, or other fibrotic processes.

Based on the cell type-, tissue- and disease stage-dependent specialties, lncRNA may also present as biomarkers for clinical diagnosis in renal diseases (Brandenburger et al., 2018; Cheng et al., 2019; Li et al., 2019; Liu et al., 2019b; Loganathan et al., 2020). Interestingly, lncRNAs are relevant biomarkers for disease due to their existence with proteins or in vesicles in the extracellular space under pathological conditions (Teng and Ghoshal, 2015; Ellinger et al., 2016; Zhang et al., 2017c; E, S., Costa et al., 2018; Sarfi et al., 2019). Studies have demonstrated that circulating lncRNAs in body fluid, lncRNA GAS8-AS1, H19, metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), and HOTAIR may be used as promising biomarkers to predict the early progression of cancers (Zhang et al., 2016, 2017b; Du et al., 2018a). Notably, the lncRNA expression profiles in urine also contribute to the early detection of acute T cell-mediated rejection of renal allografts (Lorenzen et al., 2015b), highlighting the importance of lncRNAs in T cell-mediated immune response during renal injuries (Hu et al., 2013).

The modulation of lncRNAs on renal fibrosis is a promising therapeutic target for fibrosis. However, it remains largely unexplored. The low expression amounts, the less conservation between species, the functional complexity, and the difficulty in modifying structures and locations of lncRNA in nuclear or cytoplasmic compartments have halted the development of lncRNA therapies.

Nevertheless, new technologies such as CRISPR/Cas9 editing (Wang et al., 2019; Horlbeck et al., 2020), Gapmer antisense

oligonucleotide-mediated lncRNA silencing (Castanotto et al., 2015; Kuespert et al., 2020), plasmid/vector-delivery short hairpin RNAs (shRNAs) (Zhu et al., 2019; Yao et al., 2020) and ultrasound-mediated gene transfer method (Zhou et al., 2015a; Feng et al., 2018; Sun et al., 2018; Zhang et al., 2019c) may represent the novel strategies to modulate the expression and function of lncRNA in kidney diseases in the future.

AUTHOR CONTRIBUTIONS

Y-YG, J-YD, and X-RH wrote the manuscript, X-SL and H-YL revised and edited the manuscript. All authors contributed to the discussion of this manuscript.

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REFERENCES

Arvaniti, E., Moulos, P., Vakrakou, A., Chatziantoniou, C., Chadjichristos, C., Kavvadas, P., et al. (2016). Whole-transcriptome analysis of UUO mouse model of renal fibrosis reveals new molecular players in kidney diseases. *Sci. Rep.* 6:26235.

Batista, P. J., and Chang, H. Y. (2013). Long noncoding RNAs: cellular address codes in development and disease. *Cell* 152, 1298–1307. doi: 10.1016/j.cell.2013.02.012

Bijkerk, R., Au, Y. W., Stam, W., Duijs, J., Koudijs, A., Lievers, E., et al. (2019). Long Non-coding RNAs rian and miat mediate myofibroblast formation in kidney fibrosis. *Front. Pharmacol.* 10:215. doi: 10.3389/fphar.2019.00215

Bonafoux, D., and Lee, W.-C. (2009). Strategies for TGF-beta modulation: a review of recent patents. *Expert Opin. Ther. Patents* 19, 1759–1769. doi: 10.1517/13543770903397400

Border, W. A., Okuda, S., Languino, L. R., Sporn, M. B., and Ruoslahti, E. (1990). Suppression of experimental glomerulonephritis by antiserum against transforming growth factor beta 1. *Nature* 346, 371–374. doi: 10.1038/346371a0

Brandenburger, T., Salgado Somoza, A., Devaux, Y., and Lorenzen, J. M. (2018). Noncoding RNAs in acute kidney injury. *Kidney Int.* 94, 870–881. doi: 10.1016/j.kint.2018.06.033

Brockdorff, N., Ashworth, A., Kay, G. F., Cooper, P., Smith, S., Mccabe, V. M., et al. (1991). Conservation of position and exclusive expression of mouse Xist from the inactive X chromosome. *Nature* 351, 329–331. doi: 10.1038/351329a0

Brown, C. J., Ballabio, A., Rupert, J. L., Lafreniere, R. G., Grompe, M., Tonlorenzi, R., et al. (1991). A gene from the region of the human X inactivation centre is expressed exclusively from the inactive X chromosome. *Nature* 349, 38–44. doi: 10.1038/349038a0

Cao, H. Y., Li, D., Wang, Y. P., Lu, H. X., Sun, J., and Li, H. B. (2020a). Clinical significance of reduced expression of lncRNA TUG1 in the peripheral blood of systemic lupus erythematosus patients. *Int. J. Rheum. Dis.* 23, 428–434. doi: 10.1111/1756-185x.13786

Cao, H. Y., Li, D., Wang, Y. P., Lu, H. X., Sun, J., and Li, H. B. (2020b). The protection of NF-κB inhibition on kidney injury of systemic lupus erythematosus mice may be correlated with lncRNA TUG1. *Kaohsiung J. Med. Sci.* 36, 354–362. doi: 10.1002/kjm2.12183

Castanotto, D., Lin, M., Kowolik, C., Wang, L., Ren, X.-Q., Soifer, H. S., et al. (2015). A cytoplasmic pathway for gapmer antisense oligonucleotide-mediated gene silencing in mammalian cells. *Nucleic Acids Res.* 43, 9350–9361. doi: 10.1093/nar/gkv964

Che, H., Wang, Y., Li, H., Li, Y., Sahil, A., Lv, J., et al. (2020). Melatonin alleviates cardiac fibrosis via inhibiting lncRNA MALAT1/miR-141-mediated NLRP3 inflammasome and TGF-β1/Smads signaling in diabetic cardiomyopathy. *Faseb J.* 34, 5282–5298. doi: 10.1096/fj.201902692r

Chen, H. Y., Huang, X. R., Wang, W., Li, J. H., Heuchel, R. L., Chung, A. C., et al. (2011). The protective role of Smad7 in diabetic kidney disease: mechanism and therapeutic potential. *Diabetes* 60, 590–601. doi: 10.2337/db10-0403

Chen, W., Zhang, L., Zhou, Z.-Q., Ren, Y.-Q., Sun, L.-N., Man, Y.-L., et al. (2018). Effects of Long Non-Coding RNA LINC00963 on renal interstitial fibrosis and oxidative stress of rats with chronic renal failure via the foxo signaling pathway. *Cell. Physiol. Biochem. Int. J. Exp. Cell. Physiol. Biochem. Pharmacol.* 46, 815–828. doi: 10.1159/000488739

Chen, W., Zhou, Z.-Q., Ren, Y.-Q., Zhang, L., Sun, L.-N., Man, Y.-L., et al. (2019). Effects of long non-coding RNA LINC00667 on renal tubular epithelial cell proliferation, apoptosis and renal fibrosis via the miR-19b-3p/LINC00667/CTGF signaling pathway in chronic renal failure. *Cell. Signal.* 54, 102–114. doi: 10.1016/j.cellsig.2018.10.016

Chen, Y. C., Kuo, P. Y., Chou, Y. C., Chong, H. E., Hsieh, Y. T., Yang, M. L., et al. (2020). Up-regulated expression of pro-apoptotic long noncoding RNA lincRNA-p21 with enhanced cell apoptosis in lupus nephritis. *Int. J. Mol. Sci.* 22:301. doi: 10.3390/ijms22010301

Cheng, G., Liu, D., Liang, H., Yang, H., Chen, K., and Zhang, X. (2019). A cluster of long non-coding RNAs exhibit diagnostic and prognostic values in renal cell carcinoma. *Aging (Albany NY)* 11, 9597–9615. doi: 10.18632/aging.102407 doi: 10.18632/aging.102407

Cho, M. E., Smith, D. C., Branton, M. H., Penzak, S. R., and Kopp, J. B. (2007). Pirfenidone slows renal function decline in patients with focal segmental glomerulosclerosis. *Clin. J. Am. Soc. Nephrol.* 2, 906–913. doi: 10.2215/cjn.01050207

Chung, A. C., Huang, X. R., Zhou, L., Heuchel, R., Lai, K. N., and Lan, H. Y. (2009). Disruption of the Smad7 gene promotes renal fibrosis and inflammation in unilateral ureteral obstruction (UUO) in mice. *Nephrol. Dial. Transplant* 24, 1443–1454. doi: 10.1093/ndt/gfn699

Chung, A. C., and Lan, H. Y. (2011). Chemokines in renal injury. *J. Am. Soc. Nephrol.* 22, 802–809. doi: 10.1681/asn.2010050510

Chung, A. C., Zhang, H., Kong, Y. Z., Tan, J. J., Huang, X. R., Kopp, J. B., et al. (2010). Advanced glycation end-products induce tubular CTGF via TGF-beta-independent Smad3 signaling. *J. Am. Soc. Nephrol.* 21, 249–260. doi: 10.1681/asn.2009010018

Dai, X. Y., Zhou, L., Huang, X. R., Fu, P., and Lan, H. Y. (2015). Smad7 protects against chronic aristolochic acid nephropathy in mice. *Oncotarget* 6, 11930–11944. doi: 10.18632/oncotarget.3718

Derynck, R., and Zhang, Y. E. (2003). Smad-dependent and Smad-independent pathways in TGF-beta family signalling. *Nature* 425, 577–584. doi: 10.1038/nature02006

Du, L., Duan, W., Jiang, X., Zhao, L., Li, J., Wang, R., et al. (2018a). Cell-free lncRNA expression signatures in urine serve as novel non-invasive biomarkers for diagnosis and recurrence prediction of bladder cancer. *J. Cell. Mol. Med.* 22, 2838–2845. doi: 10.1111/jcmm.13578

Du, N., Xu, Z., Gao, M., Liu, P., Sun, B., and Cao, X. (2018b). Combination of Ginsenoside Rg1 and Astragaloside IV reduces oxidative stress and inhibits TGF-beta1/Smads signaling cascade on renal fibrosis in rats with diabetic nephropathy. *Drug Des. Devel. Ther.* 12, 3517–3524. doi: 10.2147/dddt.171286 doi: 10.2147/dddt.s171286

E, S., Costa, M. C., Kurc, S., Drożdż, A., Cortez-Dias, N., and Enguita, F. J. (2018). The circulating non-coding RNA landscape for biomarker research: lessons and prospects from cardiovascular diseases. *Acta Pharmacol. Sin.* 39, 1085–1099. doi: 10.1038/aps.2018.35

Ellinger, J., Gevensleben, H., Müller, S. C., and Dietrich, D. (2016). The emerging role of non-coding circulating RNA as a biomarker in renal cell carcinoma. *Expert Rev. Mol. Diagn.* 16, 1059–1065. doi: 10.1080/14737159.2016.1239531

Fan, J. M., Ng, Y. Y., Hill, P. A., Nikolic-Paterson, D. J., Mu, W., Atkins, R. C., et al. (1999). Transforming growth factor-beta regulates tubular epithelial-myofibroblast transdifferentiation in vitro. *Kidney Int.* 56, 1455–1467. doi: 10.1046/j.1523-1755.1999.00656.x

Feng, M., Tang, P. M., Huang, X. R., Sun, S. F., You, Y. K., Xiao, J., et al. (2018). TGF-beta mediates renal fibrosis via the Smad3-Erbb4-IR long noncoding RNA axis. *Mol. Ther.* 26, 148–161. doi: 10.1016/j.ymthe.2017.09.024

Feng, X., Zhao, J., Ding, J., Shen, X., Zhou, J., and Xu, Z. (2019). LncRNA Blnc1 expression and its effect on renal fibrosis in diabetic nephropathy. *Am. J. Trans. Res.* 11, 5664–5672.

Flynn, R. A., and Chang, H. Y. (2014). Long noncoding RNAs in cell-fate programming and reprogramming. *Cell Stem Cell* 14, 752–761. doi: 10.1016/j.stem.2014.05.014

Gao, J., Wang, W., Wang, F., and Guo, C. (2018). LncRNA-NR_033515 promotes proliferation, fibrogenesis and epithelial-to-mesenchymal transition by targeting miR-743b-5p in diabetic nephropathy. *Biomed. Pharmacother.* 106, 543–552. doi: 10.1016/j.biopha.2018.06.104

Gao, Y., Chen, Z.-Y., Wang, Y., Liu, Y., Ma, J.-X., and Li, Y.-K. (2017). Long non-coding RNA ASncmtRNA-2 is upregulated in diabetic kidneys and high glucose-treated mesangial cells. *Exp. Ther. Med.* 13, 581–587. doi: 10.3892/etm.2017.4027

Glassock, R. J., Warnock, D. G., and Delanaye, P. (2017). The global burden of chronic kidney disease: estimates, variability and pitfalls. *Nat. Rev. Nephrol.* 13, 104–114. doi: 10.1038/nrneph.2016.163

Gu, Y.-Y., Liu, X.-S., Huang, X.-R., Yu, X.-Q., and Lan, H.-Y. (2020a). Diverse role of TGF-β in kidney disease. *Front. Cell Dev. Biol.* 8:123. doi: 10.3389/fcell.2020.00123

Gu, Y.-Y., Liu, X.-S., Huang, X.-R., Yu, X.-Q., and Lan, H.-Y. (2020b). TGF-β in renal fibrosis: triumphs and challenges. *Future Med. Chem.* 12, 853–866. doi: 10.4155/fmc-2020-0005

Gu, Y. Y., Lu, F. H., Huang, X. R., Zhang, L., Mao, W., Yu, X. Q., et al. (2020c). Non-Coding RNAs as biomarkers and therapeutic targets for diabetic kidney disease. *Front. Pharmacol.* 11:583528. doi: 10.3389/fphar.2020.583528

Györfi, A. H., Matei, A.-E., and Distler, J. H. W. (2018). Targeting TGF-β signaling for the treatment of fibrosis. *Matrix Biol. J. Int. Soc. Matrix Biol.* 6, 8–27. doi: 10.1016/j.matbio.2017.12.016

Han, R., Hu, S., Qin, W., Shi, J., Zeng, C., Bao, H., et al. (2019). Upregulated long noncoding RNA LOC105375913 induces tubulointerstitial fibrosis in focal segmental glomerulosclerosis. *Sci. Rep.* 9:716.

Hangauer, M. J., Vaughn, I. W., and Mcmanus, M. T. (2013). Pervasive transcription of the human genome produces thousands of previously unidentified long intergenic noncoding RNAs. *PLoS Genet.* 9:e1003569. doi: 10.1371/journal.pgen.1003569

Horlbeck, M. A., Liu, S. J., Chang, H. Y., Lim, D. A., and Weissman, J. S. (2020). Fitness effects of CRISPR/Cas9-targeting of long noncoding RNA genes. *Nat. Biotechnol.* 38, 573–576. doi: 10.1038/s41587-020-0428-0

Hou, C. C., Wang, W., Huang, X. R., Fu, P., Chen, T. H., Sheikh-Hamad, D., et al. (2005). Ultrasound-microbubble-mediated gene transfer of inducible Smad7 blocks transforming growth factor-beta signaling and fibrosis in rat remnant kidney. *Am. J. Pathol.* 166, 761–771. doi: 10.1016/s0002-9440(10)62297-3

Hu, G., Tang, Q., Sharma, S., Yu, F., Escobar, T. M., Muljo, S. A., et al. (2013). Expression and regulation of intergenic long noncoding RNAs during T cell development and differentiation. *Nat. Immunol.* 14, 1190–1198. doi: 10.1038/ni.2712

Hu, M., Wang, R., Li, X., Fan, M., Lin, J., Zhen, J., et al. (2017). LncRNA MALAT1 is dysregulated in diabetic nephropathy and involved in high glucose-induced podocyte injury via its interplay with β-catenin. *J. Cell. Mol. Med.* 21, 2732–2747. doi: 10.1111/jcmm.13189

Hu, S. Y., Jia, X. Y., Li, J. N., Zheng, X., Ao, J., Liu, G., et al. (2016). T cell infiltration is associated with kidney injury in patients with anti-glomerular basement membrane disease. *Sci. China Life Sci.* 59, 1282–1289. doi: 10.1007/s11427-016-5030-9

Huang, S., Xu, Y., Ge, X., Xu, B., Peng, W., Jiang, X., et al. (2019). Long noncoding RNA NEAT1 accelerates the proliferation and fibrosis in diabetic nephropathy through activating Akt/mTOR signaling pathway. *J. Cell. Physiol.* 234, 11200–11207. doi: 10.1002/jcp.27770

Huang, W., Lan, X., Li, X., Wang, D., Sun, Y., Wang, Q., et al. (2017). Long non-coding RNA PVT1 promote LPS-induced septic acute kidney injury by regulating TNFα and JNK/NF-κB pathways in HK-2 cells. *Int. Immunopharmacol.* 47, 134–140. doi: 10.1016/j.intimp.2017.03.030

Huang, X. R., Chung, A. C., Wang, X. J., Lai, K. N., and Lan, H. Y. (2008a). Mice overexpressing latent TGF-beta1 are protected against renal fibrosis in obstructive kidney disease. *Am. J. Physiol. Renal. Physiol.* 295, F118–F127.

Huang, X. R., Chung, A. C., Zhou, L., Wang, X. J., and Lan, H. Y. (2008b). Latent TGF-beta1 protects against crescentic glomerulonephritis. *J. Am. Soc. Nephrol.* 19, 233–242. doi: 10.1681/asn.2007040484

Isaka, Y. (2018). Targeting TGF-beta signaling in kidney fibrosis. *Int. J. Mol. Sci.* 19:2532. doi: 10.3390/ijms19092532

Iwano, M., Plieth, D., Danoff, T. M., Xue, C., Okada, H., and Neilson, E. G. (2002). Evidence that fibroblasts derive from epithelium during tissue fibrosis. *J. Clin. Invest.* 110, 341–350. doi: 10.1172/jci0215518

Jiang, H., Wu, F., Jiang, N., Gao, J., and Zhang, J. (2019). Reconstruction and analysis of competitive endogenous RNA network reveals regulatory role of long non−coding RNAs in hepatic fibrosis. *Mol. Med. Rep.* 20, 4091–4100.

Jiang, X., and Zhang, F. (2017). Long noncoding RNA: a new contributor and potential therapeutic target in fibrosis. *Epigenomics* 9, 1233–1241. doi: 10.2217/epi-2017-0020

Ju, W., Ogawa, A., Heyer, J., Nierhof, D., Yu, L., Kucherlapati, R., et al. (2006). Deletion of Smad2 in mouse liver reveals novel functions in hepatocyte growth and differentiation. *Mol. Cell Biol.* 26, 654–667. doi: 10.1128/mcb.26.2.654-667.2006

Ka, S. M., Huang, X. R., Lan, H. Y., Tsai, P. Y., Yang, S. M., Shui, H. A., et al. (2007). Smad7 gene therapy ameliorates an autoimmune crescentic glomerulonephritis in mice. *J. Am. Soc. Nephrol.* 18, 1777–1788. doi: 10.1681/asn.2006080901

Kinsey, G. R., and Okusa, M. D. (2014). Expanding role of T cells in acute kidney injury. *Curr. Opin. Nephrol. Hypertens* 23, 9–16. doi: 10.1097/01.mnh.0000436695.29173.de

Kölling, M., Genschel, C., Kaucsar, T., Hübner, A., Rong, S., Schmitt, R., et al. (2018). Hypoxia-induced long non-coding RNA Malat1 is dispensable for renal ischemia/reperfusion-injury. *Sci. Rep.* 8:3438.

Kopp, J. B., Factor, V. M., Mozes, M., Nagy, P., Sanderson, N., Bottinger, E. P., et al. (1996). Transgenic mice with increased plasma levels of TGF-beta 1 develop progressive renal disease. *Lab. Invest* 74, 991–1003.

Kuespert, S., Heydn, R., Peters, S., Wirkert, E., Meyer, A.-L., Siebörger, M., et al. (2020). Antisense oligonucleotide in LNA-gapmer design targeting TGFBR2-a key single gene target for safe and effective inhibition of TGFβ signaling. *Int. J. Mol. Sci.* 21:1952. doi: 10.3390/ijms21061952

Lan, H. Y. (2008). Smad7 as a therapeutic agent for chronic kidney diseases. *Front. Biosci.* 13:4984–4992. doi: 10.2741/3057

Lan, H. Y. (2011). Diverse roles of TGF-beta/Smads in renal fibrosis and inflammation. *Int. J. Biol. Sci.* 7, 1056–1067. doi: 10.7150/ijbs.7.1056

Lan, H. Y. (2012a). Smads as therapeutic targets for chronic kidney disease. *Kidney Res. Clin. Pract.* 31, 4–11. doi: 10.1016/j.krcp.2011.12.001

Lan, H. Y. (2012b). Transforming growth factor-beta/Smad signalling in diabetic nephropathy. *Clin. Exp. Pharmacol. Physiol.* 39, 731–738. doi: 10.1111/j.1440-1681.2011.05663.x

Lan, H. Y., and Chung, A. C. (2012). TGF-beta/Smad signaling in kidney disease. *Semin. Nephrol.* 32, 236–243. doi: 10.1016/j.semnephrol.2012.04.002

Lan, H. Y., Mu, W., Tomita, N., Huang, X. R., Li, J. H., Zhu, H. J., et al. (2003). Inhibition of renal fibrosis by gene transfer of inducible Smad7 using ultrasound-microbubble system in rat UUO model. *J. Am. Soc. Nephrol.* 14, 1535–1548. doi: 10.1097/01.asn.0000067632.04658.b8

Li, A., Peng, R., Sun, Y., Liu, H., Peng, H., and Zhang, Z. (2018). LincRNA 1700020I14Rik alleviates cell proliferation and fibrosis in diabetic nephropathy via miR-34a-5p/Sirt1/HIF-1α signaling. *Cell Death Dis.* 9:461.

Li, J., Qu, X., Yao, J., Caruana, G., Ricardo, S. D., Yamamoto, Y., et al. (2010). Blockade of endothelial-mesenchymal transition by a Smad3 inhibitor

delays the early development of streptozotocin-induced diabetic nephropathy. *Diabetes* 59, 2612–2624. doi: 10.2337/db09-1631

Li, J. H., Zhu, H. J., Huang, X. R., Lai, K. N., Johnson, R. J., and Lan, H. Y. (2002). Smad7 inhibits fibrotic effect of TGF-Beta on renal tubular epithelial cells by blocking Smad2 activation. *J. Am. Soc. Nephrol.* 13, 1464–1472. doi: 10.1097/01.asn.0000014252.37680.e4

Li, M. O., and Flavell, R. A. (2008). TGF-beta: a master of all T cell trades. *Cell* 134, 392–404. doi: 10.1016/j.cell.2008.07.025

Li, N., Cui, Y., Yin, M., and Liu, F. (2019). Screening potential prognostic biomarkers of long non-coding RNAs for predicting the risk of chronic kidney disease. *Braz. J. Med. Biol. Res.* 52:e8333.

Li, N., Jia, T., and Li, Y. R. (2020). LncRNA NEAT1 accelerates the occurrence and development of diabetic nephropathy by sponging miR-23c. *Eur. Rev. Med. Pharmacol. Sci.* 24, 1325–1337.

Liao, Z., Ye, Z., Xue, Z., Wu, L., Ouyang, Y., Yao, C., et al. (2019). Identification of renal long non-coding RNA RP11-2B6.2 as a positive regulator of type I interferon signaling pathway in lupus nephritis. *Front. Immunol.* 10:975. doi: 10.3389/fimmu.2019.00975

Ling, L., Tan, Z., Zhang, C., Gui, S., Hu, Y., and Chen, L. (2018). Long noncoding RNA ENSRNOG00000037522 is involved in the podocyte epithelial−mesenchymal transition in diabetic rats. *Int. J. Mol. Med.* 41, 2704–2714.

Liu, B., Qiang, L., Wang, G. D., Duan, Q., and Liu, J. (2019a). LncRNA MALAT1 facilities high glucose induced endothelial to mesenchymal transition and fibrosis via targeting miR-145/ZEB2 axis. *Eur. Rev. Med. Pharmacol. Sci.* 23, 3478–3486.

Liu, G. X., Li, Y. Q., Huang, X. R., Wei, L., Chen, H. Y., Shi, Y. J., et al. (2013). Disruption of Smad7 promotes ANG II-mediated renal inflammation and fibrosis via Sp1-TGF-beta/Smad3-NF.kappaB-dependent mechanisms in mice. *PLoS One* 8:e53573. doi: 10.1371/journal.pone.0053573

Liu, G. X., Li, Y. Q., Huang, X. R., Wei, L. H., Zhang, Y., Feng, M., et al. (2014). Smad7 inhibits AngII-mediated hypertensive nephropathy in a mouse model of hypertension. *Clin. Sci. (Lond)* 127, 195–208. doi: 10.1042/cs20130706

Liu, Y. (2011). Cellular and molecular mechanisms of renal fibrosis. *Nat. Rev. Nephrol.* 7:684. doi: 10.1038/nrneph.2011.149

Liu, Z., Huang, X. R., and Lan, H. Y. (2012). Smad3 mediates ANG II-induced hypertensive kidney disease in mice. *Am. J. Physiol. Renal. Physiol.* 302, F986–F997.

Liu, Z., Wang, Y., Shu, S., Cai, J., Tang, C., and Dong, Z. (2019b). Non-coding RNAs in kidney injury and repair. *Am. J. Physiol. Cell Physiol.* 317, C177–C188.

Lodyga, M., and Hinz, B. (2019). TGF-β1 - A truly transforming growth factor in fibrosis and immunity. *Semin. Cell Dev. Biol.* 101, 123–139. doi: 10.1016/j.semcdb.2019.12.010

Loeffler, I., Liebisch, M., Allert, S., Kunisch, E., Kinne, R. W., and Wolf, G. (2018). FSP1-specific SMAD2 knockout in renal tubular, endothelial, and interstitial cells reduces fibrosis and epithelial-to-mesenchymal transition in murine STZ-induced diabetic nephropathy. *Cell Tissue Res.* 372, 115–133. doi: 10.1007/s00441-017-2754-1

Loganathan, T. S., Sulaiman, S. A., Abdul Murad, N. A., Shah, S. A., Abdul Gafor, A. H., Jamal, R., et al. (2020). Interactions among non-coding RNAs in diabetic nephropathy. *Front. Pharmacol.* 11:191. doi: 10.3389/fphar.2020.00191

Lopez-Hernandez, F. J., and Lopez-Novoa, J. M. (2012). Role of TGF-beta in chronic kidney disease: an integration of tubular, glomerular and vascular effects. *Cell Tissue Res.* 347, 141–154. doi: 10.1007/s00441-011-1275-6

Lorenzen, J. M., Schauerte, C., Kielstein, J. T., Hübner, A., Martino, F., Fiedler, J., et al. (2015a). Circulating long noncoding RNATapSaki is a predictor of mortality in critically ill patients with acute kidney injury. *Clin. Chem.* 61, 191–201. doi: 10.1373/clinchem.2014.230359

Lorenzen, J. M., Schauerte, C., Kölling, M., Hübner, A., Knapp, M., Haller, H., et al. (2015b). Long noncoding RNAs in urine are detectable and may enable early detection of acute t cell-mediated rejection of renal allografts. *Clin. Chem.* 61, 1505–1514. doi: 10.1373/clinchem.2015.243600

Lu, S., Dong, L., Jing, X., Gen-Yang, C., Zhang-Suo, L., and Zhan-Zheng, Z. (2020). Abnormal lncRNA CCAT1/microRNA-155/SIRT1 axis promoted inflammatory response and apoptosis of tubular epithelial cells in LPS caused acute kidney injury. *Mitochondrion* 53, 76–90. doi: 10.1016/j.mito.2020.03.010 doi: 10.1016/j.mito.2020.03.010

Ludwig-Portugall, I., and Kurts, C. (2014). T cell isolation from mouse kidneys. *Methods Mol. Biol.* 1193, 27–35. doi: 10.1007/978-1-4939-1212-4_4

Mack, M., and Yanagita, M. (2015). Origin of myofibroblasts and cellular events triggering fibrosis. *Kidney Int.* 87, 297–307. doi: 10.1038/ki.2014.287

Majo, J., Klinkhammer, B. M., Boor, P., and Tiniakos, D. (2019). Pathology and natural history of organ fibrosis. *Curr. Opin. Pharmacol.* 49, 82–89. doi: 10.1016/j.coph.2019.09.009

March, J. T., Golshirazi, G., Cernisova, V., Carr, H., Leong, Y., Lu-Nguyen, N., et al. (2018). Targeting TGFβ signaling to address fibrosis using antisense oligonucleotides. *Biomedicines* 6:74. doi: 10.3390/biomedicines6030074

Meng, X. M. (2019). Inflammatory mediators and renal fibrosis. *Adv. Exp. Med. Biol.* 1165, 381–406. doi: 10.1007/978-981-13-8871-2_18

Meng, X. M., Huang, X. R., Xiao, J., Chung, A. C., Qin, W., Chen, H. Y., et al. (2012). Disruption of Smad4 impairs TGF-beta/Smad3 and Smad7 transcriptional regulation during renal inflammation and fibrosis *in vivo* and *in vitro*. *Kidney Int.* 81, 266–279. doi: 10.1038/ki.2011.327

Meng, X. M., Nikolic-Paterson, D. J., and Lan, H. Y. (2014). Inflammatory processes in renal fibrosis. *Nat. Rev. Nephrol.* 10, 493–503. doi: 10.1038/nrneph.2014.114

Meng, X. M., Nikolic-Paterson, D. J., and Lan, H. Y. (2016a). TGF-beta: the master regulator of fibrosis. *Nat. Rev. Nephrol.* 12, 325–338. doi: 10.1038/nrneph.2016.48 doi: 10.1038/nrneph.2016.48

Meng, X. M., Wang, S., Huang, X. R., Yang, C., Xiao, J., Zhang, Y., et al. (2016b). Inflammatory macrophages can transdifferentiate into myofibroblasts during renal fibrosis. *Cell Death Dis.* 7:e2495. doi: 10.1038/cddis.2016.402

Meng, X. M., Zhang, Y., Huang, X. R., Ren, G. L., Li, J., and Lan, H. Y. (2015). Treatment of renal fibrosis by rebalancing TGF-beta/Smad signaling with the combination of asiatic acid and naringenin. *Oncotarget* 6, 36984–36997. doi: 10.18632/oncotarget.6100

Morishita, Y., Yoshizawa, H., Watanabe, M., Ishibashi, K., Muto, S., Kusano, E., et al. (2014). siRNAs targeted to Smad4 prevent renal fibrosis *in vivo*. *Sci. Rep.* 4:6424.

Ng, Y. Y., Hou, C. C., Wang, W., Huang, X. R., and Lan, H. Y. (2005). Blockade of NFkappaB activation and renal inflammation by ultrasound-mediated gene transfer of Smad7 in rat remnant kidney. *Kidney Int. Suppl.* 67, S83–S91.

Nie, Y., Li, S., Yi, Y., Su, W., Chai, X., Jia, D., et al. (2014). Effects of astragalus injection on the TGFbeta/Smad pathway in the kidney in type 2 diabetic mice. *BMC Complement Altern Med.* 14:148.

Ohno, S. (1972). So much "junk" DNA in our genome. . *Brookhaven Symp. Biol.* 23, 366–370.

Pang, X., Feng, G., Shang, W., Liu, L., Li, J., Feng, Y., et al. (2019). Inhibition of lncRNA MEG3 protects renal tubular from hypoxia-induced kidney injury in acute renal allografts by regulating miR-181b/TNF-α signaling pathway. *J. Cell. Biochem.* 120, 12822–12831. doi: 10.1002/jcb.28553

Peng, W., Huang, S., Shen, L., Tang, Y., Li, H., and Shi, Y. (2019). Long noncoding RNA NONHSAG053901 promotes diabetic nephropathy via stimulating Egr-1/TGF-β-mediated renal inflammation. *J. Cell Physiol.* 234, 18492–18503. doi: 10.1002/jcp.28485

Provenzano, M., Coppolino, G., De Nicola, L., Serra, R., Garofalo, C., Andreucci, M., et al. (2019). Unraveling cardiovascular risk in renal patients: a new take on old tale. *Front. Cell Dev. Biol.* 7:314. doi: 10.3389/fcell.2019.00314

Pu, Y., Zhao, H., Wu, X., Mei, M., and Shen, B. (2020). The long noncoding RNA Ptprd-IR is a novel molecular target for TGF-β1-mediated nephritis. *Int. J. Biochem. Cell Biol.* 122:105742. doi: 10.1016/j.biocel.2020.105742

Puthanveetil, P., Chen, S., Feng, B., Gautam, A., and Chakrabarti, S. (2015). Long non-coding RNA MALAT1 regulates hyperglycaemia induced inflammatory process in the endothelial cells. *J. Cell. Mol. Med.* 19, 1418–1425. doi: 10.1111/jcmm.12576

Quinn, J. J., and Chang, H. Y. (2016). Unique features of long non-coding RNA biogenesis and function. *Nat. Rev. Genet.* 17, 47–62. doi: 10.1038/nrg.2015.10

Ren, G.-L., Zhu, J., Li, J., and Meng, X.-M. (2019). Noncoding RNAs in acute kidney injury. *J. Cell. Physiol.* 234, 2266–2276. doi: 10.1002/jcp.27203

Riaz, F., and Li, D. (2019). Non-coding RNA associated competitive endogenous rna regulatory network: novel therapeutic approach in liver fibrosis. *Curr. Gene. Ther.* 19, 305–317. doi: 10.2174/1566523219666191107113046

Ruiz-Ortega, M., Rayego-Mateos, S., Lamas, S., Ortiz, A., and Rodrigues-Diez, R. R. (2020). Targeting the progression of chronic kidney disease. *Nat. Rev. Nephrol.* 16, 269–288.

Sarfi, M., Abbastabar, M., and Khalili, E. (2019). Long noncoding RNAs biomarker-based cancer assessment. *J. Cell Physiol.* 234, 16971–16986. doi: 10.1002/jcp.28417

Schiffer, M., Bitzer, M., Roberts, I. S., Kopp, J. B., Ten Dijke, P., Mundel, P., et al. (2001). Apoptosis in podocytes induced by TGF-beta and Smad7. *J. Clin. Invest.* 108, 807–816. doi: 10.1172/jci200112367

Schiffer, M., Schiffer, L. E., Gupta, A., Shaw, A. S., Roberts, I. S. D., Mundel, P., et al. (2002). Inhibitory smads and tgf-Beta signaling in glomerular cells. *J. Am. Soc. Nephrol. JASN* 13, 2657–2666. doi: 10.1097/01.asn.0000033276.06451.50

Sharma, K., Ix, J. H., Mathew, A. V., Cho, M., Pflueger, A., Dunn, S. R., et al. (2011). Pirfenidone for diabetic nephropathy. *J. Am. Soc. Nephrol. JASN* 22, 1144–1151.

Shen, J., Liu, L., Zhang, F., Gu, J., and Pan, G. (2019). LncRNA TapSAKI promotes inflammation injury in HK-2 cells and urine derived sepsis-induced kidney injury. *J. Pharm. Pharmacol.* 71, 839–848. doi: 10.1111/jphp.13049

Sun, J., Zhang, S., Shi, B., Zheng, D., and Shi, J. (2017). Transcriptome identified lncRNAs associated with renal fibrosis in UUO Rat Model. *Front. Physiol.* 8:658. doi: 10.3389/fphys.2017.00658

Sun, S. F., Tang, P. M. K., Feng, M., Xiao, J., Huang, X. R., Li, P., et al. (2018). Novel lncRNA Erbb4-IR promotes diabetic kidney injury in db/db mice by targeting miR-29b. *Diabetes* 67, 731–744. doi: 10.2337/db17-0816

Tang, P. M., Tang, P. C., Chung, J. Y., and Lan, H. Y. (2017). TGF-beta1 signaling in kidney disease: from Smads to long non-coding RNAs. *Noncoding RNA Res.* 2, 68–73. doi: 10.1016/j.ncrna.2017.04.001

Tang, P. M., Zhang, Y. Y., and Lan, H. Y. (2018a). LncRNAs in TGF-beta-driven tissue fibrosis. *Noncoding RNA* 4:26. doi: 10.3390/ncrna4040026

Tang, P. M., Zhang, Y. Y., Mak, T. S., Tang, P. C., Huang, X. R., and Lan, H. Y. (2018b). Transforming growth factor-beta signalling in renal fibrosis: from Smads to non-coding RNAs. *J. Physiol.* 596, 3493–3503. doi: 10.1113/jp274492

Teng, K. Y., and Ghoshal, K. (2015). Role of Noncoding RNAs as biomarker and therapeutic targets for liver fibrosis. *Gene. Expr.* 16, 155–162. doi: 10.3727/105221615x14399878166078

Tian, H., Wu, M., Zhou, P., Huang, C., Ye, C., and Wang, L. (2018). The long non-coding RNA MALAT1 is increased in renal ischemia-reperfusion injury and inhibits hypoxia-induced inflammation. *Renal. Failure* 40, 527–533. doi: 10.1080/0886022x.2018.1487863

Trachtman, H., Fervenza, F. C., Gipson, D. S., Heering, P., Jayne, D. R., Peters, H., et al. (2011). A phase 1, single-dose study of fresolimumab, an anti-TGF-beta antibody, in treatment-resistant primary focal segmental glomerulosclerosis. *Kidney Int.* 79, 1236–1243. doi: 10.1038/ki.2011.33

Tsuchida, K., Zhu, Y., Siva, S., Dunn, S. R., and Sharma, K. (2003). Role of Smad4 on TGF-beta-induced extracellular matrix stimulation in mesangial cells. *Kidney Int.* 63, 2000–2009. doi: 10.1046/j.1523-1755.2003.00009.x

Van der Hauwaert, C., Glowacki, F., Pottier, N., and Cauffiez, C. (2019). Non-Coding RNAs as new therapeutic targets in the context of renal fibrosis. *Int. J. Mol. Sci.* 20:1977. doi: 10.3390/ijms20081977

Vincenti, F., Fervenza, F. C., Campbell, K. N., Diaz, M., Gesualdo, L., Nelson, P., et al. (2017). A phase 2, double-blind, placebo-controlled, randomized study of fresolimumab in patients with steroid-resistant primary focal segmental glomerulosclerosis. *Kidney Int. Rep.* 2, 800–810. doi: 10.1016/j.ekir.2017.03.011

Voelker, J., Berg, P. H., Sheetz, M., Duffin, K., Shen, T., Moser, B., et al. (2017). Anti-TGF-beta1 antibody therapy in patients with diabetic nephropathy. *J. Am. Soc. Nephrol.* 28, 953–962. doi: 10.1681/asn.2015111230

Wang, C., Lu, T., Emanuel, G., Babcock, H. P., and Zhuang, X. (2019). Imaging-based pooled CRISPR screening reveals regulators of lncRNA localization. *Proc. Natl. Acad. Sci. U.S.A.* 116, 10842–10851. doi: 10.1073/pnas.1903808116

Wang, J., Pan, J., Li, H., Long, J., Fang, F., Chen, J., et al. (2018a). lncRNA ZEB1-AS1 was suppressed by p53 for renal fibrosis in diabetic nephropathy. *Mol. Ther. Nucleic Acids* 12, 741–750. doi: 10.1016/j.omtn.2018.07.012

Wang, J., Song, J., Li, Y., Shao, J., Xie, Z., and Sun, K. (2020a). Down-regulation of LncRNA CRNDE aggravates kidney injury via increasing MiR-181a-5p in sepsis. *Int. Immunopharmacol.* 79:105933. doi: 10.1016/j.intimp.2019.105933

Wang, M., Wang, S., Yao, D., Yan, Q., and Lu, W. (2016a). A novel long non-coding RNA CYP4B1-PS1-001 regulates proliferation and fibrosis in diabetic nephropathy. *Mol. Cell Endocrinol.* 426, 136–145. doi: 10.1016/j.mce.2016.02.020

Wang, M., Yao, D., Wang, S., Yan, Q., and Lu, W. (2016b). Long non-coding RNA ENSMUST00000147869 protects mesangial cells from proliferation and fibrosis induced by diabetic nephropathy. *Endocrine* 54, 81–92. doi: 10.1007/s12020-016-0950-5

Wang, P., Luo, M. L., Song, E., Zhou, Z., Ma, T., Wang, J., et al. (2018b). Long noncoding RNA lnc-TSI inhibits renal fibrogenesis by negatively regulating the TGF-beta/Smad3 pathway. *Sci. Transl. Med.* 10:eaat2039. doi: 10.1126/scitranslmed.aat2039

Wang, S., Chen, X., Wang, M., Yao, D., Chen, T., Yan, Q., et al. (2018c). Long non-coding RNA CYP4B1-PS1-001 inhibits proliferation and fibrosis in diabetic nephropathy by interacting with nucleolin. *Cell Physiol. Biochem.* 49, 2174–2187. doi: 10.1159/000493821

Wang, W., Huang, X. R., Canlas, E., Oka, K., Truong, L. D., Deng, C., et al. (2006). Essential role of Smad3 in angiotensin II-induced vascular fibrosis. *Circ. Res.* 98, 1032–1039. doi: 10.1161/01.res.0000218782.52610.dc

Wang, Y. Y., Jiang, H., Pan, J., Huang, X. R., Wang, Y. C., Huang, H. F., et al. (2017). Macrophage-to-Myofibroblast transition contributes to interstitial fibrosis in chronic renal allograft injury. *J. Am. Soc. Nephrol.* 28, 2053–2067. doi: 10.1681/asn.2016050573

Wang, Z., Zhang, B., Chen, Z., He, Y., Ru, F., Liu, P., et al. (2020b). The long noncoding RNA myocardial infarction-associated transcript modulates the epithelial-mesenchymal transition in renal interstitial fibrosis. *Life Sci.* 241:117187. doi: 10.1016/j.lfs.2019.117187

Wu, C. F., Chiang, W. C., Lai, C. F., Chang, F. C., Chen, Y. T., Chou, Y. H., et al. (2013). Transforming growth factor beta-1 stimulates profibrotic epithelial signaling to activate pericyte-myofibroblast transition in obstructive kidney fibrosis. *Am. J. Pathol.* 182, 118–131. doi: 10.1016/j.ajpath.2012.09.009

Xiao, X., Yuan, Q., Chen, Y., Huang, Z., Fang, X., Zhang, H., et al. (2019). LncRNA ENST00000453774.1 contributes to oxidative stress defense dependent on autophagy mediation to reduce extracellular matrix and alleviate renal fibrosis. *J. Cell. Physiol.* 234, 9130–9143. doi: 10.1002/jcp.27590

Xie, H., Xue, J. D., Chao, F., Jin, Y. F., and Fu, Q. (2016). Long non-coding RNA-H19 antagonism protects against renal fibrosis. *Oncotarget* 7, 51473–51481. doi: 10.18632/oncotarget.10444

Xu, B. H., Sheng, J., You, Y. K., Huang, X. R., Ma, R. C. W., Wang, Q., et al. (2020). Deletion of Smad3 prevents renal fibrosis and inflammation in type 2 diabetic nephropathy. *Metabolism* 103:154013. doi: 10.1016/j.metabol.2019.154013

Xue, L., Liu, L., Huang, J., Wen, J., Yang, R., Bo, L., et al. (2017). Tumor necrosis factor-like weak inducer of apoptosis activates type i interferon signals in lupus nephritis. *Biomed. Res. Int.* 2017:4927376.

Xue, R., Li, Y., Li, X., Ma, J., An, C., and Ma, Z. (2019). miR-185 affected the EMT, cell viability, and proliferation via DNMT1/MEG3 pathway in TGF-β1-induced renal fibrosis. *Cell Biol. Int.* 43, 1152–1162. doi: 10.1002/cbin.11046

Yang, F., Chung, A. C., Huang, X. R., and Lan, H. Y. (2009). Angiotensin II induces connective tissue growth factor and collagen I expression via transforming growth factor-beta-dependent and -independent Smad pathways: the role of Smad3. *Hypertension* 54, 877–884. doi: 10.1161/hypertensionaha.109.136531

Yang, F., Huang, X. R., Chung, A. C., Hou, C. C., Lai, K. N., and Lan, H. Y. (2010). Essential role for Smad3 in angiotensin II-induced tubular epithelial-mesenchymal transition. *J. Pathol.* 221, 390–401.

Yang, Y. L., Hu, F., Xue, M., Jia, Y. J., Zheng, Z. J., Li, Y., et al. (2019). Early growth response protein-1 upregulates long noncoding RNA Arid2-IR to promote extracellular matrix production in diabetic kidney disease. *Am. J. Physiol. Cell Physiol.* 316, C340–C352.

Yang, Y. L., Xue, M., Jia, Y. J., Hu, F., Zheng, Z. J., Wang, L., et al. (2020). Long noncoding RNA NEAT1 is involved in the protective effect of Klotho on renal tubular epithelial cells in diabetic kidney disease through the ERK1/2 signaling pathway. *Exp. Mol. Med.* 52, 266–280. doi: 10.1038/s12276-020-0381-5

Yao, L., Zhou, B., You, L., Hu, H., and Xie, R. (2020). LncRNA MIAT/miR-133a-3p axis regulates atrial fibrillation and atrial fibrillation-induced myocardial fibrosis. *Mol. Biol. Rep.* 47, 2605–2617. doi: 10.1007/s11033-020-05347-0

Yi, H., Peng, R., Zhang, L. Y., Sun, Y., Peng, H. M., Liu, H. D., et al. (2017). LincRNA-Gm4419 knockdown ameliorates NF-κB/NLRP3 inflammasome-mediated inflammation in diabetic nephropathy. *Cell Death Dis.* 8:e2583. doi: 10.1038/cddis.2016.451

Yoshimura, A., and Muto, G. (2011). TGF-beta function in immune suppression. *Curr. Top Microbiol. Immunol.* 350, 127–147. doi: 10.1007/82_2010_87

Yuan, Q., Tan, R. J., and Liu, Y. (2019). Myofibroblast in kidney fibrosis: origin, activation, and regulation. *Adv. Exp. Med. Biol.* 1165, 253–283. doi: 10.1007/978-981-13-8871-2_12

Zeisberg, E. M., Potenta, S. E., Sugimoto, H., Zeisberg, M., and Kalluri, R. (2008). Fibroblasts in kidney fibrosis emerge via endothelial-to-mesenchymal transition. *J. Am. Soc. Nephrol.* 19, 2282–2287. doi: 10.1681/asn.2008050513

Zhang, C., Yuan, J., Hu, H., Chen, W., Liu, M., Zhang, J., et al. (2017a). Long non-coding RNA CHCHD4P4 promotes epithelial-mesenchymal transition and inhibits cell proliferation in calcium oxalate-induced kidney damage. *Braz. J. Med. Biol. Res.* 51:e6536.

Zhang, D., Liu, X., Wei, B., Qiao, G., Jiang, T., and Chen, Z. (2017b). Plasma lncRNA GAS8-AS1 as a potential biomarker of papillary thyroid carcinoma in chinese patients. *Int. J. Endocrinol.* 2017:2645904.

Zhang, J., Jiang, T., Liang, X., Shu, S., Xiang, X., Zhang, W., et al. (2019a). lncRNA MALAT1 mediated high glucose-induced HK-2 cell epithelial-to-mesenchymal transition and injury. *J. Physiol. Biochem.* 75, 443–452. doi: 10.1007/s13105-019-00688-2

Zhang, K., Luo, Z., Zhang, Y., Zhang, L., Wu, L., Liu, L., et al. (2016). Circulating lncRNA H19 in plasma as a novel biomarker for breast cancer. *Cancer Biomarkers* 17, 187–194. doi: 10.3233/cbm-160630

Zhang, L., Zhao, S., and Zhu, Y. (2020a). Long noncoding RNA growth arrest-specific transcript 5 alleviates renal fibrosis in diabetic nephropathy by downregulating matrix metalloproteinase 9 through recruitment of enhancer of zeste homolog 2. *Faseb J.* 34, 2703–2714. doi: 10.1096/fj.201901380rr

Zhang, L. H., Xiao, B., Zhong, M., Li, Q., Chen, J. Y., Huang, J. R., et al. (2020b). LncRNA NEAT1 accelerates renal mesangial cell injury via modulating the miR-146b/TRAF6/NF-κB axis in lupus nephritis. *Cell Tissue Res.* 382, 627–638. doi: 10.1007/s00441-020-03248-z

Zhang, P., Sun, Y., Peng, R., Chen, W., Fu, X., Zhang, L., et al. (2019b). Long non-coding RNA Rpph1 promotes inflammation and proliferation of mesangial cells in diabetic nephropathy via an interaction with Gal-3. *Cell Death Dis.* 10:526.

Zhang, T. N., Li, D., Xia, J., Wu, Q. J., Wen, R., Yang, N., et al. (2017c). Non-coding RNA: a potential biomarker and therapeutic target for sepsis. *Oncotarget* 8, 91765–91778. doi: 10.18632/oncotarget.21766

Zhang, Y., Meng, X. M., Huang, X. R., and Lan, H. Y. (2018). The preventive and therapeutic implication for renal fibrosis by targetting TGF-beta/Smad3 signaling. *Clin. Sci. (Lond)* 132, 1403–1415. doi: 10.1042/cs20180243

Zhang, Y. Y., Tang, P. M., Tang, P. C., Xiao, J., Huang, X. R., Yu, C., et al. (2019c). LRNA9884, a Novel Smad3-dependent long noncoding rna, promotes diabetic kidney injury in db/db mice via enhancing MCP-1-dependent renal inflammation. *Diabetes* 68, 1485–1498. doi: 10.2337/db18-1075

Zhao, D., Liu, Z., and Zhang, H. (2019). The protective effect of the TUG1/miR−197/MAPK1 axis on lipopolysaccharide−induced podocyte injury. *Mol. Med. Rep.* 20, 49–56.

Zhao, T., Sun, S., Zhang, H., Huang, X., Yan, M., Dong, X., et al. (2016). Therapeutic effects of tangshen formula on diabetic nephropathy in rats. *PLoS One* 11:e0147693. doi: 10.1371/journal.pone.0147693

Zhao, T. T., Zhang, H. J., Lu, X. G., Huang, X. R., Zhang, W. K., Wang, H., et al. (2014). Chaihuang-Yishen granule inhibits diabetic kidney disease in rats through blocking TGF-beta/Smad3 signaling. *PLoS One* 9:e90807. doi: 10.1371/journal.pone.0090807

Zhou, H., Gao, L., Yu, Z. H., Hong, S. J., Zhang, Z. W., and Qiu, Z. Z. (2019). LncRNA HOTAIR promotes renal interstitial fibrosis by regulating Notch1 pathway via the modulation of miR-124. *Nephrology (Carlton)* 24, 472–480. doi: 10.1111/nep.13394

Zhou, J., and Jiang, H. (2019). Livin is involved in TGF-beta1-induced renal tubular epithelial-mesenchymal transition through lncRNA-ATB. *Ann. Transl. Med.* 7:463. doi: 10.21037/atm.2019.08.29

Zhou, L., Fu, P., Huang, X. R., Liu, F., Chung, A. C., Lai, K. N., et al. (2010). Mechanism of chronic aristolochic acid nephropathy: role of Smad3. *Am. J. Physiol. Renal. Physiol.* 298, F1006–F1017.

Zhou, Q., Chung, A. C., Huang, X. R., Dong, Y., Yu, X., and Lan, H. Y. (2014). Identification of novel long noncoding RNAs associated with TGF-beta/Smad3-mediated renal inflammation and fibrosis by RNA sequencing. *Am. J. Pathol.* 184, 409–417. doi: 10.1016/j.ajpath.2013.10.007

Zhou, Q., Huang, X. R., Yu, J., Yu, X., and Lan, H. Y. (2015a). Long Noncoding RNA Arid2-IR Is a novel therapeutic target for renal inflammation. *Mol. Ther.* 23, 1034–1043. doi: 10.1038/mt.2015.31

Zhou, Q., Xiong, Y., Huang, X. R., Tang, P., Yu, X., and Lan, H. Y. (2015b). Identification of genes associated with Smad3-dependent Renal Injury by RNA-seq-based transcriptome analysis. *Sci. Rep.* 5:17901.

Zhou, S. G., Zhang, W., Ma, H. J., Guo, Z. Y., and Xu, Y. (2018). Silencing of LncRNA TCONS_00088786 reduces renal fibrosis through miR-132. *Eur. Rev. Med. Pharmacol. Sci.* 22, 166–173.

Zhu, Y., Ni, T., Lin, J., Zhang, C., Zheng, L., and Luo, M. (2019). Long non-coding RNA H19, a negative regulator of microRNA-148b-3p, participates in hypoxia stress in human hepatic sinusoidal endothelial cells via NOX4 and eNOS/NO signaling. *Biochimie* 163, 128–136. doi: 10.1016/j.biochi.2019.04.006

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Kidney Biopsy in Patients With Monoclonal Gammopathy: A Multicenter Retrospective Cohort Study

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Objectives: To analyze the clinical characteristics and renal pathological manifestations of patients with monoclonal gammopathy (MG) and kidney injury.

Methods: This was a multicenter retrospective cohort study conducted at four tertiary hospitals in China. The study population comprised patients with MG admitted from January 1 2013 to December 31 2020. Hospitalization records, laboratory data, and kidney biopsy reports of all patients were collected from the electronic hospital information systems. The study outcomes included kidney disease progression and major hemorrhagic complications after kidney biopsy.

Results: We identified 1,164 patients with MG, 782 (67.2%) of whom had underlying kidney injury. Of 101 patients who underwent kidney biopsy, 16 had malignant neoplasms. Amyloid nephropathy was the most common finding (n = 34, 33.7%), followed by membranous nephropathy (n = 18, 17.8%) and membranoproliferative nephritis (n = 8, 7.9%). Among 85 patients with non-malignant hematologic conditions who underwent kidney biopsy, 43 had MG of renal significance (MGRS) related lesions and 42 had MG-unrelated lesions. The risk of kidney disease progression was higher in patients with kidney injury than in patients without kidney injury.

Conclusion: Among patients with MG and kidney injury, only 12.9% underwent kidney biopsy and more than 40% of these patients had MG-unrelated lesions. A kidney biopsy is safe and essential to maximize the possibility of correct diagnosis for patients with clinically suspected MG of renal significance (MGRS).

Keywords: kidney biopsy, monoclonal gammopathy, monoclonal gammopathy of undetermined significance, monoclonal gammopathy of renal significance, amyloid nephropathy

INTRODUCTION

Monoclonal gammopathy (MG) is defined as the presence of a monoclonal immunoglobulin (MIg) in serum resulting from the clonal proliferation of Ig-producing plasma cells or B-lymphocytes (1, 2). Monoclonal gammopathies are a group of disorders ranging from non-malignant small clonal proliferations to malignant neoplasms of plasma cells or B-lymphocytes (3, 4). MG of undetermined significance (MGUS) is usually a premalignant condition, is not associated with any organ damage attributable to the MIg, and does not require treatment (5, 6).

The MIg or its fragments (light chains and/or heavy chains) are secreted into the blood and subsequently filtered by the glomerulus before entering the urine. The kidneys are commonly involved in such hematologic conditions and there are various types of renal disease (7). Kidney damage secondary to MIg in the absence of hematologic malignancy has been increasingly recognized and is called MG of renal significance (MGRS) (8). This term was introduced by the International Kidney and Monoclonal Gammopathy Research Group (IKMG) in 2012 and updated to include all B-cell and plasma cell proliferative disorders that produce a nephrotoxic MIg by the new IKMG consensus in 2019 (9). However, the underlying B-cell or plasma cell clone does not cause tumor complications or meet any current hematologic criteria for specific therapy. Once the hematologic condition progresses to overt multiple myeloma (MM), Waldenström macroglobulinemia (WM), advanced-stage chronic lymphocytic leukemia, or malignant lymphoma (as defined by their respective established disease criteria), these diseases are no longer considered MGRS and affected patients are managed according to disease-specific protocols.

Chronic kidney disease (CKD) is a leading public health problem worldwide. The global estimated prevalence of CKD is 13.4% (11.7–15.1%) (10). During the past decades, the prevalence of CKD and MG has significantly increased, especially among the elderly (11–13). Therefore, it is conceivable that some hospitalized patients with MG also have CKD, but kidney damage is not associated with MIg. Given the different disease management strategies, it is very important to distinguish MGRS from other types of CKD in these patients (14, 15). However, there is uncertainty about which patients should undergo kidney biopsy and when this should be performed among nephrologists and hematologists. Knowledge about clinicopathological features of those patients with MG and kidney injury who needs kidney biopsy is still limited.

In this retrospective cohort study, we described the clinical characteristics of MG patients with kidney injury, the spectrum of kidney biopsy findings in these patients, and the clinical outcomes of these patients. We also evaluated the risk of hemorrhagic complications and the potential benefit of kidney biopsy in these patients.

METHODS

Study Design, Population, and Data Source

This was a retrospective multicenter cohort study conducted at four tertiary hospitals in China (Nanfang hospital, Guangzhou; Guilin Medical University Affiliated Hospital, Guilin; The First People's Hospital of Foshan, Foshan; The second people's hospital of Shenzhen, Shenzhen). The study cohort comprised patients admitted to a participating hospital from January 1, 2013, to December 31, 2020, who had at least one positive serum immunofixation result during hospitalization. The admission date of the hospitalization during which the patient was first diagnosed with MG was considered the baseline for analysis.

The study centers were asked to export the hospitalization records, laboratory data, and kidney biopsy reports of all patients from the electronic hospital information systems. Hospitalization records consisted of patients' age, sex, date of admission, diagnosis code at admission and discharge, and in-hospital death. Laboratory data included the value and time of patients' serum and urinary tests. Kidney biopsy reports included histologic diagnosis and detailed histologic reports based on light microscopy, electron microscopy, and immunofluorescence assays. The specimens were regularly stained with hematoxylin-eosin, periodic acid Schiff, periodic acid-silver methenamine, and Masson's trichrome, and tested for IgA, IgG, IgM, C3, C4, C1q, and κ/λ light chains by immunofluorescence assays.

The exported data from all study centers were pooled and cleaned at the National Clinical Research Center for Kidney Disease in Guangzhou. The Medical Ethics Committee of Nanfang Hospital approved the study protocol (NFYY-2015-073).

Identification of MGRS

All patients were divided into two groups (with and without kidney injury) based on their kidney function and urinary test results at baseline for more detailed analysis. Patients were considered to have kidney injury at baseline if they met any of the following inclusion criteria: urinary protein $\geq$ 1+, estimated glomerular filtration rate (eGFR) $\leq$ 60 ml/min/1.73 m^2 (CKD-EPI equation), or daily urinary protein excretion $>$ 0.5 g/24 h.

Among MG patients without hematologic malignancy who underwent kidney biopsy, lesions were considered to be associated with MGRS based on the consensus guideline issued by the IKMG. C3 glomerulopathy and thrombotic microangiopathy are not associated with renal deposition of MIg. Other MGRS-related lesions are caused by deposition of MIgs, fragments thereof, or various aggregation products. Patients with thrombotic microangiopathy were considered to have an MGRS-related lesion if no other obvious cause of thrombotic microangiopathy (such as atypical hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, drugs, or underlying autoimmune disease) was identified.

Study Outcome

The primary outcome was kidney disease progression, which was defined as progression to end-stage renal disease (sustained

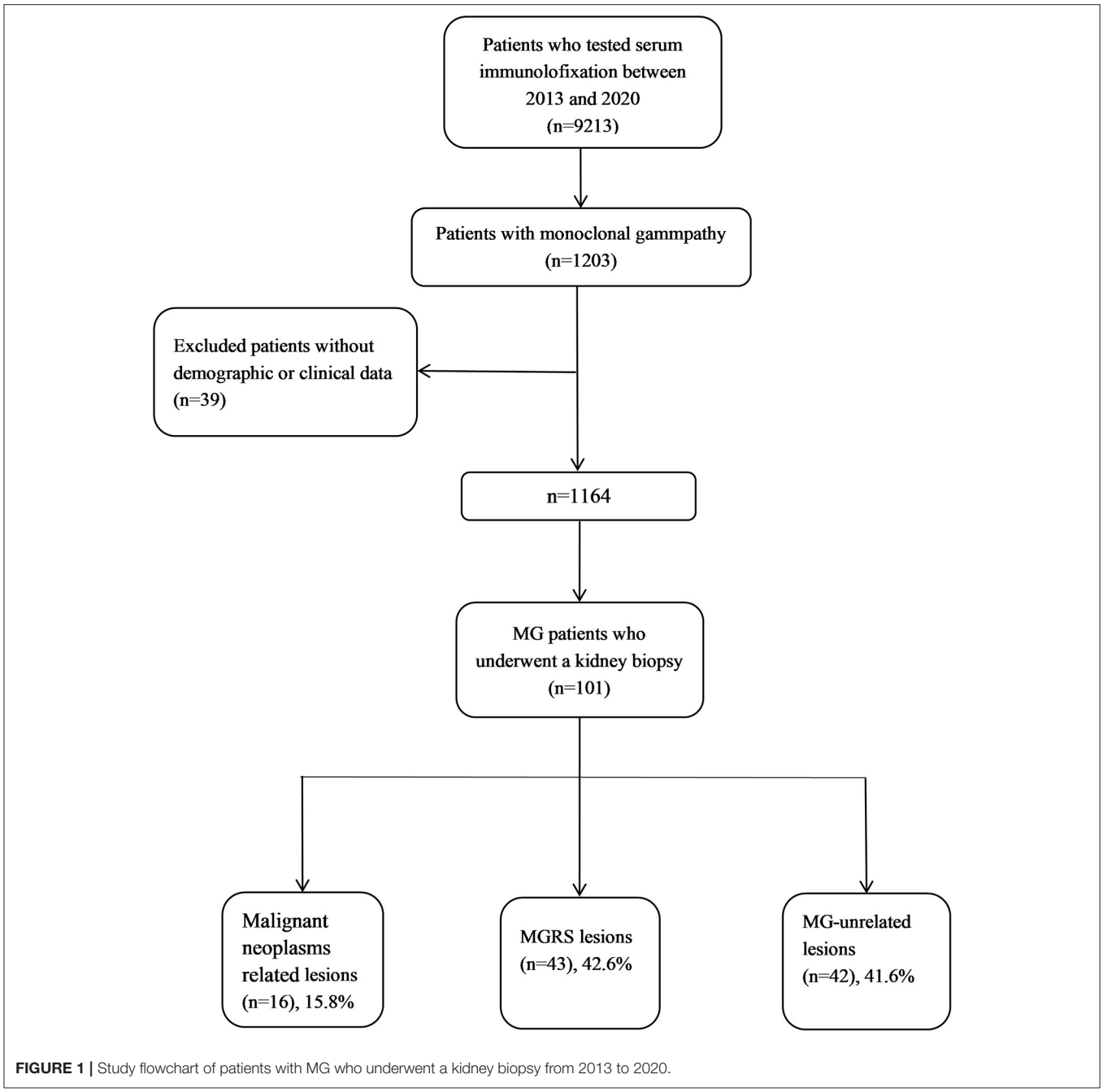

FIGURE 1 | Study flowchart of patients with MG who underwent a kidney biopsy from 2013 to 2020.

eGFR < 15 ml/min/1.73 m^2 or the need for maintenance renal replacement therapy) or a permanent 30% reduction in the eGFR relative to the initial level at biopsy. The secondary outcome was major hemorrhagic complications, which were defined as hemorrhagic complications requiring blood transfusion or other invasive procedures after kidney biopsy.

Statistical Analysis

The eGFR was calculated using the CKD-Epidemiology Collaboration equation. SPSS 22.0 statistical software was used. All P values are two-sided. $P < 0.05$ was considered statistically significant. Continuous data were expressed as mean ± standard deviation. Categorical variables were presented as number and percentage. Survival analysis was performed by generating Kaplan-Meier curves and using the log-rank test. Only patients followed up for more than 3 months were included in survival analysis.

RESULTS

Study Population

Among 9,213 hospitalized patients who underwent a serum immunofixation test during hospitalization, 1,203 had positive results. After excluding 39 patients with missing demographic

TABLE 1 | Baseline characteristics of patients with MG stratified by the status of kidney injury.

Characteristic	Overall (n = 1,164)	With kidney injury (n = 782)	Without kidney injury (n = 382)	P
Male	711 (61.1%)	481 (61.5%)	230 (60.2%)	0.669
Age (year)	61.2 ± 12.4	62.1 ± 12.3	59.4 ± 12.5	0.001
Hypertension	290 (24.9%)	231 (29.5%)	59 (15.4%)	<0.001
Diabetes	120 (10.3%)	90 (11.5%)	30 (7.9%)	0.054
Hepatitis B	35 (3.0%)	25 (3.2%)	10 (2.6%)	0.587
Serum studies				
Albumin (g/l)	31.2 ± 7.4	29.9 ± 7.5	33.8 ± 6.3	<0.001
Creatinine (μmo/l)	166.3 ± 268.2	216.1 ± 315.3	64.3 ± 16.8	<0.001
eGFR (ml/min per 1.73 m^2)	71.4 ± 36.7	58.5 ± 36.8	97.8 ± 17.1	<0.001
eGFR<60 (ml/min per 1.73 m^2)	401 (34.5%)	401 (51.3%)	0 (0)	<0.001
κ/λ	1.81 (0.70–7.42)	1.71 (0.70–6.08)	2.10 (0.80–9.81)	0.543
Urinary studies				
Urinary protein (g/d)	0.82 (0.20–3.04)	1.44 (0.35–4.15)	0.11 (0.07–0.15)	<0.001
Proteinuria >1.5 g/d	166 (39.6%)	166 (48.0%)	0 (0)	<0.001
Hematuria	407 (38.8%)	407 (56.3%)	0 (0)	<0.001
κ/λ	1.42 (0.49–2.68)	1.42 (0.49–2.68)	/	/
Hematologic studies				
Clonal subtype				
Ig isotype				
IgA(+)	305 (26.2%)	212 (27.1%)	93 (24.3%)	0.314
IgG(+)	638 (54.8%)	416 (53.2%)	222 (58.1%)	0.113
IgM(+)	128 (11.0%)	78 (10.0%)	50 (13.1%)	0.111
IgA/IgG/IgM(+)	19 (1.6%)	14 (1.8%)	5 (1.3%)	0.543
IgA&IgG&IgM(−)	74 (6.4%)	62 (7.9%)	12 (3.1%)	0.002
Ig light chain				
Kappa(+)	498 (42.8%)	321 (41.0%)	177 (46.3%)	0.087
Lambda(+)	480 (41.2%)	333 (42.6%)	147 (38.5%)	0.182
Kappa&Lambda(+)	20 (1.7%)	12 (1.5%)	8 (2.1%)	0.49
Kappa&Lambda(−)	166 (14.3%)	116 (14.8%)	50 (13.1%)	0.424
Received BM biopsy	377 (29.0%)	261 (33.4%)	76 (19.9%)	<0.001

Values for continuous variables are described as mean ± SD or median (IQR) depending on the distribution, and for categoric variables described as count (%).

or clinical data, we identified 1,164 patients who met the inclusion criteria (**Figure 1**). Of these patients, 782 (67.2%, 782/1,164) had underlying kidney injury, only 101 (12.9%, 101/782) of whom underwent kidney biopsy. The level of serum creatinine, daily excretion of urinary protein, and proportions of patients with hypertension and hematuria were higher among patients with kidney injury than among patients without kidney injury (**Table 1**). Age, the ratio of serum total light chains (κ to λ), and the proportions of male patients, patients with diabetes, and patients with hepatitis B did not significantly differ between the two groups. The clonal subtype of MIg, including immunoglobulin isotype and immunoglobulin light chain, determined by serum immunofixation is also shown in **Table 1**. Light chain only was observed with a higher frequency in MG patients with kidney injury (62/782, 7.9% vs. 12/382, 3.1%, MG patients with kidney injury vs. MG patients without kidney injury) and kidney biopsy group (43/101, 42.6 vs. 19/681, 2.8%, kidney biopsy group vs. no kidney biopsy group).

Clinical Characteristics and Pathological Manifestations of MG Patients Who Underwent Kidney Biopsy

Among patients with MG and kidney injury, 101 underwent kidney biopsy. **Table 2** summarizes the demographic and laboratory data of patients who underwent kidney biopsy and those who did not. Patients with kidney injury who underwent kidney biopsy were younger, had higher levels of serum creatinine and daily excretion of urinary protein and had a lower initial eGFR and serum albumin level than patients with kidney injury who did not undergo kidney biopsy.

Of 101 patients who underwent kidney biopsy, 16 (15.8%) were diagnosed with malignant neoplasms (15 MM and 1 smoldering MM). Among 85 patients with non-malignant hematologic conditions, 43 had MGRS-related lesions and 42 had MG-unrelated lesions (**Table 3**). There was no significant difference in the average age, level of serum creatinine and daily excretion of urinary protein, the ratio of serum total light

TABLE 2 | Baseline characteristics of MG patients with kidney injury stratified by the status of kidney biopsy.

Characteristic	Kidney biopsy ($n = 101$)	Without kidney biopsy ($n = 681$)	*P*
Male	68 (67.3%)	413 (60.6%)	0.198
Age (year)	56.2 ± 12.0	62.9 ± 12.1	<0.001
Hypertension	36 (35.6%)	195 (28.6%)	0.15
Diabetes	17 (16.8%)	73 (10.7%)	0.072
Hepatitis B	8 (7.9%)	17 (2.5%)	0.004
Serum studies			
Albumin (g/l)	25.8 ± 8.4	30.5 ± 7.2	<0.001
Creatinine (μmo/l)	167.1 ± 182.3	223.3 ± 330.0	0.012
eGFR (ml/min per 1.73 m^2)	62.7 ± 33.5	57.9 ± 37.3	0.182
eGFR<60 (ml/min per 1.73 m^2)	46 (45.5%)	355 (52.1%)	0.217
κ/λ	1.35 (0.72–2.12)	1.83 (0.68–9.91)	<0.001
Urinary studies			
Urinary protein (g/d)	4.94 (1.67–7.07)	1.10 (0.33–3.27)	<0.001
Proteinuria >1.5 g/d	38 (77.6%)	128 (43.1%)	<0.001
Hematuria	72 (71.3%)	335 (53.9%)	0.001
κ/λ	1.42 (0.49–2.68)	/	/
Clonal subtype			
Ig isotype			
IgA(+)	18 (17.8%)	194 (28.5%)	0.024
IgG(+)	30 (29.7%)	386 (56.7%)	<0.001
IgM(+)	10 (9.9%)	68 (10.0%)	0.979
IgA/IgG/IgM(+)	0 (0)	14 (2.1%)	0.146
IgA&IgG&IgM(−)	43 (42.6%)	19 (2.8%)	<0.001
Ig light chain			
Kappa(+)	26 (25.7%)	295 (43.3%)	0.001
Lambda(+)	59 (58.4%)	274 (40.2%)	0.001
Kappa&Lambda(+)	0 (0)	12 (1.8%)	0.179
Kappa&Lambda(−)	16 (15.8%)	100 (14.7%)	0.76
Received BM biopsy	60 (59.4%)	201 (29.5%)	<0.001

Values for continuous variables are described as mean ± SD or median (IQR) depending on the distribution, and for categoric variables described as count (%).

chains (κ to λ) between patients with MGRS-related and MG-unrelated lesions.

As shown in **Table 4**, amyloid nephropathy was the most common MGRS-related lesion, followed by membranoproliferative glomerulonephritis. Eighteen patients were diagnosed with membranous nephropathy, two of whom had MGRS-related lesions and 16 of whom had MG-unrelated lesions. Membranous nephropathy was the most common MG-unrelated lesion. Four patients were diagnosed with diabetic glomerulosclerosis, all of whom had preexisting diabetes mellitus and were considered to have MG-unrelated lesions. The types of the light chain (κ and λ) present were consistent between the serum immunofixation and tissue immunofluorescence assays in 40% of patients. Consistent with the heterogeneity, IgG subclass staining differed across various pathologic manifestions groups in MG patients (**Supplementary Table 1**).

Study Outcomes

Among 782 patients with MG and kidney injury, 280 (35.8%, 280/782) were followed up for more than 3 months and included in the primary outcome analysis. Over a median of 13 months (interquartile range, 8–22 months) of follow-up, kidney disease progression (the primary outcome) occurred in 58 (20.7%, 58/280) patients. Among 85 non-maligancy MG patients who have received kidney biopsy, kidney disease progression occurred in 1 of 43 (2.3%) patients with MGRS-related lesions and 3 of 42 (7.1%) patients with MG-unrelated lesions. Kaplan-Meier curves in **Figure 2** showed that the risk of kidney disease progression was higher in MG patients with kidney injury than in MG patients without kidney injury ($P < 0.0001$). However, the risk of kidney disease progression significantly differ between MG patients who underwent kidney biopsy and those who did not ($P = 0.03$) (**Supplementary Figure 1**). Of two patients who suffered major hemorrhagic complications after a kidney biopsy, one underwent blood transfusion and the other underwent intra-arterial embolization.

DISCUSSION

In this multicenter retrospective cohort of 1,164 patients with MG, 782 (67.2%, 782/1,164) had underlying kidney injury. Among them, only 101 (12.9%, 101/782) underwent kidney

TABLE 3 | Baseline characteristics of patients with MG with kidney injury who underwent a kidney biopsy.

Characteristic	Kidney biopsy not relevant with malignant neoplasms ($n = 85$)	MGRS-related lesions ($n = 43$)	MG-unrelated lesions ($n = 42$)	*P*
Male	60 (70.6%)	26 (60.5%)	34 (81.0%)	0.038
Age (year)	55.8 ± 12.4	57.7 ± 11.0	53.9 ± 13.6	0.161
Hypertension	32 (37.6%)	12 (27.9%)	20 (47.6%)	0.061
Diabetes	15 (17.6%)	4 (9.3%)	11 (26.2%)	0.041
Hepatitis B	7 (8.2%)	3 (7.0%)	4 (9.5%)	0.669
Serum studies				
Albumin (g/l)	24.9 ± 8.2	23.6 ± 8.3	26.2 ± 8.0	0.139
Creatinine (μmo/l)	160.1 ± 155.9	166.4 ± 175.3	153.5 ± 135.0	0.705
eGFR (ml/min per 1.73 m^2)	63.7 ± 33.9	60.0 ± 33.3	67.5 ± 34.5	0.309
eGFR<60 (ml/min per 1.73 m^2)	39 (45.9%)	24 (55.8%)	15 (35.7%)	0.063
κ/λ	1.35 (0.73–1.91)	0.78 (0.51–1.70)	1.60 (1.26–2.25)	0.57
Urinary studies				
Urinary protein (g/d)	5.64 (2.03–7.07)	5.91 (3.57–7.21)	3.37 (1.59–7.96)	0.44
Proteinuria >1.5 g/d	32 (82.1%)	18 (85.7%)	14 (77.8%)	0.52
Hematuria	60 (70.6%)	30 (69.8%)	30 (71.4%)	0.867
κ/λ	1.36 (0.53–2.2)	0.69 (0.21–1.42)	1.98 (1.47–3.77)	0.478
Hematologic studies				
Clonal subtype				
Ig isotype				
IgA(+)	15 (17.6%)	7 (16.3%)	8 (19.0%)	0.738
IgG(+)	24 (28.2%)	13 (30.2%)	11 (26.2%)	0.679
IgM(+)	10 (11.8%)	3 (7.0%)	7 (16.7%)	0.166
IgA/IgG/IgM(+)	0 (0)	0 (0)	0 (0)	/
IgA&IgG&IgM(−)	36 (42.4%)	20 (46.5%)	16 (38.1%)	0.432
Ig light chain				
Kappa(+)	21 (24.7%)	7 (16.3%)	14 (33.3%)	0.068
Lambda(+)	51 (60.0%)	29 (67.4%)	22 (52.4%)	0.156
Kappa&Lambda(+)	0 (0)	0 (0)	0 (0)	/
Kappa&Lambda(−)	13 (15.3%)	7 (16.3%)	6 (14.3%)	0.799
Received BM biopsy	49 (57.6%)	27 (62.8%)	22 (52.4%)	0.332

Values for continuous variables are described as mean ± SD or median (IQR) depending on the distribution, and for categoric variables described as count (%).

biopsy and 2 (2.0%, 2/101) suffered from major hemorrhagic complications. Kidney lesions were not related to MG in more than 40% (49.4%, 42/85) of these patients. Kidney biopsy was a safe and simple procedure for pathological diagnosis and prognosis. In our cohort, amyloid nephropathy was found to be the most common MGRS-related lesion, while membranous nephropathy was the most common MG-unrelated lesion. Given that the therapeutic regimens of MGRS and other types of kidney disease are different, our findings suggest that kidney biopsy is essential and can provide clinicians with useful information to guide subsequent treatment.

Over the last decades, the prevalence of CKD and MG has increased dramatically in China. CKD is prevalent in hospitalized patients with MG. Therefore, patients can simultaneously have CKD and MG without a direct causal relationship between the two disorders. The kidney biopsy is the gold standard for diagnosis of most kidney diseases however the procedure was underperformed in our country. There was only 12.9% (101/782) of our study population with kidney biopsy. Except for patients with absolute contraindications (i.e., severe thrombocytopenia), the main reasons why most patients with MG and kidney disease did not undergo kidney biopsy may be that (1) patients with MG and kidney injury were diagnosed with MGRS by default and kidney biopsy was arbitrarily considered unnecessary, and (2) patients refused to undergo kidney biopsy because it is invasive and the risk of hemorrhagic complications might be higher in patients with hematologic disease. Our study showed that kidney biopsy is safe and the risk of massive hemorrhage in MG patients is similar to that reported in general patients (16, 17). More importantly, the major clinical characteristics, such as age, level of serum creatinine and urinary protein, the proportion of hematuria, were not comparable between group with MGRS-related lesions and group with MG-unrelated lesions in our study patients, indicating that the clinical parameters could not be a

TABLE 4 | Classification of pathological manifestations of MG patients who underwent kidney biopsy.

Malignant neoplasms related lesions (*N* = 16)	*n* (%)	MGRS-related lesions (*N* = 43)	*n* (%)	MG-unrelated lesions (*N* = 42)	*n* (%)
Amyloid nephropathy	8 (50.0%)	Amyloid nephropathy	26 (60.5%)	MN	16 (38.1%)
Glomerular minor lesion	2 (12.5%)	MPGN	4 (9.3%)	Diabetic glomerulosclerosis	4 (9.5%)
FSGS	1 (6.3%)	MN	2 (4.7%)	IgA nephropathy	3 (7.1%)
EPGN	1 (6.3%)	Mesangioproliferative GN	2 (4.7%)	FSGS	3 (7.1%)
MPGN	1 (6.3%)	Mesangial nodular sclerosing glomerulopathy	2 (4.7%)	MPGN	3 (7.1%)
LCDD	1 (6.3%)	Subacute tubulointerstitial nephritis	2 (4.7%)	Glomerular minor lesion	3 (7.1%)
HCDD	1 (6.3%)	C3 GN	1 (2.3%)	Proliferative-sclerosing GN	3 (7.1%)
TMA	1 (6.3%)	EPGN	1 (2.3%)	Subacute tubulointerstitial nephritis	2 (4.8%)
		LCDD	1 (2.3%)	Hypertensive renal disease	1 (2.4%)
		TMA	1 (2.3%)	LN	1 (2.4%)
		Proliferative-sclerosing GN	1 (2.3%)	EPGN	1 (2.4%)
				Mesangioproliferative GN	1 (2.4%)
				Hepatitis B virus-associated nephritis	1 (2.4%)

FSGS, focal segmental glomurular sclerosis; MN, membranous nephropathy; TMA, thrombotic microangiopathy; MPGN, membranoproliferative glomerulonephritis; LCDD, light chain deposition disease; HCDD, heavy chain deposition disease; EPGN, endocapillary proliferative glomerulonephritis; LN, lupus nephritis.

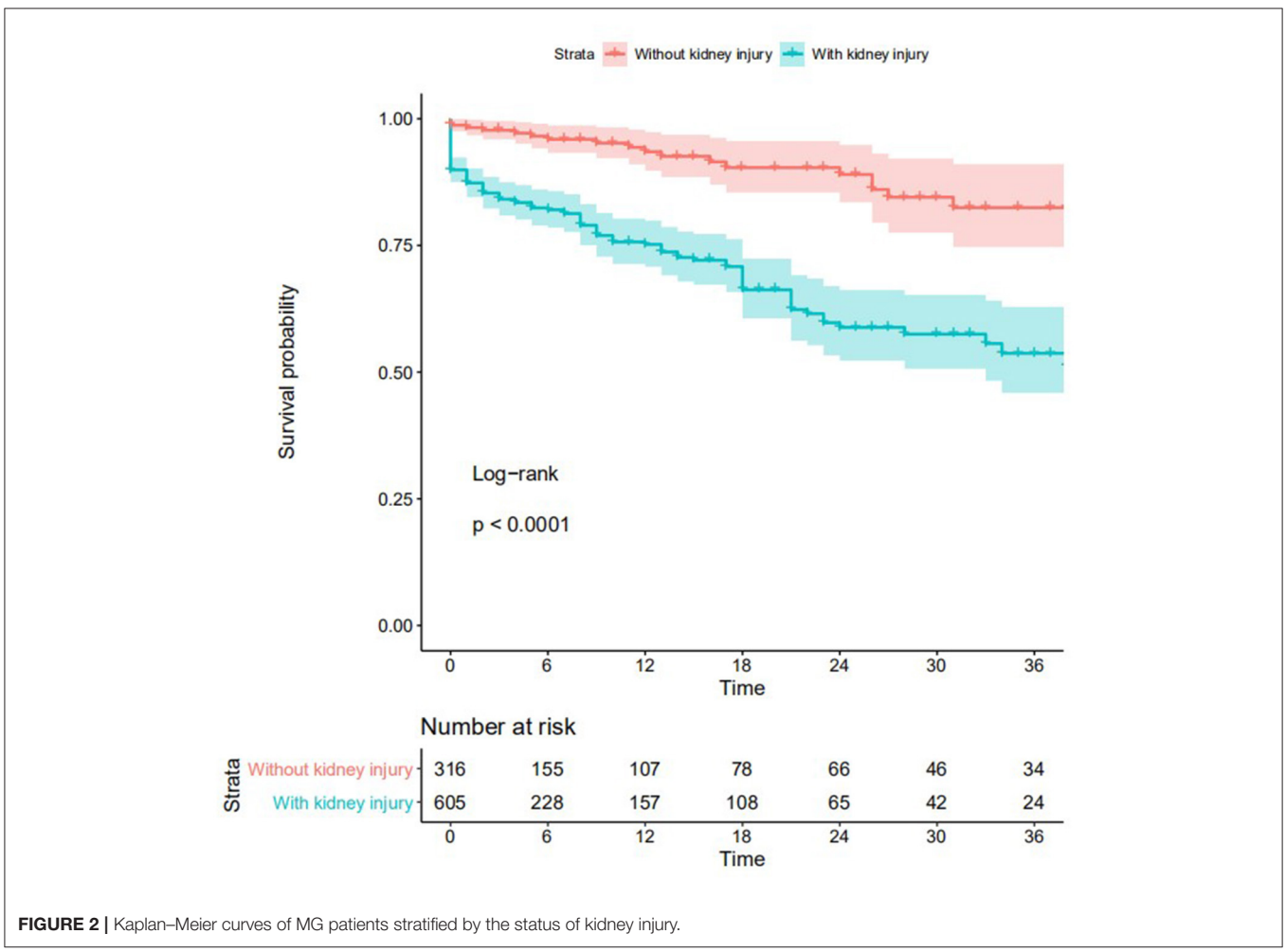

FIGURE 2 | Kaplan–Meier curves of MG patients stratified by the status of kidney injury.

predictor of finding the MGRS-related lesion. Establishing an MGRS diagnosis hinges on kidney biopsy as it is the only way to demonstrate the presence of monoclonal immunoglobulin deposits in the renal area.

In the IKMG consensus, MGUS does not require treatment, while specific management usually employing chemotherapy toward the pathologic clone and type of kidney injury is recommended for MGRS due to the nephrotoxicity of monoclonal immunoglobulin. MGRS is complex and heterogeneous concerning clinical, pathogenetic, pathologic, and prognostic findings, therefore, a close collaboration between several specialties is required for optimal patient treatment and management. Treatment of MGRS intends to eliminate the underlying clonal plasma cell or B-cell population, and thereby decrease or abolish production of the offending MIg (18). This is the most effective intervention achieved by stem cell transplantation, chemotherapy, and targeted therapeutic regimens developed for MM, acute lymphoblastic leukemia, and B-cell lymphoproliferative disorders (19, 20). According to Ravindran's investigation (3), 13.7% of newly diagnosed MG had chronic kidney diseases, indicating without a kidney biopsy, it is very likely that MG patients with MG-unrelated lesions will be presumably considered as MGRS and treated accordingly. Therefore, clinicians should comprehensively evaluate patients with suspected MGRS, including complete assessment of their renal function and nephrologists should have a low threshold to perform a kidney biopsy.

Consistent with the results of previous studies, amyloid nephropathy was the most common type of MGRS-related lesions in our study, followed by membranoproliferative glomerulonephritis (21). Due to the nephrotoxic potential of MIgs owing to their unique physicochemical properties, the renal disease develops differently in the setting of monoclonal gammopathies (22). Patients with MIg could behave as MGUS present with no sign of renal impairment, however, few studies reported the exact proportion of MG patients with renal impairment. Several small studies showed that kidney injury was observed in 14–58% of patients with specific types of MG while our MG cohort contains 67.2% (782/1,164) renal dysfunction individuals (3, 23, 24). Other patients with MIg show diverse pathological manifestations upon renal biopsy known as MIg-related renal diseases, such as diseases of the glomerulus (amyloid light-chain amyloidosis and MIg deposition disease) and tubules (proximal tubular disorders and cast nephropathy) (25). Most MIg-associated renal diseases develop due to direct deposition of nephrotoxic MIg or its light or heavy chain fragments in various renal tissue compartments. The MIg-related disease is diagnosed based on kidney biopsy and immunofluorescence studies that identify monotypic immunoglobulin deposits (although these are minimal in patients with C3 glomerulopathy and thrombotic microangiopathy).

Furthermore, among patients who underwent kidney biopsy, 18(17.8%, 18/101) and 3(3.3%, 3/101) were diagnosed with membranous nephropathy and IgA nephropathy, respectively. This is unsurprising because these two disorders are the most common type of biopsy-identified glomerulonephritis in general patients (26). Among the 18 patients with membranous nephropathy, 16 (89%, 16/18) with positive PLA2R staining and negative for κ and λ light chains were considered to have MG-unrelated lesions. Meanwhile, the two other patients with negative PLA2R staining and positive for IgG3 and κ or λ light chains were considered to have MG-related membranous nephropathy. Treatment of primary and secondary membranous nephropathy differs. Similarly, treatment of MGRS and primary IgA nephropathy differs. These patients may benefit from accurate pathological diagnosis by kidney biopsy. Although the Kaplan-Meier curves did show a worse prognosis in kidney biopsy group, it may result from selection bias as patients who underwent kidney biopsy had more severe impaired function. Also, long-term follow-up and monitoring are required for some patients who cannot be fully diagnosed after a kidney biopsy. A total of seven MG patients were diagnosed with membranoproliferative glomerulonephritis with high clinical suspicion of MGRS in our study. However, the manifestation of kidney damage (proteinuria/hematuria) in 2 of 7 patients disappeared spontaneously during follow-up, indicating that the kidney lesion was related to other causes (such as infection) rather than to MGRS.

Besides, a higher frequency of light chain only subtype (IgA&IgG&IgM(−)) was observed in MG patients with kidney injury and kidney biopsy group. Light chain excretion, following by deposition and crystallization in kidney, could results in tubular nephropathy. The finding made by Klomjit and coworkers demonstrated a higher concentration of free serum light chain in MG patients with kidney biopsy who tended to have more severe CKD. However, whether the concentration differs among MG subclasses and to what extent it affects the kidney function needs further evacuation.

The investigation conducted in Mayo Clinic depicted that proteinuria ≥1.5 g/d, hematuria and elevated free light chain ratio are potential predictor for MGRS. However, in our cohort, with free light chain ratio inaccessiblem, the clinical measurements, including ratio of κ and λ, proteinuria, hematuria and total light chain showed no differences between patients with MGRS-related lesions and with MG-unrelated lesions. We tend to accounted it to the selection bias in the subcohort since kidney biopsy is more likely to be applied in patients with more severe CKD.

Our study with a large cohort has comprehensively described the clinical characteristics and spectrum of kidney biopsy findings in patients with MG and renal injury. Most previous studies focused on a specific type of MGRS, such as amyloidosis or light chain deposition disease, but without kidney biopsy findings. Furthermore, our cohort is unique because patients with hematologic conditions (MM and WM) were analyzed separately, suggested that we could more accurately describe the characteristics of MGRS and MG-unrelated kidney disease. Finally, this was a multicenter study and the sample size was relatively large. There are some limitations to our study. First, underlying inherent selection bias could not be addressed due to the retrospective nature of this study. Second, important clinical indicators such as free light chain analysis, morphologic assessment, peripheral blood flow

cytometry, and myeloma fluorescent *in situ* hybridization were unavailable for most patients. Thus, the correlations between these clinical indicators and MRGS could not be analyzed.

Only 12.9% of MG patients with kidney injury underwent kidney biopsy and more than 40% of these patients had MG-unrelated lesions, among which amyloid nephropathy accounting for the most. Combined the fact that renal insufficiency sigficantly worson the kidney survival in MG patients and the relative low rate of complication after kidney biopsy, we conclude that kidney biopsy is a safe and essential procedure to maximize the possibility of correct diagnosis, and should be encouraged for patients with suspected MGRS.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Medical Ethics Committee of Nanfang Hospital (NFYY-2015-073). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

GW and SN contributed to the study design and result interpretation. GW and DC took the lead in drafting the manuscript and received major funding for the study. SN, MW, and QW obtained and analyzed the data. SN, MW, YK, JO, and NJ prepared and cleaned the data. XZ, FL, YC, XL, and RC contributed to the data collection. MZ and DC contributed to revising the manuscript. All authors contributed to the interpretation of data, provided critical revisions to the manuscript, and approved the final draft.

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SUPPLEMENTARY MATERIAL

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REFERENCES

1. Kyle RA, Therneau TM, Rajkumar SV, Offord JR, Larson DR, Plevak MF, et al. A long-term study of prognosis in monoclonal gammopathy of undetermined significance. *N Engl J Med.* (2002) 346:564–9. doi: 10.1056/NEJMoa011332023
2. Fermand JP, Bridoux F, Dispenzieri A, Jaccard A, Kyle RA, Leung N, et al. Monoclonal gammopathy of clinical significance: a novel concept with therapeutic implications. *Blood.* (2018) 132:1478–85. doi: 10.1182/blood-2018-04-839480
3. Chaudhry HM, Mauermann ML, Rajkumar SV. Monoclonal gammopathy-associated peripheral neuropathy: diagnosis and management. *Mayo Clin Proc.* (2017) 92:838–50. doi: 10.1016/j.mayocp.2017.02.003
4. International Myeloma Working G. Criteria for the classification of monoclonal gammopathies, multiple myeloma and related disorders: a report of the international myeloma working group. *Br J Haematol.* (2003) 121:749–57. doi: 10.1046/j.1365-2141.2003.04355.x
5. Rajkumar SV, Dimopoulos MA, Palumbo A, Blade J, Merlini G, Mateos MV, et al. International myeloma working Group updated criteria for the diagnosis of multiple myeloma. *Lancet Oncol.* (2014) 15:e538–48. doi: 10.1016/S1470-2045(14)70442-5
6. Kyle RA, Durie BG, Rajkumar SV, Landgren O, Blade J, Merlini G, et al. Monoclonal gammopathy of undetermined significance (MGUS) and smoldering (asymptomatic) multiple myeloma: IMWG consensus perspectives risk factors for progression and guidelines for monitoring and management. *Leukemia.* (2010) 24:1121–7. doi: 10.1038/leu.2010.60
7. Perazella MA, Finkel KW. American society of nephrology onco-nephology forum. paraprotein-related kidney disease: attack of the killer M proteins. *Clin J Am Soc Nephrol.* (2016) 11:2256–9. doi: 10.2215/CJN.02960316
8. Leung N, Bridoux F, Hutchison CA, Nasr SH, Cockwell P, Fermand JP, et al. Monoclonal gammopathy of renal significance: when MGUS is no longer undetermined or insignificant. *Blood.* (2012) 120:4292–5. doi: 10.1182/blood-2012-07-445304
9. Leung N, Bridoux F, Batuman V, Chaidos A, Cockwell P, D'Agati VD, et al. The evaluation of monoclonal gammopathy of renal significance: a consensus report of the International Kidney and Monoclonal Gammopathy Research Group. *Nat Rev Nephrol.* (2019) 15:45–59. doi: 10.1038/s41581-018-0077-4
10. Hill NR, Fatoba ST, Oke JL, Hirst JA, O'Callaghan CA, Lasserson DS, et al. Global prevalence of chronic kidney disease - a systematic review and meta-analysis. *PLoS ONE.* (2016) 11:e0158765. doi: 10.1371/journal.pone.0158765
11. Kyle RA, Therneau TM, Rajkumar SV, Larson DR, Plevak MF, Offord JR, et al. Prevalence of monoclonal gammopathy of undetermined significance. *N Engl J Med.* (2006) 354:1362–9. doi: 10.1056/NEJMoa054494
12. Saran R, Robinson B, Abbott KC, Agodoa LYC, Bragg-Gresham J, Balkrishnan R, et al. US renal data system 2018 annual data report: epidemiology of kidney disease in the United States. *Am J Kidney Dis.* (2019) 73(3 Suppl 1):A7–8. doi: 10.1053/j.ajkd.2019.01.001
13. Atkin C, Reddy-Kolanu V, Drayson MT, Sapey E, Richter AG. The prevalence and significance of monoclonal gammopathy of undetermined significance in acute medical admissions. *Br J Haematol.* (2020) 189:1127–35. doi: 10.1111/bjh.16487
14. Chauvet S, Bridoux F, Ecotière L, Javaugue V, Sirac C, Arnulf B, et al. Kidney diseases associated with monoclonal immunoglobulin M-secreting B-cell lymphoproliferative disorders: a case series of 35 patients. *Am J Kidney Dis.* (2015) 66:756–67. doi: 10.1053/j.ajkd.2015.03.035

15. Paueksakon P, Revelo MP, Horn RG, Shappell S, Fogo AB. Monoclonal gammopathy: significance and possible causality in renal disease. *Am J Kidney Dis.* (2003) 42:87–95. doi: 10.1016/S0272-6386(03)00412-8
16. Soares SM, Fervenza FC, Lager DJ, Gertz MA, Cosio FG, Leung N. Bleeding complications after transcutaneous kidney biopsy in patients with systemic amyloidosis: single-center experience in 101 patients. *Am J Kidney Dis.* (2008) 52:1079–83. doi: 10.1053/j.ajkd.2008.05.022
17. Fish R, Pinney J, Jain P, Addison C, Jones C, Jayawardene S, et al. The incidence of major hemorrhagic complications after renal biopsies in patients with monoclonal gammopathies. *Clin J Am Soc Nephrol.* (2010) 5:1977–80. doi: 10.2215/CJN.00650110
18. Fermand JP, Bridoux F, Kyle RA, Kastritis E, Weiss BM, Cook MA, et al. How I treat monoclonal gammopathy of renal significance (MGRS). *Blood*. (2013) 122:3583–90. doi: 10.1182/blood-2013-05-495929
19. Röllig C, Knop S, Bornhäuser M. Multiple myeloma. *Lancet*. (2015) 385:2197–208. doi: 10.1016/S0140-6736(14)60493-1
20. Gertz MA. Immunoglobulin light chain amyloidosis: 2020 update on diagnosis, prognosis, and treatment. *Am J Hematol.* (2020) 95:848–60. doi: 10.1002/ajh.25819
21. Klomjit N, Leung N, Fervenza F, Sethi S, Zand L. Rate and predictors of finding monoclonal gammopathy of renal significance (MGRS) lesions on kidney biopsy in patients with monoclonal gammopathy. *J Am Soc Nephrol.* (2020) 31:2400–11. doi: 10.1681/ASN.2020010054
22. Bridoux F, Leung N, Hutchison CA, Touchard G, Sethi S, Fermand JP, et al. Diagnosis of monoclonal gammopathy of renal significance. *Kidney Int.* (2015) 87:698–711. doi: 10.1038/ki.2014.408
23. Nasr SH, Satoskar A, Markowitz GS, Valeri AM, Appel GB, Stokes MB, et al. Proliferative glomerulonephritis with monoclonal IgG deposits. *J Am Soc Nephrol.* (2009) 20:2055–64. doi: 10.1681/ASN.2009010110
24. Heilman RL, Velosa JA, Holley KE, Offord KP, Kyle RA. Long-term follow-up and response to chemotherapy in patients with light-chain deposition disease. *Am J Kidney Dis.* (1992) 20:34–41. doi: 10.1016/S0272-6386(12)80314-3
25. Doshi M, Lahoti A, Danesh FR, Batuman V, Sanders PW, American Society of Nephrology Onco-Nephrology Forum. Paraprotein-related kidney disease: kidney injury from paraproteins-what determines the site of injury? *Clin J Am Soc Nephrol.* (2016) 11:2288–94. doi: 10.2215/CJN.02560316
26. Jin B, Zeng C, Ge Y, Le W, Xie H, Chen H, et al. The spectrum of biopsy-proven kidney diseases in elderly Chinese patients. *Nephrol Dial Transplant.* (2014) 29:2251–9. doi: 10.1093/ndt/gfu239

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ORIGINAL RESEARCH
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Novel Immune Cell Subsets Exhibit Different Associations With Vascular Outcomes in Chronic Kidney Disease Patients—Identifying Potential Biomarkers

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Background and Aims: Alterations in novel immune cell subsets, such as angiogenic T cells (Tang), senescent T cells ($CD4^+CD28^{null}$), and monocyte subsets are associated with impaired vascular homeostasis in several inflammatory conditions. However, mediators underlying vascular deterioration in chronic kidney disease (CKD) are poorly characterized. This study assessed their role in the vascular deterioration of CKD using a broad spectrum of surrogate markers ranging from altered functionality to overt calcification.

Methods: Tang ($CD3^+CD31^+CXCR4^+$), $CD4^+CD28^{null}$ cells, and monocytes [CD14/CD16 subsets and angiotensin-converting enzyme (ACE) expression] were measured in peripheral blood by flow cytometry in 33 CKD stage 5 patients undergoing peritoneal dialysis (CKD5-PD) and 15 healthy controls (HCs). Analyses were replicated in a hemodialysis cohort. Vascular surrogate markers (including adventitial vasa vasorum, pulse wave velocity, intima-media thickness, and vascular calcification) were assessed by appropriate imaging methods.

Results: In CKD5-PD, decreased Tang levels ($p < 0.001$) were unrelated to clinical features or traditional cardiovascular (CV) risk factors but correlated negatively with troponin T levels ($r = -0.550$, $p = 0.003$). Instead, $CD4^+CD28^{null}$ frequency was increased ($p < 0.001$), especially in those with vascular calcifications. Quantitative and qualitative differences were also observed within the monocyte pool, a shift toward $CD16^+$ subsets and ACE expression being found in CKD. Equivalent results were observed in the replication cohort. Each subset associated distinctly with adverse vascular outcomes in univariate and multivariate analyses: while Tang depletion

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was linked to poor vascular function and subclinical atherosclerosis, increases in $CD4^+CD28^{null}$ were associated with overt vascular thickening and calcification. Monocytes were not independently associated with vascular outcomes in CKD patients.

Conclusions: Novel T cell and monocyte subsets are altered in CKD. Altered T-cell subpopulations, but not monocytes, exhibited distinct associations with different vascular outcomes in CKD. Tang are emerging biomarkers of subclinical vascular deterioration in CKD.

Keywords: atherosclerosis, vasa vasorum, CKD, inflammation, vascular outcomes

INTRODUCTION

Chronic kidney disease (CKD) is hallmarked by chronic systemic inflammation, which not only plays a role in aggravating renal damage, but it also contributes to the development of comorbidities, such as cardiovascular (CV) disease (1, 2).

Immune dysregulation is known to be involved in vascular traits such as endothelial damage, vascular dysfunction, or atherosclerotic plaque development, thus leading to CV disease (3). Although both innate and adaptive immune responses are thought to be involved, the exact mediators are yet to be characterized. Even though a crucial role for T cells and monocytes has been largely hypothesized (4, 5), it has not been until recently that specific, novel subsets of T cells and monocytes were documented to have dedicated functions in vascular homeostasis.

Within the T-cell compartment, angiogenic T cells (Tang) were described as a subset of naive T cells with key roles in vasculogenesis and vascular repair due to their functional cooperation with endothelial progenitor cells (6). As such, Tang have been proven to promote endothelial cell proliferation and function *in vitro* and trigger vessel formation and repair *in vivo* (6). Of note, a decreased frequency of circulating Tang has been reported in several conditions in association with adverse CV outcomes (7–11). However, Tang levels have not been characterized in CKD patients yet. On the other hand, $CD4^+CD28^{null}$ cells are known to be a biomarker of immunosenescence, and their noxious, cytotoxic-mediated effects on the vasculature have been described *in vitro* and *in vivo*, including endothelial cell apoptosis as well as plaque destabilization and rupture (12–14).

Moreover, recent breakthroughs on monocyte biology need to be considered. First, although classically considered a unique and relative uniform population, monocytes are now recognized as a mixture of phenotypical and functionally different subsets, including at least three categories based on their CD14/CD16 surface expression: classical ($CD14^+CD16^-$), intermediate ($CD14^+CD16^+$), and non-classical ($CD14^{low}CD16^+$) (15, 16). Since these subsets differ in terms of their ability to elicit cytokine responses, antigen presentation, or phagocytosis (15, 17), it is tempting to speculate that they may have different effects on vascular homeostasis. In addition, changes in the expression of other markers, such as angiotensin-converting enzyme (ACE), have been reported (18, 19) and linked to changes in monocyte activities (19). Even though the role of monocytes in vascular homeostasis has been a topic of major interest, how this monocyte heterogeneity relates to vascular outcomes in CKD remains unknown (20).

Emerging evidence points to an important role of these subsets in vascular homeostasis in other conditions. However, evidence is lacking in CKD. First, most of the studies have focused on individual cell populations, hence making it difficult to compare the effects of the different subsets. Second, vascular involvement in CKD is a complex scenario with multiple stages and pathogenic circuits. Then, focusing on a single entity, such as medial calcification or atherosclerosis does not allow to draw firm conclusions on the global picture. Overcoming these limitations will help not only to gain understanding toward these potential disease mediators but will also pave the ground for their potential use as biomarkers in the clinical setting. Therefore, the main aim of this study was to evaluate potential alterations of different novel immune cell subsets, previously related to vascular homeostasis, as well as their associations with a broad spectrum of vascular surrogate markers, ranging from subclinical atherosclerosis to overt vascular calcification, in CKD patients.

MATERIALS AND METHODS

Ethics Statement

Approval for the study was obtained from the institutional review board (Comité de Ética Regional de Investigación Clínica, reference PI17/02181) in compliance with the Declaration of Helsinki. All participants gave a written informed consent prior to their inclusion in the study.

Study Participants

Our study involved 33 CKD stage 5 patients undergoing peritoneal dialysis (CKD5-PD) recruited from the Peritoneal Dialysis Outpatient Clinic [Unidad de Gestión Clínica de Nefrología, Hospital Universitario Central de Asturias (HUCA), Spain]. A group of 15 individuals with normal renal function from the general population were recruited as healthy controls (HCs). Additionally, 16 CKD stage 5 patients on hemodialysis (CKD5-HD) (Hemodialysis Outpatient Clinic, HUCA) and six HCs were independently recruited as a replication cohort (**Supplementary Table 1**). Exclusion criteria were immunosuppressive treatment, pregnancy, diagnosis of immune-mediated disease, cancer or diabetes mellitus, recent (<3 months) or current infections, previous CV disease, abdominal aneurysm or intermittent claudication, or previous

carotid surgery. The burden of traditional CV risk factors was assessed by the SCORE algorithm (low-risk charts) according to the European Society of Cardiology (ESC) guidelines (21). The SCORE algorithm integrates information about total cholesterol levels, blood pressure (systolic), smoking status, age, and gender, hence deriving an estimated figure of CV risk (10-year risk for fatal CV). The estimated risk is used in clinical practice to stratify patients [based on ESC guidelines (21)] into categories of risk for preventive pharmacological and non-pharmacological interventions. The SCORE algorithm has been validated in European real-world populations.

Blood samples were obtained by venipuncture (during a clinical appointment before dialysis). Automated serum biochemical parameters, lipid analysis, and complete blood counts were performed on all the participants at the Laboratorio de Medicina (HUCA) by means of routine laboratory methods. Serum samples were stored at −80°C until further analyses.

Analysis of Peripheral Blood Mononuclear Cells

Peripheral blood samples were immediately processed to obtain peripheral blood mononuclear cells (PBMCs) by centrifugation (1,900 rpm, 20 min) on density gradients (Lymphosep, Biowest, Germany).

The analysis of PBMCs was performed by flow cytometry. First, PBMCs were treated with FcR Blocking Reagent (Miltenyi Biotec, Germany) for 20 min at 4°C to avoid unspecific antibody FcR binding. Then, cells were incubated with CD14 FITC (Immunostep, Spain), CD16 APC-Cy7 (BioLegend, Germany), and ACE APC (Miltenyi Biotec) or CD3 PerCP-Cy-5, 5 (Tonbo Biosciences, Belgium), CD184 PE-Cy7 (BD Biosciences, Germany), CD31 FITC (BD Biosciences), CD4 PE (Immunostep), and CD28 APC-Cy7 (Thermo Fischer, Germany) or corresponding isotype antibodies for 30 min at 4°C protected from light. Next, cells were washed twice with PBS and analyzed by flow cytometry [FACS Canto II (BD Biosciences) with FACS Diva 6.5 software].

Then, "live gate" excluding debris and no cellular events was designed. Lymphocyte and monocyte regions were defined according to their forward scatter (FSC)/side scatter (SSC) features. Gating was performed based on the signal provided by the isotype controls (**Supplementary Figure 1**). Tang were defined as previously described (9). In brief, lymphocytes were evaluated for CD3 expression, and those $CD31^+CD184^+$ within the $CD3^+$ gate were considered Tang. Tang were further subdivided into $CD4^+$Tang and $CD8^+$ Tang subsets (**Figure 1A**). $CD3^+$ cells were evaluated for CD4 and CD8 expression, and $CD4^+$ cells lacking CD28 expression were defined as $CD4^+CD28^{null}$ cells (22) (**Figure 1A**). For the analysis of monocytes, events contained in the monocyte region (FSC/SSC) were evaluated for their CD14 and CD16 expression, and subsets were defined as follows: classical ($CD14^+CD16^-$), intermediate ($CD14^+CD16^+$), and non-classical ($CD14^{low}CD16^+$) monocytes. All monocytes were contained within the FSC/SSC-defined monocyte region. Moreover, no lymphocytes were found within this region, as these cells were all characterized by a $CD14^-$ expression (**Figure 2A**). No monocytes were observed within the lymphocyte gate either. Finally, ACE expression was evaluated in the whole monocyte region as well as in each of the different monocyte subsets; ACE^+ events within each population were calculated (**Figure 2A**). Absolute levels were computed by applying the lymphocyte/monocyte counts obtained in the automated blood cell counts.

Quantification of Circulating Cytokines

Circulating interleukin (IL)-10, IL-6, IL-2, tumor necrosis factor (TNF)α and interferon (IFN)γ levels were measured in serum samples using a bead-based multiplex assay (BiolegendPlex, BioLegend), following the protocol provided by the manufacturer. Samples were analyzed in a FACS Canto II flow cytometer (BD Biosciences) under FACS Diva 6. The detection limits were 1.2 pg/ml (IL-10 and IL-2) or 2.4 pg/ml (IL-6, TNFα and IFNγ).

Angiotensin-Converting Enzyme Gene Expression Analysis

The gene expression of ACE was analyzed in total RNA extracted from PBMCs by using TRI reagent (Sigma-Aldrich). After reverse transcription using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems), quantitative real-time PCR (qPCR) reactions were performed in triplicate using the Stratagene Mx3005P QPCR System (Agilent Technologies), Fast Start Universal Probe Master (Roche), and pre-developed assays for qPCR [ACE: Hs00174179_m1 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH): Hs99999905_m1; Thermo-Fisher Scientific]. ACE expression was quantified as relative units to GAPDH expression by comparing threshold cycles using the $^{\Delta\Delta}$Ct method.

Surrogate Vascular Outcomes

Abdominal aortic calcification [Kauppila Index (KI)] was quantified in lateral lumbar X-rays. Carotid–femoral pulse wave velocity (PWV) was measured using Complior Analyze (ALAM Medical) according to the manufacturer's instructions. Results are the average of three optimal measurements.

B-mode ultrasound was performed by an experienced radiologist using a Toshiba-Aplio XG machine (Toshiba American Medical Systems). To evaluate left carotid intima-media thickness (cIMT) as well as carotid and femoral plaque presence (either cIMT >1.5 mm or a focal thickening going over into the arterial lumen by at least 50% of the surrounding cIMT value) (23), all subjects, laid in a supine position, underwent a prior axial examination of the extracranial carotid artery followed by a longitudinal exploration.

Plaques were described by number, location, dimension, echogenicity, and homogeneity (stable and unstable).

Adventitial vasa vasorum (aVV), the plexus of microvessels surrounding the adventitial layer, were quantified following a similar strategy to that validated by Arcidiacono et al. (24) but avoiding the use of a contrast agent. Indeed, the very sensitive Superb Microvascular Imaging Ultrasound (Toshiba Aplio 500), designed for the detection of small-diameter blood vessel flow,

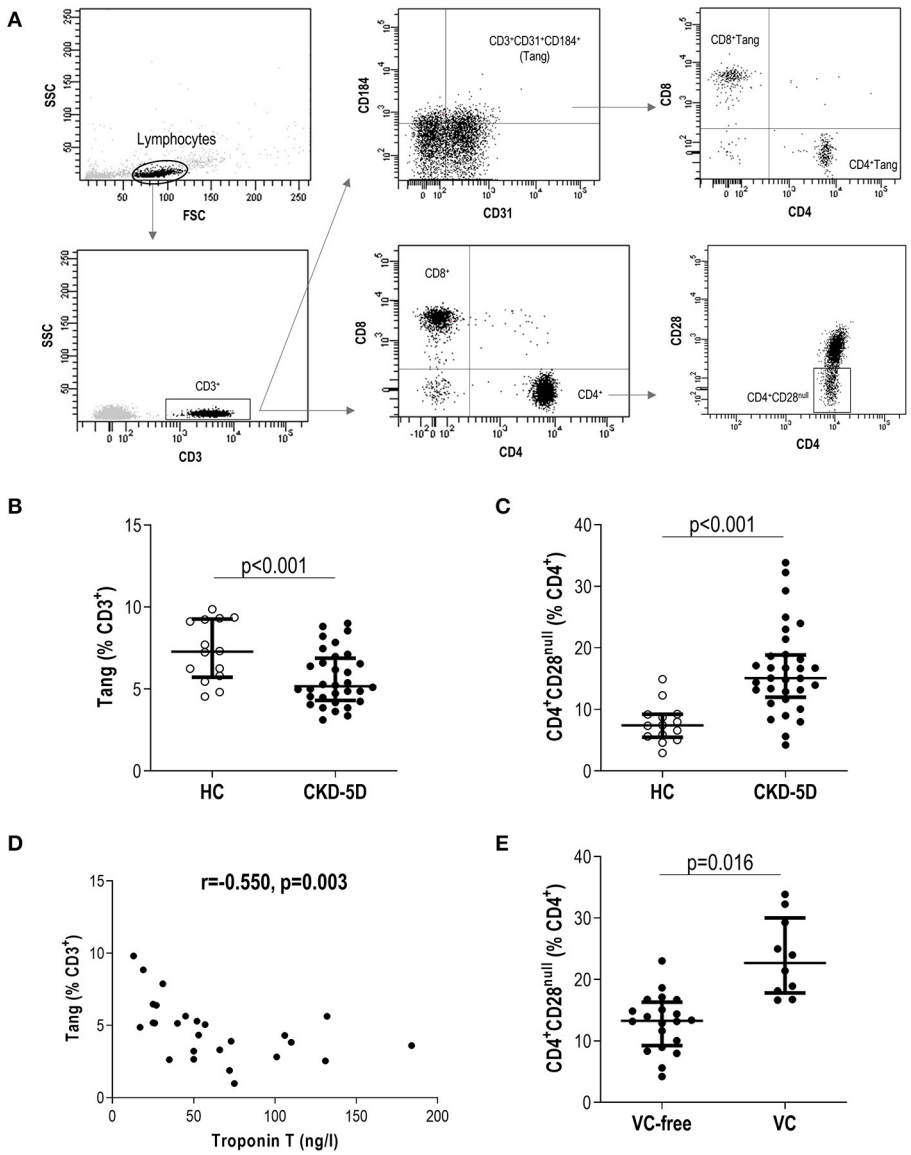

FIGURE 1 | Analysis of novel T-cell subsets in chronic kidney disease (CKD). **(A)** Gating strategy for the identification and quantification of $CD3^+$, $CD4^+$, $CD8^+$, angiogenic T cells (Tang), $CD4^+$Tang, $CD8^+$Tang, and $CD4^+CD28^{null}$ subsets by flow cytometry. Dot-plots are shown from a representative patient. Gating was based on isotype controls (**Supplementary Figure 1**). **(B)** Tang (g = 1.92) and (**C**) $CD4^+CD28^{null}$ (g = 2.10) levels were compared between healthy control (HC) (open dots) and chronic kidney disease stage 5 patients undergoing peritoneal dialysis (CKD5-PD) (black dots). **(D)** Association between Tang levels and those of serum Troponin T in CKD5-PD. Correlation was assessed by Spearman's rank test. **(E)** Association between $CD4^+CD28^{null}$ cells and presence of vascular calcification (VC) (g = 1.62). In scatter plots, each dot represents one individual. Upper and lower bars represent 75th and 25th percentiles, and medium bars correspond to the median values. Differences between groups were assessed by Mann–Whitney *U* tests, and size effects were calculated with Hedge's g statistic.

was used. Vasa vasorum density was quantified in the left carotid artery and, for the first time, in the femoral arteries of HC and CKD5-PD (**Supplementary Figure 2**). The ImageJ program was used for the quantification of aVV density of the adventitial vessels, identified by an experienced radiologist, as depicted in representative **Supplementary Figure 2**. All surrogate vascular measurements were performed by an operator blinded to the study participants.

Statistical Analyses

Continuous variables were expressed as median [interquartile range (IQR)] or mean ± standard deviation according to the distribution of the variables. Categorical variables were summarized as *n* (%). Differences between groups were assessed by Mann–Whitney *U* tests or Student *T* tests, as appropriate. Hedge's g statistic was calculated in order to evaluate size effects (values g > 0.6 and g > 0.80 were considered of medium effect

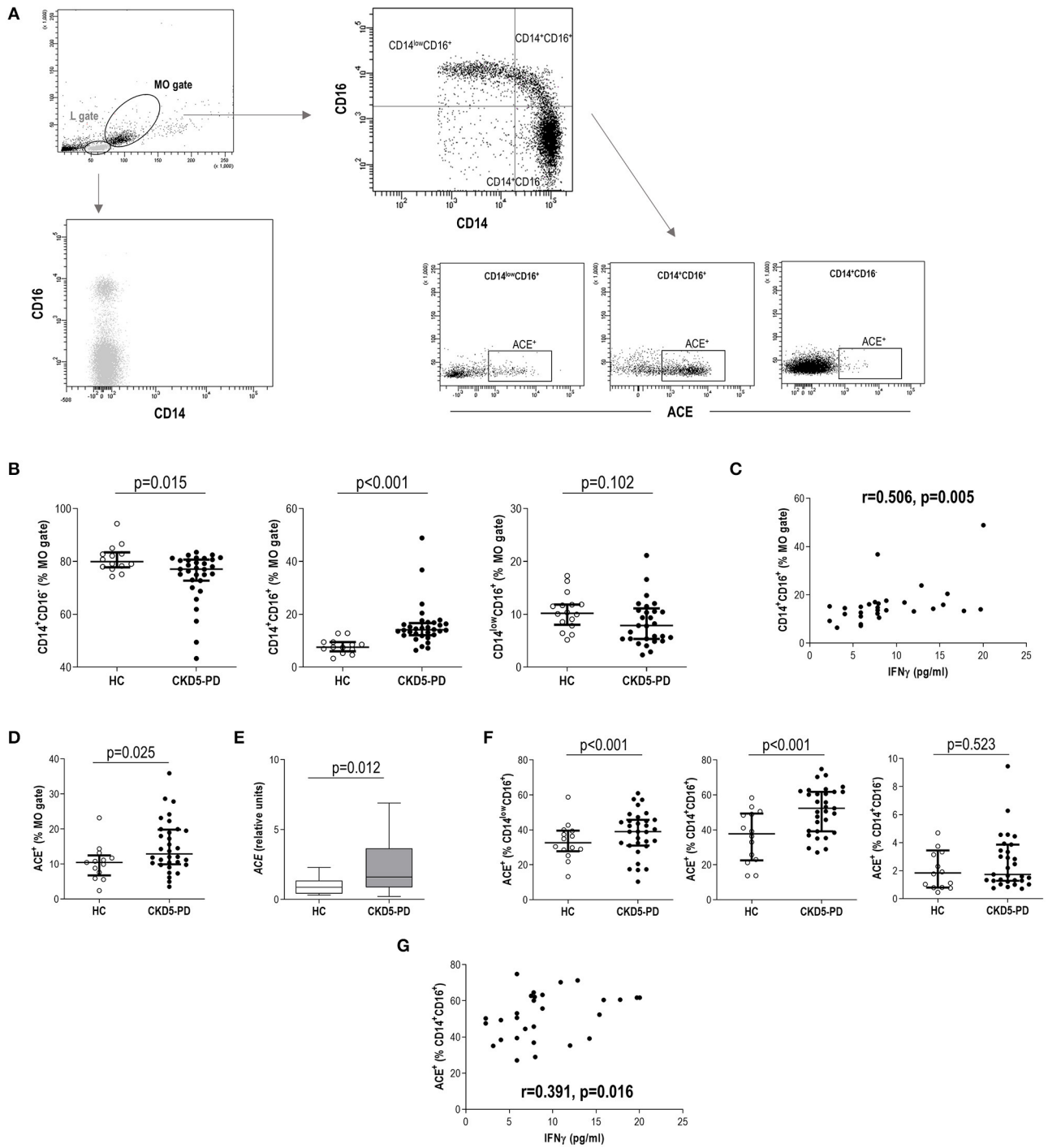

FIGURE 2 | Analysis of monocyte subsets and angiotensin-converting enzyme (ACE) expression in chronic kidney disease (CKD). **(A)** Gating strategy for the identification and quantification of monocyte subsets and their ACE expression. Dot-plots are shown from a representative patient. Monocytes were initially gated from their forward scatter (FSC)/side scatter (SSC) properties (MO gate). Monocytes were not contained within lymphocytes (L gate), which was only composed of $CD14^{-}$ cells. **(B)** Intermediate (g = 0.91) and classical (g = 0.72) monocyte subsets were found to be altered in chronic kidney disease stage 5 patients undergoing peritoneal dialysis (CKD5-PD) (black dots) compared to healthy controls (HCs) (open dots). **(C)** Association between intermediate monocyte levels and those of serum interferon (IFN)γ in CKD5-PD. **(D)** The expression of ACE within the total monocyte gate was analyzed by flow cytometry **(A)**, and increased expression was observed in CKD5-PD (g = 0.90). This was confirmed by gene expression analysis on peripheral blood mononuclear cells (PBMCs) [**(E)**, g = 0.63]. **(F)** The ACE expression was further evaluated on each monocyte subset and compared between HC (open dots) and CKD5-PD (black dots) (non-classical, g = 0.95, and intermediate, g = 1.25). **(G)** Association between ACE^{+} intermediate monocyte and IFNγ serum levels in CKD5-PD. In scatter plots, each dot represents one individual. Upper and lower bars represent 75th and 25th percentiles, and medium bars correspond to the median values. Differences between groups were assessed by Mann–Whitney *U* tests, and size effects were calculated with Hedge's g statistic. Correlations were assessed by Spearman's rank test.

and large effect, respectively) (25). The associations between continuous variables were evaluated by correlations (Spearman's rank test) or linear regression models, either univariate or multivariate adjusted by confounders. B coefficients and 95% confidence intervals (CIs) were computed. A p-value < 0.050 was considered statistically significant. Statistical analyses were performed with SPSS 24.0 and GraphPad Prism 8.0 for Windows.

RESULTS

Novel T-Cell Subsets in Chronic Kidney Disease

A total of 33 CKD5-PD and 15 HCs were recruited for this study (**Table 1**). Tang ($CD3^+CD31^+CD184^+$) levels in peripheral blood were evaluated by flow cytometry, and a highly significant lower Tang frequency was observed in CKD5-PD (**Figure 1B**). No differences were found in the frequency of $CD4^+$ [46.55% (21.07) vs. 48.66% (19.45), $p = 0.518$] and $CD8^+$ subsets [42.20% (21.01) vs. 41.09% (18.16), $p = 0.258$] within the Tang pool between patients and controls. As a consequence, both $CD4^+$Tang [1.88% (1.16) vs. 3.43% (1.80), $p < 0.001$, g = 1.82] and $CD8^+$Tang [2.02% (0.88) vs. 3.11% (1.72), $p < 0.001$, g = 1.15] were found to be decreased in CKD5-PD. Moreover, $CD4^+CD28^{null}$ cells were also evaluated, and CKD5-PD exhibited a marked increase in this cell population compared to HC (**Figure 1C**). These differences were maintained when evaluated within the total lymphocyte region [Tang: 1.68% (1.05) vs. 3.38% (1.44), $p < 0.001$, g = 0.92; and $CD4^+CD28^{null}$: 9.06% (4.16) vs. 5.09% (2.52), $p < 0.001$, g = 1.10]. Importantly, equivalent results were observed when the absolute levels were computed [Tang: 2.38 (2.29) vs. 7.00 (4.99)·10^3/μl, $p < 0.001$, g = 2.30; $CD4^+CD28^{null}$: 11.65 (8.83) vs. 5.82 (10.34)·10^3/μl, $p = 0.035$, g = 0.70]. Similarly, no differences were observed in the $CD3^+$ [HC: 46.25% (17.39) vs. CKD: 42.26% (29.55), $p = 0.447$), $CD4^+$ (61.86% (12.55) vs. 60.99% (14.81), $p = 0.645$] or $CD8^+$ subsets [33.86% (12.95) vs. 34.85% (12.00), $p = 0.800$].

Next, in order to gain insights into the origin of these alterations, their associations with laboratory parameters and clinical features were analyzed. Whereas, Tang levels were associated with those of total cholesterol ($r = -0.665$, $p = 0.005$) and low-density lipoprotein (LDL) cholesterol ($r = -0.372$, $p = 0.021$) in HC, these associations were not found in patients ($r = -0.037$, $p = 0.853$ and $r = -0.203$, $p = 0.301$, respectively). Interestingly, Tang levels showed an inverse correlation with serum troponin T (TnT) concentration in CKD5-PD (**Figure 1D**). Regarding $CD4^+CD28^{null}$ cells, patients with vascular calcifications exhibited higher levels of this subset than their calcification-free counterparts (**Figure 1E**). No associations were observed for Tang or $CD4^+CD28^{null}$ cells with the rest of biochemical parameters examined in either HCs or patients [all $p > 0.050$, or with time on dialysis or medications in patients (all $p > 0.050$)]. Glucocorticoid usage was not observed to have an effect on these subpopulations (Tang: $p = 0.356$, $CD4^+CD28^{null}$: $p = 0.239$). Excluding patients under glucocorticoid treatment yielded equivalent results. Similarly, no associations were observed between Tang or $CD4^+CD28^{null}$ cells with the levels of circulating cytokines (**Supplementary Tables 1, 2**).

Finally, we recruited a replication cohort consisting of 16 CKD5-HD and six age- and gender-matched controls (**Supplementary Tables 3, 4**) in order to validate our results. The independent analysis of this cohort confirmed the differences observed in peritoneal dialysis patients for these cell subsets (**Supplementary Figure 2**). No major differences in blood cell counts and clinical parameters were observed between CKD5-HD and their peritoneal dialysis counterparts.

These results suggest that CKD is hallmarked by distinct alterations of T-cell subsets.

Monocyte Subsets and Angiotensin-Converting Enzyme Expression

The analysis of monocyte subsets (**Figure 2A**) revealed an increased frequency of intermediate monocytes in CKD5-PD at the expense of a reduction in the classical group compared to HC (**Figure 2B**). Equivalent results were observed when absolute levels were calculated [intermediate: 7.67 (5.61) vs. 3.89 (3.71)·10^3/μl, $p < 0.001$, g = 0.89; classical: 31.22 (17.88) vs. 42.18 (18.35)·10^3/μl, $p = 0.035$, g = 0.62; non-classical: 3.90 (4.10) vs. 4.10 (2.28)·10^3/μl, $p = 0.696$]. Intermediate monocytes were found to be positively correlated with IFNγ serum levels (**Figure 2C**), whereas no other associations were observed with the rest of cytokines analyzed. No effect was observed for medications (all $p > 0.050$), including glucocorticoids (intermediate: $p = 0.128$, classical: $p = 0.318$, non-classical: $p = 0.564$). Excluding patients under glucocorticoid treatment yielded equivalent results.

Additionally, the surface expression of ACE was analyzed in monocytes. Monocytes from CKD5-PD exhibited an increased ACE expression (**Figure 2D**). This increase was confirmed at the gene expression level by RT-PCR (**Figure 2E**). Interestingly, the ACE expression was not uniform within the monocyte pool, but differences were noted among subsets. It was mainly expressed by intermediate and non-classical monocytes, whereas their classical counterparts exhibited a negligible expression (**Figure 2A**). Moreover, increased expression was observed in CKD5-PD compared to controls in both intermediate and non-classical subsets compared to HC (**Figure 2F**). Of note, ACE expression (analyzed by flow cytometry) on intermediate monocytes was found to be positively correlated with IFNγ serum levels (**Figure 2G**).

Furthermore, equivalent analyses were performed in our replication cohort, and similar results were obtained (**Supplementary Figure 3**).

All these findings suggest that quantitative and qualitative differences characterize the monocyte pool in CKD patients, linked at least in part to IFNγ levels.

Novel Immune Cell Subsets and Vascular Surrogate Markers

Next, vascular surrogate markers (including aVV, PWV, cIMT) and Kauppila scores were measured in 17 CKD5-PD

TABLE 1 | Demographical, laboratory, and clinical parameters of individuals recruited for this study.

	HC ($n = 15$)	CKD5-PD ($n = 33$)	*P*-value
Age, years, mean (range)	48.00 (22.00–68.00)	55.00 (21.00–77.00)	0.070
Sex, women/men	10/5	13/20	0.080
Laboratory parameters			
Albumin, mg/dl	44.17 ± 1.95	34.37 ± 4.35	<0.001
Urea, mg/dl	33.46 ± 8.08	132.66 ± 43.76	<0.001
Creatinine, mg/dl	0.78 ± 0.17	7.81 ± 2.72	<0.001
Plasma Ca, mmol/l	2.34 ± 0.07	2.17 ± 0.16	<0.001
Plasma phosphate, mmol/l	1.04 ± 0.18	1.64 ± 0.42	<0.001
PTH, pg/ml	49.60 ± 15.52	371.03 ± 198.61	<0.001
Total cholesterol, mg/dl	202.25 ± 49.20	159.32 ± 40.28	0.005
HDL cholesterol, mg/dl	67.63 ± 13.14	59.00 ± 24.52	0.017
LDL cholesterol, mg/dl	113.00 ± 38.38	74.71 ± 38.56	0.002
Triglycerides, mg/dl	65.53 ± 25.54	131.82 ± 75.33	0.003
25(OH)-vitamin D, ng/ml	32.93 ± 15.38	10.10 ± 6.89	<0.001
CRP, mg/dl	0.14 ± 0.07	0.64 ± 0.86	0.002
Troponin T, ng/l	5.33 ± 3.45	61.48 ± 41.82	<0.001
Blood cell counts ($10^3/\mu l$) median (IQR)			
Leukocytes	5.67 (2.06)	6.25 (3.59)	0.380
Neutrophils	2.94 (1.15)	3.52 (2.27)	0.075
Lymphocytes	1.96 (0.91)	1.22 (0.85)	0.022
Monocytes	0.44 (0.19)	0.56 (0.23)	0.071
Eosinophils	0.11 (0.10)	0.23 (0.20)	0.002
Basophils	0.04 (0.02)	0.05 (0.05)	0.109
Clinical features			
Vascular calcifications, n (%)		18 (54.5)	
Kauppila score		7.88 ± 8.98	
Time on dialysis, months (median (IQR)		15.00 (16.00)	
Systolic blood pressure, mm Hg		133.88 ± 16.04	
Diastolic blood pressure, mm Hg		81.05 ± 10.70	
Treatments, n (%)			
Paricalcitol		15 (45.4)	
Phosphate binders		18 (54.5)	
Statins		21 (63.6)	
Methylprednisolone‡		9 (27.22)	
Epo		24 (72.7)	

Variables were summarized as mean ± SD or n (%), unless otherwise stated. Differences were assessed by Student t tests, Mann–Whitney U tests, or χ^2 tests, as appropriate. ‡ *8/9 patients were under low-dose glucocorticoids (<5 mg/day).*

CKD5-PD, chronic kidney disease stage 5 patients undergoing peritoneal dialysis; CRP, C-reactive protein; HC, healthy control; HDL cholesterol, high-density lipoprotein cholesterol; IQR, interquartile range; LDL cholesterol, low-density lipoprotein cholesterol.

and 14 HCs. No differences in demographical, clinical, medications, or blood counts were observed between patients with and without vascular assessments, thus indicating that the subset analyzed was representative of the whole population (**Supplementary Table 5**). Of note, patients exhibited higher carotid aVV expansion and a trend in femoral location, together with higher PWV and cIMT values than HCs (**Table 2**).

In order to evaluate whether immune cell populations could be regarded as biomarkers of subclinical CV endpoints, correlations with these assessments were performed (**Table 3**). Interestingly, Tang were strongly correlated with aVV and PWV measurements. Importantly, these associations were maintained after correcting for the effect of traditional CV risk factors (**Table 4**). On the other hand, $CD4^+CD28^{null}$ cells were found to be associated with cIMT and Kauppila score (**Table 3**). This association was maintained [β (95% CI), $p = 0.883$ (0.357, 1.409), $p = 0.003$] in a multivariate model after adjusting for traditional CV risk factors (entered as the SCORE) and time on dialysis as confounders. Finally, intermediate monocytes were correlated to PWV and vascular calcification (**Table 3**), although correlations were observed to be weaker than those of T-cell subsets. These associations were not observed after controlling for confounders (both $p > 0.050$). Of note, no associations with other T-cell subsets ($CD3^+$, $CD4^+$, or $CD8^+$) or leukocyte subsets were

TABLE 2 | Vascular surrogate markers in CKD and HC.

	HC ($n = 14$)	CKD5-PD ($n = 17$)	*P*-values
Carotid aVV, number (n vasa)	0.00 (2.00)	3.00 (7.00)	<0.050
Carotid aVV, area (% mm^2/mm)	0.00 (2.16)	2.61 (8.84)	0.023
Femoral aVV, number (n vasa)	0.00 (1.25)	1.00 (3.50)	0.245
Femoral aVV, area (% mm^2/mm)	0.00 (1.57)	0.89 (4.67)	0.121
PWV, m/s	7.20 (1.38)	8.70 (2.82)	0.008
cIMT, mm	0.60 (0.20)	0.75 (0.32)	0.045

Variables were summarized as median (interquartile range) and compared by Mann–Whitney U tests.

aVV, adventitial vasa vasorum; cIMT, carotid intima-media thickness; CKD5-PD, chronic kidney disease stage 5 patients undergoing peritoneal dialysis; HC, healthy control; PWV, pulse wave velocity.

observed (**Supplementary Table 6**), thus ruling out a potential confounding effect and emphasizing the specific role of the previous cell populations in vascular repair.

Taken together, distinct altered immune cell populations are related to different vascular surrogate outcomes in CKD. Tang were independently associated with subclinical atherosclerosis and vascular functionality even after adjusting for traditional risk factors.

DISCUSSION

Although systemic inflammation has been described to contribute to vascular outcomes in CKD, the exact mediators are yet to be characterized. A growing body of evidence has identified novel immune cell subsets that play a role in vascular homeostasis and damage in different scenarios. CKD patients suffer a profound vascular deterioration, with different mechanisms being involved. Delineating the associations of the different immune mediators with the distinct adverse vascular scenarios is of major interest. In the present study, we addressed the analysis of some of these immune cell subsets in CKD. Our findings revealed marked alterations within T cells subsets, hallmarked by a Tang decrease and elevated $CD4^+CD28^{null}$ cells, as well as within the monocyte pool, characterized by a shift toward $CD16^+$ subsets and enhanced ACE expression. These alterations exhibited distinct associations with vascular outcomes in patients under peritoneal dialysis, Tang depletion being related to a poor vascular functionality and subclinical atherosclerosis, whereas senescent $CD4^+$ T cells were associated with overt vascular wall thickening and calcification. Of note, these alterations were found in CKD5 patients undergoing peritoneal or hemodialysis compared to their control populations, thus suggesting that these phenomena may be related to the CKD stage itself. To the best of our knowledge, this is the first study not only to present a joint characterization of these subsets in CKD but also to evaluate their associations with a broad range of surrogate vascular outcomes in this condition.

One of the most remarkable findings of our work was the analysis of the Tang population. Tang are known to carry out vascular protective actions (6), and Tang depletion has been linked to CV outcomes in several immune-mediated conditions (7–11). The results herein presented expand the current knowledge in a two-fold manner. First, Tang depletion was observed beyond autoimmunity, hence strengthening the role of Tang as potential mediators of vascular homeostasis in a broad range of disorders, rather than being an immune disease-specific mechanism. Importantly, both $CD4^+$ and $CD8^+$ Tang subsets were found to be diminished, pointing to a strong quantitative effect on the Tang subset rather than a qualitative effect within the Tang composition. Second, decreased Tang have been linked to subclinical CV disease surrogate markers (aVV and PWV), that is, with the first signs of vascular impairments, but not with more advanced vascular traits (such as vascular calcification). In fact, in healthy individuals with zero risk for the classical atherosclerotic risk factors, left carotid aVV increased with age, the natural determinant of a higher prevalence of atheromatous lesions, in parallel with increases in cIMT within the normal range, thus supporting the accuracy and sensitivity of aVV as an earlier marker of subclinical atherosclerosis (24). Furthermore, in experimental hypercholesterolemia, increases in the density of coronary aVV precede epicardial endothelial dysfunction (26). Accordingly, type 2 diabetic patients present a higher left carotid adventitial neovascularization when compared to controls, with the highest aVV density in patients with retinopathy (angiogenesis) despite similar cIMT (27). Also, increases in aVV and in cIMT also occur in the right carotid artery of CKD stages 3–4 and dialysis patients with higher neovascularization at the earlier CKD stages (28).

Similarly, PWV is a well-established early marker of arterial stiffness (29). Since CKD patients exhibited both atherosclerosis and arteriosclerosis (30, 31), finding a mediator linked to the subclinical stage of both processes is of major relevance. Furthermore, this is supported by the strong association with TnT levels in CKD, a well-known biomarker of subclinical CV damage with predictive ability in CKD (32, 33). Therefore, a role for Tang to stratify CV risk may be considered. Interestingly, aVV expansion has been reported to be dependent on hypoxia-inducible factor (HIF)-1 and vascular endothelial growth factor (VEGF) production (34, 35), these molecules being also linked to Tang mobilization (6). Importantly, current literature points to an association between aVV sprouting and T-cell adventitial accumulation, rather than monocytes or other subsets (36–38). These notions are in line with our findings comparing the distinct vascular surrogate markers. Taken together, these results may point to Tang as potential biomarkers of subclinical vascular impairment in CKD.

Furthermore, Tang depletion was not associated with the burden of traditional CV risk factors, and they were confirmed to be independent predictors of vascular surrogate markers. Therefore, this reinforces the idea that Tang could provide additional information as biomarkers. This is further supported by the fact that Tang depletion seemed to be a uniform observation in CKD patients, that is, linked to the disease itself and not to specific disease features. Since current algorithms

TABLE 3 | Associations between subclinical CV outcomes and immune cell subsets in CKD5-PD.

	Number of neovasa (carotid)	Number of neovasa (femoral)	aVV area (carotid)	aVV area (femoral)	PWV	cIMT	Kauppila
T cells							
Tang (% lymphocytes)	***r* = −0.640** ***p* = 0.006**	*r* = −0.194 *p* = 0.456	***r* = −0.813** ***p* < 0.001**	***r* = −0.542** ***p* = 0.025**	***r* = −0.734** ***p* < 0.001**	*r* = −0.029 *p* = 0.921	*r* = −0.359 *p* = 0.060
$CD4^+CD28^{null}$ (% lymphocytes)	*r* = 0.333 *p* = 0.192	*r* = 0.088 *p* = 0.737	*r* = 0.395 *p* = 0.117	*r* = 0.395 *p* = 0.117	*r* = 0.435 *p* = 0.093	***r* = 0.566** ***p* = 0.035**	***r* = 0.581** ***p* < 0.001**
Monocytes							
Non-classical (% monocytes)	*r* = 0.286 *p* = 0.265	*r* = −0.325 *p* = 0.203	*r* = 0.505 *p* = 0.039	*r* = 0.069 *p* = 0.794	*r* = 0.271 *p* = 0.310	*r* = −0.237 *p* = 0.415	*r* = 0.217 *p* = 0.258
ACE^+ Non-classical (% non-classical)	*r* = −0.123 *p* = 0.637	*r* = −0.085 *p* = 0.744	*r* = −0.114 *p* = 0.662	*r* = −0.040 *p* = 0.879	*r* = 0.055 *p* = 0.841	*r* = 0.059 *p* = 0.842	*r* = −0.214 *p* = 0.266
Intermediate (% monocytes)	*r* = 0.294 *p* = 0.252	*r* = 0.167 *p* = 0.522	*r* = 0.472 *p* = 0.086	*r* = 0.378 *p* = 0.135	***r* = 0.576** ***p* = 0.020**	*r* = 0.171 *p* = 0.558	***r* = 0.388** ***p* = 0.037**
ACE^+ intermediate (% intermediate)	*r* = 0.158 *p* = 0.545	*r* = −0.166 *p* = 0.106	*r* = 0.406 *p* = 0.106	*r* = 0.159 *p* = 0.542	*r* = 0.175 *p* = 0.516	*r* = −0.183 *p* = 0.532	*r* = 0.017 *p* = 0.930
Classical (% monocytes)	*r* = −0.299 *p* = 0.243	*r* = 0.065 *p* = 0.804	***r* = −0.559** ***p* = 0.043**	*r* = −0.345 *p* = 0.174	*r* = −0.473 *p* = 0.064	*r* = 0.088 *p* = 0.765	*r* = −0.279 *p* = 0.142
ACE^+ classical (% classical)	*r* = 0.257 *p* = 0.319	*r* = −0.013 *p* = 0.961	*r* = 0.262 *p* = 0.310	*r* = 0.148 *p* = 0.572	*r* = 0.251 *p* = 0.349	*r* = −0.083 *p* = 0.777	*r* = 0.326 *p* = 0.090

Correlations were assessed by Spearman's rank tests. Correlations reaching statistical significance were highlighted in bold.
ACE, angiotensin-converting enzyme; aVV, adventitial vasa vasorum; cIMT, carotid intima-media thickness; CKD5-PD, chronic kidney disease stage 5 patients undergoing peritoneal dialysis; CV, cardiovascular; PWV, pulse wave velocity; Tang, angiogenic T cells.

TABLE 4 | Tang as predictors of subclinical CV outcomes.

	Number of neovasa (carotid)	Number of neovasa (femoral)	aVV area (carotid)	aVV area (femoral)	PWV
Univariate					
Tang	−0.505 (−2.281, −0.485) *p* = 0.004	−0.293 (−1.085, 0.116) *p* = 0.110	−0.523 (−3.819, −0.862) *p* = 0.003	−0.466 (−2.936, −0.477) *p* = 0.008	−0.663 (−1.40, −0.682) *p* < 0.001
Multivariate					
Tang	−0.662 (−6.309, −0.582) *p* = 0.022	0.161 (−1.290, 2.263) *p* = 0.566	−2.313 (−10.870, −0.409) *p* = 0.036	−0.393 (−6.963, −0.010) *p* = 0.048	−0.437 (−3.390, −0.141) *p* = 0.010
SCORE	−0.270 (−2.055, 0.702) *p* = 0.310	0.540 (−0.071, 1.640) *p* = 0.069	0.025 (−2.404, 2.633) *p* = 0.924	0.411 (−0.258, 3.423) *p* = 0.086	0.368 (−0.241, 1.545) *p* = 0.139

The associations between Tang frequency and subclinical CV outcomes were analyzed by linear regression in univariate or multivariate models adjusted for the burden of traditional CV risk factors (entered as the SCORE). The unstandardized regression coefficient (B) and (95% CI) with the corresponding p-values were computed.
aVV, adventitial vasa vasorum; CV, cardiovascular; PWV, pulse wave velocity; Tang, angiogenic T cells.

solely based on traditional risk factors fail to achieve an appropriate risk stratification in CKD (39–41), these findings may support the use of Tang as an additional instrument in the clinical setting. Larger studies to explore the ability of Tang to reclassify CKD patients to appropriate risk categories are warranted.

Additionally, the analysis of T-cell subsets revealed that CKD patients were hallmarked by a $CD4^+CD28^{null}$ expansion. This subset was strongly correlated with the extent of vascular calcification, whereas no associations with subclinical features were noted. Interestingly, $CD4^+CD28^{null}$ are terminally differentiated cells that result from a cumulative T-cell activation, leading to T cells exhaustion (42, 43). As a consequence, they are originated after a long exposure to pro-senescent stimuli. This notion may underlie their association with vascular calcification, as it is the result of an evolutive, cumulative process, thus supporting their association with a harder endpoint than subclinical vascular outcomes, as observed for Tang.

Taken together, these lines of evidence highlight that T cells may act as a double-edged sword for vascular outcomes in inflammatory conditions. Despite being highly heterogeneous, T cells have been largely considered to prompt vascular impair or endothelial damage progression. The observation that early T-cell accumulation precedes vascular lesions further supported this idea. However, the findings from our group (8, 9) and others (7, 10, 44) support the existence of T cell-mediated protective mechanisms for vascular homeostasis. Disruptions of these mechanisms or an altered balance with the deleterious effects

mediated by other T-cell populations may explain the occurrence of vascular events (45). Then, the role of T cells in this scenario may be more complex than initially conceived. Consequently, the protective effects of some T-cell subsets may be taken into account, especially when the use of immunomodulating agents is considered.

Of note, despite our results showing profound changes in monocyte subsets in CKD patients, only intermediate monocytes were related to vascular surrogate markers, such correlations being weaker than those retrieved for T cells and being interfered by potential confounders. These results may sound counterintuitive, since monocytes are pivotal for the development of some vascular events. Moreover, the lack of association with clinical features leads us to think that monocyte alterations may be related to the immunopathogenesis of the CKD itself, rather than to vascular outcomes. Interestingly, the notion that a Th1 predominance has been documented in CKD (46, 47) aligns with this point. The fact that the disturbances of the circulating monocytes seemed to be homogeneous across all patients independently of clinical features (even between dialysis modalities) prompted us to hypothesize that these alterations may appear earlier in the continuum of CKD stages, thus being a common trait of its pathogenesis. This may explain why these changes were so consistent across individuals. Importantly, animal models have also confirmed an early shift in monocyte subsets during the initial steps leading to CKD (48). Therefore, it may be conceivable that monocytes become activated in previous stages, thus eliciting an inflammatory response by activating adaptive responses at a later stage. Emerging evidence supports this "progressive" model, with an initial involvement of innate pathways that fuel an aberrant activation of adaptive responses in the final phases of CKD. These lines of evidence may account for the lack of associations between monocyte disturbances and vascular outcomes in CKD stage 5.

In conclusion, novel immune cell subsets are related to vascular surrogate markers in CKD patients. T cells emerge as double-edged swords in the associations between vascular outcomes and CKD, with Tang depletion being associated with poor vascular functionality and subclinical atherosclerosis, whereas $CD4^{+}CD28^{null}$ were related to calcification. On the contrary, profound quantitative and qualitative changes were found within the monocyte compartment, but only intermediate monocytes were slightly related to vascular outcomes in univariate analyses. Our proof-of-concept study suggested for the first time a potential role for Tang in CKD, hence paving the ground for functional studies to unveil the mechanisms underlying Tang depletion in CKD. However, this study comes with some limitations that should be remarked, including reduced sample size and cross-sectional design. Our patient population was homogeneous and reflected a real-world CKD population undergoing dialysis. However, patients with diabetes were excluded due to the autoimmune background, which might have a differential effect on these populations, independently of that of the CKD. Further studies with appropriate control populations are needed to evaluate whether the alterations herein reported could be observed in diabetic CKD patients. The inclusion of an independent validation cohort strengthens the consistency of our findings. It must be noted that our study found validation in the independent comparisons of each CKD5 cohort with its respective control group, but no comparisons were made between dialysis modalities, as this felt beyond the study aims. Having found a similar picture in these analysis, our findings could be considered to be a general effect of the CKD stage itself, although a potential effect of the dialysis modality should not be ruled out. However, whether these alterations may be extended to other CKD stages needs to be confirmed in future studies. Similarly, whether dialysis modality may influence these alterations cannot be addressed in the present study. Importantly, the findings related to the vascular outcomes were only analyzed in patients undergoing PD, so it remains unclear whether these results can be transferred to patients under hemodialysis. Furthermore, larger, long-term prospective studies are warranted to evaluate whether these subsets could be considered predictive biomarkers that could assist in the clinical setting.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comité de Ética Regional de Investigación Clínica. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JR-C and NC-L performed most of the experimental procedures and carried out the statistical analyses. CU and BM-C carried out some experimental procedures. CR-S, ES-A, and MR-G were in charge of patients' recruitment and clinical data collection. JR-C, NC-L, MN-D, MA, BF-M, JC-A, AS, and AD contributed to the data analysis interpretation and discussion of the results. JR-C, NC-L, and AS drafted the manuscript. AD conceived the study, designed the protocols, and edited the manuscript. All authors read the manuscript, revised it for intellectual content, approved the final version, and agreed to be accountable for all aspects of the work.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.618286/full#supplementary-material

REFERENCES

1. Lam CWK. 2. Inflammation, cytokines chemokines in chronic kidney disease. *EJIFCC*. (2009) 20:12–20.
2. Silverstein DM. Inflammation in chronic kidney disease: role in the progression of renal and cardiovascular disease. *Pediatr Nephrol*. (2009) 24:1445–52. doi: 10.1007/s00467-008-1046-0
3. Libby P, Loscalzo J, Ridker PM, Farkouh ME, Hsue PY, Fuster V, et al. Inflammation, immunity, and infection in atherothrombosis: JACC review topic of the week. *J Am Coll Cardiol*. (2018) 72:2071–81. doi: 10.1016/j.jacc.2018.08.1043
4. Tabas I, Lichtman AH. Monocyte-macrophages and T cells in atherosclerosis. *Immunity*. (2017) 47:621–34. doi: 10.1016/j.immuni.2017.09.008
5. Padgett LE, Araujo DJ, Hedrick CC, Olingy CE. Functional crosstalk between T cells and monocytes in cancer and atherosclerosis, *J Leukoc Biol*. (2020) 108:297–308. doi: 10.1002/JLB.1MIR0420-076R
6. Hur J, Yang H-M, Yoon C-H, Lee C-S, Park K-W, Kim J-H, et al. Identification of a novel role of T cells in postnatal vasculogenesis: characterization of endothelial progenitor cell colonies. *Circulation*. (2007) 116:1671–82. doi: 10.1161/CIRCULATIONAHA.107.694778
7. Cavazzana I, Piantoni S, Sciatti E, Fredi M, Taraborelli M, Bonadei I, et al. Relationship between endothelial dysfunction, videocapillaroscopy and circulating $CD3^+CD31^+CXCR4^+$ lymphocytes in systemic lupus erythematosus without cardiovascular risk factors. *Lupus*. (2019) 28:210–6. doi: 10.1177/0961203318821161
8. López P, Rodríguez-Carrio J, Martínez-Zapico A, Caminal-Montero L, Suarez A. Senescent profile of angiogenic T cells from systemic lupus erythematosus patients. *J Leukoc Biol*. (2016) 99:405–12. doi: 10.1189/jlb.5HI0215-042R
9. Rodríguez-Carrio J, Alperi-López M, López P, Alonso-Castro S, Ballina-García FJ, Suárez A. Angiogenic T cells are decreased in rheumatoid arthritis patients. *Ann Rheum Dis*. (2014) 287:e244. doi: 10.1016/j.atherosclerosis.2019.06.749
10. Rouhl RPW, Mertens AECS, van Oostenbrugge RJ, Damoiseaux JGMC, Debrus-Palmans LL, Henskens LHG, et al. Angiogenic T-cells and putative endothelial progenitor cells in hypertension-related cerebral small vessel disease. *Stroke*. (2012) 43:256–8. doi: 10.1161/STROKEAHA.111.632208
11. Manetti M, Pratesi S, Romano E, Bellando-Randone S, Rosa I, Guiducci S, et al. Angiogenic T cell expansion correlates with severity of peripheral vascular damage in systemic sclerosis. *PLoS ONE*. (2017) 12:e0183102. doi: 10.1371/journal.pone.0183102
12. Dumitriu IE. The life (and death) of $CD4^+$ $CD28^{(null)}$ T cells in inflammatory diseases. *Immunology*. (2015) 146:185–93. doi: 10.1111/imm.12506
13. Nakajima T, Schulte S, Warrington KJ, Kopecky SL, Frye RL, Goronzy JJ, et al. T-cell-mediated lysis of endothelial cells in acute coronary syndromes. *Circulation*. (2002) 105:570–5. doi: 10.1161/hc0502.103348
14. Thewissen M, Somers V, Hellings N, Fraussen J, Damoiseaux J, Stinissen P. $CD4^+CD28^{null}$ T cells in autoimmune disease: pathogenic features and decreased susceptibility to immunoregulation. *J Immunol*. (2007) 179:6514–23. doi: 10.4049/jimmunol.179.10.6514
15. Guilliams M, Mildner A, Yona S. Developmental and functional heterogeneity of monocytes. *Immunity*. (2018) 49:595–613. doi: 10.1016/j.immuni.2018.10.005
16. Sprangers S, de Vries TJ, Everts V. Monocyte heterogeneity: consequences for monocyte-derived immune cells. *J Immunol Res*. (2016) 2016:1–10. doi: 10.1155/2016/1475435
17. Stansfield BK, Ingram DA. Clinical significance of monocyte heterogeneity. *Clin Trans Med*. (2015) 4:e5. doi: 10.1186/s40169-014-0040-3
18. Ryu JH, Vuk-Pavlović Z, Rohrbach MS. Monocyte heterogeneity in angiotensin-converting enzyme induction mediated by autologous T lymphocytes. *Clin Exp Immunol*. (1992) 88:288–94. doi: 10.1111/j.1365-2249.1992.tb03075.x
19. Cao D-Y, Saito S, Veiras LC, Okwan-Duodu D, Bernstein EA, Giani JF, et al. Role of angiotensin-converting enzyme in myeloid cell immune responses. *Cell Mol Biol Lett*. (2020) 25:31. doi: 10.1186/s11658-020-00225-w
20. Rogacev KS, Heine GH. Human monocyte heterogeneity–a nephrological perspective. *Néphrol Thér*. (2010) 6:219–25. doi: 10.1016/j.nephro.2010.01.008
21. Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL, et al. European Guidelines on cardiovascular disease prevention in clinical practice. *Eur Heart J*. (2016)37:2315–81.
22. Rodríguez-Carrio J, Alperi-López M, López P, Alonso-Castro S, Ballina-García FJ, Suárez A, TNFα polymorphism as marker of immunosenescence for rheumatoid arthritis patients. *Exp Gerontol*. (2015) 61:123–9. doi: 10.1016/j.exger.2014.12.009
23. Touboul P-J, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, et al. Mannheim carotid intima-media thickness consensus (2004–2006). *Cerebrovasc Dis*. (2007) 23:75–80. doi: 10.1159/000097034
24. Arcidiacono MV, Rubinat E, Borras M, Betriu A, Trujillano J, Vidal T, et al. Left carotid adventitial vasa vasorum signal correlates directly with age and with left carotid intima-media thickness in individuals without atheromatous risk factors. *Cardiovasc Ultrasound*. (2015) 13:20. doi: 10.1186/s12947-015-0014-7
25. Nakagawa S, Cuthill IC. Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biol Rev*. (2007) 82:591–605. doi: 10.1111/j.1469-185X.2007.00027.x
26. Herrmann J. Coronary vasa vasorum neovascularization precedes epicardial endothelial dysfunction in experimental hypercholesterolemia. *Cardiovasc Res*. (2001) 51:762–6. doi: 10.1016/S0008-6363(01)00347-9
27. Arcidiacono MV, Traveset A, Rubinat E, Ortega E, Betriu A, Hernández M, et al. Microangiopathy of large artery wall: a neglected complication of diabetes mellitus. *Atherosclerosis*. (2013) 228:142–7. doi: 10.1016/j.atherosclerosis.2013.02.011
28. Arcidiacono MV, Martinez-Alonso M, Belart M, Vilar A, Martín M, Craver L, et al. High levels of hemoglobin promote carotid adventitial vasa vasorum neoangiogenesis in chronic kidney disease. *Mediators Inflamm*. (2017) 2017:1–11. doi: 10.1155/2017/3795142
29. Safar ME, London GM. Therapeutic studies and arterial stiffness in hypertension. *J Hypertens*. (2000) 18:1527–35. doi: 10.1097/00004872-200018110-00001
30. Drüeke TB, Massy ZA. Atherosclerosis in CKD: differences from the general population. *Nat Rev Nephrol*. (2010) 6:723–35. doi: 10.1038/nrneph.2010.143
31. London GM, Drüeke TB. Atherosclerosis and arteriosclerosis in chronic renal failure. *Kidney Int*. (1997) 51:1678–95. doi: 10.1038/ki.1997.233
32. Roos A, Bandstein N, Lundbäck M, Hammarsten O, Ljung R, Holzmann MJ. Stable high-sensitivity cardiac troponin T levels and outcomes in patients with chest pain. *J Am Coll Cardiol*. (2017) 70:2226–36. doi: 10.1016/j.jacc.2017.08.064
33. Kanderian AS, Francis GS. Cardiac troponins and chronic kidney disease. *Kidney Int*. (2006) 69:1112–4. doi: 10.1038/sj.ki.5000174
34. Kuwahara F, Kai H, Tokuda K, Shibata R, Kusaba K, Tahara N, et al. Hypoxia-inducible factor-1α/vascular endothelial growth factor pathway for adventitial vasa vasorum formation in hypertensive rat aorta. *Hypertension*. (2002) 39:46–50. doi: 10.1161/hy1201.097200

35. Mulligan-Kehoe MJ, Simons M. Vasa vasorum in normal and diseased arteries. *Circulation*. (2014) 129:2557–66. doi: 10.1161/CIRCULATIONAHA.113.007189
36. Moos MPW, John N, Gräbner R, Noßmann S, Günther B, Vollandt R, et al. The lamina adventitia is the major site of immune cell accumulation in standard chow-fed apolipoprotein E–deficient mice. *Arterioscler Thromb Vasc Biol*. (2005) 25:2386–91. doi: 10.1161/01.ATV.0000187470.31662.fe
37. Schwartz CJ, Mitchell JR. Cellular infiltration of the human arterial adventitia associated with atheromatous plaques. *Circulation*. (1962) 26:73–8. doi: 10.1161/01.CIR.26.1.73
38. Watanabe M, Sangawa A, Sasaki Y, Yamashita M, Tanaka-Shintani M, Shintaku M, et al. Distribution of inflammatory cells in adventitia changed with advancing atherosclerosis of human coronary artery. *J Atheroscler Thromb*. (2007) 14:325–31. doi: 10.5551/jat.E489
39. Weiner DE, Tighiouart H, Elsayed EF, Griffith JL, Salem DN, Levey AS, et al. The Framingham predictive instrument in chronic kidney disease. *J Am Coll Cardiol*. (2007) 50:217–24. doi: 10.1016/j.jacc.2007.03.037
40. Muntner P, He J, Astor BC, Folsom AR, Coresh J. Traditional and nontraditional risk factors predict coronary heart disease in chronic kidney disease: results from the atherosclerosis risk in communities study. *J Am Soc Nephrol*. (2005) 16:529–38. doi: 10.1681/ASN.2004080656
41. Shlipak MG, Fried LF, Cushman M, Manolio TA, Peterson D, Stehman-Breen C, et al. Cardiovascular mortality risk in chronic kidney disease. *JAMA*. (2005)293:1737. doi: 10.1001/jama.293.14.1737
42. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. *Nat Rev Immunol*. (2015) 15:486–99. doi: 10.1038/nri3862
43. Maly K, Schirmer M. The story of CD4 $^{+}$ CD28 – T cells revisited: solved or still ongoing? *J Immunol Res*. (2015) 2015:1–11. doi: 10.1155/2015/348746
44. Alunno A, Ibba-Manneschi L, Bistoni O, Cipriani S, Topini F, Gerli R, et al. Angiogenic T cells in primary Sjögren's syndrome: a double-edged sword? *Clin Exp Rheumatol*. (2019) 37 (Suppl 1):36–41.
45. Mallat Z, Taleb S, Ait-Oufella H, Tedgui A. The role of adaptive T cell immunity in atherosclerosis: Fig. 1. *J Lipid Res*. (2009) 50:S364–9. doi: 10.1194/jlr.R800092-JLR200
46. Azadegan-Dehkordi F, Bagheri N, Shirzad H, Rafieian-Kopaei M. The role of Th1 and Th17 cells in glomerulonephritis. *J Nephropathol*. (2015) 4:32–7. doi: 10.12860/jnp.2015.07
47. Kitching AR, Katerelos M, Mudge SJ, Tipping PG, Power DA, Holdsworth SR. Interleukin-10 inhibits experimental mesangial proliferative glomerulonephritis. *Clin Exp Immunol*. (2002) 128:36–43. doi: 10.1046/j.1365-2249.2002.01793.x
48. Tecklenborg J, Clayton D, Siebert S, Coley SM. The role of the immune system in kidney disease. *Clin Exp Immunol*. (2018) 192:142–50. doi: 10.1111/cei.13119

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Impacts of COVID-19 Pandemic on Psychological Well-Being of Older Chronic Kidney Disease Patients

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COVID-19 pandemic has been a major global issue, its eventual influences on the population welfare, global markets, public security, and everyday activities remain uncertain. Indeed, the pandemic has arisen a significant global threat. Its psychological impact is predicted to be severe and enduring, but the absolute magnitude is still largely unclear. Chronic kidney disease (CKD) is a complication markedly contributes to the mortality of COVID-19 cases, meanwhile several studies have demonstrated the high frequency and seriousness of the COVID-19 in CKD patients receiving dialysis. Importantly, the influence of COVID-19 among CKD patients without dialysis is still largely unexplored. Thus, we systemically summarized how mental health affects the spreading of COVID-19 to virtually worldwide, covering perspectives from several countries across a wide range of fields and clinical contexts. This review aims to provide the latest details and reveal potential concerns on the public health including psychological well-being of the older patients with CKD.

Keywords: COVID-19 pandemic, psychological well-being, aging-old age-seniors, immune system, chronic kidney disease

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INTRODUCTION

Psychological well-being (PWB) is fundamentally equivalent to other phrases that apply to desirable psychological operations, including pleasure or fulfillment. It is not essential or valuable to consider the fundamental differences between all these phrases (1). Psychological well-being means being on good terms with others and leading a purposeful and meaningful life (2). It was found that people with positive psychological well-being are more carefree and enjoy a more vibrant and comfortable life (3). However, nearly 25% of people with chronic conditions experienced psychological problems related to COVID-19, particularly CKD patients (4, 5). Currently, personalized treatment should be the norm in handling CKD patients (6–10). Because of COVID-19, it seems to be far more critical that this approach be pursued to minimize the possibility of excessive or insufficient treatment and reduce the likelihood of developing a prejudice (11). This applies to COVID-19 as people's psychological well-being experienced the most significant impact during the pandemic. The ones with stable psychological well-being were in a better state than those whose well-being was below par (3). As there have been constant interruptions to everyday life owing to social distancing, which has been imposed to minimize the transmission of COVID-19, precedent hazards to public mental health were observed (12). The

risk of COVID-19 severe complications and poor prognosis is higher for CKD patients, particularly those who undergo chronic dialysis therapy, including higher rates of hospitalization, intensive-care unit admission, mechanical ventilation, and death (13). The well-being of patients has been a significant issue during the pandemic considering the mental effects on even ordinary healthy people were more critical than expected (14). Hence, the impact on the psychological well-being of older CKD patients will be studied.

INTERACTION BETWEEN COVID-19 AND PSYCHOLOGICAL WELL-BEING

The findings of the research conducted by Moreno et al. (15) are diverse (**Table 1**), possibly due to variations in the methodology adopted, the venues of the analysis, and the fact that the research takes place during the pandemic. Possible consequences of modifications to health resources on accessibility and reliability and performance of psychiatric services throughout the COVID-19 pandemic (16). Phobic anxiety, impulse purchase, and television addiction, all linked to psychological disruptions, insomnia, exhaustion, and consciousness deterioration, have been documented, and digital networking has been linked to heightened anxiety and depression-associated anxiety (17–19).

The illustration (**Table 1**) shows the possible effects of modifications to health resources on psychiatric services throughout the COVID-19 pandemic. It further describes the reliability and impact of these adjustments in resources amid the COVID-19 pandemic (13). Numerous people worldwide are now feeling stress and paranoia, particularly the elderly or people with existing health issues and even active and energetic youths. The anxiety is about the novel coronavirus, which the technical term is severe acute respiratory syndrome coronavirus (SARS-CoV-2) (20). This hideous virus induces a lethal respiratory condition known COVID-19, which brings fever, severe chest infections, and breathlessness (occasional lack of taste and smell or digestive troubles). COVID-19 is a disease that can escalate quickly; in certain instances, it can be fatal (21). Therefore, the psychological well-being of CKD patients during the period of COVID-19 should be concerned. The current situation is difficult for everyone in public, particularly for the older people who are existing mental health issues; such as anxiety and depression-associated anxiety that are more vulnerable to major medical problems related to coronavirus infection and the emergence of COVID-19 pulmonary disease with possibly catastrophic results (22).

EFFECTS OF LOCKDOWN ON HUMANS AMID COVID-19

Lockdown may also lead to pressure, resentment, and intensified harmful activities like internet gambling. In earlier outbreaks, the older person affected by lockdown had a higher likelihood of experiencing psychological problems and sorrow (23, 24). It has been observed that the number of elderly resorting to counseling services because of psychological distress has increased (25, 26). Local personal networks and experiences with other inhabitants, relatives, and caregivers due to isolation could also contribute to depression, immobility, and an inactive lifestyle among citizens, adding to their solitude. Solitude as well as social alienation have been associated with worse psychological health (for example, stress, despair, and neurological damage) along with the reduced quality of life (for example, weaker motor control, poorer heart health, sleep disturbance, and loss of strength) and increased death rates. Forced alienation may very well contribute to an inactive lifestyle, yet a person's lifestyle is crucial to reducing physical, mental, and societal medical issues (27, 28).

Based on current evidence from past disease outbreaks and new data from the recent episode, it is anticipated that mental morbidity will eventually increase. Also, such morbidity may escalate afterward and last longer than the external harmful effects of the outbreak (29). Such a pattern is shown in various aspects throughout this edition, which states that the initial stages of the epidemic did not automatically trigger a rise in psychological well-being sessions. Nevertheless, transition to the current constraints introduced by COVID-19 has added burdens to the field of psychological well-being (30). Moreover, the predicted rise in psychiatric illness, which could lead to more suicidal behavior, is more likely to emerge during and after the outbreak, as the financial crisis, local mental health services, human weaknesses, and the harsh truth of radically transformed habits converge (31).

HEALTH EFFECTS OF COVID-19: WORLDWIDE SITUATION

The World Health Organization states that the global transmission of COVID-19 is accelerated bit by bit. As shown by the data updated on 18 February 2020, the total number of confirmed instances had reached over 72,000, with almost 1,900 coming from China (32). The total number of deaths from COVID-19 is estimated to be more significant since the estimates vary from country to country. Although the virus affects everyone, assuming that all factors are identical, evidence has consistently shown that the death rate is higher among older individuals and people with complications (3, 33). The case fatality rate (CFR) of individuals aged 70 was between 0.3 and 3.5%. These figures are lower than the 8% CFR in patients between 70 and 79 and ∼15% in patients over 80 in China. As for Italy, empirical studies indicate the average age of patients dying from COVID-19 was 80, with CFR rising above 70 years of age; 12.5% (34–43), 19.7% (44–53), and 22.7% (over 90) (54). A study found that found the subjects to have obtained COVID-19-related information once in a while from the following channels: online (including sites, online news, and internet networks, such as Facebook and Twitter), acquaintances, traditional media (including television, newspapers, and radio), structured activities on COVID-19 (be it online or face-to-face), medical workers in healthcare environments, colleagues, and families. This research concluded that about 80% of the subjects received COVID-19 information online (55).

TABLE 1 | Implications for medical resources changes on availability, efficiency, and output of psychological treatment during the COVID-19 pandemic.

	Possible negative consequences	Possible positive consequences
The healthcare system's primary emphasis on the detection, reduction, and control of COVID-19.	• Primary educational emphasis on physical wellbeing; emphasis on social distancing rather than bodily distancing while remaining linked. • Redistribution of services to meet physical wellbeing treatment demands; reduced face-to-face interactions among and inside care units; bodily and psychological toll on medical staff; personnel deficiencies in medical services.	• Knowledge about the psychological implications of COVID-19 has the potential to raise the public's general psychological health awareness; the chance to highlight the significance of self-care, recovery measures, and household assistance; enhancement of funding for psychological health care from non-profit or private institutions; and multidisciplinary initiatives to activate support groups, using innovations to enable swift, scalable, and effective team interaction and collaboration inside and among teams (for example, psychological well-being, and basic treatment). • Improve bodily and psychological well-being through behavioral changes, the implementation of low-barrier destigmatized psychosocial assessments, counseling programs, and a student-to-student framework.
Controlled admission to other kinds of medical services as a critical component of COVID-19 management	Triage procedures that prioritize acute patients only resulted in a reduction in hospital visits (such as those for administering or distributing of drugs), emergency department visits, inpatient treatment, and pharmaceutical accessibility; community psychoeducation, community cognitive treatment, and mutual help initiatives being eliminated or scaled down; options for cardiometabolic and detrimental impact screening being reduced, overall inpatient spaces being reduced; hospital entry restrictions; reduced hospitalizations; hasty departure to mitigate the possibility of healthcare facility-related infection, particularly for those who have been hospitalized involuntarily.	Re-evaluation of the effective provision distribution, data retention regulations, and payment for telehealth and multimedia, virtual medical services, and choices for in-home care; availability strategies (for example, web channels), health policy, privacy rules, flexible drug coverage, including the usage of restricted drugs; creation of digital platforms for community outpatient therapies; controlling techniques that are less resistant to risk; less overcrowding in inpatient wards; re-evaluation of the duration of inpatient stays that are required; reassessment of the demand of forced medical services

Source: Moreno et al. (15).

Psychological symptoms and illnesses can occur as an adjunct to an unavoidable disease incident. These can appear at an active stage or later in time. The outbreak itself is a traumatic event, but it is significantly more disturbing to work as a medical practitioner to cope with such a severe disease (56). In America, the fatality rate in New York City for patients 75 years old or older was over 1,500 per 100,000 people. Advanced age and complications such as coronary disease, diabetes, chronic lung ailments, and persistent kidney dysfunction tend to increase the dangers of COVID-19. In many more existing patients with renal failure, where the health care system may evaluate employees, The CKD patients should consider the risks of death from COVID-19 in assessing the risks and benefits of treatment options (57).

ANALYZING THE PSYCHOLOGICAL ISSUES CAUSED BY COVID-19

Controversial expectations, coping with significant shortcomings in the resources for screening and therapy and the protection of patients and health care professionals from infection, the pressure of current overall well-being measures that place restrictions on personal autonomy, tremendous and rising financial troubles, and contradictory instructions from professionals are some of the major factors which would inevitably contribute to endless difficulty and heightened threats of Covid-19-related psychological disorder (58, 59). Healthcare practitioners have a major role in resolving these serious consequences as part of the pandemic response (60, 61).

Wide analysis of poor mental health has proven that tremendous problems are widespread in infected communities, an observation that is likely to be replicated in populations affected by the Covid-19 pandemic. Owing to these challenges, numerous people refuse to give in to therapy. Some individuals have developed new attributes. Considering all factors, in "standard" disastrous incidents, technological failures, and deliberate events of massive destruction, a major concern is post-traumatic stress disorder (PTSD) arising from exposure to trauma. Contagious illnesses, such as dangerous virus infection, may not follow existing trauma frameworks required to study PTSD, but other psychological issues such as stress and anxiety can arise (62). Several communities could be more vulnerable than others in coping with the psychosocial effects of disease outbreaks. Particularly, people who suffer from the disease, those at increased risk for infection (including the elderly, people with lower resistance, and those residing in community surroundings), and people with prior medical, emotional, or drug use problems are at heightened risk for antagonistic psychosocial outcomes (63).

OLDER CKD PATIENTS DURING COVID-19 PANDEMIC

The COVID-19 pandemic revealed the notorious vulnerability of the monetary system and the overwhelming ramifications for

TABLE 2 | Description of studies on older CKD patients negative psychological impact during COVID-19 in the review.

References	Country	Methods	Participants/ Sample	Prevalence	Impact on Psychological Well-Being
Lee et al. (52)	Western Pennsylvania and New Mexico	Phone survey	$N = 49$ participants, mean age: 56 years; gender: male 53%.	• (1) 27% of the participants had clinical levels of depressive symptoms, but only 12% had anxiety meeting clinical criteria. • (2) About 33% of participants reported poor sleep quality over the last month. Perceived stress was high in about 30% of participants, and 85% felt overwhelmed by difficulties with COVID-19, although 41% felt that things were pretty/very often going their way.	• (1) Anxiety; • (2) Depressive symptoms.
Sousa et al. (91)	Portugal	• Mixed method: (1) quantitative method from medical records, • (2) qualitative method semi-structured interviews	$N = 20$, mean age: 66.9 (±11.9); gender: male 55%.	• (1) Impact on family relationships (70%); fear of being infected due to high-risk condition (70%); increased emotional distress (55%); fear of getting infected in the dialysis unit (55%); difficulty adjusting to the contingency plan at the dialysis unit (55%); altered self-esteem and autonomy (40%). • (2) Impacts on disease and treatment-related health behaviors (25–55%), decreased physical activity (55%); management of dietary recommendations (35%); management of fluid restrictions (25%); need for nephrologist consultation (25%). • (3) Positive impacts (40%), personal growth (40%); increased social support (30%). • (4) Coping strategies (35-80%). • (5) Adherence to the protection measures at home (35%); engage in indoor and outdoor leisure activities (80%); seeking social support for instrumental use (65%); adherence to the protection measures at the dialysis clinic (55%); using social media and/or telephone to communicate (50%); religious coping (45%); seeking social support for emotional use (40%); avoidance(40%).	• (1) Emotional stress include anxiety; • Social distrust; • (3) Somatization.
Yang et al. (93)	China	(1) Survey	N= 273, mean age: 59.9 (+/− 14.4); gender: male 41.4%.	(1) Nonspecific psychiatric morbidity 45.8% by using General Health Questionniare-28 (GHQ-28) (2) Clinical concern (19.4%) by using Impact of Events Scale–Revised (IES-R) (3) Kidney Disease Quality of Life (KDQOL) and KDQOL-36 Short Form (SF) were significantly improved when compared with the intiital study ($p = 0.006$ and $p = 0.031$, respectively) (4) General Health Questionnaire-28 (GHQ-28) and Impact of Events Scale–Revised (IES-R) did not have significant change, but there were improvement in somatic symptoms (p= 0.006); anxiety and insomnia (p= 0.005); and intrusion (p=0.049)	(1) Somatic symptoms (2) Anxiety (3) Insomnia (4) Duration of hemodiaglysis may affect mental health, QoL, or health status.
Barutcu Atas et al. (49)	Turkey	(1) Survey	$N = 106$, mean age: 44.2 (±13.3), gender: male 61.3%.	• (1) High-perceived stress (49, 46.2%), • (2) Poor sleep quality (51, 48.1%) • (3) Insomina (40, 37.7%) • (4) Anxiety (25, 23.6%) • (5) Depression (47, 44.3%) • (6) Regression analyses revealed that high-perceived stress is an independent predictor of anxiety and depression.	• (1) Stress, • (2) Sleeping quality, • (3) Anxiety, • (4) Depression.

our financial structure should new tactics not be adopted to tailor medical services to specific patient subgroups. The massive group of elderly and feeble people over 65 poses a public health concern. According to the latest data from the Istituto Superiore di Sanità of Italy, COVID-19 tends to be more deadly among elderly patients: 96.4% of deaths were over 60 years of age. People aged 70 or above account for 35.5% of instances as statistics were classified by age level, whereas participants aged over 80 accounts for 52.3%. Patients with renal disease are an elderly group that is especially susceptible to infection and carries a higher risk of death than the average person. The massive group of elderly and feeble people over 65 poses a public health concern. The COVID-19 pandemic revealed the notorious vulnerability of the monetary system and the overwhelming ramifications for our financial structure should new tactics not be adopted to tailor medical services to specific patient subgroups (64).

Given that most patients with CKD are seniors, who experience biological deterioration of renal function and are more vulnerable to renal disease, COVID-19 emerges as a pertinent issue because of the heightened risk of comorbidities and fatality in patients with chronic renal disease (65). Moreover, specific antiviral and immunosuppressive approaches to combat COVID-19 infection have been hampered by severe renal damage. The combination of age and chronic renal disease is most likely a possible cause of COVID-19 in immunosuppressive activity. Immunol senescence is a condition that occurs in older adults and is accompanied by weakened responsive and inherent immune function (66). Numerous changes have occurred, including thymic involution, a reduction in naïve T-cells and progenitor B-cells, and a reduction in the production of MHC class II on macrophages. Among chronic renal disease cases, a significant immunosuppressive condition has been observed as well: (i) diminished granulocyte and monocyte/macrophage phagocytic activity; (ii) reduced antigen-presenting potential of antigen-presenting cells; (iii) loss of antigen-presenting dendritic cells; (iv) weakened B lymphocyte numbers and immune generating ability; (v) reduction in naïve and central memory $CD4^+$ and $CD8^+$ T lymphocytes; (vi) disrupted cell-mediated resistance. Because of such considerations, older CKD patients must fully adhere to the guidelines of the Ministry of Health and Nephrological Scientific Societies for COVID-19 reduction (34–53, 67–92).

CHALLENGES AND RECOMMENDATIONS CONCERNING THE PSYCHOLOGICAL IMPACT AMONG CKD PATIENTS

Under the outbreak, further attention to be paid to public health, both physical and psychological, to help communities during this challenging period (71–73). The COVID-19 outbreak has brought many extra challenges to the study, planning, and management of health (4, 74, 75). The problems of COVID-19 mental health and bureaucratic responses to the outbreak are not exactly unprecedented. Past mental health deficiencies could become more deep-rooted and considerably more difficult to tackle (76, 77). Evidence from all over the world of shifts in individuals' mental health, possibly attributable to the COVID-19 outbreak, has been hindered by the use of residence assessments, distorted or unverifiable mental health metrics, and the lack of other pre-COVID-19 conventional knowledge to measure the transition, be it among individuals or throughout the whole population (78, 79, 93–95).

One study showed elevated rates of mental illness among US adults in 2020 compared with 2018, and the increase was the most significant among young adults aged between 18 and 24 and females (19). Legislators, politicians, and specialized agencies require accurate information on the shifts in mental health associated with the outbreak so that decisions are backed by knowledge on the extent of transitions in individuals' mental health and vulnerability to psychiatric problems (80). In such a crisis, the ability to track and address psychosocial needs during proper consultation in clinical care is severely constrained by the immense complexity of household regulation. Strategies for telemedicine are given to psychosocial institutions and are increasingly distributed in stimulating environments (81). As far as COVID-19 is concerned, psychosocial evaluation and monitoring may include concerns linked to COVID-19 stress factors (e.g., exposures to infected materials, infected family members, loss of loved ones, and segregation) and further mishaps (e.g., economic hardship) (82, 83).

Psychosocial impacts include depression, stress, psychiatric disturbances, insomnia, heightened drug use and aggressive actions at home, and signs of vulnerability (84) (**Table 2**), such as previous physical or emotional disorders. Some individuals may need guidance regarding structured psychological health evaluation and treatment. Others benefit from ongoing counseling to enhance health and facilitate adjustment (e.g., psychoeducation or cognitive behavior approaches) (85, 86). Given the increasing financial crisis and the multiple threats of this outbreak, self-destructive thoughts may emerge, which entails a timely meeting with professionals or a recommendation for possible crisis psychiatric hospitalization (87, 88). At the gentler end of the psychosocial spectrum, a large amount of interaction between patients, families, and the wider populace could be better structured by presenting evidence on typical reactions to this form of resistance and by drawing attention to what people can do in the middle of severe circumstances (89, 90).

In a multivariable study, Varshney et al. (48) found that advanced age, prolonged illness, the existence of breathing troubles, and absence of community backup have a significant correlation with unusual mental effects of COVID-19 on individuals with chronic disease. Once they need to go outside for provisions, elderly CKD patients and those living on their own become especially susceptible. Throughout the COVID-19 epidemic, numerous CKD patients in the early to later phases of kidney disease might have testing arrangements postponed. Failure to detect significant development of CKD has profound implications for both the patient and the community (**Figure 1**) (64, 91).

Healthcare practitioners may provide guidelines for mental stress and adjustment (such as planning exercises and timetabling) (92), connect patients with psychosocial health departments, and encourage patients to pursue adequate mental health assistance as needed (34). Nadler et al. (35) noted that

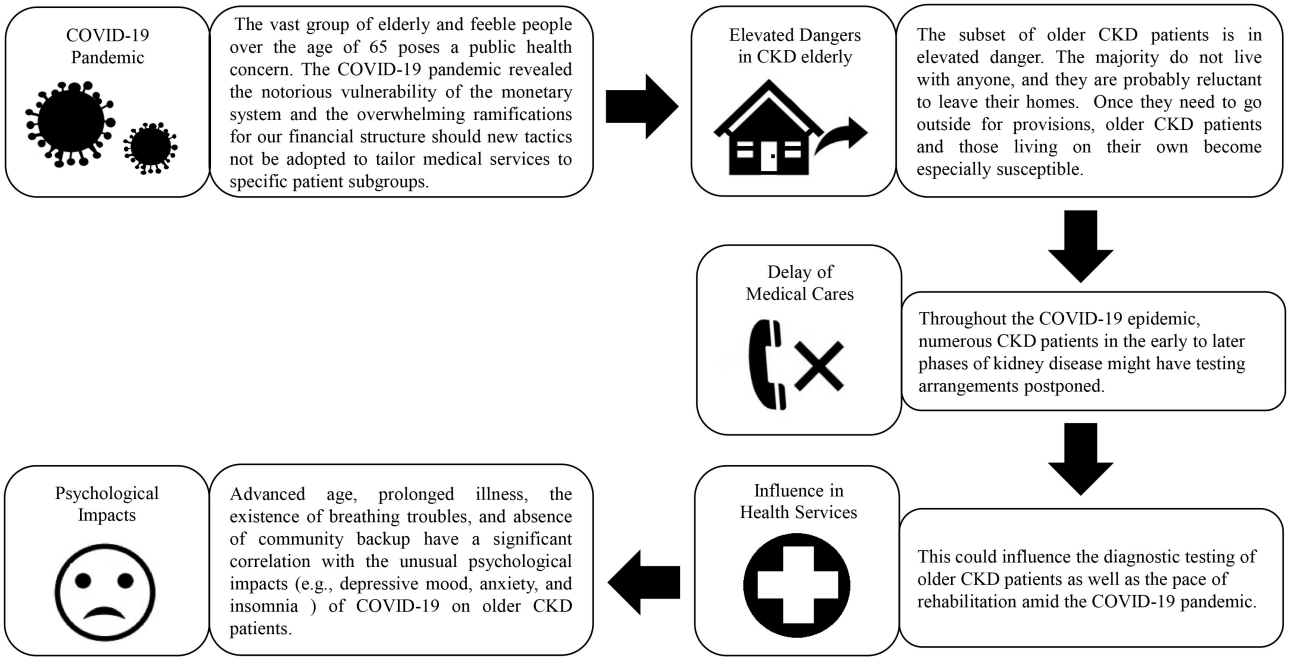

FIGURE 1 | During COVID-19, the phenomenon has a detrimental psychological impact on CKD patients. Source: Coppolino et al. (64) and Varshney et al. (48).

because the caregivers typically lessen their children's discomfort, transparent talks should be encouraged to discuss children's reactions and issues. As far as health care providers themselves are concerned, the innovative concept of SARS-CoV-2, preliminary screening, minimal treatment options, insufficient PPE and other medical resources, prolonged unresolved pressures, and other related risks are sources of stress and could potentially overwhelm systems (36, 37). SARS-CoV-2 is spread from humans to humans through direct contact with an infected person *via* nasal spills or touching contaminated substances. Maturity and chronic illness have been identified as possible causes of severe disease and death (38). Ghinai et al. (39) confirmed that SARS-CoV-2, which resulted in the condition currently known as COVID-19, had been disseminated across China and 26 more countries as of 18 February 2020. Advanced age, being female, extended illness, breathing symptoms, and lack of social support were essentially related to the peculiar mental impact of COVID-19 on patients with renal impairment. Patients aged 34 or older were more likely to suffer from psychiatric disorders due to the recent outbreak (40, 41). This result invalidates an Indian study where young adults encountered more significant psychological problems due to COVID-19. This discrepancy may be attributed to the incorrect assumption that COVID-19 is not as accurate in younger individuals (42, 43).

Self-care offered by providers, like mental healthcare providers, requires training on disease and risks (44), tracking someone's pressure reaction, as well as seeking adequate assistance with personal and occupational responsibilities and issues, such as professional mental health (22, 45, 46). Healthcare systems must handle the burden of subcontractors and comprehensive operations by evaluating reactions and implementation, modifying projects and plans, adjusting expectations, and designing tools to deliver psychosocial assistance based on the circumstances (47).

DISCUSSION

The health care system must offer coaching and instruction on psychosocial problems to healthcare service administrators, emergency personnel, and health care providers. Mental health and emergency response systems must work together to identify, establish and allocate evidence-based resources such as disaster-related mental health, psychological well-being crisis and referral, special patient needs, and alarm and distress treatment. Risk consultation initiatives should resolve the difficulty of emerging problems, such as legislation, vaccination affordability and sufficiency, and the need for evidence-based arrangements related to disease outbreaks, and tackle various psychosocial considerations. Psychological well-being practitioners may strengthen perceptions that can be expressed through supporting the experts. The COVID-19 episode has a devastating impact on personal and collective welfare and care work. Despite health concerns, ultimately, medical treatment practitioners have a significant role in tracking psychosocial needs and delivering psychosocial assistance to their patients, providers of therapeutic services, and social initiatives that should be integrated into overall pandemic healthcare.

COVID-19 has contributed to increased recognized risk factors for mental health problems. In addition to weirdness and insecurity, quarantine and physical isolation can lead to significant alienation, lack of income, delays, limited access to core domains, increased exposure to alcohol and internet betting, and decreased family and community assistance, especially in

more vulnerable people (21). The COVID-19 outbreak also provides a significant barrier to involvement in preliminary testing for older adults with tumors who are currently underserved in oncology and other clinical tests. Testing or possible admission to such therapeutic initial testing has been completed or based on several assessment projects worldwide.

CONCLUSION

COVID-19 is an evolving and rapidly growing disease that warrants personalized attention and assessment depending on the incidence of the infection. When humanity is dealing with the outbreak and working to find ways to effectively distribute cancer treatment to more mature patients, it is necessary to intervene to protect the vulnerable and counter the prolonged detrimental consequences in this age group. Since this is unlikely the last outbreak in human history, it is crucial to embrace this opportunity to discover facts and formulate strategies for any possible scenarios. It should also be understood that previous studies could contribute to a range of uses depending on the stage of the outbreak. Overall, it is especially critical that older individuals practice social distancing. Nevertheless, the scientific evidence of the experiences of older adults has been minimal so far. To understand the impact of the outbreak on more mature people and to develop viable arrangements, it is vital to examine how older individuals respond to quarantine measures and identify the difficulties and frustrations faced by older people and patients with CKD.

AUTHOR CONTRIBUTIONS

AC and PT carried out the outline of this manuscript. AC wrote the manuscript with support from JH and JL. JH and JL gave valuable comments and suggestion. PT helped to supervise the whole manuscript with his professional advances in Chronic Kidney Disease. AC and PT linked up the situation of nowadays older CKD patients during COVID-19 and their risk factors of psychological well-being. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Shanthanna H, Bhatia A, Radhakrishna M, Belley-Cote E, Vanniyasingam T, Thabane L, et al. Interventional pain management for chronic pain: a survey of physicians in Canada. *Canad J Anesthesia.* (2020) 67:343–52. doi: 10.1007/s12630-019-01547-w
2. Salehinejad MA, Majidinezhad M, Ghanavati E, Kouestanian S, Vicario CM, Nitsche MA, et al. Negative impact of COVID-19 pandemic on sleep quantitative parameters, quality, and circadian alignment: implications for health and psychological well-being. *EXCLI J.* (2020) 19:1297. doi: 10.1101/2020.07.09.20149138
3. Torales J, O'Higgins M, Castaldelli-Maia JM, Ventriglio A. The outbreak of COVID-19 coronavirus and its impact on global mental health. *Int J Soc Psychiatry.* (2020) 66:317–20. doi: 10.1177/0020764020915212
4. Heid AR, Cartwright F, Wilson-Genderson M, Pruchno R. Challenges experienced by older people during the initial months of the COVID-19 pandemic. *COVID-19 Res.* (2021) 61:48–58. doi: 10.1093/geront/gnaa138
5. Singhai K, Swami MK, Nebhinani N, Rastogi A, Jude E. Psychological adaptive difficulties and their management during COVID-19 pandemic in people with diabetes mellitus. *Diabetes Metabolic Syndr Clin Res Rev.* (2020) 14:1603–5. doi: 10.1016/j.dsx.2020.08.025
6. Tang PMK, Nikolic-Paterson DJ, Lan HY. Macrophages: versatile players in renal inflammation and fibrosis. *Nat Rev Nephrol.* (2019) 15:144–58. doi: 10.1038/s41581-019-0110-2
7. Tang PMK, Zhang YY, Hung JSC, Chung JYF, Huang XR, To KF, et al. DPP4/CD32b/NF-κB circuit: a novel druggable target for inhibiting CRP-driven diabetic nephropathy. *Mol Ther.* (2021) 29:365–75. doi: 10.1016/j.ymthe.2020.08.017
8. Tang PCT, Chan ASW, Zhang CB, Córdoba CAG, Zhang YY, To KF, et al. TGF-β1 signaling: immune dynamics of chronic kidney diseases. *Front Med.* (2021) 8:628519. doi: 10.3389/fmed.2021.628519
9. Tang PMK, Zhang YY, Xiao J, Tang PCT, Chung JYF, Li J, et al. Neural transcription factor Pou4f1 promotes renal fibrosis via macrophage–myofibroblast transition. *Proc Natl Acad Sci USA.* (2020) 117:20741–52. doi: 10.1073/pnas.1917663117
10. Tang PCT, Zhang YY, Chan MKK, Lam WWY, Chung JYF, Kang W, et al. The emerging role of innate immunity in chronic kidney diseases. *Int J Mol Sci.* (2020) 21:4018. doi: 10.3390/ijms21114018
11. Martel AL, Abolmaesumi P, Stoyanov D, Mateus D, Zuluaga MA, Zhou SK, et al. Medical image computing and computer assisted intervention – MICCAI 2020: 23rd International Conference, Lima, Peru, October 4–8, 2020. In: *Proceedings, Part IV. Lecture Notes in Computer Science 12264.* (2020). doi: 10.1007/978-3-030-59713-9
12. Xiong J, Lipsitz O, Nasri F, Lui LM, Gill H, Phan L, et al. Impact of COVID-19 pandemic on mental health in the general population: a systematic review. *J Affect Disord.* (2020) 277:55–64. doi: 10.1016/j.jad.2020.08.001
13. Yamada T, Mikami T, Chopra N, Miyashita H, Chernyavsky S, Miyashita S. Patients with chronic kidney disease have a poorer prognosis of coronavirus disease 2019 (COVID-19): an experience in New York City. *Int Urol Nephrol.* (2020) 52:1405–6. doi: 10.1007/s11255-020-02494-y
14. Bostan S, Erdem R, Öztürk YE, Kiliç T, Yilmaz A. The effect of COVID-19 pandemic on the Turkish society. *Electronic J General Med.* (2020) 17:em237. doi: 10.29333/ejgm/7944
15. Moreno C, Wykes T, Galderisi S, Nordentoft M, Crossley N, Jones N, et al. How mental health care should change as a consequence of the COVID-19 pandemic. *Lancet Psychiatry.* (2020) 7:813–24. doi: 10.1016/S2215-0366(20)30307-2
16. Strang P, Bergstrom J, Martinsson L, Lundstrom S. Dying from COVID-19: loneliness, end-of-life discussions, and support for patients and their families in nursing homes and hospitals. A national register study. *J Pain Symptom Manage.* (2020) 60:e2–13. doi: 10.1016/j.jpainsymman.2020.07.020
17. Simoes e Silva AC, Miranda AS, Rocha NP, Teixeira AL. Neuropsychiatric disorders in chronic kidney disease. *Front Pharmacol.* (2019) 10:932. doi: 10.1055/b-006-161146
18. Rigoli F. The link between coronavirus, anxiety, and religious beliefs in the United States and United Kingdom. *PsyArXiv [Preprint].* (2020). doi: 10.31234/osf.io/wykeq

19. Singu S, Acharya A, Challagundla K, Byrareddy SN. Impact of social determinants of health on the emerging COVID-19 pandemic in the United States. *Front Public Health.* (2020) 8:406. doi: 10.3389/fpubh.2020.00406
20. Elbeddini A, Wen CX, Tayefehchamani Y, To A. Mental health issues impacting pharmacists during COVID-19. *J Pharmaceutical Policy Prac.* (2020) 13:1–6. doi: 10.1186/s40545-020-00252-0
21. Chew QH, Wei KC, Vasoo S, Chua HC, Sim K. Narrative synthesis of psychological and coping responses towards emerging infectious disease outbreaks in the general population: practical considerations for the COVID-19 pandemic. *Singapore Med J.* (2020) 61:350–6. doi: 10.11622/smedj.2020046
22. Chudasama YV, Gillies CL, Zaccardi F, Coles B, Davies MJ, Seidu S, et al. Impact of COVID-19 on routine care for chronic diseases: a global survey of views from healthcare professionals. *Diabetes Metabolic Syndr Clin Res Rev.* (2020) 14:965–7. doi: 10.1016/j.dsx.2020.06.042
23. Ballivian J, Alcaide ML, Cecchini D, Jones DL, Abbamonte JM, Cassetti I. Impact of COVID−19-related stress and lockdown on mental health among people living with HIV in Argentina. *J Acquired Immune Deficiency Syndr.* (2020) 85:475–82. doi: 10.1097/QAI.0000000000002493
24. Dickerson J, Kelly B, Lockyer B, Bridges S, Cartwright C, Willan K, et al. Experiences of lockdown during the Covid-19 pandemic: descriptive findings from a survey of families in the Born in Bradford study. *Wellcome Open Res.* (2020) 5:228. doi: 10.12688/wellcomeopenres.16317.1
25. Goethals L, Barth N, Guyot J, Hupin D, Celarier T, Bongue B. Impact of home quarantine on physical activity among older adults living at home during the COVID-19 pandemic: qualitative interview study. *JMIR Aging.* (2020) 3:e19007. doi: 10.2196/19007
26. Louvardi M, Pelekasis P, Chrousos GP, Darviri C. Mental health in chronic disease patients during the COVID-19 quarantine in Greece. *Palliative Support Care.* (2020) 18:394–9. doi: 10.1017/S1478951520000528
27. Vindegaard N, Benros ME. COVID-19 pandemic and mental health consequences: systematic review of the current evidence. *Brain Behav Immun.* (2020) 89:531–42. doi: 10.1016/j.bbi.2020.05.048
28. Santini ZI, Jose PE, Cornwell EY, Koyanagi A, Nielsen L, Hinrichsen C, et al. Social disconnectedness, perceived isolation, and symptoms of depression and anxiety among older Americans (NSHAP): a longitudinal mediation analysis. *Lancet Public Health.* (2020) 5:e62–70. doi: 10.1016/S2468-2667(19)30230-0
29. Holmes EA, O'Connor RC, Perry VH, Tracey I, Wessely S, Arseneault L, et al. Multidisciplinary research priorities for the COVID-19 pandemic: a call for action for mental health science. *Lancet Psychiatry.* (2020) 7:547. doi: 10.1016/S2215-0366(20)30168-1
30. Paradise AW, Kernis MH. Self-esteem and psychological well-being: implications of fragile self-esteem. *J Soc Clin Psychol.* (2002) 21:345–61. doi: 10.1521/jscp.21.4.345.22598
31. Chang AY, Cullen MR, Harrington RA, Barry M. The impact of novel coronavirus COVID-19 on noncommunicable disease patients and health systems: a review. *J Internal Med.* (2020) 289:450–62. doi: 10.1111/joim.13184
32. Korczyn AD. Dementia in the COVID-19 period. *J Alzheimer's Dis.* (2020) 75:1071. doi: 10.3233/JAD-200609
33. Velayudhan L, Aarsland D, Ballard C. Mental health of people living with dementia in care homes during COVID-19 pandemic. *Int Psychogeriatrics.* (2020) 32:1253–4. doi: 10.1017/S1041610220001088
34. Li Y, Mutchler JE. Older adults and the economic impact of the COVID-19 pandemic. *J Aging Soc Policy.* (2020) 32:477–87. doi: 10.1080/08959420.2020.1773191
35. Nadler MB, Barry A, Murphy T, Prince R, Elliott M. Strategies to support health care providers during the COVID-19 pandemic. *CMA*192 *J.* (2020) 192:E522. doi: 10.1503/cmaj.75499
36. McGinty EE, Presskreischer R, Han H, Barry CL. Psychological distress and loneliness reported by US adults in 2018 and April 2020. *JAMA.* (2020) 324:93–4. doi: 10.1001/jama.2020.9740
37. Seyahi E, Poyraz BC, Sut N, Akdogan S, Hamuryudan V. The psychological state and changes in the routine of the patients with rheumatic diseases during the coronavirus disease (COVID-19) outbreak in Turkey: a web-based cross-sectional survey. *Rheumatol Int.* (2020) 40:1229–38. doi: 10.1007/s00296-020-04626-0
38. Keng A, Brown EE, Rostas A, Rajji TK, Pollock BG, Mulsant BH, et al. Effectively caring for individuals with behavioral and psychological symptoms of dementia during the COVID-19 pandemic. *Front Psychiatry.* (2020) 11:573367. doi: 10.3389/fpsyt.2020.573367
39. Ghinai I, McPherson TD, Hunter JC, Kirking HL, Christiansen D, Joshi K, et al. First known person-to-person transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the USA. *Lancet.* (2020) 395:1137–44. doi: 10.1016/S0140-6736(20)30607-3
40. Gavin B, Lyne J, McNicholas F. Mental health and the COVID-19 pandemic. *Irish J Psychol Med.* (2020) 37:156–8. doi: 10.1017/ipm.2020.72
41. Guan WJ, Liang WH, He JX, Zhong NS. Cardiovascular comorbidity and its impact on patients with COVID-19. *Euro Respir J.* (2020) 55:2001227. doi: 10.1183/13993003.01227-2020
42. Birditt KS, Turkelson A, Fingerman KL, Polenick CA, Oya A. Age differences in stress, life changes, and social ties during the COVID-19 pandemic: implications for psychological well-being. *Gerontologist.* (2020) 61:205–16. doi: 10.1093/geront/gnaa204
43. Addis SG, Nega AD, Miretu DG. Psychological impact of COVID-19 pandemic on chronic disease patients in Dessie town government and private hospitals, Northeast Ethiopia. *Diabetes Metabolic Syndr Clin Res Rev.* (2021) 15:129–35. doi: 10.1016/j.dsx.2020.12.019
44. Pasay-An E. Exploring the vulnerability of frontline nurses to COVID-19 and its impact on perceived stress. *J Taibah Univ Med Sci.* (2020) 15:404–9. doi: 10.1016/j.jtumed.2020.07.003
45. Lodha P, De Sousa. A. Mental health perspectives of COVID-19 and the emerging role of digital mental health and telepsychiatry. *Arch Med Health Sci.* (2020) 8:133. doi: 10.4103/amhs.amhs_82_20
46. Koenig HG. Maintaining health and well-being by putting faith into action during the COVID-19 pandemic. *J Religion Health.* (2020) 59:2205–14. doi: 10.1007/s10943-020-01035-2
47. Greenberg N, Docherty M, Gnanapragasam S, Wessely S. Managing mental health challenges faced by healthcare workers during covid-19 pandemic. *BMJ.* (2020) 368:M1211. doi: 10.1136/bmj.m1211
48. Varshney M, Parel JT, Raizada N, Sarin SK. Initial psychological impact of COVID-19 and its correlates in Indian Community: an online (FEEL-COVID) survey. *PLoS ONE.* (2020) 15:e0233874. doi: 10.1371/journal.pone.0233874
49. Barutcu Atas D, Aydin Sunbul E, Velioglu A, Tuglular S. The association between perceived stress with sleep quality, insomnia, anxiety and depression in kidney transplant recipients during Covid-19 pandemic. *PLoS ONE.* (2021) 16:e0248117. doi: 10.1371/journal.pone.0248117
50. Hernández-García I, Giménez-Júlvez T. Assessment of health information about COVID-19 prevention on the internet: infodemiological study. *JMIR Public Health Surv.* (2020) 6:e18717. doi: 10.2196/18717
51. Kobia F, Gitaka J. COVID-19: are Africa's diagnostic challenges blunting response effectiveness? *AAS Open Res.* (2020) 3:4. doi: 10.12688/aasopenres.13061.1
52. Lee J, Steel J, Roumelioti ME, Erickson S, Myaskovsky L, Yabes JG, et al. Psychosocial impact of COVID-19 pandemic on patients with end-stage kidney disease on hemodialysis. *Kidney360.* (2020) 1:1390–7. doi: 10.34067/KID.0004662020
53. Levey AS, Atkins R, Coresh J, Cohen EP, Collins AJ, Eckardt KU, et al. Chronic kidney disease as a global public health problem: approaches and initiatives – a position statement from kidney disease improving global outcomes. *Kidney Int.* (2007) 72:247–59. doi: 10.1038/sj.ki.5002343
54. World Health Organization. *Coronavirus Disease (COVID-19):* Weekly Epidemiological (2020). Available online at: https://apps.who.int/iris/bitstream/handle/10665/333905/nCoV-weekly-sitrep1-eng.pdf
55. Ko NY, Lu WH, Chen YL, Li DJ, Wang PW, Hsu ST, et al. COVID-19-related information sources and psychological well-being: an online survey study in Taiwan. *Brain Behav Immunity.* (2020) 87:153–4. doi: 10.1016/j.bbi.2020.05.019
56. AlAteeq DA, Aljhani S, Althiyabi I, Majzoub S. Mental health among healthcare providers during coronavirus disease (COVID-19) outbreak in Saudi Arabia. *J Infection Public Health.* (2020) 13:1432–7. doi: 10.1016/j.jiph.2020.08.013
57. Battisti NML, Mislang AR, Cooper L, O'Donovan A, Audisio RA, Cheung KL, et al. Adapting care for older cancer patients during the COVID-19 pandemic:

Recommendations from the International Society of Geriatric Oncology (SIOG) COVID-19 Working Group. *J Geriatric Oncol*. (2020) 11:1190–8. doi: 10.1016/j.jgo.2020.07.008
58. Sanyaolu A, Okorie C, Marinkovic A, Patidar R, Younis K, Desai P, et al. Comorbidity and its impact on patients with COVID-19. *SN Comprehens Clin Med*. (2020) 2:1069–76. doi: 10.1007/s42399-020-00363-4
59. Vik-Mo AO, Giil LM, Borda MG, Ballard C, Aarsland D. The individual course of neuropsychiatric symptoms in people with Alzheimer's and Lewy body dementia: 12-year longitudinal cohort study. *Br J Psychiatry*. (2020) 216:43–8. doi: 10.1192/bjp.2019.195
60. Selvin E, Juraschek SP. Diabetes epidemiology in the COVID-19 pandemic. *Diabetes Care*. (2020) 43:1690–4. doi: 10.2337/dc20-1295
61. Tan BY, Chew NW, Lee GK, Jing M, Goh Y, Yeo LL, et al. Psychological impact of the COVID-19 pandemic on health care workers in Singapore. *Ann Internal Med*. (2020) 173:317–20. doi: 10.7326/M20-1083
62. Caqueo-Urízar A, Urzúa A, Aragón-Caqueo D, Charles CH, El-Khatib Z, Otu A, et al. Mental health and the COVID-19 pandemic in Chile. *Psychol Trauma Theory Res Prac Policy*. (2020) 12:521–3. doi: 10.1037/tra0000753
63. Alonso-Lana S, Marquié M, Ruiz A, Boada M. Cognitive and Neuropsychiatric Manifestations of COVID-19 and Effects on Elderly Individuals With Dementia. *Front. Aging Neurosci*. (2020) 12:588872. doi: 10.3389/fnagi.2020.588872
64. Coppolino G, Presta P, Nicotera R, Placida G, Vita C, Carullo N, et al. COVID-19 and renal disease in elderly patients. *Geriatric Care*. (2020) 6. doi: 10.4081/gc.2020.9029
65. Coresh J, Astor BC, Greene T, Eknoyan G, Levey AS. Prevalence of chronic kidney disease and decreased kidney function in the adult US population. Third National Health Nutrition Examination Survey. *Am J Kidney Dis*. (2003) 41:1–2. doi: 10.1053/ajkd.2003.50007
66. U. S. Renal Data System. *Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States*. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD (2013).
67. Eckardt KU, Berns JS, Rocco MV, Kasiske BL. Definition and classification of CKD: the debate should be about patient prognosis – a position statement from KDOQI and KDIGO. *Am J Kidney Dis*. (2009) 53:915–20. doi: 10.1053/j.ajkd.2009.04.001
68. Fehrman-Ekholm I, Skeppholm L. Renal function in the elderly (>70 years old) measured by means of iohexol clearance, serum creatinine, serum urea and estimated clearance. *Scand J Urol Nephrol*. (2004) 38:73–7. doi: 10.1080/00365590310015750
69. Dowling TC, Wang ES, Ferrucci L, Sorkin JD. Glomerular filtration rate equations overestimate creatinine clearance in older individuals enrolled in the Baltimore longitudinal study on aging: impact on renal drug dosing. *Pharmacotherapy*. (2013) 33:912–21. doi: 10.1002/phar.1282
70. Koppe L, Klich A, Dubourg L, Ecochard R, Hadj-Aissa A. Performance of creatinine-based equations compared in older patients. *J Nephrol*. (2013) 26:716–23. doi: 10.5301/jn.5000297
71. Janiri D, Petracca M, Moccia L, Tricoli L, Piano C, Bove F, et al. COVID-19 pandemic and psychiatric symptoms: the impact on Parkinson's disease in the elderly. *Front Psychiatry*. (2020) 11:1306. doi: 10.3389/fpsyt.2020.581144
72. Hartmann-Boyce J, Morris E, Goyder C, Kinton J, Perring J, Nunan D, et al. Diabetes and COVID-19: risks, management, and learnings from other national disasters. *Diabetes Care*. (2020) 43:1695–703. doi: 10.2337/dc20-1192
73. Li SY, Tang YS, Chan YJ, Tarng DC. Impact of the COVID-19 pandemic on the management of patients with end-stage renal disease. *J Chin Med Assoc*. (2020) 83:628. doi: 10.1097/JCMA.0000000000000356
74. Pierce M, Hope H, Ford T, Hatch S, Hotopf M, John A, et al. Mental health before and during the COVID-19 pandemic: a longitudinal probability sample survey of the UK population. *Lancet Psychiatry*. (2020) 7:883–92. doi: 10.1016/S2215-0366(20)30308-4
75. Rapelli G, Lopez G, Donato S, Pagani AF, Parise M, Bertoni A, et al. A postcard from Italy: challenges and psychosocial resources of partners living with and without a chronic disease During COVID-19 epidemic. *Front Psychol*. (2020) 11:3559. doi: 10.3389/fpsyg.2020.567522
76. Huppert FA. Psychological well-being: evidence regarding its causes and consequences. *Appl Psychol Health Well Being*. (2009) 1:137–64. doi: 10.1111/j.1758-0854.2009.01008.x
77. Helmich RC, Bloem BR. The impact of the COVID-19 pandemic on Parkinson's disease: hidden sorrows and emerging opportunities. *J Parkinson's Dis*. (2020) 10:351. doi: 10.3233/JPD-202038
78. Bambra C, Riordan R, Ford J, Matthews F. The COVID-19 pandemic and health inequalities. *J Epidemiol Commun Health*. (2020) 74:964–8. doi: 10.1136/jech-2020-214401
79. Godinic D, Obrenovic B, Khudaykulov A. Effects of economic uncertainty on mental health in the COVID-19 pandemic context: social identity disturbance, job uncertainty and psychological well-being model. *Int J Innov Econ Dev*. (2020) 6:61–74. doi: 10.18775/ijied.1849-7551-7020.2015.61.2005
80. Bebbington PE, McManus S. Revisiting the one in four: the prevalence of psychiatric disorder in the population of England 2000–2014. *Br J Psychiatry*. (2020) 216:55–7. doi: 10.1192/bjp.2019.196
81. Curigliano G, Cardoso MJ, Poortmans P, Gentilini O, Pravettoni G, Mazzocco K, et al. Recommendations for triage, prioritization and treatment of breast cancer patients during the COVID-19 pandemic. *Breast*. (2020) 52:8–16. doi: 10.1016/j.breast.2020.04.006
82. Philip M, Mahakalka CC, Kapl MN, Kshirsagar S, Shukla A. Mental and behavioral changes during COVID 19 pandemic and how to deal with it. *J Crit Rev*. (2020) 7:1105–12. doi: 10.31838/jcr.07.08.233
83. Nouri S, Khoong EC, Lyles CR, Karliner L. Addressing equity in telemedicine for chronic disease management during the Covid-19 pandemic. *NEJM Catalyst Innov Care Deliv*. (2020) 1:1–13. doi: 10.1056/CAT.20.0123
84. Cullen W, Gulati G, Kelly BD. Mental health in the Covid-19 pandemic. *QJM Int J Med*. (2020) 113:311–2. doi: 10.1093/qjmed/hcaa110
85. Pfefferbaum B, North CS. Mental health and the Covid-19 pandemic. *New Engl J Med*. (2020) 383:510–2. doi: 10.1056/NEJMp2008017
86. Sultana A, Sharma R, Hossain MM, Bhattacharya S, Purohit N. *Burnout among healthcare providers during COVID-19 pandemic: challenges and evidence-based interventions*. SocArXiv 4hxga, Center for Open Science (2020). doi: 10.31235/osf.io/4hxga
87. Beaglehole R, Epping-Jordan J, Patel V, Chopra M, Ebrahim S, Kidd M, et al. Improving the prevention and management of chronic disease in low-income and middle-income countries: a priority for primary health care. *Lancet*. (2008) 372:940–9. doi: 10.1016/S0140-6736(08)61404-X
88. Maugeri G, Castrogiovanni P, Battaglia G, Pippi R, D'Agata V, Palma A, et al. The impact of physical activity on psychological health during Covid-19 pandemic in Italy. *Heliyon*. (2020) 6:e04315. doi: 10.1016/j.heliyon.2020.e04315
89. McGlinchey E, Hitch C, Butter S, McCaughey L, Berry E, Armour C. Understanding the lived experiences of healthcare professionals during the COVID-19 pandemic: an interpretative phenomenological analysis. *PsyArXiv [Preprint]*. (2020). doi: 10.31234/osf.io/7cvzj
90. Mirsky JB, Horn DM. Chronic disease management in the COVID-19 era. *Am J Manag Care*. (2020) 26:329–30. doi: 10.37765/ajmc.2020.43838
91. Sousa H, Ribeiro O, Costa E, Frontini R, Paúl C, Amado L, et al. Being on hemodialysis during the COVID-19 outbreak: a mixed-methods' study exploring the impacts on dialysis adequacy, analytical data, patients' experiences. *Semin Dial*. (2021) 34:66–76. doi: 10.1111/sdi.12914
92. Guo Y, Cheng C, Zeng Y, Li Y, Zhu M, Yang W, et al. Mental health disorders and associated risk factors in quarantined adults during the COVID-19 outbreak in China: cross-sectional study. *J Med Internet Res*. (2020) 22:e20328. doi: 10.2196/20328
93. Yang ZH, Pan XT, Chen Y, Wang L, Chen QX, Zhu Y, et al. Psychological profiles of Chinese patients with hemodialysis during the panic of coronavirus disease 2019. *Front Psychiatry*. (2021) 12:157. doi: 10.3389/fpsyt.2021.616016
94. Spoorthy MS, Pratapa SK, Mahant S. Mental health problems faced by healthcare workers due to the COVID-19 pandemic–a review. *Asian J Psychiatry*. (2020) 51:102119. doi: 10.1016/j.ajp.2020.102119

95. Sharma H, Verma S. Preservation of physical and mental health amid COVID-19 pandemic: recommendations from the existing evidence of disease outbreaks. *Int J Acad Med.* (2020) 6:76. doi: 10.4103/IJAM.IJAM_47_20

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Generation and Characterization of Mouse Models of C3 Glomerulonephritis With CFI D288G and P467S Mutations

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C3 glomerulopathy (C3GP) is a disease entity caused by abnormality of the complement alternative pathway (AP) and characterized by C3 deposition in glomeruli. Many variations or mutations of complement factors are believed to underlie the susceptibility to C3GP, but there is a lack of experimental evidence. We have recently reported a patient with C3 glomerulonephritis (C3GN) and compound heterozygosity of two novel variations in the complement factor (CFI). Here, we generated a mouse model to mimic the CFI variations for studying pathogenicity of CFI variations in C3GN development. We used the CRISPR/Cas9 system to make mutant mouse lines that carried D288G and P467S mutations in CFI, respectively, and crossed them to generate mice with compound heterozygosity of CFI D288G and P467S. The mice were all normal in either SPF (specific pathogen free) or regular environment. When treated with lipopolysaccharides (LPS), a bacterial endotoxin that mimics infection and sepsis, the mice developed albuminuria, kidney function impairment, and C3 glomerular deposition at levels comparable with the wild-type mice. The mice with other genotypes concerning CFI D288G and P467S were also tested in parallel. Unexpectedly, we found that the D288G homozygotes all developed severe mesangial deposition of C3 in the LPS model, indicating that CFI D288G variation was involved in the C3 deposition, a key feature of C3GN. The mouse lines generated in the present study can be used to further study the role of CFI variations in C3GN development; in addition, they may be used to screen and test infections and environmental factors capable of triggering C3GN.

Keywords: complement factor I, amino acid variations, C3 glomerulopathy, mouse model, lipopolysaccharides

INTRODUCTION

C3 glomerulopathy (C3GP) which included Dense Deposit Disease (DDD) and C3 glomerulonephritis(C3GN) depending on the location of electronic density is a rare disease characterized by predominant C3 deposition in glomeruli and membranoproliferation in glomeruli (Fakhouri et al., 2010). Dysregulation of complement alterative pathway (AP) plays an important role in the pathogenesis of C3GP (Pickering et al., 2013; Schena et al., 2020),

and the genetic variations or mutations in complement regulators have been implicated in AP dysregulation. For instance, homozygous or heterozygous mutations in the regulatory complement proteins factor H (CFH), factor I (CFI), C3, MCP (CD46), complement Factor B, and CFHRs are found in patients with C3GP (Smith et al., 2019). In addition to C3GP, AP abnormality is also implicated in thrombotic microangiopathy (TMA), including atypical hemolytic uremic syndrome (aHUS), as suggested by mutations in C3, CFI, CD46, and CFH found in patients with aHUS (Feitz et al., 2018). AP is constitutively active and serves as an immune surveillance and effector system operating in circulation and on cell surfaces. The activity of AP is tightly regulated to prevent the damage of self-cells in the body.

Although the variations/mutations in the complement regulators are believed to predispose the carriers to the C3GP and TMA, there are very few animal models that mimic the variations and prove their roles in the disease development, particularly for the single missense variations. Such animal models are important because the variations/mutations identified in the complement regulators in C3GP patients are not necessarily responsible for the diseases. First of all, the genetic defects in the complement-related genes have been identified only in a portion (∼25%) of the patients with C3GP (Bu et al., 2016; Smith et al., 2019); secondly, the functional consequences of the defects (especially those of single missense mutation) on the proteins have essentially not been tested; and thirdly, other known and unknown factors (e.g., variants of other genes, autoantibodies, etc.) may co-exist with the identified variations/mutations of complement regulators and actually interfere with the AP and cause the C3GP independently of the variations/mutations in the complement regulators (Ozaltin et al., 2013; Noris et al., 2019).

We have recently reported a case of C3GN combined with TMA in renal allograft (Wen et al., 2018). The gene test showed that this patient has two novel CFI gene variations, c.848A > G in exon 6 and c.1339C > T in exon 11, which resulted in D283G in the Ldlra domain (low-density lipoprotein receptor domain class A) and P447S in the trypsin-like serine protease domain, respectively. The c.848A > G allele was from his father and c.1339C > T from the mother. These two novel CFI variants presumably underlie the development of C3GN/MTA in the patient, which was likely triggered by a lung infection (Wen et al., 2018).

In the present study, we generated the CFI D288G and P467S compound mutations in mice that mimic the CFI variations in the patient. We found that these mice did not develop spontaneous C3GP and responded to LPS treatment similarly to wild-type mice concerning proteinuria, C3 glomerular deposition, circulating C3, and mesangial expansion. Mice with other genotypes of D288G and P467S mutations exhibited similar responses except for D288G homozygotes that had a severe C3 deposition, mesangial expansion, and reduced circulating C3, indicating that D288G affects CFI activity. These mouse models can be used for C3GN and MTA research and for screening and testing infections and environmental agents that can trigger C3GN.

MATERIALS AND METHODS

Generation of CFI D288G and P467S Mutations in Mice Using CRISPR/Cas9

To create a CFI D288G mutant model using CRISPR/Cas9-mediated gene editing, we acquired from Ensembl[1] the Cfi genome information, Cfi-001 ENSMUST00000077918, which has 14 exons, with the ATG start codon in exon 1 and TGA stop codon in exon 14 (**Supplementary Figure 1**). The Cfi gene targeting for CFI D288G and P467S was designed based on this information. To make CFI D288G point mutation in mice, two sgRNAs targeting the intron 6-7 and Exon 6 of Cfi gene were, respectively, constructed and transcribed *in vitro*. The donor vector with the Cfi-D288G fragment was designed and prepared. sgRNA sequences: 5′ sgRNA (5′–3′): ACCAATACAAGTGTAATGGTG; PAM: AGG; and 3′ sgRNA (5′–3′): ACATATGTGTGATGTGCACG; PAM: TGG. For P467S mutation, sgRNAs and donor vector were similarly prepared. For each mutation, the corresponding Cas9 mRNA, sgRNA, and donor were co-injected into mouse zygotes, and the zygotes were transferred into the oviduct of pseudopregnant ICR females at 0.5 dpc. The F0 mice were born 19∼21 days after implantation, and those with desired mutations were identified by sequencing the PCR product from mouse tail DNA. The F0 mice which had a copy of the point mutation of D288G or P467S in CFI were identified by genotyping following the method described below (**Supplementary Figure 2**). Next, the F0 mice were crossed with C57BL/6J mouse to generate heterozygotes. These two mouse lines were crossed (CFI-D288G$^{+/m}$ x CFI-P467S$^{+/m}$ to generate 1) CFI-D288G$^{m/m}$ homozygotes, 2) CFI-D288G$^{+/m}$ heterozygotes, 3) CFI-P467S$^{m/m}$ homozygotes, 4) CFI-P467S$^{+/m}$ heterozygotes, 5) CFI-D288G$^{+/m}$;P467S$^{+/m}$ compound heterozygotes (cHet) mice for further studies. The wildtype pups obtained from the crosses were used as controls. The generation of the mouse models and their breeding were conducted in the mouse facility of the Nanjing Biomedical Research Institute of Nanjing University. The treatment, sample collection, and sacrifice of the mice were performed in the mouse analysis laboratory at Jinling Hospital, Nanjing Univeristy School of Medicine.

Thermal Cycling Condition of PCR

Most PCR amplifications were performed with a regular condition: incubated at 95°C/5 min, followed by 35 cycles of 95°C/30 s, 55–60°C/30 s, 72°C/5 min for 35 cycles, and then held at 10°C. For primer 1, 3, 5, and 7 (**Supplementary Table 1**), touch-down PCR was performed with following thermal cycling condition: 95°C/5 min, followed by 20 cycles of 98°C/30 s, 65°C/30 s (−0.5°C each cycle), and 72°C/45 s, and followed by another 20 cycles of 98°C/30 s, 55°C/30 s, and 72°C/45 s for 35 cycles, and then 72°C/5 min.

LPS Treatment of Mice

LPS treatment was performed following our previous study (Lang et al., 2019). Briefly, LPS (Sigma Aldrich, MO, United States)

[1] www.ensembl.org

was dissolved in saline at a concentration of 1 mg/ml, and was injected intraperitoneally at the dose of 10 mg/kg. Twenty-four hours later, the second injection at the same dose was performed. Twelve hours after the second injection, the spot urine samples and kidney biopsies were collected from the mice for further analyses.

Measurement of Urinary Albumin/Creatinine Ratio

We measured the urinary albumin and creatinine levels using the ELISA kit, Albuwell M (Exocell, Philadelphia, United States), and the QuantiChrom^{TM} Creatinine Assay Kit (Bioassay systems, CA, United States), respectively, following the manufacturer's instructions. Urinary albuminuria levels were expressed as albumin/creatinine ratio (uACR, μg/mg).

Animal Sacrifice, Perfusion, and Kidney Tissue Collection

Mice were briefly anesthetized by inhaling Isoflurane in a chamber, followed by intraperitoneal injection of ketamine/xylazine hydrochloride solution (dose of ketamine, 100 mg/kg; xylazine, 10 mg/kg body weight). When mice were completely anesthetized, they were killed by perfusion with PBS buffer through left ventricle after blood collection from orbital sinus. Kidneys were then excised and cut into two halves, each of which was embedded in OCT compound and snap frozen with liquid nitrogen for immunofluorescence staining, and in 4% paraformaldehyde (PFA) followed by paraffin embedding for PAS staining, respectively.

PAS Staining and Pathological Scoring

We used the same method for PAS staining as described previously (Wu et al., 2015). Glomerular mesangial expansion was scored as follow: 0, no any expansion; (1) 1–10% glomeruli with mild expansion; (2) 11–25% glomeruli with moderate expansion; (3) 26–50% glomeruli with severe expansion; (4) 50% glomeruli being sclerotic. Twenty glomeruli were examined and scored for each mouse. A pathologist who was blinded to the sample identities performed the evaluation and scoring.

Immunofluorescence Staining

Sections of 5-μm thickness of mouse frozen kidney tissues were blocked with 10% FBS and incubated with primary antibodies labeled with FITC, rabbit polyclonal anti-C3, IgG, and IgA (DAKO, United States). The images were captured under the Leica microscope (DM5000B). For quantification of C3 deposition, we used ImageJ (the National Institutes of Health, United States) to determine the intensity of staining in glomeruli. For IgG and IgA, a pathologist blinded to the sample identities examined the glomeruli and scored staining intensity by 0 (no staining), 1 (weak staining), and 2 (positive staining).

Measurement of Serum C3 of the Mice

Serum C3 levels were measured using the ELISA kit for serum C3 measurement (the Cloud-clone Inc, Wuhan, China, SEA861Mu). We performed the measurement following the manual instruction.

Measurement of Serum Creatinine, BUN, and Albumin of the Mice

We performed serum creatinine, BUN and albumin measurements for the mice following the methods that we have described previously (Wu et al., 2015).

CD3 and CD68 Immunohistochemical Staining of Kidney Tissues

Immunohistochemical staining of CD3 (Novocastra, NCL-L-CD3-565) and CD68 (Dako, Anti-Human CD68 clone PG-M1 M0876) was performed on mouse kidney sections following the method described (Wu et al., 2015).

Statistical Analyses

The data are presented as the mean ± SD. The results were analyzed using GraphPad Prism 6 software (GraphPad Software Inc., CA, United States). The differences between two groups were analyzed using a two-tailed Student's *t*-test. Based on whether the assumption of normal distribution and homogeneity of variance was met or not, ANOVA or Kruskal-Wallis test was used for statistical analyses, followed by *post hoc* Dunn's test.

Study Approval

The animals and experimental procedures were approved by the Institutional Animal Care and Use Committee of Jinling Hospital, Nanjing University School of Medicine, China (2015NZGKJ-057).

RESULTS

Determination of Point Mutations to Be Made in Mouse CFI That Mimic the Patient's Variations

To map the patient's CFI variations to the mouse CFI genes, we aligned the amino acid residue sequences of human and mouse CFI (**Figure 1A**). The two amino acid residues of human CFI, D283, and P447, which were mutated in the patient, are conserved in mouse (D288, P467). The corresponding nucleotide sequence of mouse Cfi gene and the point mutations (c.848A > G and c.1339C > T) to be made according to the patient's CFI are shown in **Figure 1B**. We followed the procedure described in the Methods to generate mice of various genotypes concerning CFI-D288G and -P467S mutations.

CFI-D288G/P467S cHet Mice Were Normal

The CFI-D288G/P467S cHet mice were born with the expected Mendelian ratio in the breeding, indicating that the compound heterozygosity of CFI-D288G/P467S did not cause embryonic lethality. These mice grew normally with a body weight comparable with wild-type littermates

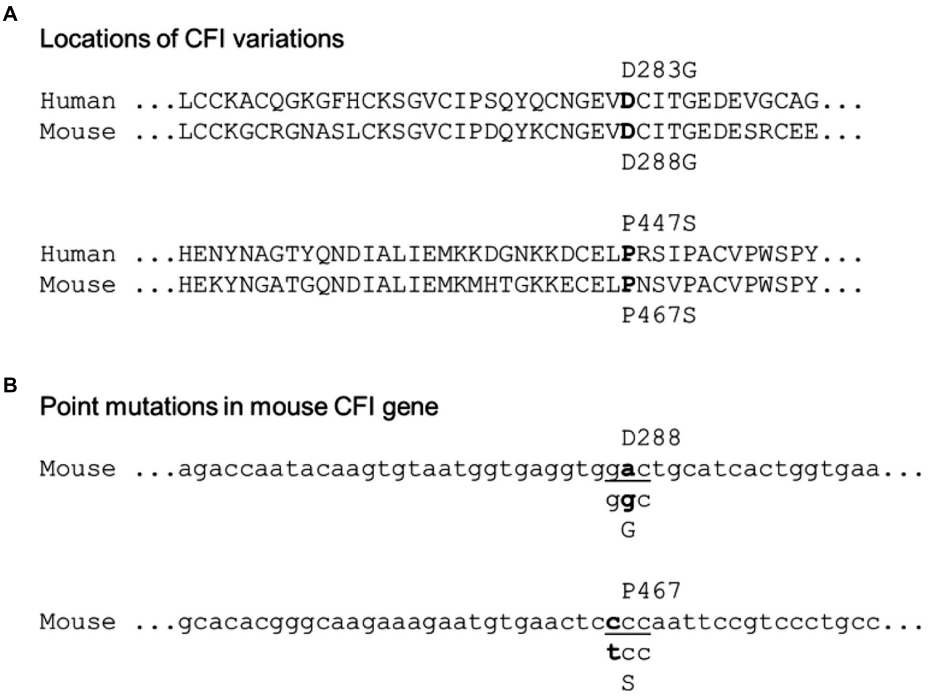

FIGURE 1 | Localization of the patient's CFI variations in mouse counterpart. **(A)** Alignment of human and mouse CFI amino acid sequences near the variations. The human D283 vs. mouse D288 and human P477 vs. mouse P467 are indicated. **(B)** Design of point mutations in CFI gene to be made in mice: CFI D288 codon "gac" to be swapped to "ggc" for glycine (G), and P467 codon "ccc" to "tcc" for serine (S).

and had no observable abnormalities, e.g., albuminuria, as determined by albumin/creatinine ratio (ACR) of spot urine, at a specific pathogen free (SPF) environment. We also left the mice in a regular, non-SPF environment, allowing certain potential pathogens in the environment to trigger C3GN in the mice. However, after exposure to the regular environment for more than a month, the mice were still normal and did not develop albuminuria (the mice were 12 weeks old at the time). The mice with other genotypes, including D288G heterozygotes, D288G homozygotes, P467S heterozygotes, and P467S homozygotes, were also normal as shown by lack of proteinuria (**Figure 2**).

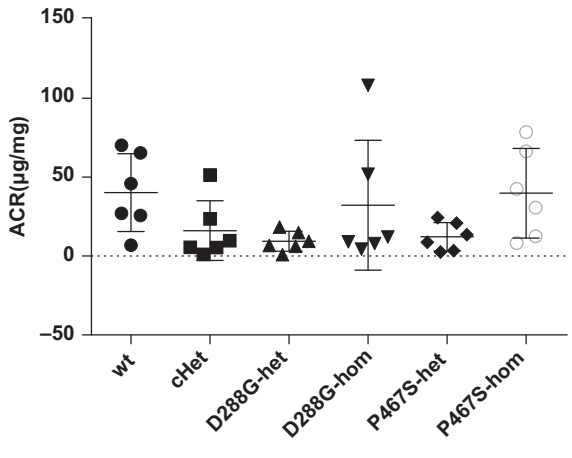

FIGURE 2 | The CFI D288G/P467S cHet mice and the mice with other genotypes did not develop proteinuria spontaneously at the age of 12 weeks. Urinary albumin/creatinine ratio (μg/mg) was measured for the mice in the groups of mice as indicated, and they all had a normal level of urinary albumin. Wt, wildtype; cHet, compound D288G/P467S heterozygotes; D288G-Het, D288G heterozygotes; D288G-Hom, D288G homozygotes; P467S-Het, P467S heterozygotes; P467S-Hom, P467S homozygotes. $n = 6$ for all groups. No statistically significant differences were observed among the groups according to the Kruskal-Wallis test ($p = 0.079$).

CFI-D288G/P467S cHet Mice Responded to LPS Treatment Similarly to Wild-Type Mice

Patients with susceptibility to C3 and TMA can be fine under a normal condition, but can develop the disease with certain stimuli from environment, e.g., infections. Unfortunately, the exact factors capable of triggering the disease are not known. We then chose and tested LPS, an agent that mimics infection and sepsis, and hoped that it would induce C3GN in the CFI-D288G/P467S cHet mice.

We found that all the mice developed proteinuria at the comparable levels (**Figure 3**). The success of the LPS model was further demonstrated by the increased serum BUN, serum creatinine and decreased serum albumin after LPS treatment (**Supplementary Figure 3**). cHet mice were expected to have a higher level of proteinuria because they mimicked the patient

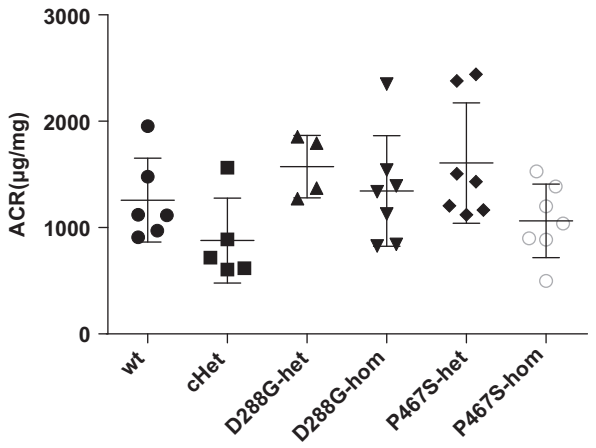

FIGURE 3 | The albuminuria of mice of CFI-D288G/P467S cHet and other genotypes after LPS treatment. Thirty-six hours after the first 10 mg/kg LPS injection, spot urine samples were collected and subjected to ACR measurement. Wt, $n = 6$; cHet, $n = 5$; D288G-Het, $n = 4$; D288G-Hom, $n = 7$; P467S-Het, $n = 7$; and P467S-Hom, $n = 7$. ANOVA analysis was performed and showed no statistically significant differences among the groups ($p = 0.071$).

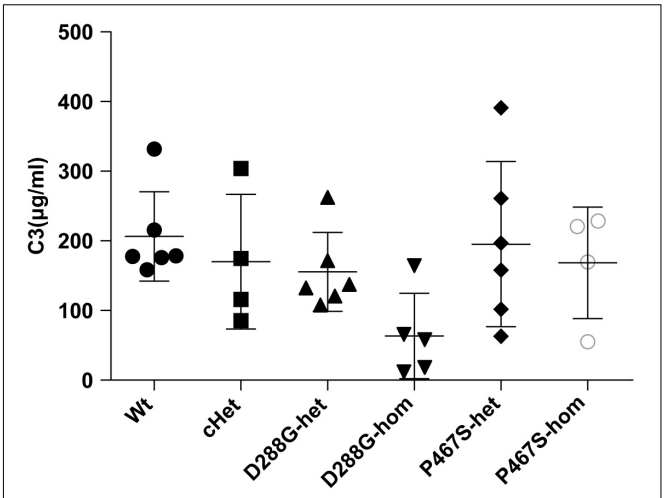

FIGURE 5 | Comparisons of the circulating C3 levels of the groups of mice with different genotypes under the treatment of LPS. The serum C3 level of the D288G homozygote group was lower than that of the other groups, but the differences did not reach any statistical significance ($p = 0.114$) according to the ANOVA test. Wt, $n = 6$; cHet, $n = 4$; D288G-het, $n = 6$; D288G-hom, $n = 5$; P467S-het, $n = 6$; P467S-hom, $n = 4$.

of C3GN. However, their proteinuria was comparable with that of wild-type and other mice. Consistently, the serum creatinine, BUN, and albumin levels of the cHet mice were also similar to wildtype and other mice (**Supplementary Figure 4**).

We performed C3 immunofluorescence staining on the kidney sections of the mice, and found that cHet mice had a weak mesangial deposition of C3 at levels similar to wild-type mice (**Figure 4**). We also measured the circulating C3 in the mice and found that cHet mice had a similar level of serum C3 to that of wildtype mice (**Figure 5**). Additionally, we performed IgG and IgA staining, and found similar levels of IgG intensity between cHet and wild-type mice (**Figure 6**). IgA staining was negative for all of them (data not shown). In the inspection of inflammatory cells in the kidney sections stained with CD3 and CD68 antibodies, we did not find overt infiltration of T cells and macrophage cells in all the groups of mice (**Supplementary Figure 5**). No red blood cells were found in the sediment of urine of all the groups (data not shown), precluding hematuria in the mice. In the pathological examination of the kidneys, no thrombotic microangiopathy was found in any mice (data not shown).

CFI-D288G Homozygotes Developed C3GN Phenotypes After LPS Treatment

In the above examinations, we noticed that CFI-D288G homozygotes had a C3 deposition in glomeruli that was much

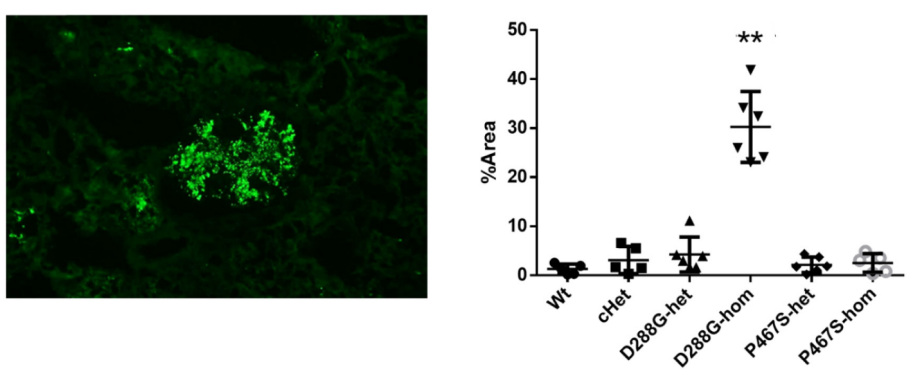

FIGURE 4 | Examination of C3 deposition in the kidneys of the mice treated by LPS. **(A)** An example of C3 staining pattern in mesangium. **(B)** Quantification and comparison of C3 staining intensity in the mice in the 6 groups. Wt, $n = 5$; cHet, $n = 5$; D288G-het, $n = 6$; D288G-hom, $n = 6$; P467S-het, $n = 6$; P467S-hom, $n = 5$. $^{**}p = 0.0012$ vs. wt according to the Kruskal-Wallis test with Dunn's multiple comparisons.

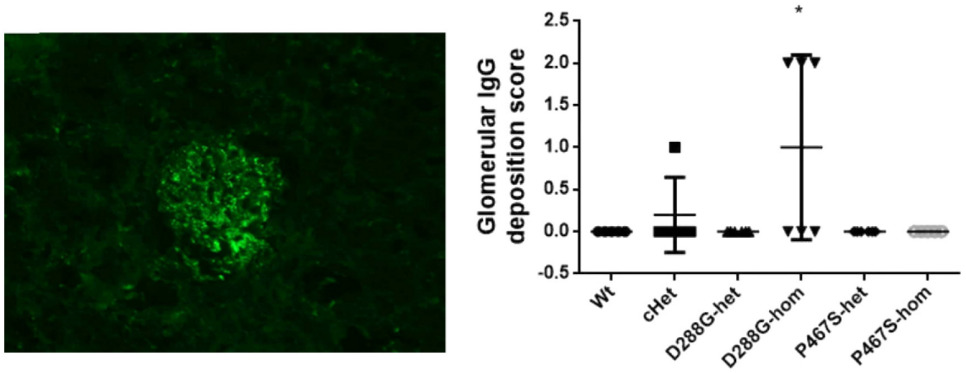

FIGURE 6 | Examination of IgG deposition in the kidneys of the mice treated by LPS. An example of IgG staining pattern in a glomerulus (left), and the quantification and comparison of IgG intensity in the mice in the 6 groups. Wt, $n = 5$; cHet, $n = 5$; D288G-het, $n = 6$; D288G-hom, $n = 6$; P467S-het, $n = 6$; P467S-hom, $n = 5$. *$p = 0.052$ vs. wt according to the Kruskal-Wallis test.

more severe than that of mice in the other groups in the LPS model. As shown in **Figure 4**, we performed semi-quantitative analysis of the staining intensity of each mouse using ImageJ and compared the results across the groups. We found that D288G homozygotes had a much higher intensity of C3 in glomeruli than the other mice ($p = 0.0012$ vs. wt, **Figure 4**). In the IgG staining, half of the D288G homozygotes were positive while the other half were negative. IgG staining was negative in all the mice in the other groups except that one mouse in the cHET group showed weak positive staining (**Figure 5**). C3 and IgG staining in tubules and blood vessels was minimal. In the serum C3 measurement, there was no difference between the groups ($p = 0.114$, **Figure 6**).

In the PAS staining of kidney sections, mild mesangial expansion was noted in some mice in each group (**Figures 7A,B**), however, there was no statistical difference in severity of mesangial expansion across the groups of mice (**Figure 7C**).

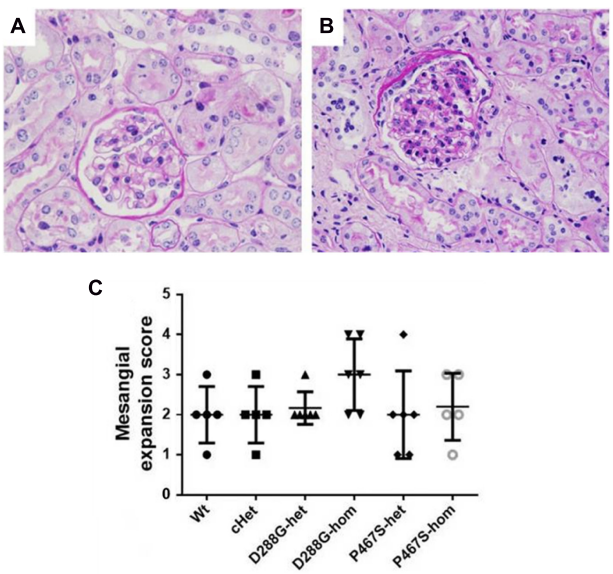

FIGURE 7 | Mesangial expansion in the mice of CFI-D288G/P467S cHet and other genotypes after LPS treatment. **(A,B)** PAS staining showing normal glomeruli in mouse kidney **(A)** and a glomerulus with mesangial expansion **(B)** (x600). **(C)** Quantification of mesangial expansion in the 6 groups of mice. Wt, $n = 5$; cHet, $n = 5$; D288G-het, $n = 6$; D288G-hom, $n = 6$; P467S-het, $n = 6$; P467S-hom, $n = 5$. Kruskal-Wallis test was used for statistical analysis, showing no significance among groups ($p = 0.307$), although the D288G homozygote group tended to have a higher score than the other groups.

DISCUSSION

In the present study, we generated the first animal models of human CFI variants, and found that D288G and P467S compound heterozygous mice that mimicked the CFI variations in a previously reported patient did not develop spontaneous C3GN. With LPS treatment, the D288G/P467S cHet mice developed proteinuria, mesangial expansion and C3 deposition at levels similar to the mice of wild-type. Unexpectedly, the D288G homozygotes all had much more severe mesangial C3 deposition compared with the mice in the other groups. It is possible that with proper treatment, the D288G/P467S cHet mouse model of the patient's CFI variations would also be induced to develop C3GN or MTA. Therefore, these mouse models can be used for further study of the role of the CFI variations in C3GN and MTA development. In addition, we propose that these mouse lines could be used as a tool for screening of infections and environmental agents that are capable of triggering C3GN and TMA in individuals with CFI variations, and the feasibility of such screening has been clearly demonstrated with the model of LPS which successfully induced C3GN phenotypes in the D288G homozygotes mice. The environmental agents can be various drugs, chemicals, pollutants and even food.

At present, a number of variations in CFI have been reported in patients with C3GP and aHUS (Fremeaux-Bacchi et al., 2004; Caprioli et al., 2006). Pathogenic variations of CFI are localized at several conserved domains, including the "low-density lipoprotein receptor domain class A" (Ldlra) and "the trypsin-like serine protease domain" (Tryp) (Servais et al., 2007;

Leroy et al., 2011) (HGMD database)[2]. The two novel CFI variations (D283G and P447S) in the patient that we reported previously (Wen et al., 2018) are also localized to these two domains, respectively, and thus likely underlie the pathogenesis of C3GP and TMA in the patient. We therefore expected that the mice with the compound heterozygosity of D288G and P467S (cHet) as in the patient would develop C3GN and/or TMA. However, the cHet mice did not differ from the mice of wild-type and other genotypes essentially under normal conditions and LPS treatment.

Although we did not observe glomerular C3 deposition in the D288G/P467S cHet mice under the particular experimental conditions in this study, the potential of compound and heterozygous D288G and P467S variations to cause glomerular C3 deposition cannot be precluded. There might be several reasons for the failure of the cHet mice to develop glomerular C3 deposition in the LPS treatment. First of all, modeling complement variations in animals may not be easy if additional factors, which are absent in mice, are required for the onset of disease in patients. For instance, in humans, even the nonsense mutations that lead to CFI deficiency do not fully penetrate, leaving a portion of individuals with the mutations free of the disease (Fremeaux-Bacchi et al., 2004). In fact, most genetic variants have a low penetrance and require additional variations/mutations or other factors, e.g., thrombomodulin (Schena et al., 2020), to cause a disease. Secondly, mice may tolerate CFI deficiency better than humans due to certain differences of the complement system between mice and humans (Pouw et al., 2015). For example, CFI deficiency can cause C3GN in humans (Servais et al., 2007), whereas CFI knockout does not cause spontaneous glomerulonephritis and proteinuria, but does result in mesangial C3 deposition and expansion in a portion of the knockout mice (Rose et al., 2008). Thirdly, an appropriate trigger of the disease might be critical for the onset of the disease for a given variation. LPS may not best mimic the lung infection that caused C3GP in the patient that we reported previously (Wen et al., 2018), thus requiring the right pathogens to be identified and used to treat the cHet mice for glomerular C3 deposition. Unfortunately, we did not have the information of how the infection occurred to the patient.

Interestingly, in contrast with the cHet and other genotypes, D288G homozygotes developed clear and severe C3 deposition in mesangium in the treatment of LPS. This resembles the CFI knockout mice without LPS treatment (Rose et al., 2008), suggesting that D288G homozygosity and LPS acted in concert to alter complement alternative pathway, resulting in elevation of complement activity. Mutations in the Ldlra domain of Cfi have been found in patients with C3GP (Servais et al., 2007; Leroy et al., 2011), suggesting that the homozygous D288G mutation in the mice could impair CFI activity and be responsible for the increased severity of glomerular lesions in the D288G homozygote mice in LPS treatment. On the other hand, the D288G homozygotes did not exhibit a more severe proteinuria than other mice after LPS treatment. It is known that LPS can directly act on podocytes as a ligand for TLR4, resulting in podocyte cytoskeletal injury and massive proteinuria in mice, and this direct proteinuria-inducing effect of LPS should be stronger than that of C3GP triggered by LPS, causing the D288G homozygote group to have similar proteinuria as that in the other groups.

Glomerular C3 deposition occurred in the D288G homozygotes but not the D288G/P467S cHet mice. We speculate that the D288G mutation may cause a greater loss of CFI activity than P467S mutation although further experiments are required to prove it. It is possible that D288G homozygosity, but not D288G/P467S cHet, reaches a threshold of CFI activity loss, which leads to C3GN development. Thus, the homozygous D288G mice could be a more sensitive model than D288G/P467S cHet and other genotypes in detecting C3GN-inducing factors. However, the cHet mice may have the potential to detect C3GN inducers as well. Further studies are required toward this direction.

Our present study has suggested that the CFI variations were responsible for the C3GN on the renal allograft of our patient. According to our study, we consider that he could receive a second renal transplantation. His transplanted kidney could be safe as long as he avoids infections and exposure to C3GN-inducing factors. If an infection or exposure does occur, an immediate and most effective treatment should be followed to minimize the activation of the C3 system to avoid damage to his renal allograft.

One limitation of the present study was the relatively low number of mice of each genotype that were employed for the studies. A larger number of mice for each group with different genotypes would reveal clearer and more convincing results in the study. Another limitation is that the time period for observing the spontaneous development of C3GN in the mice was not long enough. Spontaneous C3GN might be observed in old mice.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by the Institutional Animal Care and Use Committee of Jinling Hospital, Nanjing University School of Medicine, China (2015NZGKJ-057).

AUTHOR CONTRIBUTIONS

JW conceived the study. JW, HS, MZ, JZ, and SS designed the experiments. HS, MZ, JW, DZ, XZ, FX, and WQ performed the

[2]http://www.hgmd.cf.ac.uk/

experiments. SS, JW, HS, MZ, and XL interpreted data and wrote the manuscript. All authors have reviewed and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.649801/full#supplementary-material

REFERENCES

Bu, F., Borsa, N. G., Jones, M. B., Takanami, E., Nishimura, C., Hauer, J. J., et al. (2016). High-Throughput genetic testing for thrombotic microangiopathies and C3 glomerulopathies. *J. Am. Soc. Nephrol. JASN* 27, 1245–1253. doi: 10.1681/asn.2015040385

Caprioli, J., Noris, M., Brioschi, S., Pianetti, G., Castelletti, F., Bettinaglio, P., et al. (2006). Genetics of HUS: the impact of MCP, CFH, and IF mutations on clinical presentation, response to treatment, and outcome. *Blood* 108, 1267–1279. doi: 10.1182/blood-2005-10-007252

Fakhouri, F., Fremeaux-Bacchi, V., Noel, L. H., Cook, H. T., and Pickering, M. C. (2010). C3 glomerulopathy: a new classification. *Nat. Rev. Nephrol.* 6, 494–499. doi: 10.1038/nrneph.2010.85

Feitz, W. J. C., van de Kar, N., Orth-Holler, D., van den Heuvel, L. P. J. W., and Licht, C. (2018). The genetics of atypical hemolytic uremic syndrome. *Medizinische Genetik* 30, 400–409.

Fremeaux-Bacchi, V., Dragon-Durey, M. A., Blouin, J., Vigneau, C., Kuypers, D., Boudailliez, B., et al. (2004). Complement factor I: a susceptibility gene for atypical haemolytic uraemic syndrome. *J. Med. Genet.* 41:e84. doi: 10.1136/jmg.2004.019083

Lang, Y., Zhao, Y., Zheng, C., Lu, Y., Wu, J., Zhu, X., et al. (2019). MiR-30 family prevents uPAR-ITGB3 signaling activation through calcineurin-NFATC pathway to protect podocytes. *Cell Death Dis.* 10:401.

Leroy, V., Fremeaux-Bacchi, V., Peuchmaur, M., Baudouin, V., Deschênes, G., Macher, M. A., et al. (2011). Membranoproliferative glomerulonephritis with C3NeF and genetic complement dysregulation. *Pediatric Nephrol.* 26, 419–424. doi: 10.1007/s00467-010-1734-4

Noris, M., Donadelli, R., and Remuzzi, G. (2019). Autoimmune abnormalities of the alternative complement pathway in membranoproliferative glomerulonephritis and C3 glomerulopathy. *Pediatric Nephrol.* 34, 1311–1323. doi: 10.1007/s00467-018-3989-0

Ozaltin, F., Li, B., Rauhauser, A., An, S. W., Soylemezoglu, O., Gonul, I. I., et al. (2013). DGKE variants cause a glomerular microangiopathy that mimics membranoproliferative GN. *J. Am. Soc. Nephrol. JASN* 24, 377–384. doi: 10.1681/asn.2012090903

Pickering, M. C., D'Agati, V. D., Nester, C. M., Smith, R. J., Haas, M., Appel, G. B., et al. (2013). C3 glomerulopathy: consensus report. *Kidney Int.* 84, 1079–1089.

Pouw, R. B., Vredevoogd, D. W., Kuijpers, T. W., Wouters, D., et al. (2015). Of mice and men: the factor H protein family and complement regulation. *Mol. Immunol.* 67, 12–20. doi: 10.1016/j.molimm.2015.03.011

Rose, K. L., Paixao-Cavalcante, D., Fish, J., Manderson, A. P., Malik, T. H., Bygrave, A. E., et al. (2008). Factor I is required for the development of membranoproliferative glomerulonephritis in factor H-deficient mice. *J. Clin. Invest.* 118, 608–618.

Schena, F. P., Esposito, P., and Rossini, M. (2020). A narrative review on C3 glomerulopathy: a rare renal disease. *Int. J. Mol. Sci.* 21:525. doi: 10.3390/ijms21020525

Servais, A., Fremeaux-Bacchi, V., Lequintrec, M., Salomon, R., Blouin, J., Knebelmann, B., et al. (2007). Primary glomerulonephritis with isolated C3 deposits: a new entity which shares common genetic risk factors with haemolytic uraemic syndrome. *J. Med. Genet.* 44, 193–199. doi: 10.1136/jmg.2006.045328

Smith, R. J. H., Appel, G. B., Blom, A. M., Cook, H. T., D'Agati, V. D., Fakhouri, F., et al. (2019). C3 glomerulopathy - understanding a rare complement-driven renal disease. *Nat. Rev. Nephrol.* 15, 129–143. doi: 10.1038/s41581-018-0107-2

Wen, J., Wang, W., Xu, F., Sun, J., Chen, J., and Ni, X. (2018). C3 glomerulonephritis and thrombotic microangiopathy of renal allograft after pulmonary infection in a male with concomitant two complement factor I gene variations: a case report. *BMC Nephrol.* 19:148. doi: 10.1186/s12882-018-0952-z

Wu, J., Zheng, C., Wang, X., Yun, S., Zhao, Y., Liu, L., et al. (2015). MicroRNA-30 family members regulate calcium/calcineurin signaling in podocytes. *J. Clin. Invest.* 125, 4091–4106. doi: 10.1172/jci81061

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frontiers in Physiology

ORIGINAL RESEARCH
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Renal Inflammation and Innate Immune Activation Underlie the Transition From Gentamicin-Induced Acute Kidney Injury to Renal Fibrosis

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Subjects recovering from acute kidney injury (AKI) are at risk of developing chronic kidney disease (CKD). The mechanisms underlying this transition are unclear and may involve sustained activation of renal innate immunity, with resulting renal inflammation and fibrosis. We investigated whether the NF-κB system and/or the NLRP3 inflammasome pathway remain activated after the resolution of AKI induced by gentamicin (GT) treatment, thus favoring the development of CKD. Male Munich-Wistar rats received daily subcutaneous injections of GT, 80 mg/kg, for 9 days. Control rats received vehicle only (NC). Rats were studied at 1, 30, and 180 days after GT treatment was ceased. On Day 1, glomerular ischemia (ISCH), tubular necrosis, albuminuria, creatinine retention, and tubular dysfunction were noted, in association with prominent renal infiltration by macrophages and myofibroblasts, along with increased renal abundance of TLR4, IL-6, and IL1β. Regression of functional and structural changes occurred on Day 30. However, the renal content of IL-1β was still elevated at this time, while the local renin-angiotensin system remained activated, and interstitial fibrosis became evident. On Day 180, recurring albuminuria and mild glomerulosclerosis were seen, along with ISCH and unabated interstitial fibrosis, whereas macrophage infiltration was still evident. GT-induced AKI activates innate immunity and promotes renal inflammation. Persistence of these abnormalities provides a plausible explanation for the transition of AKI to CKD observed in a growing number of patients.

Keywords: acute kidney injury, chronic kidney disease, gentamicin, innate immunity, NF-κB

INTRODUCTION

Recent evidence indicates that, after recovering from widespread renal cellular necrosis and inflammation, a substantial fraction of patients who overcome acute kidney injury (AKI) develop an insidious process of renal interstitial collagen accumulation that eventuates in renal fibrosis (RF) and chronic kidney disease (CKD). The mechanisms underlying this

transition from AKI to CKD are incompletely understood, and likely involve a complex interplay between renal infiltration by inflammatory cells and activation of innate immunity, adaptive immunity, and the local renal-angiotensin system (Hoste and Kellum, 2007; Chawla, 2011; Black et al., 2018; Sato and Yanagita, 2018).

Inflammatory phenomena are key to the development of kidney damage in both ischemic and nephrotoxic insults, the two most common causes of AKI. Early infiltration by inflammatory cells, along with increased expression of proinflammatory cytokines and adhesion molecules, has been reported in experimental AKI (Han and Bonventre, 2004; Kurts et al., 2013; Jang and Rabb, 2015; Li et al., 2018; Sato and Yanagita, 2018). Accordingly, AKI can be prevented by macrophage or lymphocyte depletion, as well as by cytokine inhibition (Han and Bonventre, 2004; Gonçalves et al., 2011; Zhang et al., 2013; Li et al., 2018). Inflammatory events also play a central role in the pathogenesis of RF and CKD, as shown with models as diverse as 5/6 renal ablation (Nx; Floege et al., 1992; Eddy, 1996; Fanelli et al., 2017; Foresto-Neto et al., 2018), diabetic nephropathy (Mezzano et al., 2004; Foresto-Neto et al., 2020), adriamycin nephropathy (Faustino et al., 2018), and unilateral ureteral obstruction (Esteban et al., 2004; Vilaysane et al., 2010), as well as in the AKI-CKD transition (Venkatachalam et al., 2015; Black et al., 2018; Yang, 2019).

In recent years, the role of innate immunity, particularly the NF-κB and NLRP3 inflammasome pathways, in the inflammatory process associated with AKI has become apparent (Iyer et al., 2009; Jang and Rabb, 2015; Li et al., 2018). Likewise, the prominent pathogenic role of innate immunity in CKD has been evidenced in a variety of experimental models (Jo et al., 2006; Fujihara et al., 2007; Iyer et al., 2009; Castoldi et al., 2012; Kim et al., 2013; Fanelli et al., 2017; Faustino et al., 2018; Zambom et al., 2019; Foresto-Neto et al., 2020).

In principle, activation of innate immunity by membrane debris and biomolecules released after renal ischemia/reperfusion (I/R) or toxic cell damage should be self-limited and cease once tubular regeneration is completed. However, if innate immunity activation persists, the resulting inflammation could lead to continuing renal damage. We hypothesized that, once set in motion in the context of AKI, the NF-κB system and/or the NLRP3 inflammasome pathway remain activated even after the acute event is resolved, thus sustaining an insidious process of renal inflammation that eventuates in RF, completing a complex transition to CKD.

To avoid the confounding effects of I/R-associated hypoxia (Nizet and Johnson, 2009; Mishra et al., 2015), thus focusing on the impact of cell injury on innate immunity, we utilized the model of nephrotoxic AKI by gentamicin (GT). Cytotoxicity caused by GT was shown to be associated with intense renal inflammation and NF-κB activation, along with residual renal accumulation of collagen 30 days after GT treatment (Nakajima et al., 1994; Geleilete et al., 2002; Volpini et al., 2004). However, whether GT-induced activation of renal innate immunity, inflammation, and fibrosis persists and progresses to CKD after longer periods of time has not been verified.

MATERIALS AND METHODS

Experimental Protocol

All experimental procedures were approved by the Research Ethics Committee of the Faculty of Medicine of University of São Paulo (CEP-FMUSP, process no. 057/16). All experiments were performed in strict conformity with institutional guidelines and with international standards for manipulation and care of laboratory animals.

Sixty-three adult male Munich-Wistar rats, weighing 250–280 g, were obtained from a local facility at the Faculty of Medicine, University of São Paulo. The animals were maintained at 22 ± 1°C and 60 ± 5% relative air humidity, under an artificial 12:12 h light-dark cycle. Rats were fed regular chow containing 22% protein (Nuvital, Curitiba, Brazil) and *ad libitum* water.

The rats were divided into two groups: NC (N = 31), rats receiving subcutaneous injection of 0,15 M saline solution and GT (N = 32), rats receiving daily subcutaneous injections of GT (Gentatec-Chemitec, São Paulo, Brazil), 80 mg/kg/day, during 9 days. This protocol of GT administration standardized previously (Nakajima et al., 1994; Geleilete et al., 2002; Volpini et al., 2004) was calibrated to ensure that a toxic but nonlethal amount of GT reaches the kidneys, thus promoting tubular cell necrosis and AKI. Pretreatment body weights (267 ± 5 in NC and 265 ± 2 in GT) and albuminuria (1.6 ± 0.1 in NC and 1.5 ± 0.1 in GT) were similar between the two groups. NC and GT rats were studied 1 day (Day 1, 10 NC and 10 GT rats), 30 days (Day 30, 10 NC and 10 GT rats), and 180 days (Day 180, 11 NC and 12 GT rats) after these 9-day treatments were ceased. At each time point, body weight (BW) and systolic blood pressure were determined using an automated optoelectronic device (BP 2000 Blood Pressure Analysis System, Visitech Systems, EUA). All rats were preconditioned to remain calm during the procedure. In addition, animals were kept for 24 h in metabolic cages for urine collection for measurement of albumin and creatinine excretion.

On Days 1, 30, and 180, rats were anesthetized with ketamine (50 mg/kg im.) and xylazine (10 mg/kg im.). Blood samples were taken from the abdominal aorta for measurement of serum creatinine and plasma sodium and potassium concentrations. The kidneys were retrogradely perfused *in situ* through the abdominal aorta with cold saline to remove blood from renal vessels. The right kidney was excised, instantly frozen in liquid nitrogen, and stored at −80°C for subsequent analysis. The left kidney was retrogradely perfused *in situ* with Duboscq-Brazil solution for fixation. The renal tissue was then weighed, cut in two mid-coronal slices, post-fixed in buffered 10% formaldehyde solution, and embedded in paraffin, using standard sequential techniques, for histomorphometric and immunohistochemical analysis, performed in 4-μm-thick sections. Rats were killed instantly by the kidney perfusion-fixation procedure.

Biochemical and Enzymatic Analysis

Urinary albumin was determined by radial immunodiffusion (Mancini et al., 1965) using a polyclonal rabbit anti-albumin

antibody (#0855715, MP Biomedicals LLC, United States). Serum and urine creatinine concentrations were measured by a colorimetric assay kit (Labtest Diagnostic, Sao Paulo, Brazil). The urine albumin/creatinine ratio was expressed in mg/mg.

Histomorphometric Analysis

The morphometric evaluations were performed in a blinded manner by a single observer. The extent of glomerular injury was estimated by determining the percentage of glomeruli with either ischemic (ISCH) or sclerotic lesions (GS) in sections stained by the Periodic acid-Schiff reaction (Fujihara et al., 1994). Glomerular ischemia was defined as a collapse of the glomerular tuft, with decrease in the tuft volume, closure of the capillary loops, wrinkling of the basement membrane, and enlargement of the Bowman's space. GS was characterized by deposition of hyaline material in a segment of the glomerular tuft, with consequent occlusion of capillary loops.

Immunohistochemical Analysis

Renal slices were mounted on glass slides coated with 6% silane. The following primary antibodies were employed as: monoclonal mouse anti-ED-1 (#MCA341R, Serotec, Oxford, United Kingdom) for macrophages, polyclonal rabbit anti-Mannose receptor (CD206, #Ab64693, Abcam, Cambridge, United Kingdom) for M2 macrophages (anti-inflammatory phenotype), monoclonal mouse anti-α-smooth muscle actin (α-SMA; #A2547, Sigma-Aldrich, Saint Louis, MO), polyclonal rabbit anti-collagen type 1 (#34710, Abcam, Cambridge, United Kingdom), polyclonal rabbit anti-fibronectin (#Ab2413, Abcam, Cambridge, United Kingdom), and polyclonal rabbit anti-angiotensin II (AngII; #T4007, Peninsula Laboratories, San Carlos, CA) for AngII-positive cells. The immunohistochemical techniques used in this study were described in detail in the previous studies (Arias et al., 2013; Foresto-Neto et al., 2018; Zambom et al., 2019). The interstitial density of macrophages and AngII-positive cells was evaluated in a blinded manner at ×400 magnification. For each section, 25 microscopic fields (corresponding to a total area of 0.08 mm^2) were examined. Results were expressed in cells/mm^2. The percentage of cortical interstitial area occupied by collagen-1 and fibronectin was estimated by a point-counting technique (Jepsen and Mortensen, 1979).

Total Protein Extraction

Kidney proteins were extracted using lysis buffer (#89900, Thermo Scientific, Rockford, IL) with protease and phosphatase inhibitor (Roche, Mannheim, Germany). Protein concentration was determined with the bicinchoninic acid method, using homogenate aliquots containing 100 μg of protein.

Western Blot Assays

A 100 μg aliquot of renal homogenate was mixed in 2 × Laemmli buffer and denatured at 96°C for 5 min. For the specific nuclear fraction analysis, the pre-prepared samples were not denatured. Protein separation was performed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. For each blot, samples from two rats per group were loaded so that the intensity of each band (factored by its respective housekeeping band) could always be reliably compared among groups. Seven rats per group per time point were analyzed in this way. The separated proteins were transferred to a nitrocellulose membrane, which was incubated with 5% non-fat milk or 5% BSA in tris-buffered saline for 2 h at room temperature to block nonspecific binding. The membrane was then incubated overnight at 4°C with primary antibodies for: monoclonal mouse anti-β-actin, 1:5,000 (#A2228, Sigma-Aldrich, Saint Louis, MO); polyclonal rabbit anti-TLR4 (#Sc30002, Santa Cruz Biotechnology, Dallas, TX); monoclonal mouse anti-caspase-1 (casp-1), 1:1,000 (#Sc56036, Santa Cruz Biotechnology, Dallas, TX); monoclonal mouse anti-interleukin 6 (IL-6), 1:1,000 (#Ab9324, Abcam, Cambridge, United Kingdom); and monoclonal mouse anti-αSMA, 1:1,000 (#A2547, Sigma-Aldrich, Saint Louis, MO). After rinsing with Tris-buffered saline Tween 20 buffer, membranes were incubated with secondary antibodies labeled with HRP. Immunostained bands were detected using a chemiluminescence kit (Thermo Scientific, Rockford, United States) and were further analyzed by densitometry with a gel documentation system and the Uvisoft-Uviband Max software (Uvitec Cambridge, Cambridge, United Kingdom).

ELISA Analysis

The renal contents of KIM-1 and IL-1β were determined using a commercial ELISA kit (R&D Systems, Minneapolis, MN). The analyses were performed following rigorously the manufacturer's instructions.

Statistical Analysis

Statistical differences among groups were assessed by one-way ANOVA, with pairwise post-test comparisons according to the Tukey's method (Wallenstein et al., 1980). The differences were considered significant at $p < 0.05$. Results were expressed as means ± *SE*. All calculations were performed using the GraphPad Prism 6.01 software.

RESULTS

Although body weight was not significantly changed by GT on Day 1 (258 ± 3 g vs. 262 ± 8 g in NC, $p > 0.05$), growth was limited on Day 30 (281 ± 6 g vs. 298 ± 10 g, respectively, $p < 0.05$). On Day 180, body growth was similar between groups. Whereas group GT remained normotensive on Day 1 and Day 30, BP was modestly but significantly ($p < 0.05$) elevated on Day 180 compared to Day 1 (**Figure 1A**). GT rats developed a marked increase in the albumin excretion rate on Day 1 (21.4 ± 3.0 mg/24 h vs. 3.5 ± 0.6 in control, $p < 0.05$). Albuminuria regressed on Day 30 (2.9 ± 0.6 mg/24 h vs. 2.3 ± 0.4 in control, $p > 0.05$), but returned on Day 180 (25.0 ± 5.5 mg/24 h vs. 7.5 ± 2.1 in control, $p < 0.05$). Parallel changes were noted in the albumin/creatinine ratio (**Figure 1B**). Serum creatinine was prominently elevated on Day 1, returned to control on Day 30, and remained at normal levels on Day 180 (**Figure 1C**).

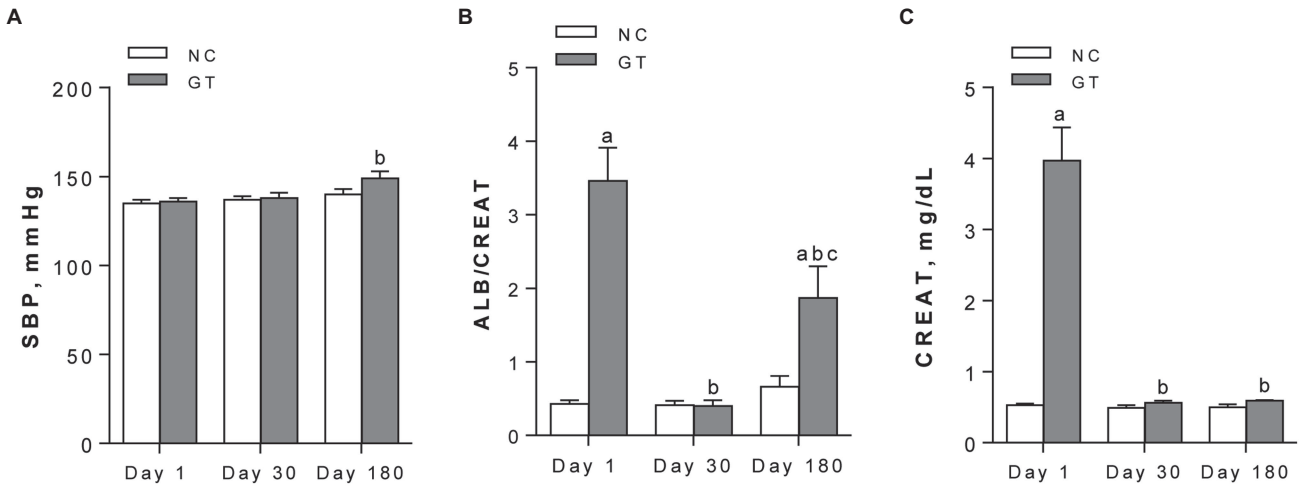

FIGURE 1 | Systolic blood pressure **(A)**, urine albumin/creatinine ratios, in mg/mg **(B)**, and serum creatinine **(C)** assessed on Days 1, 30, and 180 after GT treatment in groups NC and GT. Results expressed as means ± *SE*. $^{a}p < 0.05$ vs. NC; $^{b}p < 0.05$ vs. Day 1; $^{c}p < 0.05$ vs. Day 30.

On Day 1, GT rats exhibited a slight but significant decrease in serum sodium levels, while a marked increase in the fractional excretion of sodium (FE_{Na+}) and potassium (FE_{K+}) was observed. Both parameters returned to control on Day 30 and remained at these levels on Day 180 (**Figures 2A–D**). Urine osmolality was markedly reduced in GT rats on Day 1, returning to control levels from Day 30 on **Figure 2E**. The renal content of KIM-1 was strikingly increased on Day 1 ($p < 0.0001$), and declined progressively on Days 30 and 180, though remaining slightly but significantly increased above control levels (**Figure 2F**).

On Day 1, GT-treated rats exhibited widespread acute tubular necrosis (ATN), with cell lysis and cellular debris in most tubular lumina. Glomerulosclerosis (GS), with segmental areas of mesangial expansion and capillary loop occlusion, as well as glomerular ischemia (ISCH), with collapse of the entire tuft and closure of capillary loops, were also observed. Representative microphotographs of ATN, ISCH, and GS, along with the frequency of ISCH and GS on Days 1, 30, and 180, are shown in **Figure 3**. Widespread ATN was observed on Day 1, but complete tubular cell regeneration was evident on Days 30 and 180. Percent ISCH was slightly and not significantly increased on Days 1 and 30 but was clearly elevated on Day 180 ($p < 0.001$). The frequency of GS was markedly increased on Day 1, regressed on Day 30, but returned to abnormally high levels on Day 180.

Representative microphotographs of renal tissue stained by immunohistochemistry, focusing on the expression of inflammatory components along the study, are shown in **Figure 4**, whereas the corresponding quantitative analyses are given in **Figure 5**. On Day 1, GT rats exhibited intense renal interstitial infiltration by macrophages. Of note, M2-type anti-inflammatory cells constituted only a small fraction of the infiltrating macrophages. AngII-positive cells and myofibroblasts were also conspicuously present at this phase. On Day 30, the intensity of macrophage infiltration was attenuated, but the anti-inflammatory M2 phenotype now represented nearly half of its composition. The presence of myofibroblasts was also strongly reduced at this time, while that of AngII-positive cells remained unabated. On Day 180, low-grade renal interstitial macrophage infiltration was still observed, whereas myofibroblasts had nearly returned to control levels. By contrast, the number of AngII-positive cells remained at the same elevated levels as observed on Days 1 and 30 ($p < 0.0001$ vs. NC).

Representative microphotographs showing renal tissue stained by immunohistochemistry for collagen-1 and fibronectin are shown in **Figure 4**. Renal deposition of both molecules exhibited a progressive increase along the study, becoming significantly elevated compared to NC, and indicating that an insidious process of fibrosis took place in the renal tissue (**Figure 5**).

The renal contents of TLR4, IL-6, the active form of caspase-1, and IL-1β were all increased in GT rats on Day 1 (**Figure 6**). All these parameters returned to normal on Day 30, except for IL-1β. On Day 180, IL-1β remained significantly increased compared to controls, whereas IL-6 and caspase-1 were again elevated.

DISCUSSION

As shown previously (Nakajima et al., 1994; Geleilete et al., 2002; Volpini et al., 2004), GT rats developed nephrotoxic AKI, with limited body growth, marked creatinine retention, and tubular damage, characterized by clear histologic evidence of cellular necrosis, along with marked increase in the renal content of KIM-1. These tubular structural changes were accompanied by severe functional limitation, with a striking increase in the fractional excretion of sodium and potassium, as well as a drastic fall in urine osmolality. In addition, a marked increase in the urine albumin/creatinine ratio and low-grade sclerosing tuft lesions indicated that the glomeruli were also damaged.

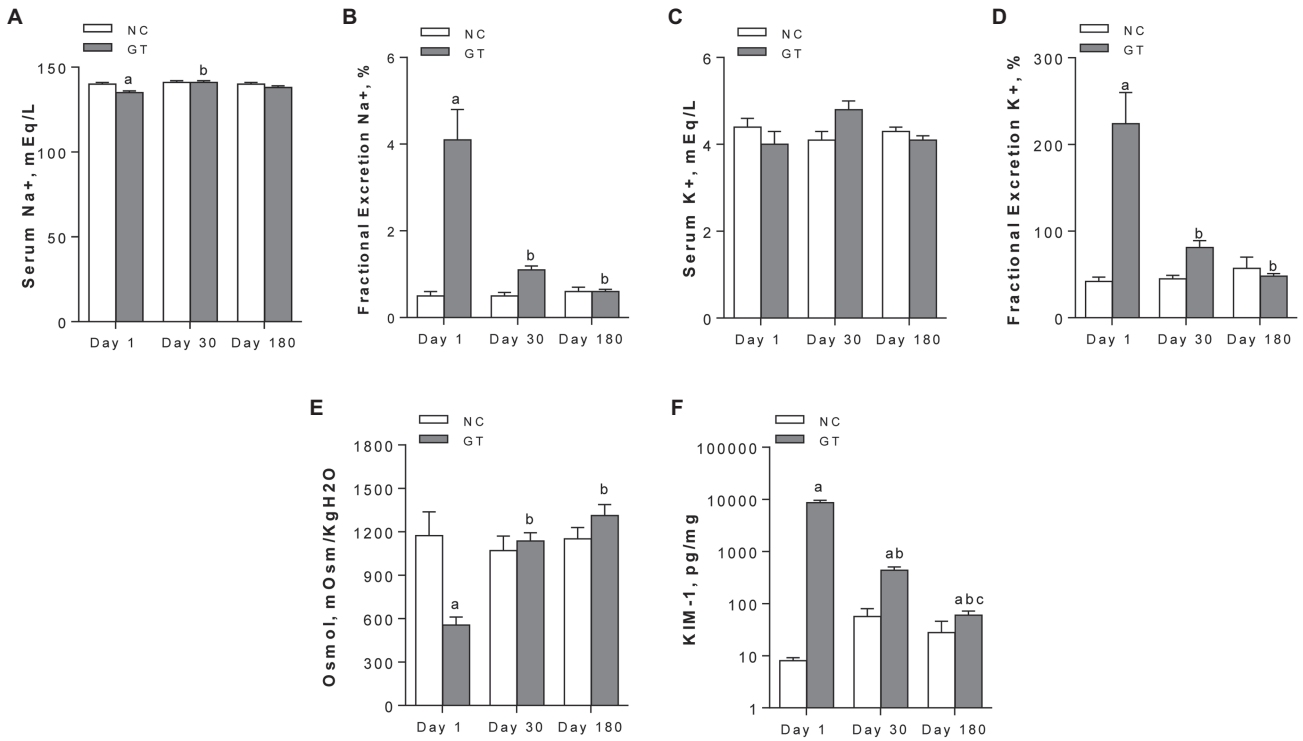

FIGURE 2 | Serum concentration **(A)** and fractional excretion **(B)** of sodium; serum concentration **(C)** and fractional excretion **(D)** of potassium; and urinary osmolality **(E)** and renal content of KIM-1 **(F)**. Results expressed as means ± *SE*. [a]$p < 0.05$ vs. NC; [b]$p < 0.05$ vs. Day 1; [c]$p < 0.05$ vs. Day 30.

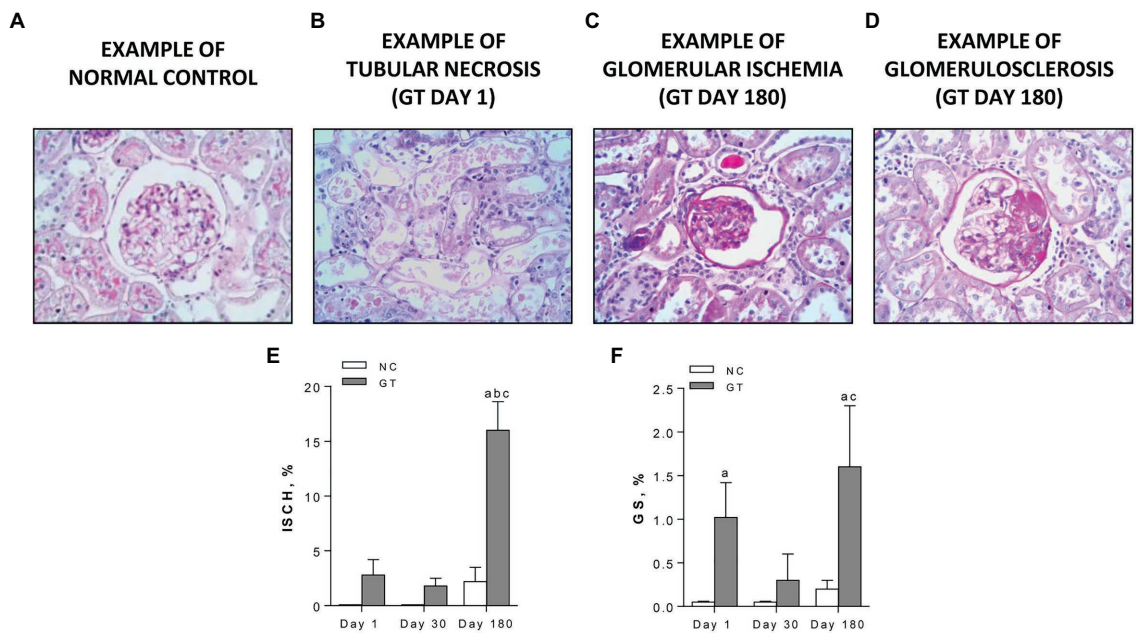

FIGURE 3 | Representative microphotographs of renal lesions (PAS-stained, 400×) **(A)** normal glomerulus; **(B)** widespread acute tubular necrosis, present on Day 1 only, with tubular profiles nearly completely devoid of cells and filled by necrotic material; **(C)** a representative ischemic (ISCH) glomerulus, showing tuft collapse, wrinkled basement membrane, and closure of capillary loops, on Day 180; **(D)** a representative glomerulus with a segmental sclerotic lesion (GS), with occlusion of capillary loops by a hyaline material, on Day 180; and **(E,F)** quantitative analysis of ISCH and GS, respectively, in groups NC and GT. Results expressed as means ± *SE*. [a]$p < 0.05$ vs. NC, [b]$p < 0.05$ vs. Day 1, and [c]$p < 0.05$ vs. Day 30.

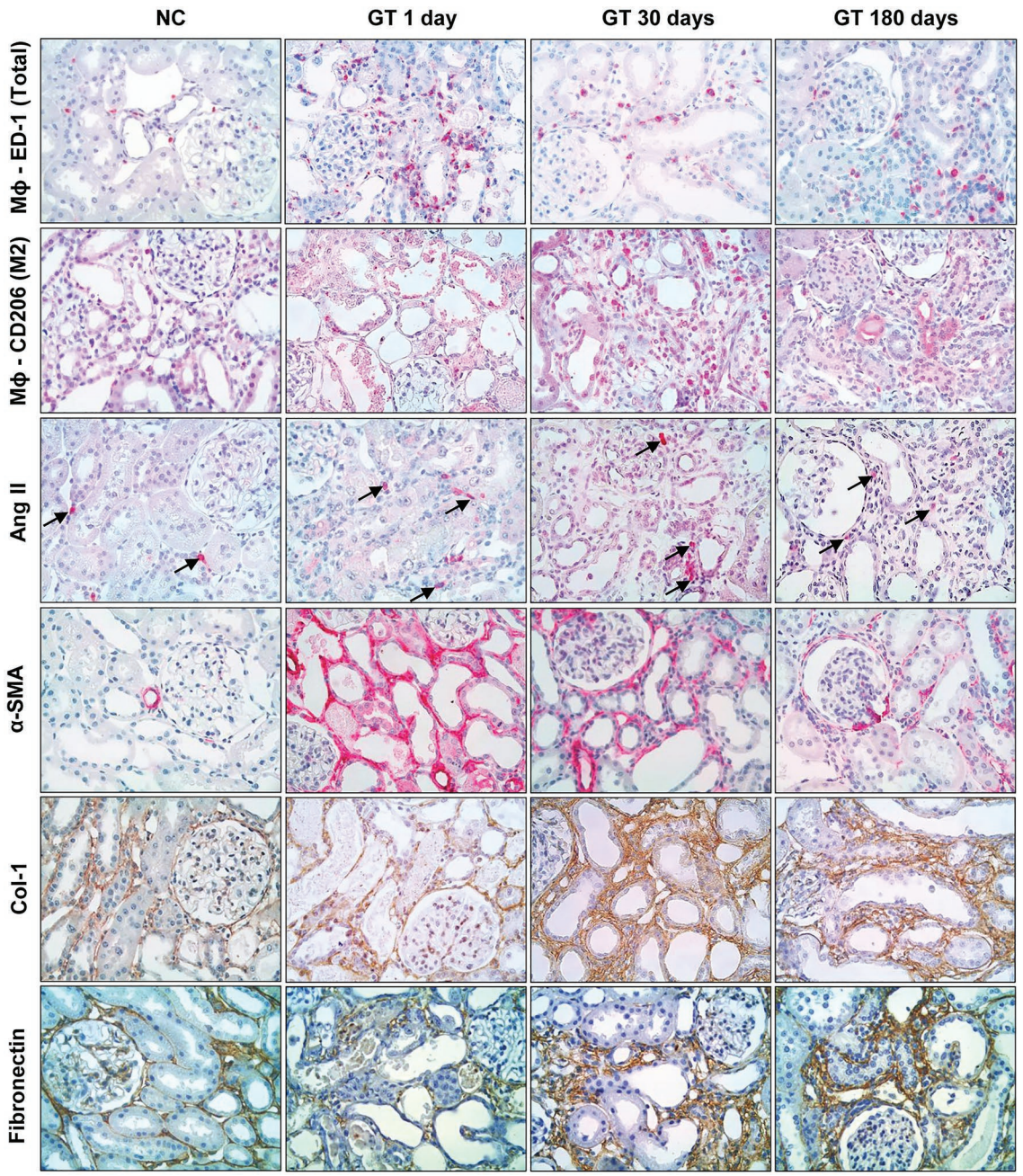

FIGURE 4 | Representative microphotographs (in immunohistochemically stained sections, 400×) of renal interstitial infiltration by total macrophages (ED-1-positive cells); M2 macrophages (CD206-positive cells); AngII-positive cells; α-SMA-positive cells; and renal interstitial deposition of Collagen-1 and Fibronectin in groups NC and GT on Days 1, 30, and 180.

An intense macrophage infiltration was shown on Day 1. Only few of these cells were CD206-positive, indicating that the M2 subtype did not play a role at this acute phase, and that macrophages followed a predominantly proinflammatory behavior, as observed in the previous studies of this and other AKI models (Geleilete et al., 2002; Jo et al., 2006). Cells staining positively for α-SMA, presumably myofibroblasts, were also detected at this stage, in agreement with the previous studies of either the GT (Geleilete et al., 2002) or the I/R model (Forbes et al., 2000). Infiltration by cells staining positively for Ang II was also observed, indicating local activation of the renin-angiotensin system (RAS), not previously shown in association with GT nephrotoxicity. This observation is consistent with the observed overexpression of α-SMA, and with the report of a beneficial effect of losartan in experimental GT nephrotoxicity (Heeba, 2011). Together, these findings reinforce

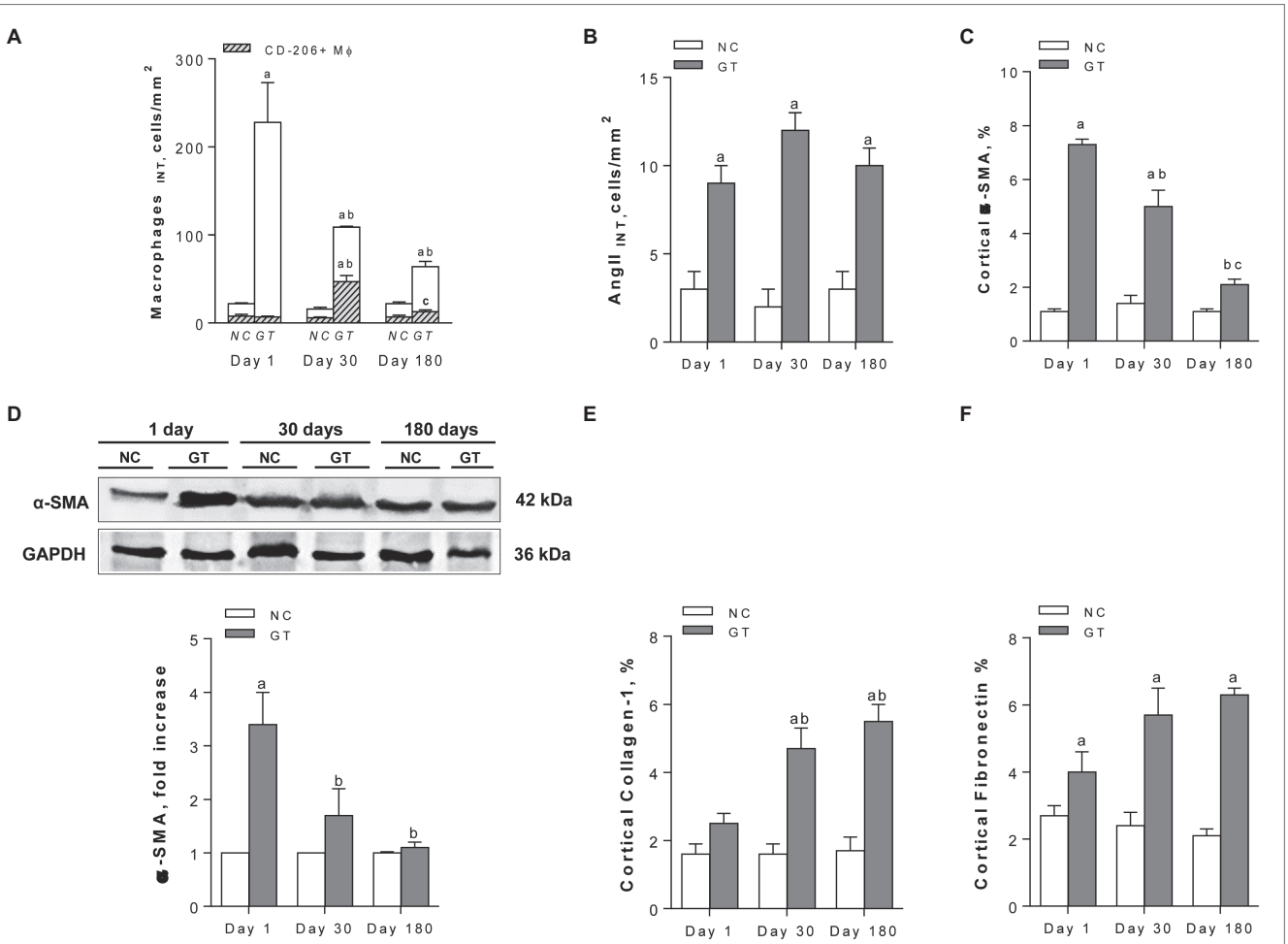

FIGURE 5 | Quantitative analysis. **(A)** interstitial macrophages, with the CD206-positive fraction represented by the hatched areas; **(B)** interstitial AngII-positive cells; **(C)** percent cortical α-SMA-positive area (by immunohistochemistry); **(D)** renal abundance of α-SMA (by Western blot); and **(E,F)** percent Collagen-1- and Fibronectin-positive cortical areas, respectively (by immunohistochemistry), in groups NC and GT on Days 1, 30, and 180. Western blot analysis included seven animals per group. Results expressed as means ± *SE*. $^{a}p < 0.05$ vs. NC, $^{b}p < 0.05$ vs. Day 1, and $^{c}p < 0.05$ vs. Day 30.

the notion that ATN, whether resulting from I/R or toxicity, is associated with exuberant renal inflammation (Forbes et al., 2000; Geleilete et al., 2002; Iyer et al., 2009). Of note, renal fibronectin accumulation was also observed, indicating that incipient renal fibrosis was already present at this early stage.

The renal inflammation observed on Day 1 in rats that received GT was associated with a marked increase in the abundance of IL-6, one of the main NF-κB targets. This finding is consistent with that of Volpini et al. (2004), who reported activation of the NF-κB system 5 days after GT administration. In addition, treatment with GT led to an increase in the renal abundance of TLR4, caspase-1, and IL-1β, suggesting that the NLRP3 inflammasome pathway was also activated. Since what was measured was total IL-1β, it cannot be established with certainty whether the renal content of its active form was also increased. However, as the renal abundance of caspase-1 was also augmented, it is more than likely that this was indeed the case.

Taken together, these results indicate that a strong activation of innate renal immunity occurred on Day 1, encompassing both the NF-κB and NLRP3 pathways. The mechanisms that triggered this process have not been determined. Cell remains, such as membrane debris and DNA fragments, can function as danger-associated molecular patterns (DAMPs), binding to TLRs and activating both the NF-κB and NLRP3 inflammatory pathways (Anders et al., 2004; Iyer et al., 2009; Martinon et al., 2009; Lorenz et al., 2014). In addition, the NF-κB system may have been activated by AngII and/or by the presence of oxidative stress, as demonstrated in other models (Ruiz-Ortega et al., 2000; Mezzano et al., 2004; Ji et al., 2009; Heeba, 2011). Irrespective of the triggering factors, this process helps to explain the development of the intense renal inflammatory process that involved the kidneys during the acute phase of GT toxicity. It is noteworthy that all these changes took place only 1 day after the GT injections, suggesting that they constitute a fast response to cell damage/destruction.

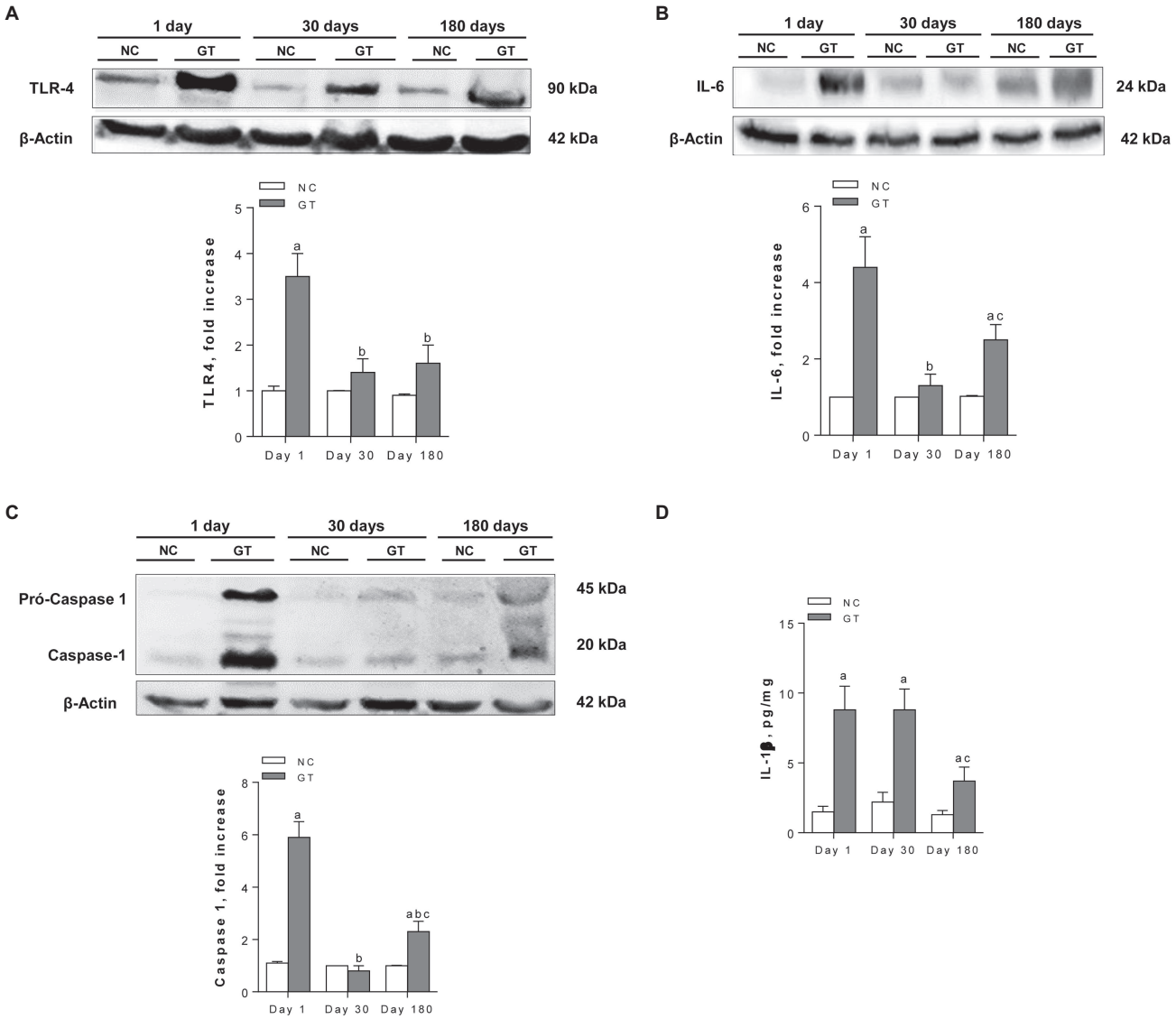

FIGURE 6 | Representative Western blots and quantitative assessment of the abundance of Toll-like receptor 4 **(A)**, Interleukin-6 **(B)**, Caspase-1 **(C)**, and renal content of IL-1β by ELISA **(D)** in groups NC and GT on Days 1, 30, and 180. Western blot analysis included seven animals per group. Results expressed as means ± *SE*. $^{a}p < 0.05$ vs. NC, $^{b}p < 0.05$ vs. Day 1, and $^{c}p < 0.05$ vs. Day 30.

The structural changes observed on Day 1 had completely regressed 30 days after GT administration. The glomerular sclerosing lesions nearly disappeared, indicating that they had reflected readily reversible processes, such as mesangial matrix deposition, with no permanent structural damage. The associated functional changes also regressed, with normalization of plasma creatinine and albuminuria. There was also full recovery from tubular necrosis, with cell regeneration and return of the renal KIM-1 content to normal values. Tubular function was also restored, with normalization of fractional sodium and potassium excretion, in addition to full recovery of urine concentrating ability.

Renal inflammation also regressed on Day 30. Macrophage infiltration lost intensity, while subtype 2 (CD-206-positive) anti-inflammatory cells now represented 45% of the total cell count, compared to 6% on Day 1. Likewise, myofibroblast infiltration declined to values no longer significantly different from those observed in NC. Together, these findings seemed to indicate that, 1 month after the GT insult, the intense inflammatory process observed on Day 1 had ceased and was on the way to complete resolution. However, this regression was only partial. The density of Ang II-positive cells remained as high as observed on Day 1. In addition, collagen type-1 deposition, which was not significantly increased on Day 1, was now clearly augmented, accompanied by a similar increase in fibronectin accumulation, each taking up a much larger fraction of the renal cortex than in control rats. Innate immunity also remained activated as: although the renal contents of caspase-1 and TLR-4 were normalized, IL-1β and the p65

moiety remained at levels similar to those observed on Day 1. Thus, a silent inflammatory process, associated with, and likely fueled by, an equally quiescent activation of innate immunity, was operative at this time point.

In the present study, rats acutely exposed to the nephrotoxic effects of GT were followed for an unprecedented 180 days after treatment. At the end of this period, only residual signs of tubular injury remained, as indicated by a decrease of urinary KIM-1 to levels only slightly above control, while the fractional excretion of sodium and potassium, as well as urinary osmolality, stayed at control levels. However, several signs of an insidious CKD were now apparent. Although serum creatinine remained close to normal, glomerular sclerosing lesions, which had regressed on Day 30, now reappeared; a considerable fraction of the glomeruli exhibited changes characteristic of ischemia; and albuminuria returned with the same intensity observed on Day 1. These findings indicate that, despite the apparent regression observed on Day 30, the glomerular injury initiated by GT administration remained active.

The renal inflammation observed on Day 1 also appeared to have completely subsided on Day 180, with macrophages (both total and CD206-positive) and myofibroblasts down to normal values. However, other findings indicated that low-grade renal inflammation persisted. Cortical infiltration by AngII-positive cells remained unabated, indicating continued RAS activation. Moreover, renal accumulation of collagen-1 and fibronectin was still evident, indicating persistence of renal fibrosis. As to innate immunity, the renal content of IL-1β was still abnormally high, albeit at more modest levels than initially observed, while the abundance of caspase-1, which had returned to normal on Day 30, was once again heightened. On the other hand, the abundance of IL-6 was still significantly elevated compared to NC values, consistent with persistent activation of the NF-κB system.

The development of insidious renal inflammation following resolution of an acute insult has been reported in our laboratory and elsewhere utilizing differing experimental models of CKD. Rodríguez-Iturbe et al. showed that rats undergoing temporary treatment with L-NAME developed permanent renal inflammation and salt-sensitive arterial hypertension (Rodríguez-Iturbe et al., 2012; Franco et al., 2013). In our study, renal inflammation, along with increased local AngII production, may help to explain why hypertension developed after AKI had subsided. It must be noted that angiotensin II produced outside the afferent arteriole acts much as a proinflammatory cytokine, rather than promoting hypertension by a systemic effect. This dichotomy helps to explain the dissociation between blood pressure and the amount of cells staining positively for angiotensin II, observed in this and in the previous studies (Mattar et al., 2007; Franco et al., 2013).

Slowly progressive nephropathy after acute injury was also shown by Anderson et al. (1988), who described the development of progressive glomerulosclerosis after treatment with puromycin aminonucleoside that led to short-lived massive proteinuria. Likewise, Fujihara et al. (2006) and Mattar et al. (2007) showed that rats subjected for a short period to NO inhibition by L-NAME developed progressive glomerulosclerosis associated with renal interstitial fibrosis. The reasons for these transitions have not been elucidated. In the present study, CKD could result from a marked reduction in the number of nephrons, leading to overload of the remaining glomeruli and intracapillary hypertension, as previously demonstrated for other CKD models (Anderson et al., 1988; Fujihara et al., 2000, 2007). However, this explanation seems unlikely, since creatinine retention was not observed on Days 30 and 180. Moreover, the intensity of glomerular damage was only moderate, less than 2% of glomeruli showing sclerosing lesions. An attractive possibility is the lasting activation of the local RAS, previously demonstrated in other CKD models (Fujihara et al., 2006; Mattar et al., 2007), which could help explain both glomerular and tubulointerstitial injury. The mechanism of continued RAS activation after recovery from GT-induced AKI is uncertain. It is noteworthy that evidence of equally persistent NF-κB and NLRP3/IL-1β activation was also observed in GT rats. AngII can activate the NF-κB system by degrading the I-κB inhibitory factor (Ruiz-Ortega et al., 2000; Douillette et al., 2006). Conversely, activation of the NF-κB system stimulates the expression of the AT1 receptor (Klahr and Morrissey, 2000; Luo et al., 2015). Similar interactions have been shown between the NF-κB and the NLRP3/IL-1β systems (Solt et al., 2007; Verstrepen et al., 2008; Zambom et al., 2019). These reciprocal effects can help explain the lasting activation of these systems, as well as the perpetuation of renal fibrosis in GT-treated rats. An additional possibility is represented by the transformation of endothelial cells and/or macrophages into myofibroblasts, a process observed in experimental models of fibrosis, such as adriamycin nephropathy, unilateral ureteral obstruction, and, most pertinent to our findings, AKI to CKD transition (Li et al., 2007; Tang et al., 2020).

In summary, rats that received GT for 9 days showed evident signs of AKI, with tubular necrosis, loss of renal function, and intense renal inflammation. Although structural and functional changes had regressed 30 days later, some important elements of inflammation persisted, along with activation of the RAS, the NF-κB system, and the NLRP3 pathway. One hundred and eighty days after the administration of GT, albuminuria, glomerular injury, and interstitial fibrosis had recurred, accompanied by evidence of sustained activation of the RAS, the NF-κB system, and the NLRP3/IL-1β pathway. These observations indicate that long-term renal fibrosis does occur after gentamicin-induced renal injury, and may be mediated, and perpetuated, by continuing renal activation of the RAS and of innate immunity. Targeting the NF-κB and/or the NLRP3 inflammasome systems may represent a new strategy in the effort to prevent the progression of renal fibrosis and, in particular, the AKI-CKD transition.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine of University of São Paulo.

AUTHOR CONTRIBUTIONS

AA, FZ, OF-N, KO, VA, SA, AS, DM, CF, and RZ carried out the experiments and analyzed the data. RZ, CF, and NC conceived the research project. All authors contributed to the article and approved the submitted version.

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REFERENCES

Anders, H. J., Banas, B., and Schlondorff, D. (2004). Signaling danger: toll-like receptors and their potential roles in kidney disease. *J. Am. Soc. Nephrol.* 15, 854–867. doi: 10.1097/01.asn.0000121781.89599.16

Anderson, S., Diamond, J. R., Karnovsky, M. J., and Brenner, B. M. (1988). Mechanisms underlying transition from acute glomerular injury to late glomerular sclerosis in a rat model of nephrotic syndrome. *J. Clin. Invest.* 82, 1757–1768. doi: 10.1172/JCI113789

Arias, S. C., Valente, C. P., Machado, F. G., Fanelli, C., Origassa, C. S., de Brito, T., et al. (2013). Regression of albuminuria and hypertension and arrest of severe renal injury by a losartan-hydrochlorothiazide association in a model of very advanced nephropathy. *PLoS One* 8:e56215. doi: 10.1371/journal.pone.0056215

Black, L. M., Lever, J. M., Traylor, A. M., Chen, B., Yang, Z., Esman, S. K., et al. (2018). Divergent effects of AKI to CKD models on inflammation and fibrosis. *Am. J. Physiol. Ren. Physiol.* 315, F1107–F1118. doi: 10.1152/ajprenal.00179.2018

Castoldi, A., Braga, T. T., Correa-Costa, M., Aguiar, C. F., Bassi, Ê., Correa-Silva, R., et al. (2012). TLR2, TLR4 and the MYD88 signaling pathway are crucial for neutrophil migration in acute kidney injury induced by sepsis. *PLoS One* 7:e37584. doi: 10.1371/journal.pone.0037584

Chawla, L. S. (2011). Acute kidney injury leading to chronic kidney disease and long-term outcomes of acute kidney injury: the best opportunity to mitigate acute kidney injury? *Contrib. Nephrol.* 174, 182–190. doi: 10.1159/000329396

Douillette, A., Bibeau-Poirier, A., Gravel, S. P., Clément, J. F., Chénard, V., Moreau, P., et al. (2006). The proinflammatory actions of angiotensin II are dependent on p65 phosphorylation by the IkappaB kinase complex. *J. Biol. Chem.* 281, 13275–13284. doi: 10.1074/jbc.M512815200

Eddy, A. A. (1996). Molecular insights into renal interstitial fibrosis. *J. Am. Soc. Nephrol.* 7, 2495–2508. doi: 10.1681/ASN.V7122495

Esteban, V., Lorenzo, O., Rupérez, M., Suzuki, Y., Mezzano, S., Blanco, J., et al. (2004). Angiotensin II, via AT1 and AT2 receptors and NF-kappaB pathway, regulates the inflammatory response in unilateral ureteral obstruction. *J. Am. Soc. Nephrol.* 15, 1514–1529. doi: 10.1097/01.asn.0000130564.75008.f5

Fanelli, C., Arias, S. C. A., Machado, F. G., Okuma, J. K., Malheiros, D. M. A. C., Azevedo, H., et al. (2017). Innate and adaptive immunity are progressively activated in parallel with renal injury in the 5/6 renal ablation model. *Sci. Rep.* 7:3192. doi: 10.1038/s41598-017-02915-6

Faustino, V. D., Arias, S. C. A., Ferreira Ávila, V., Foresto-Neto, O., Zambom, F. F. F., Machado, F. G., et al. (2018). Simultaneous activation of innate and adaptive immunity participates in the development of renal injury in a model of heavy proteinuria. *Biosci. Rep.* 38:BSR20180762. doi: 10.1042/BSR20180762

Floege, J., Burns, M. W., Alpers, C. E., Yoshimura, A., Pritzl, P., Gordon, K., et al. (1992). Glomerular cell proliferation and PDGF expression precede glomerulosclerosis in the remnant kidney model. *Kidney Int.* 41, 297–309. doi: 10.1038/ki.1992.42

Forbes, J. M., Hewitson, T. D., Becker, G. J., and Jones, C. L. (2000). Ischemic acute renal failure: long-term histology of cell and matrix changes in the rat. *Kidney Int.* 57, 2375–2385. doi: 10.1046/j.1523-1755.2000.00097.x

Foresto-Neto, O., Albino, A. H., Arias, S. C. A., Faustino, V. D., Zambom, F. F. F., Cenedeze, M. A., et al. (2020). NF-κB system is chronically activated and promotes glomerular injury in experimental Type 1 diabetic kidney disease. *Front. Physiol.* 11:84. doi: 10.3389/fphys.2020.00084

Foresto-Neto, O., Ávila, V. F., Arias, S. C. A., Zambom, F. F. F., Rempel, L. C. T., Faustino, V. D., et al. (2018). NLRP3 inflammasome inhibition ameliorates tubulointerstitial injury in the remnant kidney model. *Lab. Investig.* 98, 773–782. doi: 10.1038/s41374-018-0029-4

Franco, M., Tapia, E., Bautista, R., Pacheco, U., Santamaria, J., Quiroz, Y., et al. (2013). Impaired pressure natriuresis resulting in salt-sensitive hypertension is caused by tubulointerstitial immune cell infiltration in the kidney. *Am. J. Physiol. Ren. Physiol.* 304, F982–F990. doi: 10.1152/ajprenal.00463.2012

Fujihara, C. K., Antunes, G. R., Mattar, A. L., Malheiros, D. M., Vieira, J. M., and Zatz, R. (2007). Chronic inhibition of nuclear factor-kappaB attenuates renal injury in the 5/6 renal ablation model. *Am. J. Physiol. Ren. Physiol.* 292, F92–F99. doi: 10.1152/ajprenal.00184.2006

Fujihara, C. K., Michellazzo, S. M., de Nucci, G., and Zatz, R. (1994). Sodium excess aggravates hypertension and renal parenchymal injury in rats with chronic NO inhibition. *Am. J. Phys.* 266, F697–F705. doi: 10.1152/ajprenal.1994.266.5.F697

Fujihara, C. K., Noronha, I. L., Malheiros, D. M. A. C., Antunes, G. R., de Oliveira, I. B., and Zatz, R. (2000). Combined mycophenolate mofetil and losartan therapy arrests established injury in the remnant kidney. *J. Am. Soc. Nephrol.* 11, 283–290. doi: 10.1681/ASN.V112283

Fujihara, C. K., Sena, C. R., Malheiros, D. M., Mattar, A. L., and Zatz, R. (2006). Short-term nitric oxide inhibition induces progressive nephropathy after regression of initial renal injury. *Am. J. Physiol. Ren. Physiol.* 290, F632–F640. doi: 10.1152/ajprenal.00259.2005

Geleilete, T. J., Melo, G. C., Costa, R. S., Volpini, R. A., Soares, T. J., and Coimbra, T. M. (2002). Role of myofibroblasts, macrophages, transforming growth factor-beta endothelin, angiotensin-II, and fibronectin in the progression of tubulointerstitial nephritis induced by gentamicin. *J. Nephrol.* 15, 633–642.

Gonçalves, G. M., Castoldi, A., Braga, T. T., and Câmara, N. O. (2011). New roles for innate immune response in acute and chronic kidney injuries. *Scand. J. Immunol.* 73, 428–435. doi: 10.1111/j.1365-3083.2011.02523.x

Han, W. K., and Bonventre, J. V. (2004). Biologic markers for the early detection of acute kidney injury. *Curr. Opin. Crit. Care* 10, 476–482. doi: 10.1097/01.ccx.0000145095.90327.f2

Heeba, G. H. (2011). Angiotensin II receptor blocker, losartan, ameliorates gentamicin-induced oxidative stress and nephrotoxicity in rats. *Pharmacology* 87, 232–240. doi: 10.1159/000325457

Hoste, E. A., and Kellum, J. A. (2007). Incidence, classification, and outcomes of acute kidney injury. *Contrib. Nephrol.* 156, 32–38. doi: 10.1159/000102013

Iyer, S. S., Pulskens, W. P., Sadler, J. J., Butter, L. M., Teske, G. J., Ulland, T. K., et al. (2009). Necrotic cells trigger a sterile inflammatory response through the Nlrp3 inflammasome. *Proc. Natl. Acad. Sci. U. S. A.* 106, 20388–20393. doi: 10.1073/pnas.0908698106

Jang, H. R., and Rabb, H. (2015). Immune cells in experimental acute kidney injury. *Nat. Rev. Nephrol.* 11, 88–101. doi: 10.1038/nrneph.2014.180

Jepsen, F. L., and Mortensen, P. B. (1979). Interstitial fibrosis of the renal cortex in minimal change lesion and its correlation with renal function.

A quantitative study. *Virchows Arch. A Pathol. Anat. Histol.* 383, 265–270. doi: 10.1007/BF00430245

Ji, Y., Liu, J., Wang, Z., and Liu, N. (2009). Angiotensin II induces inflammatory response partly via toll-like receptor 4-dependent signaling pathway in vascular smooth muscle cells. *Cell. Physiol. Biochem.* 23, 265–276. doi: 10.1159/000218173

Jo, S. K., Sung, S. A., Cho, W. Y., Go, K. J., and Kim, H. K. (2006). Macrophages contribute to the initiation of ischaemic acute renal failure in rats. *Nephrol. Dial. Transplant.* 21, 1231–1239. doi: 10.1093/ndt/gfk047

Kim, H. J., Lee, D. W., Ravichandran, K., Keys, D. O., Akcay, A., Nguyen, Q., et al. (2013). NLRP3 inflammasome knockout mice are protected against ischemic but not cisplatin-induced acute kidney injury. *J. Pharmacol. Exp. Ther.* 346, 465–472. doi: 10.1124/jpet.113.205732

Klahr, S., and Morrissey, J. J. (2000). The role of vasoactive compounds, growth factors and cytokines in the progression of renal disease. *Kidney Int. Suppl.* 75, S7–S14.

Kurts, C., Panzer, U., Anders, H. J., and Rees, A. J. (2013). The immune system and kidney disease: basic concepts and clinical implications. *Nat. Rev. Immunol.* 13, 738–753. doi: 10.1038/nri3523

Li, J., Deane, J. A., Campanale, N. V., Bertram, J. F., and Ricardo, S. D. (2007). The contribution of bone marrow-derived cells to the development of renal interstitial fibrosis. *Stem Cells* 25, 697–706. doi: 10.1634/stemcells.2006-0133

Li, J., Tang, Y., Tang, P. M. K., Jun, L., Huang, X. R., Carlsson-Skwirut, C., et al. (2018). Blocking macrophage migration inhibitory factor protects against cisplatin-induced acute kidney injury in mice. *Mol. Ther.* 26, 2523–2532. doi: 10.1016/j.ymthe.2018.07.014

Lorenz, G., Darisipudi, M. N., and Anders, H. J. (2014). Canonical and non-canonical effects of the NLRP3 inflammasome in kidney inflammation and fibrosis. *Nephrol. Dial. Transplant.* 29, 41–48. doi: 10.1093/ndt/gft332

Luo, H., Wang, X., Wang, J., Chen, C., Wang, N., Xu, Z., et al. (2015). Chronic NF-κB blockade improves renal angiotensin II type 1 receptor functions and reduces blood pressure in Zucker diabetic rats. *Cardiovasc. Diabetol.* 14:76. doi: 10.1186/s12933-015-0239-7

Mancini, G., Carbonara, A. O., and Heremans, J. F. (1965). Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry* 2, 235–254. doi: 10.1016/0019-2791(65)90004-2

Martinon, F., Mayor, A., and Tschopp, J. (2009). The inflammasomes: guardians of the body. *Annu. Rev. Immunol.* 27, 229–265. doi: 10.1146/annurev.immunol.021908.132715

Mattar, A. L., Machado, F. G., Fujihara, C. K., Malheiros, D. M., and Zatz, R. (2007). Persistent hypertension and progressive renal injury induced by salt overload after short term nitric oxide inhibition. *Clinics* 62, 749–756. doi: 10.1590/S1807-59322007000600015

Mezzano, S., Aros, C., Droguett, A., Burgos, M. E., Ardiles, L., Flores, C., et al. (2004). NF-kappaB activation and overexpression of regulated genes in human diabetic nephropathy. *Nephrol. Dial. Transplant.* 19, 2505–2512. doi: 10.1093/ndt/gfh207

Mishra, K. P., Ganju, L., and Singh, S. B. (2015). Hypoxia modulates innate immune factors: a review. *Int. Immunopharmacol.* 28, 425–428. doi: 10.1016/j.intimp.2015.07.008

Nakajima, T., Hishida, A., and Kato, A. (1994). Mechanisms for protective effects of free radical scavengers on gentamicin-mediated nephropathy in rats. *Am. J. Phys.* 266, F425–F431. doi: 10.1152/ajprenal.1994.266.3.F425

Nizet, V., and Johnson, R. S. (2009). Interdependence of hypoxic and innate immune responses. *Nat. Rev. Immunol.* 9, 609–617. doi: 10.1038/nri2607

Rodríguez-Iturbe, B., Franco, M., Tapia, E., Quiroz, Y., and Johnson, R. J. (2012). Renal inflammation, autoimmunity and salt-sensitive hypertension. *Clin. Exp. Pharmacol. Physiol.* 39, 96–103. doi: 10.1111/j.1440-1681.2011.05482.x

Ruiz-Ortega, M., Lorenzo, O., Rupérez, M., König, S., Wittig, B., and Egido, J. (2000). Angiotensin II activates nuclear transcription factor kappaB through AT(1) and AT(2) in vascular smooth muscle cells: molecular mechanisms. *Circ. Res.* 86, 1266–1272. doi: 10.1161/01.RES.86.12.1266

Sato, Y., and Yanagita, M. (2018). Immune cells and inflammation in AKI to CKD progression. *Am. J. Physiol. Ren. Physiol.* 315, F1501–F1512. doi: 10.1152/ajprenal.00195.2018

Solt, L. A., Madge, L. A., Orange, J. S., and May, M. J. (2007). Interleukin-1-induced NF-kappaB activation is NEMO-dependent but does not require IKKbeta. *J. Biol. Chem.* 282, 8724–8733. doi: 10.1074/jbc.M609613200

Tang, P. M., Zhang, Y. Y., Xiao, J., Tang, P. C., Chung, J. Y., Li, J., et al. (2020). Neural transcription factor Pou4f1 promotes renal fibrosis via macrophage-myofibroblast transition. *Proc. Natl. Acad. Sci. U. S. A.* 117, 20741–20752. doi: 10.1073/pnas.1917663117

Venkatachalam, M. A., Weinberg, J. M., Kriz, W., and Bidani, A. K. (2015). Failed tubule recovery, AKI-CKD transition, and kidney disease progression. *J. Am. Soc. Nephrol.* 26, 1765–1776. doi: 10.1681/ASN.2015010006

Verstrepen, L., Bekaert, T., Chau, T. L., Tavernier, J., Chariot, A., and Beyaert, R. (2008). TLR-4, IL-1R and TNF-R signaling to NF-kappaB: variations on a common theme. *Cell. Mol. Life Sci.* 65, 2964–2978. doi: 10.1007/s00018-008-8064-8

Vilaysane, A., Chun, J., Seamone, M. E., Wang, W., Chin, R., Hirota, S., et al. (2010). The NLRP3 inflammasome promotes renal inflammation and contributes to CKD. *J. Am. Soc. Nephrol.* 21, 1732–1744. doi: 10.1681/ASN.2010020143

Volpini, R. A., Costa, R. S., da Silva, C. G., and Coimbra, T. M. (2004). Inhibition of nuclear factor-kappaB activation attenuates tubulointerstitial nephritis induced by gentamicin. *Nephron Physiol.* 98, 97–106. doi: 10.1159/000081558

Wallenstein, S., Zucker, C. L., and Fleiss, J. L. (1980). Some statistical methods useful in circulation research. *Circ. Res.* 47, 1–9. doi: 10.1161/01.RES.47.1.1

Yang, L. (2019). How acute kidney injury contributes to renal fibrosis. *Adv. Exp. Med. Biol.* 1165, 117–142. doi: 10.1007/978-981-13-8871-2_7

Zambom, F. F. F., Oliveira, K. C., Foresto-Neto, O., Faustino, V. D., Ávila, V. F., Albino, A. H., et al. (2019). Pathogenic role of innate immunity in a model of chronic NO inhibition associated with salt overload. *Am. J. Physiol. Ren. Physiol.* 317, F1058–F1067. doi: 10.1152/ajprenal.00251.2019

Zhang, Y. X., Zhang, J. R., and Wang, Z. G. (2013). Mycophenolate mofetil affects monocyte toll-like receptor 4 signaling during mouse renal ischemia/reperfusion injury. *Chin. Med. J.* 126, 1224–1229.

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REVIEW
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Role of Macrophages and Related Cytokines in Kidney Disease

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Inflammation is a key characteristic of kidney disease, but this immune response is two-faced. In the acute phase of kidney injury, there is an activation of the immune cells to fight against the insult, contributing to kidney repair and regeneration. However, in chronic kidney diseases (CKD), immune cells that infiltrate the kidney play a deleterious role, actively participating in disease progression, and contributing to nephron loss and fibrosis. Importantly, CKD is a chronic inflammatory disease. In early CKD stages, patients present sub-clinical inflammation, activation of immune circulating cells and therefore, anti-inflammatory strategies have been proposed as a common therapeutic target for renal diseases. Recent studies have highlighted the plasticity of immune cells and the complexity of their functions. Among immune cells, monocytes/macrophages play an important role in all steps of kidney injury. However, the phenotype characterization between human and mice immune cells showed different markers; therefore the extrapolation of experimental studies in mice could not reflect human renal diseases. Here we will review the current information about the characteristics of different macrophage phenotypes, mainly focused on macrophage-related cytokines, with special attention to the chemokine CCL18, and its murine functional homolog CCL8, and the macrophage marker CD163, and their role in kidney pathology.

Keywords: macrophages, cytokines, kidney disease, CCL18, inflammation, CCL8

INTRODUCTION: ROLE OF IMMUNE CELLS IN THE ONSET AND PROGRESSION OF KIDNEY DISEASE

Renal inflammation arises as a protective response after kidney injury to fight against the initial insult and to establish tissue repair. However, if the reparative processes fail, this inflammatory response could be deleterious, participating in the kidney disease progression (1). This inflammatory response involves many different populations of immune infiltrating cells, including monocytes/macrophages, neutrophils, CD8+ and CD4+ lymphocytes, dendritic cells, mast cells and natural killer cells (2). Importantly, in chronic kidney disease (CKD), regardless of the underlying etiology, there is a persistent activation of the inflammatory response,

characterized by immune cell recruitment throughout the kidney, leading to a local overproduction of growth factors and pro-fibrotic cytokines. This altered environment could activate different cellular and molecular processes, which cause progressive nephron loss and glomerular and interstitial fibrosis, leading to end-stage kidney disease and/or premature death (1–3). CKD is emerging as an important health problem due to the absence of early diagnostic biomarkers and effective treatments. Although many *in vitro* and experimental studies have extensively characterized the distinctive macrophage phenotypes in physiological and pathological conditions, the differences between human and murine macrophages complicate the extrapolation of preclinical results into human kidney disease, being a limitation on approaching macrophages as a therapeutic target to treat human CKD. Now, we review the macrophage characteristics and phenotypes, comparing human and mice data, and focused on novel macrophage-related cytokines as biomarkers or therapeutic targets for kidney disease. We will focus in two interesting candidates, the macrophage derived chemokine CCL18, as well as its murine functional homolog *Ccl8*, and the macrophage marker CD163.

MACROPHAGE CHARACTERISTICS AND PHENOTYPES

Macrophages are innate immune cells and the main component of the mononuclear phagocyte system. Therefore, they are crucial in host defense against pathogens. Furthermore, macrophages are present in almost every organ in adult mammals, where they participate in multiple cellular processes and play a major role in the maintenance of tissue homeostasis (4). Macrophages are considered as tissue sentinels that maintain tissue integrity by eliminating senescent and dead cells, and debris (5). Moreover, they also participate in extracellular matrix (ECM) remodeling, mainly through matrix metalloproteinases (MMPs) release (6), and in the restoration of lost cells and intercellular matrices through the production of various regenerative growth factors (4).

Tissue resident macrophages (TRMs) can be classified into two different subtypes depending on their origin; one derived from circulating monocytes (7–9), and the other from embryonic precursors that are able to locally proliferate and self-renew (10, 11). Monocytes are limited to the blood compartment, the spleen and the bone marrow (12), but in response to inflammation, and guided by the cytokine milieu and/or interactions with other cells and microbial products, monocytes are quickly recruited into injured tissues and then differentiate into several specific macrophage phenotypes depending on micro-environmental signals (8, 13).

Macrophage Polarization: M1 and M2 Phenotypes

Plasticity is the hallmark feature of macrophages. The term "macrophage polarization" is used to refer to an estimate of macrophage activation status and phenotype (11). The M1/M2 polarization axis was originally defined in the 1990s (14) to describe the dichotomy in macrophage function regardless of cytokines: classically activated macrophages/M1 microbicidal macrophages and alternatively activated macrophages/M2 macrophages (**Figure 1**). M1 macrophages participate in the infection clearance and act as an initial defense barrier. The M1 phenotype is generated in response to pro-inflammatory stimuli, such as pathogen/danger-associated molecular patterns (PAMPs or DAMPs) in the presence of interferon gamma (IFN-γ) (14, 15). M1 cells are characterized by their ability to secrete significant amounts of pro-inflammatory cytokines including tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-12 (IL-12), interleukin-15 (IL-15), interleukin-18 (IL-18), and interleukin-23 (IL-23) (16–18). M2 macrophages can be a "two-edged sword." On one side, the anti-inflammatory phenotype is essential for adequate tissue repair; on the other side, it is a potential mediator of fibrosis and scarring (15). These M2 macrophages can release a different cytokine profile, such as transforming growth factor-β1 (TGF-β1), interleukin-10 (IL-10), C-C Motif Chemokine Ligand 17 (CCL17), C-C Motif Chemokine Ligand 18 (CCL18) and C-C Motif Chemokine Ligand 22 (CCL22) (**Figure 2**) (19–24). The macrophage population is currently thought to represent a continuous phenotype ranging between M1/M2 extremes and including other ill-defined populations, as commented above (**Figure 1**). Moreover, the M1/M2 classification is not representative of *in vivo* events, given that M1 and M2 stimuli do not exist alone in tissues. While a much broader spectrum of innate immune responses has been characterized, the M1/M2 axis remains the main macrophage polarization axis *in vivo* (25).

Most of the earliest studies on macrophage polarization were based on *in vitro* stimulation with different combination of cytokines. One of the most important regulators of macrophage differentiation is the colony-stimulating factor 1 receptor (CSF1R). This is a transmembrane tyrosine kinase receptor expressed on almost all mononuclear phagocytic cells (26). *Csf1r* gene deletion depleted macrophages in several tissues. Although this study demonstrates its key role in macrophages development, it also showed that some macrophages were still present, indicating the involvement of other growth factors in macrophage differentiation (27), including Granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-3 (IL-3), vascular endothelial growth factor (VEGF), and fms-like tyrosine kinase 3 ligand (Flt3L) (28–30). Macrophages respond in different ways to macrophage colony-stimulating factor (M-CSF) and GM-CSF with substantial polarizing effects. These factors have been used to study *in vitro* differentiation/polarization of macrophages (31–33). In this sense, GM-CSF activates the inflammatory program and extensive DNA methylation changes, while M-CSF-polarized cells (less differentiated state) show characteristics of anti-inflammatory repairing macrophages (33). Further research led to a sub-classification of M2 macrophages into M2a, M2b, and M2c described as wound-healing, immune complexes-related or regulatory macrophages, respectively (11, 16, 34). M2a activation induced by IL-4 or IL-13 was associated to changes in surface markers, including decreased CD14 expression, and upregulation of mannose receptor (CD206), CD209, and CD23. In M2a macrophages, pro-inflammatory

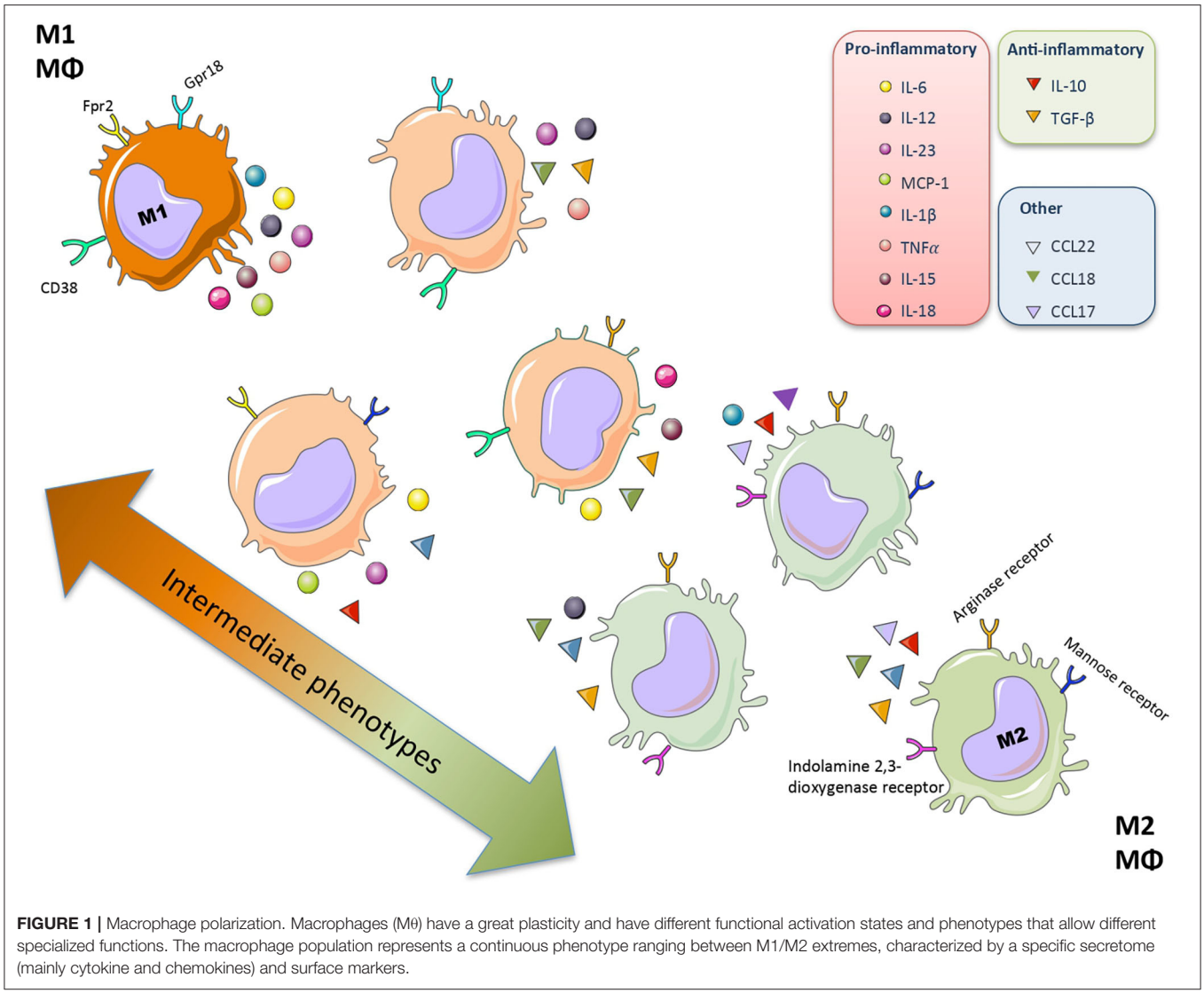

FIGURE 1 | Macrophage polarization. Macrophages (Mθ) have a great plasticity and have different functional activation states and phenotypes that allow different specialized functions. The macrophage population represents a continuous phenotype ranging between M1/M2 extremes, characterized by a specific secretome (mainly cytokine and chemokines) and surface markers.

cytokine expression is lower than in M1, but they release several factors, including CCL18 and vascular Endothelial Growth Factor (VEGF) (35). M2a can also secrete ECM components, like soluble fibronectin, and therefore their primary function is thought to be related to wound healing and tissue remodeling and repair (36). M2b cells are elicited *in vitro* by stimulation with LPS or IL-1β plus high concentrations of immune complexes. M2b macrophages are considered as functional converter of M1 cells with low IL-12 and high IL-10 production. Interestingly, they selectively produce the C-C Motif Chemokine Ligand (CCL1) (37). The M2c category includes cells stimulated with IL-10, TGF-β1 and glucocorticoids, being a heterogeneous group, but characterized by high expression of the surface marker CD163 (38). RNAseq studies have identified M2c-specific genes associated with angiogenesis, matrix remodeling, and phagocytosis, including CD163 (39). Accordingly, the analysis of the M2c macrophage-conditioned media revealed elevated production of MMPs (39). Due to the controversies in the field, a specific nomenclature for cytokine-induced macrophage polarization *in vitro* M (cytokine) have been proposed (40).

Originally, the distinction between M1 and M2 phenotypes was also based on differences in arginine metabolism (14), but recent research has unraveled the metabolic differences associated with macrophage phenotype switching (41). As an overview, and based on the premise that M1 macrophages kill pathogens and M2 resolve inflammation and tissue repair, M1 are more likely to be involved in catabolic pathways, and M2 in anabolic ones (42). Therefore, M1 tend to rely on working aerobic glycolysis likewise fatty acid biosynthesis, because of the speed of producing ATP, but it also have impaired oxidative phosphorylation (43). Moreover, in M1 macrophages, nitric oxide synthase is highly expressed, playing an essential role in the M1 function of killing pathogens, being nitric oxide the source of reactive oxygen species (ROS) with antimicrobial properties (36, 44). On the other hand, M2 are more suitable to trigger fatty acid oxidation as energy-production pathway (42). These

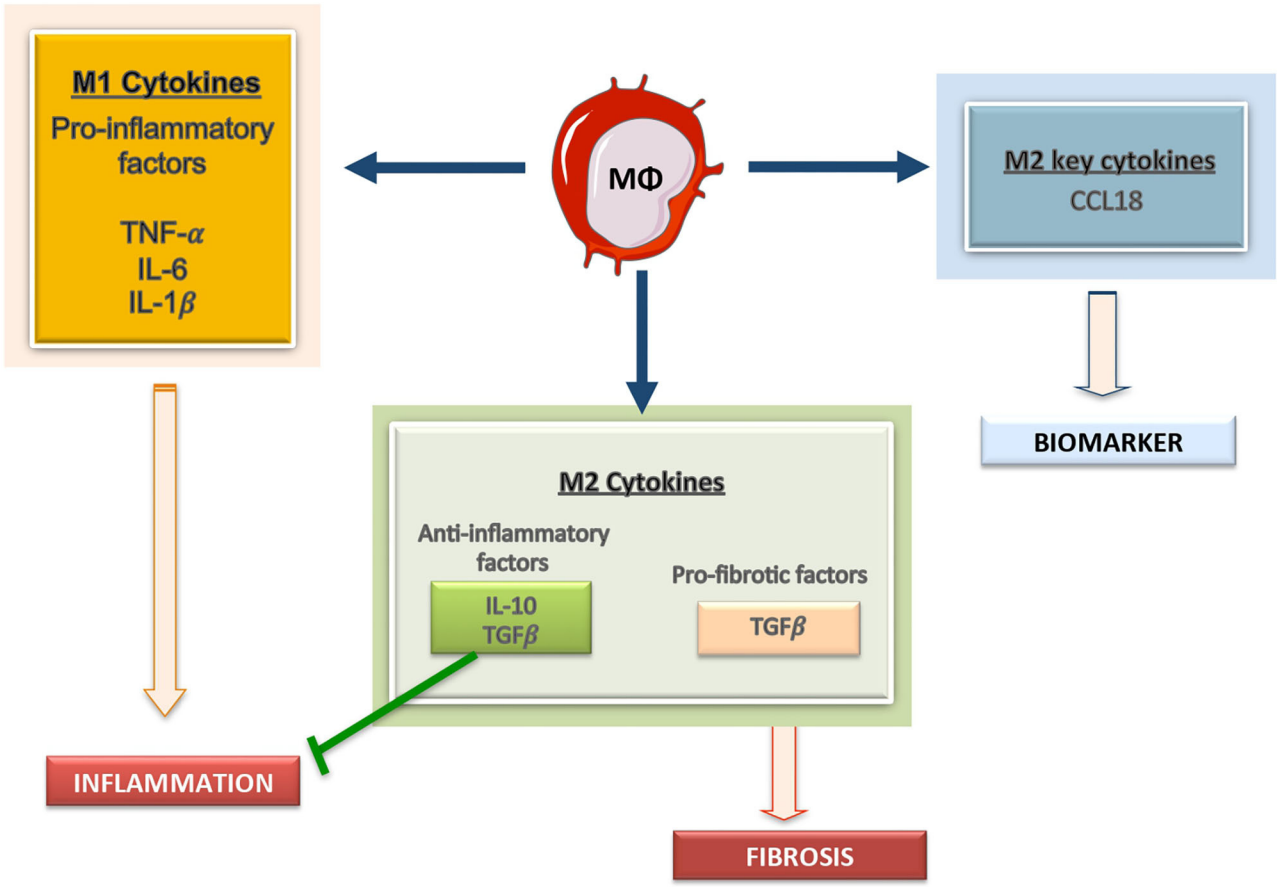

FIGURE 2 | Macrophages derived cytokines can contribute to kidney damage. Macrophages can secrete a large array of molecules, including producing pro-inflammatory mediators, including IL1, Il6, and TNF, anti-inflammatory cytokines, such as IL-10, and profibrotic factors, like TGF-β. Among the M2 macrophage related factors CCL18 is a recent described cytokine and a potential biomarker of disease progression.

macrophages secrete insulin-like growth factor 1 (IGF-1) (45), TGF-β1, and VEGF (46), as well as use arginine to produce precursors of collagen (47), thereby dampening inflammation.

Though we are aware that it may represent an oversimplification, since most of the previous literature has used the M1/M2 classification nomenclature, this terminology has been maintained in some parts of this review.

Comparison of Human and Mammalian Macrophages: Limitations, Similarities, and Future Perspectives

A limitation in the field of macrophage research is the extrapolation of preclinical studies to humans. Macrophages play a key role in the inflammatory response, but the pathogen type that infects humans can be different from other species, including rodents, as well as the molecules released by macrophages to control infections.

The studies investigating M1/M2 macrophage polarization, some of them included in the previous section, were done using mouse or human macrophages of different cell sources; including isolated circulating monocytes, bone marrow-derived cells or peritoneal macrophages, as well as established cell lines (such as THPs or RAW 264.7). A macrophage comparative study evaluating different cell sources depicted similarities between mouse and human cytokine profiles stimulated with a specific combination of cytokines, such as CXCL-10 and CXCL-11 for M (LPS,IFN) and CCL17 and CCL22 for M (IL4, IL13)-induced macrophage polarization (48). Although murine M1/M2-polarized macrophage subsets can be distinguished on the basis of combinatorial gene expression profiles, the identification of equivalent subsets in humans is still unresolved. Another key point is the specific M1/M2 macrophage subsets markers. In humans, similarly to mice, three monocyte subsets have been described according to differential expression of CD14 and CD16 on HLA-DR$^+$ cells (49). In human macrophages, CD68 is a general marker, whereas HLA-DR and CD163 are M1 and M2 markers, respectively (49, 50). Murine and human macrophages express the antigens CD68, CD11b, CSF1R, and CD163 (5). F4/80 is expressed on most tissue macrophages in the mouse and it has been extensively studied by immunohistochemistry, but has limited usefulness in humans as F4/80 is predominantly expressed on eosinophils (51). Recent research is focused on

the search of additional markers. The early growth response protein 2 (Egr2) and c-Myc have been described in murine models to identify *in vivo* polarized M2 cells. One the other hand, some *in vivo* pro-inflammatory factors have been shown to be M1 macrophage markers, such as G-protein-coupled receptor 18 (Gpr18), formyl peptide receptor 2 (Fpr2), CD40, and CD38 (52, 53). Among them, CD38 could be also a marker of M1 pro-inflammatory macrophages in humans (54). Comparative biology, together with omics technologies, such as transcriptomics, metabolomics, proteomics, and epigenomics could potentially be employed to assess similarities between mouse and human macrophages (4). Recently, the single-cell RNA sequencing across multiple mammalian species has led to the identification of CD74 and CD81 as surface markers for kidney resident macrophages (55). Future studies in human biopsies of different pathologies using cutting edge technologies will reveal human macrophages characteristics in each state of the disease.

MACROPHAGES IN RENAL DISEASES: PHENOTYPES AND FUNCTIONS

Macrophages participate in immune surveillance and in the regulation of kidney homeostasis. The macrophage response to kidney injury varies enormously depending on the nature and duration of the insult (23). Macrophages participate in the inflammatory response, both in acute kidney injury (AKI) and in CKD. They can promote kidney repair or contribute to the AKI-to-CKD transition and fibrosis, highlighting their remarkable plasticity (2, 56).

Macrophages in AKI: From Preclinical Data to Human Studies

Macrophages can actively participate in all AKI-related processes, including cell death, resolution/regeneration phase or progression to kidney fibrosis (57). Importantly, macrophage phenotypes can change along AKI, depending on disease stage and evolution. Circulating classical monocytes (CD11b+ $Ly6C^{high}$ in the mouse or $CD14^{high}CD16^{low}$ in humans) are recruited into the kidney, where they differentiate to pro-inflammatory M1 macrophages during the early phase of renal injury, in response to infection or cell damage (23). Many preclinical data suggest that M1 macrophages play a pathogenic role in the early phases of AKI. Studies done in the model of folic acid in mice showed an upregulation of cytokines and chemokines, such MCP-1/CCL2 (the main macrophage chemotactic factor) associated to the presence of M1 macrophages in the injured kidney at 48 h (58). Moreover, in ischemia-reperfusion injury (IRI) in mice M1 macrophages, identified by their high expression of iNOS, IL-12, IL-23, and Ly6C, were detected also at 48 h (59). Accordingly, depletion of kidney macrophages by liposomal clodronate (LC) at the early stages of IRI reduces AKI and improves renal repair. Furthermore, adoptive transfer of IFN-γ-stimulated macrophages in LC-treated IRI mice worsens AKI (49). Following AKI, once pathogens or injured cells are cleared up, a rapid polarization of macrophage phenotypes is necessary for tissue regeneration. During the regeneration process there is a restoration of the polarized tubular epithelium and basement membrane integrity, together with neovascularization, ensuring the recovery of tissue oxygenation by the injured microvasculature, leading to the reestablishment of the tubular cell functionality (60, 61). Therefore, a decrease in infiltrating M1 macrophages is essential to minimize injury of surrounding cells, since macrophages release cytotoxic compounds that do not distinguish self from exogenous pathogens (62, 63). Preclinical studies have described high levels of IL-4, IL-10, and IL-13, leading to a macrophage switch to M2 phenotype characterized by high expression of arginase-1 (Arg1), mannose receptor (MR, also termed CD206), chitinase-like protein (e.g., Ym1), resistin-like protein (Fizz1) and CD36 (fatty acid translocase), associated with down-regulated expression of proinflammatory markers (i.e., IL-12 and iNOS) (49). On the other hand, the prolonged presence of infiltrating macrophages in the kidney might be associated with persistent release of wound-healing growth factors, such as TGF-β1. This may turn the initial wound healing process to pathological one, resulting in further tissue damage, and contributing to the AKI-to-CKD progression, and irreversible fibrosis (64). Nevertheless, data on human macrophage phenotypes and related cytokines in healthy and AKI kidneys have been scarcely studied, limiting the translation of preclinical studies to humans. Moreover, the mechanisms enabling macrophage switch from the M1 to M2 subset remain unclear (49). Studies of human AKI biopsies have identified macrophages as the main cell type infiltrating the kidney that persist during tissue repair, being CD163 expressing -macrophages the predominant phenotype in the late phase of AKI (50, 61). Future studies in human AKI at different disease stages are needed.

Macrophages in CKD

Kidney infiltration by macrophages is common in human CKD. The magnitude of macrophage infiltration correlates with the severity of kidney injury suggesting an effector function of macrophages in CKD (19). However, the role of M1/M2 macrophages in human CKD progression is still poorly understood. In experimental progressive CKD, M1 macrophages are present in the early phases of inflammation (23) and, as this process progresses, M2 macrophages predominate to encourage repair and/or fibrosis (65), as described in unilateral ureteral obstruction (UUO) model (57, 66, 67). Some studies have suggested that the M1/M2 macrophage balance could influence CKD development (68, 69). Both M1 and M2 responses coexist during CKD (68). Indeed, M2 macrophages could originate from M1 macrophages or from proliferation/differentiation of monocytes (23, 65, 68). Some evidences suggest that macrophages can directly promote kidney fibrosis. The $CD206^{+}$ subset of M2 macrophages is strongly associated with kidney fibrosis in both human and experimental diseases (23). Indeed, bone marrow-derived M2-type pro-fibrotic macrophages are highly proliferative, which may contribute to promote kidney fibrosis in experimental models such as UUO and the Adriamycin nephropathy (70–72).

Macrophages in Hypertension and Related Kidney Damage

In experimental hypertension, M2 macrophages expressing Mouse Chitinase 3-like 3(Chi3l3)/YM1were associated with renal fibrosis (73). Macrophages may promote hypertension through the generation of M1 cytokines, such as TNF-α, IL-6, and IL-1β, and reactive oxygen species (ROS) resulting in enhanced renal sodium retention and organ damage (74, 75). In this sense, recent studies suggest that the pro-inflammatory cytokine IL-17A, produced by $CD4^+$/T and γδ-lymphocytes, but by not macrophages, plays a key role in the onset of hypertension and in hypertensive end-organ damage, such as the heart, vessels and kidneys (76, 77). However, the Th17 phenotype is sustained by interleukin-23 (IL-23), produced mainly by M1 macrophages (16), showing an interplay between macrophages and immune cells in hypertension.

NOVEL MACROPHAGE-DERIVED FACTORS AS POTENTIAL BIOMARKERS IN CKD

Macrophages can release a wide array of cytokines, which varies depending on pathological conditions. One well-known product of macrophages is MCP-1/CCL2, a chemokine driving their recruitment into injured tissues (78). Some clinical data suggest that MCP-1/CCL2 could be a biomarker of kidney fibrosis and function decline (79, 80). As commented above, M1 macrophages can produce TNF-α, IL-1β, IL-6, IL-12, IL-15, IL-18, and IL-23 whereas M2 macrophages release TGF-β1, IL-10, CCL17, CCL18, and CCL22 (**Figure 2**). Therefore, macrophage-derived biomarkers in blood and other biological fluids can reflect the activation of macrophage populations in tissues. In this sense, IL-6 is now considered an important cardiovascular risk biomarker (81). Some of these macrophage-derived cytokines can be relevant as biomarkers of kidney disease progression. Additionally, in the AKI-to-CKD transition, the evaluation of M1/M2 macrophage markers or secretome-derived factors can be used to monitor disease progression and/or remission (23). We will now focus in two interesting candidates, the macrophage derived cytokine CCL18, and the macrophage marker CD163.

CCL18 and Macrophage Functions

Chemokine (C-C motif) ligand 18 (CCL18) regulates several inflammatory and immunological processes, participating in cell recruitment (82, 83) and in phenotype transformations in cancer cells (84). This chemokine is constitutively expressed in the lung and in antigen presenting cells, such as dendritic cells and keratinocytes (83). CCL18 is one of the most highly expressed chemokines in human chronic inflammatory diseases, including allergies, fibrotic disorders and certain cancers (83). As commented before, M2 macrophages secrete high amounts of CCL18 (35). Moreover, stimulation of monocyte/macrophages with CCL18 induces an M2 spectrum macrophage phenotype (20).

CCL18 as Biomarker of Disease Progression

In some diseases, CCL18 levels are used as a biomarker of disease progression, for instance in Gaucher disease (85) idiopathic pulmonary fibrosis (86), and chronic periaortitis (87), as well as in several proliferative disorders, including breast (88) and lung (89) cancer, glioblastoma (90), bladder cancer (91), osteosarcoma (92), and prostate cancer (93, 94). However, there is scarce information in kidney diseases. Serum CCL18 levels were proposed as a biomarker of disease activity in ANCA-associated crescentic glomerulonephritis (95). In patients with CKD undergoing peritoneal dialysis treatment, CCL18 levels in peritoneal effluent correlated with progressive ultrafiltration failure and peritoneal fibrosis, suggesting that CCL18 could also be a biomarker of peritoneal damage (96).

Mouse CCL8 Is the Functional Analog of Human CCL18 and Shares CCR8 as Functional Receptor

The human CC chemokine receptor 8 (CCR8) is a seven-transmembrane-spanning G protein–coupled receptor, whose canonical ligand is Chemokine (C-C motif) ligand 1 (CCL1/I-309) (97). CCL18 was recently discovered as another CCR8 agonist with less affinity than CCL1 (98). CCR8 is expressed mostly in monocytes and thymus and acts as chemoattractant receptor for Th2 cells (99). Moreover, this receptor was found in lymphocytes in human healthy skin for preserving tissue homeostasis (100). However, CCR8 is not expressed in human kidneys in normal or pathological conditions, such as renal transplant rejection (101). In contrast, CCR8 expression was detected mainly in tumor renal cells of human renal cell carcinoma (102). Accordingly, upregulated expression of CCR8 has been described in circulating cells of patients with bladder and renal carcinoma (103).

Human CCL18 and mouse chemokine (C-C motif) ligand 8 (CCL8), named here as mCCL8, have been proposed to be functional analogs (98). The CCL18 gene has only orthologs in primates (104), whereas mouse *Ccl8* gene (also known as *Mcp-2*) lacks a human ortholog as showed by phylogenetic analysis and synteny mapping, but binds to CCR8 (105), and shares functions with CCL18 (98). Importantly, human CCL8/MCP2 is a different cytokine, exerting chemotactic actions for macrophages, and signaling through CCR1 and CCR5 (106). Therefore, to avoid confusion along the manuscript we have named mouse CCL8 here as m*Ccl8*.

Human CCL18 and Mouse CCL8 in Human and Murine Kidney Disease, Respectively

A microarray analysis of renal biopsy samples of patients with newly diagnosed ANCA-associated crescentic glomerulonephritis identified CCL18 as the most upregulated gene (95). Immunohistochemical analysis identified myeloid dendritic cells and $CD68^+$ macrophages as CCL18-producing cells, and determined that the density of $CCL18^+$ cells correlated with interstitial inflammation, crescent formation and impairment of renal function at the time of biopsy. Serum CCL18 levels also correlated with kidney disease activity, being lower in patients with immunosuppressive rescue therapy and higher in relapsing kidney disease (95). In accordance to these

data, we found that $CD163^+$/CCL18 expressing macrophages colocalized with Gremlin protein expression in another cohort of patients with ANCA-associated crescentic glomerulonephritis (107). In this study, we proposed urinary Gremlin levels as a potential biomarker of disease progression, showing a parallelism to CCL18. Despite these comprehensive studies, there are no published data on CCL18 in other human kidney diseases. In cultured human tubular cells, CCL18 increased the production of fibronectin in diabetic conditions (108). On the other hand, there is scarce information about the role of mCCL8 in preclinical kidney disease. In murine renal artery stenosis, kidney m*Ccl8* gene expression was higher than in control mice (109). However, in cultured murine tubular cells, we have found that these cells lack *Ccr8* expression and were not responsive to mouse recombinant CCL8 protein (110). In experimental folic acid-induced AKI, kidney m*Ccl8* expression was unchanged in the acute phase (24–72 h). However, kidney m*Ccl8* expression was upregulated at 7 days, a time point associated to the AKI-to-CKD transition. In UUO, kidney m*Ccl8* expression was already increased at 5 days (111), and increased progressively over time (**Figure 3**). The evaluation of M1/M2 cytokine profiles showed that kidney m*Ccl8* expression correlates to M1-related cytokine downregulation and M2 cytokine overexpression. Therefore, these data suggest a potential role of m*Ccl8* in mouse macrophage polarization toward the M2 phenotype, helping to maintain chronic inflammation and favoring kidney fibrosis.

We previously showed that increased M2 macrophage numbers associated to peritoneal CCL18 effluent concentrations in patients with CKD undergoing peritoneal dialysis (96). Moreover, in a murine model of exposure to peritoneal dialysis fluids (112) we have found that m*Ccl8* expression was increased associated to macrophage infiltration (113), supporting the concept of mouse *Ccl8* as a functional analog of human CCL18.

CD163 in Kidney Injury

CD163 is a glycosylated membrane protein, member of the scavenger receptor cysteine-rich family, also known as the hemoglobin scavenger receptor (13, 114, 115). CD163 is expressed nearly exclusively by cells of monocytic origin (e.g., monocytes, macrophages, some dendritic cells, and some tumor cells) presenting high expression levels in macrophages (13, 114, 115). By ectodomain shedding, the extracellular portion of CD163 can be released from the cell surface into circulation, in a process regulated by ADAM17/TACE (tumor necrosis factor α-converting enzyme) (116). Soluble CD163 (sCD163) is stable and measured easily in serum, and has been investigated as potential biomarker of macrophage activation in various disease contexts (117–119).

CD163 functions have been extensively reviewed (120, 121). Early studies described that CD163 expression is a hallmark of the wound healing macrophage, related to the resolution phase of inflammation after cardiopulmonary bypass surgery (122) and in human inflammatory skin disease induced by Cantharidin (123). Some studies in animal models of renal damage pointed out the key role of CD163 in the beginning and progression of renal disease (124–126). In AKI induced by experimental rhabdomyolysis increased levels of M1 macrophages were observed in the early pathological stages. These macrophages suffer a partial differentiation to M2 phenotype characterized by CD163 overexpression via HO-1 activation and IL-10 release. Moreover, peritoneal macrophages stimulated by myoglobin can induce fibrosis through the regulation of profibrotic mediators, such as TGF-β and CTGF/CCN2 (124). In cisplatin-induced nephrotoxic damage in rats increased levels of CD163 M2 macrophages associated to fibrosis were found (126). Other study in Lupus nephritis (LN) in mice described an increased $CD163^+/CD68^+$cell ratio. In addition, *Cd163* gene expression was increased in LN mice while *Ho-1*, levels were reduced, the last one associated to elevated *Bach1* and *Il-6* expression. The gene blockade of *Bach1* in mice (Bach1-deficient MRL/lpr mice) improved the loss of renal function in experimental LN assessed by BUN levels (125).

In vitro studies in human macrophages found that CD163 overexpression induces a change on the cytokine profile secretion from pro-inflammatory M1-related cytokines to M2-cytokines (127). Importantly, $CD163^+/CD68^+$ macrophages may be involved in the pathogenesis of proliferative glomerular crescents, such as in ANCA-associated glomerulonephritis or active LN. $CD163^+/CD68^+$ macrophages were found in glomerular crescents and were correlated to proteinuria and to estimated glomerular filtration rate (eGFR) (positively and negatively, respectively) (128). Moreover, two different M2 macrophages populations, $CD163^+$ and $CD206^+$, were mainly expressed in fibrous crescents and were more common in Lupus nephritis (LN) and ANCA-associated vasculitis than in IgA nephropathy and Henoch Schönlein purpura glomerulonephritis (129, 130). Patients with early stage of idiopathic membranous nephropathy had higher levels of circulating $CD14^+/CD163^+$, $CD14^+/CD163^+/CD206^+$, and $CD14^+/CD163^+/CD206^+/CD115^+$ macrophages in comparison with healthy controls (131). M2-macrophages were considered the dominant subpopulation in human LN and M2a subpopulations were associated with disease progression (132). In ANCA glomerulonephritis, urinary $CD11b^+$ and $CD163^+$ correlated with leukocyte recruitment in the kidney (133). Indeed, urinary sCD163 is a marker of human glomerulonephritis (118, 134) and active renal vasculitis (135, 136). On the other hand in LN (119), sCD163 in the urine is considered as a marker of disease activity and treatment response (137, 138). Interestingly, $CD163^+$ cells in crescents of ANCA glomerulonephritis patients colocalized with CCL18 (95) and Gremlin (107). Both CCL18 and Gremlin levels have been independently proposed as potential biomarkers of disease progression (95, 107). These two independent studies suggest that the M2 macrophage secretome could be a source of biomarkers of kidney disease progression, mainly in crescentic glomerulonephritis. Accordingly, in peritoneal dialysis patients, $CD163^+$ macrophages were present in peritoneal effluents and increased during peritonitis. Moreover, peritoneal CCL18 effluent concentrations correlated with decreased peritoneal function, showing the contribution of macrophage-derived cytokines to peritoneal damage and fibrosis (96). Despite there are few data about sCD163 in human diabetic nephropathy some studies described that sCD163 levels were also strongly

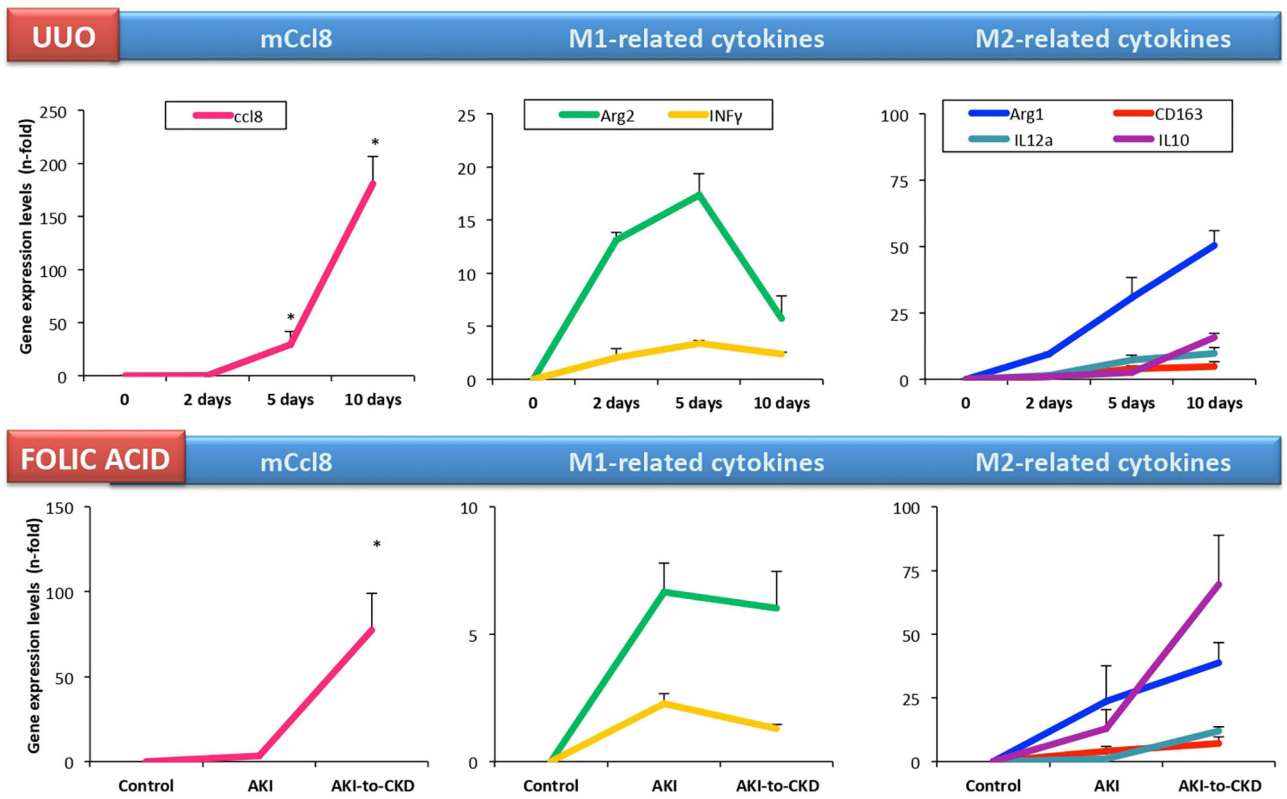

FIGURE 3 | Kidney *Ccl8* gene expression is associated to M2 cytokines in experimental kidney injury. Kidney *Ccl8* expression and M1 and M2-related factors were evaluated in CKD models: unilateral ureteral obstruction (UUO) and the acute and chronic phase of folic acid (FA) nephropathy mice [data from Cantero-Navarro et al. (110)].

associated with later development of type 2 diabetes in both lean and obese subjects, likely reflecting macrophage recruitment in the adipose tissue (117, 139–143) and in the liver (144–146), an effect associated to ADAM17/TACE-mediated shedding of TNF-α and sCD163 (116). Diabetic patients had higher numbers of circulating $CD163^+$ monocytes (147). In addition, sCD163 was also identified as a good risk biomarker of diabetic nephropathy and/or diabetic retinopathy (148). Anti-inflammatory $CD163^+$ macrophages were elevated in glomeruli from diabetic patients and were associated with pathological features such as tubular atrophy, interstitial fibrosis and glomerulosclerosis (149). In murine RAW264.7 macrophages preincubated with high glucose, calcitriol ($1,25(OH)_2D_3$) treatment blockaded M1 macrophage activation and M2 phenotype differentiation. The same result was observed in streptozotocin (STZ)-diabetic rats treated with calcitriol (150). Moreover, the uremic toxins that are accumulated in the last stage of renal disease have a role in M2 induction. A study in THP-1 cells showed that indoxil sulfate (IS) induced CD163 expression and transition to macrophages through AhR/Nrf2 activation (151). Finally, other study linked the increase of fat mass with elevated levels of sCD163, suggesting that adipose tissue macrophages play a key role in CKD proinflammatory state (140).

sCD163 was also been found in heme-related human kidney injury, such IgA nephropathy (152), intravascular hemolysis (e.g., paroxysmal nocturnal hemoglobinuria) (153), favism (154), and rhabdomyolysis AKI (124). In macrophages of patients with IgA nephropathy and macroscopic hematuria-related AKI, CD163 was associated with incomplete recovery of kidney function (152, 155) describing as the predominant subpopulations in kidney tissues, the M2a (CD206+/CD68+) and M2b (CD86+/CD68+) macrophages (130).

TARGETING MACROPHAGE RELATED PRO-INFLAMMATORY CYTOKINES AS THERAPEUTIC APPROACH FOR KIDNEY DISEASE

There have been multiple attempts to inhibit or modulate the inflammatory response to prevent or retard CKD progression. The most widely used approaches to modulate macrophage levels and/or phenotypes have been directed to M1-related cytokines, such as IL1, IL-6, and TNF-α. We now discuss preclinical studies targeting those inflammatory mediators and the key

monocyte/macrophage chemotactic factor MCP-1/CCL2, and the potential translation to humans.

Interleukin-1 Blockade in Experimental and Human Kidney Disease

The earliest studies targeting macrophage related cytokines tested IL-1 strategies. Treatment with an IL-1 receptor (IL-1R) antagonist (IL-1Ra) ameliorated experimental anti-glomerular basement membrane antibody-associated glomerulonephritis (anti-GBM) in rats (156) as well as spontaneous IgA nephropathy in ddY mice (157). Gene deletion of IL-1 Type 1 Receptor (IL1R1) or IL-1β demonstrated that IL-1β but not IL-1α contributed to crescent formation and inflammatory cell recruitment in murine anti-GBM crescentic glomerulonephritis (158). Similarly, genetic IL1R1 deletion modestly improved survival and attenuated cyst volume in experimental murine ADPKD (159). The IL-1R1 antagonist anakinra prevented nephropathy in diabetic mice (160). Accordingly, anti-IL-1β antibody attenuated the progressive loss of kidney function and preserved podocytes in murine loss of kidney mass in diabetic *db/db* mice (161). Human studies also showed beneficial effects, including improved vascular endothelial function in patients with non-dialysis-dependent CKD after 12 weeks of treatment with the IL-1 inhibitor rilonacept, whereas no changes in kidney function were observed nor expected in such a short follow-up (162). A clinical trial of the canakinumab (neutralizing antibody against IL-1β) observed a reduction in the cardiovascular event rates in atherosclerosis patients with CKD without modifying kidney function (163). However, a clinical trial of gevokizumab (antibody against IL-1β) in diabetic kidney disease was terminated prematurely, based on company priorities (164).

TNF-α Blockade

There are multiple preclinical examples of kidney protection afforded by anti-TNF strategies. Administration of a pegylated form of the soluble TNF type 1 receptor (PEG-sTNFR1) reduced renal fibrosis in experimental rat CKD induced by renal mass reduction (165) and kidney inflammation and tubular cell apoptosis in rat UUO (166). Similarly, TNF-α gene deletion or silencing attenuated kidney injury induced by high fat diet in mice by reducing fibrosis and glomerulosclerosis (167). A neutralizing anti-TNF-α antibody reduced glomerular inflammation, crescent formation, and tubulointerstitial scarring, and preserved kidney function in rat anti-GBM crescentic glomerulonephritis (168). An anti-TNF-α antibody decreased albuminuria, plasma creatinine, histopathologic changes, and kidney macrophage recruitment in an experimental type I diabetes in B6-Ins2Akita/MatbJ mice (169). Moreover, macrophage-specific TNF-α-deficient mice ($CD11b^{Cre}/TNF\text{-}\alpha^{Flox/Flox}$; C57BL/6) presented the same beneficial effects after streptozotocin-induced diabetes (169). In high fat diet-induced kidney injury, TNF-α deletion reduced kidney fibrosis, glomerulosclerosis, oxidative stress, inflammation and apoptosis (167). Despite promising preclinical results, targeting TNF-α in human kidney disease for kidney protection is controversial. A clinical trial of infliximab in LN failed in the recruitment phase (NCT00368264). However, anti-TNF-α monoclonal antibodies are used routinely to treat rheumatoid arthritis (RA), ankylosing spondylitis (AS) or psoriasis. Thus, there is mixed information on such patients and kidney disease. Some reports observed that in RA patients with CKD, anti-TNF-α drugs (adalimumab, etanercept, or infliximab) had no deletereous effect on kidney function (170) or presented slower loss of renal function (171). However, cases of AKI, focal segmental glomerulosclerosis (FSGS) or IgA nephropathy have been reported in AS, RA, or inflammatory Bowel Disease patients treated with anti-TNF-α drugs (172–176).

IL-6 Blockade in Experimental and Human Kidney Disease

Due to the key role of IL-6 in kidney diseases (177), the impact of targeting IL-6 has been studied. However, preclinical results differed for some kidney diseases. Neutralizing IL-6 (178), IL-6 receptor (IL-6R) (179) or genetic IL-6 deletion (180) decreased disease severity in different experimental models of LN in MRL-Fas^{lpr} and NZB/WF1 mice. However, anti-IL-R6 or anti-IL-6 strategies increased the severity of murine anti-GBM nephritis, while selective inhibition of IL-6 trans-signaling by sgp130Fc did not (181). Similarly, genetic IL-6 deletion did not decrease fibrosis in murine UUO (182), whereas treatment with Fc-gp130 reduced inflammation, immune cell infiltration and fibrosis in both murine UUO and IRI (65). These findings fit well with the concept that IL-6 classic signaling is anti-inflammatory and protective, whereas IL-6 trans-signaling is pro-inflammatory.

In addition to inflammation, IL-6 controls glucose metabolism and the hypothalamic-pituitary-adrenal axis among other processes which could result in deleterious effects when IL-6 is modulated (183). Therapies modulating IL-6 (olokizumab or clazakizumab) or the α subunit of its receptor (tocilizumab or sarilumab) have been used clinically to treat inflammatory diseases, such as RA, systemic lupus erythematous (SLE), diabetes and, more recently, coronavirus disease 2019 (COVID-19) (184, 185) that may develop kidney disease, but no clinical trial studied kidney function or renal injury as primary outcome. An ongoing trial is exploring the efficacy of clazakizumab to preserve kidney function in highly sensitized patients awaiting kidney transplantation (NCT03380962) or in kidney transplant receptors with late antibody-mediated rejection (NCT03444103).

MCP-1/CCL2 Blockade

MCP-1/CCL2 is the main factor driving monocyte recruitment and differentiation during inflammatory response. Therefore, MCP-1/CCL2 has been targeted in preclinical kidney disease (186). Both genetic deficiency of the MCP-1 receptor (CCR2) or MCP-1/CCL2 antagonism improved LN and vasculitis in MRL/lpr mice (187, 188). Likewise, MCP-1/CCL2 neutralizing antibody reduced glomerular macrophage infiltration and decreased crescent formation in experimental rat and murine crescentic glomerulonephritis (189, 190). Targeting MCP-1/CCL2/CCR2 was also protective in experimental diabetic nephropathy. A CCR2 antagonist (propagermanium) protected the kidneys in type 1 diabetic mice overexpressing type 2 nitric-oxide synthase (191), MCP-1/CCL2 gene deletion decreased glomerular and interstitial macrophage accumulation

and fibrosis in murine STZ-induced diabetic nephropathy (192), and the MCP-1/CCL2 antagonistic Spiegelmer mNOX-E36 (emapticap pegol) reduced the number of glomerular macrophages and improved glomerular filtration rate (GFR) in uninephrectomized *db*/*db* mice (193) and in STZ- diabetic ApoE knockout mice (194). However, in experimental murine Alport nephropathy (Col4a3-deficient mice), mNOX-E36 administration reduced glomerular and interstitial macrophage recruitment, but did not improve glomerular or interstitial histopathology or survival (195).

In humans, phase 2 clinical trials were promising in diabetic kidney disease. A selective inhibitor of CCR2 (CCX140-B) added to standard care (196) and the Spielgelmer NOX-E36 showed evidence of kidney protection in patients with type 2 diabetes and kidney disease (197). However, there are no current ongoing phase 3 trials. In this regard, the standard of care has been changed by the efficacy of sodium-glucose cotransporter-2 (SGLT2) inhibitors and any future trial should test the efficacy of new drugs on top of SGLT2 inhibition and renin-angiotensin system (RAS) blockade (198). On the other hand, there are ongoing phase 2 trials of CCX140-B (Ilacirnon) for FSGS (NCT03536754, NCT03703908).

POLARIZATION OF M1 TO M2 MACROPHAGES IN EXPERIMENTAL KIDNEY DISEASE

Inducing macrophage polarization has been also proposed as a potential approach to reduce the kidney inflammatory response (21, 199). Strategies to induce macrophage polarization, that include stimulation with cytokines, miRNAs regulation or genetic manipulation (199), have been extensively investigated in preclinical studies to reduce the inflammatory response in kidney diseases.

Cytokine-Induced Polarized Macrophages

Cell stimulation with different cytokines has been used to induce macrophage phenotype polarization and polarized cells were subsequently administered to treat experimental kidney disease (199). Administration of spleen derived macrophages (SPDM) polarized to M2 phenotype by IL-4/IL-13 stimulation *ex vivo* decreased histological and functional kidney injury as compared to M1 macrophages in experimental adriamycin nephropathy (AN) in immunodeficient mice (200). Likewise, transference of M2 macrophages generated *ex vivo* by incubation of SPDM with IL-10/TGF-β1 decreased kidney inflammation, structural injury and functional decline in the same model (201). Similarly, the administration of M1 macrophages induced by incubation with INF-γ increased tubular injury in murine IRI while M2 macrophages induced by incubation with IL-4 did not (59). In STZ-induced diabetic mice, transfusion of SPDM polarized to M2 by incubation with IL-4/IL-13 decreased tubular atrophy, glomerular hypertrophy, interstitial expansion and kidney fibrosis (202). In murine nephrotoxic nephritis, the transfusion of bone marrow-derived macrophages (BMDM) polarized to M2 phenotype ($CD206^+$) by e*x vivo* incubation with IL-4/IL-13 reduced kidney injury, proteinuria, and glomerular inflammatory cell infiltration (203). Treatment with both BMDM polarized *ex vivo* to M2 macrophages by IL-4/IL-13 as well as *in vivo* M2 polarization induced by IL-4/IL-13 injections, reduced renal crystal formation in murine experimental kidney stone disease (204). A more recent study corroborated the beneficial effects of M2 transplantation in AKI. Peritoneal macrophages isolated from mice under peritoneal dialysis were polarized to M2 by incubation with IL-4/IL-13 and then injected into the renal cortex of mice with experimental ischemia reperfusion injury (IRI). M2 macrophages administration improved kidney injury and decreased inflammation compared to those injected with non-activated (M0) macrophages (205). Finally, the intrarenal administration of an Elastin-like Polypeptide (ELP-VEGF) construct induced a clear polarization to M2 macrophage phenotype in CKD in pigs and improved renal hemodynamics and fibrosis, despite no differences in renal macrophage infiltration were found compared to control (206).

Polarized Macrophages Induced by Genetic Manipulation

Macrophages have been used successfully in cell therapy to deliver targeted therapeutic genes in models of inflammatory kidney disease. The adoptive transfer of bone-marrow-derived macrophages (BMDM) genetically modified with adenoviral particles encoding the anti-inflammatory cytokine interleukin 1 receptor antagonist (IL-1Ra) reduced kidney injury in murine anti-GBM (207). Similar results were observed using the same approach in unilateral ureteral obstruction (UUO) mice: the transference of macrophages encoding IL-1Ra after ureter obstruction reduced kidney interstitial macrophage infiltration, overall inflammation and fibrosis (208). In the same way, rat alveolar macrophages (NR8383) transfected with an adenovirus encoding IL-4 reduced albuminuria and glomerular macrophage infiltration (209). The delivery of IL-10-expressing macrophages or a dominant-negative I-KB in rat nephrotoxic nephritis also reduced kidney injury (210, 211). In murine kidney ischemia reperfusion injury (IRI), the transference of adenoviral transduced BMDM encoding Heme oxygenase-1 (HO-1)preserved kidney function (212). Similar results were observed in a rat kidney IRI. Herein, transference with adenoviral-IL-10-transduced BMDM markedly reduced albuminuria, the number of pro-inflammatory macrophages and fibrosis (210).

Macrophage Polarization Induced by miRNAs

MicroRNAs (miRNAs) are small, single-stranded non-coding RNAs (20–24 nucleotides) that regulate post-transcriptional gene expression (213). Since their discovery, multiple potential roles have been attributed to these molecules, including their participation in the inflammatory response by regulating multiple processes, including macrophage phenotype polarization (214). The miRNA expression profiles in M1 and M2 macrophages have been characterized both in human and in murine macrophages (215–217) (**Table 1**). A recent

TABLE 1 | MicroRNA and macrophage polarization.

MicroRNA	Polarization	References
miR-9	M1	(214)
miR-127		(214)
miR-155		(214)
miR-125b		(214)
miR-9-5p		(214, 218)
pre-miR21		(219)
miR-199a-5p		(220)
miRNA-19b-3p		(221)
miR-124	M2	(214)
miR-223		(214)
miR-34a		(214)
miR-132		(214)
miR-125a-5p		(214)
miR-146a		(214, 222, 223)
mature miR-21		(219)

review highlighted that miR-9, miR-127, miR-155, and miR-125b promote M1 polarization while miR-124, miR-223, miR-34a, let-7c, miR-132, miR-146a, and miR-125a-5p induce the M2 phenotype in both species (214). The relevance of some of these miRNAs in the macrophage phenotype context has been also corroborated in kidney disease with different results. miR-146a regulated local chronic inflammation in autoimmune glomerulonephritis in B6.MRLc1 mice, suggesting that it is a potential therapeutic target (224). However, genetic miR-146a deletion increased proteinuria, renal macrophage infiltration, glomerular hypertrophy and fibrosis in murine streptozotocin-induced diabetes (225). The anti-inflammatory effect of miR-146 through regulation of IL-6 expression was also demonstrated in macrophages derived from cystic fibrosis patients (222). Similarly, a transwell assay using glomerular mesangial cells from LN patients stimulated with a miR-146 mimic showed a reduction in macrophage migration (223). In the same way, although miR-9 has been reported to promote pro-inflammatory macrophage phenotype, mice transfected with lentiviral vectors expressing miR-9-5p were protected from UUO-induced kidney fibrosis through decreased infiltrating monocytes/macrophages and induction of metabolic reprogramming (218).

miR-21 is a key mediator of the anti-inflammatory response in macrophages and has been proposed to exert a dual role. Pre-miR21 polarizes macrophages to the M1 phenotype while mature miR-21 polarizes to M2 and upregulates anti-inflammatory targets, such as IL-10 (219). However, although miR-21 has been widely studied in inflammatory conditions, its role in experimental kidney disease reported controversial results. Thus, while genetic deletion of miR-21 reduced kidney fibrosis in murine UUO, IRI, Alport syndrome (226, 227) and polycystic kidney disease (228), miR-21 was also reported to protect from murine kidney IRI (229). Lademirsen sodium (RG 012), an anti–miR-21, is currently undergoing phase 2 clinical trials for Alport syndrome (NCT02855268).

Apart from the miRNAs expressed directly in macrophages, miRNAs from extracellular vesicles also influence the macrophage polarization process. For example, miR-199a-5p present in extracellular vesicles from albumin-induced tubular epithelial cells promoted the M1 macrophage phenotype in mice with high fat diet (HFD)/STZ-induced diabetes (220). Similar results were obtained in another recent study demonstrating that miRNA-19b-3p from tubular epithelial-derived exosomes promotes M1 macrophage activation in a murine endotoxemia-induced AKI (221).

CONCLUSION

Macrophages actively participate in the initial phases on kidney injury, as well as in kidney damage resolution and progression. Current pharmacological treatments that present beneficial effects in human CKD diminish macrophages infiltration in the kidney (1), including the SGLT2 inhibitors (230). Future investigations targeting macrophage polarization, and macrophage-derived cytokines could provide novel therapeutic approaches to further reduce the inflammatory response in kidney diseases.

In spite of the intensive research done, the lack of consistent nomenclature, and reliable polarization markers that are conserved between species and between *in vivo* and *in vitro* models of macrophage polarization has delayed progress in the field and complicates the extrapolation of preclinical results into human kidney disease (53). Moreover, it is necessary to develop preclinical models that truly resemble the human situation, something specially relevant in human kidney diseases due to the complex etiology of CKD (1). Some studies have been focused on comparative biology approaches on macrophages to accelerate the translation into the clinic (231). Moreover, individual variation in cytokine production in the human population has been reported (232). Novel data on transcriptional machinery of different macrophage subtypes have increased the knowledge about macrophage functions (233, 234). However, the mechanisms of genetic regulation remains unraveled. Future studies using cutting-edge technologies, including single-cell RNA sequencing, genomics, and proteomics approaches, as well as spatial transcriptomics in human biopsies are needed to improve the understanding of cytokine profiles, macrophage phenotypes and functions in different human diseases, including AKI, AKI-to-CKD transition, and CKD progression.

AUTHOR CONTRIBUTIONS

LT-S and AT-M contributed to the design of the figures. MO, LM-E, LS-S, and EC-N participated in the development of mouse models and analysis of data of the unpublished data and to the draft of some parts of the manuscript. EC-N, SR-M, TB, AS, AT-M, VM, and RR-D contributed to the draft of some parts of the manuscript. JE and AO contributed to the critical review of the manuscript and the financial support of the work.

MR-O contributed to the draft of the manuscript and financial support. All the authors have reviewed the manuscript and approved the final version.

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REFERENCES

1. Ruiz-Ortega M, Rayego-Mateos S, Lamas S, Ortiz A, Rodrigues-Diez RR. Targeting the progression of chronic kidney disease. *Nat Rev Nephrol.* (2020) 16:269–88. doi: 10.1038/s41581-019-0248-y
2. Meng XM, Nikolic-Paterson DJ, Lan HY. Inflammatory processes in renal fibrosis. *Nat Rev Nephrol.* (2014) 10:493–503. doi: 10.1038/nrneph.2014.114
3. Suzuki Y, Ruiz-Ortega M, Lorenzo O, Ruperez M, Esteban V, Egido J. Inflammation and angiotensin II. *Int J Biochem Cell Biol.* (2003) 35:881–900. doi: 10.1016/S1357-2725(02)00271-6
4. Wynn TA, Chawla A, Pollard JW. Macrophage biology in development, homeostasis and disease. *Nature.* (2013) 496:445–55. doi: 10.1038/nature12034
5. Murray PJ, Wynn TA. Protective and pathogenic functions of macrophage subsets. *Nat Rev Immunol.* (2011) 11:723–37. doi: 10.1038/nri3073
6. Zhao H, Dong Y, Tian X, Tan TK, Liu Z, Zhao Y, et al. Matrix metalloproteinases contribute to kidney fibrosis in chronic kidney diseases. *World J Nephrol.* (2013) 6:84–9. doi: 10.5527/wjn.v2.i3.84
7. Hettinger J, Richards DM, Hansson J, Barra MM, Joschko AC, Krijgsveld J, et al. Origin of monocytes and macrophages in a committed progenitor. *Nat Immunol.* (2013) 14:821–30. doi: 10.1038/ni.2638
8. Italiani P, Boraschi D. From monocytes to M1/M2 macrophages: phenotypical vs. functional differentiation. *Front Immunol.* (2014) 5:514. doi: 10.3389/fimmu.2014.00514
9. Bian Z, Gong Y, Huang T, Lee CZW, Bian L, Bai Z, et al. Deciphering human macrophage development at single-cell resolution. *Nature.* (2020) 582:571–6. doi: 10.1038/s41586-020-2316-7
10. Jenkins SJ, Ruckerl D, Cook PC, Jones LH, Finkelman FD, Van Rooijen N, et al. Local macrophage proliferation, rather than recruitment from the blood, is a signature of T H2 inflammation. *Science.* (2011) 332:1284–8. doi: 10.1126/science.1204351
11. Funes SC, Rios M, Escobar-Vera J, Kalergis AM. Implications of macrophage polarization in autoimmunity. *Immunology.* (2018) 154:186–95. doi: 10.1111/imm.12910
12. Swirski FK, Nahrendorf M, Etzrodt M, Wildgruber M, Cortez-Retamozo V, Panizzi P, et al. Identification of splenic reservoir monocytes and their deployment to inflammatory sites. *Science.* (2009) 325:612–6. doi: 10.1126/science.1175202
13. Martinez FO, Gordon S. The M1 and M2 paradigm of macrophage activation: Time for reassessment. *F1000 Prime Rep.* (2014) 6:13. doi: 10.12703/P6-13
14. Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM. M-1/M-2 macrophages and the Th1/Th2 paradigm. *J Immunol.* (2000) 164:6166–173. doi: 10.4049/jimmunol.164.12.6166
15. Ley K. M1 means kill; M2 means heal. *J Immunol.* (2017) 199:2191–3. doi: 10.4049/jimmunol.1701135
16. Martinez FO, Sica A, Mantovani A, Locati M. Macrophage activation and polarization. *Front Biosci.* (2008) 13:453–61. doi: 10.2741/2692
17. Wawrocki S, Druszczynska M, Kowalewicz-Kulbat M, Rudnicka W. Interleukin 18 (IL-18) as a target for immune intervention. *Acta Biochim Pol.* (2016) 63:59–63. doi: 10.18388/abp.2015_1153
18. Shapouri-Moghaddam A, Mohammadian S, Vazini H, Taghadosi M, Esmaeili SA, Mardani F, et al. Macrophage plasticity, polarization, and function in health and disease. *J Cell Physiol.* (2018) 233:6425–40. doi: 10.1002/jcp.26429
19. Wang Y, Harris DCH. Macrophages in renal disease. *J Am Soc Nephrol.* (2011) 22:21–7. doi: 10.1681/ASN.2010030269
20. Schraufstatter IU, Zhao M, Khaldoyanidi SK, Discipio RG. The chemokine CCL18 causes maturation of cultured monocytes to macrophages in the M2 spectrum. *Immunology.* (2012) 135:287–98. doi: 10.1111/j.1365-2567.2011.03541.x
21. Chen T, Cao Q, Wang Y, Harris DCH. M2 macrophages in kidney disease: biology, therapies, and perspectives. *Kidney Int.* (2019) 95:760–73. doi: 10.1016/j.kint.2018.10.041
22. Chen YT, Hsu H, Lin CC, Pan SY, Liu SY, Wu CF, et al. Inflammatory macrophages switch to CCL17-expressing phenotype and promote peritoneal fibrosis. *J Pathol.* (2020) 250:55–66. doi: 10.1002/path.5350
23. Tang PMK, Nikolic-Paterson DJ, Lan HY. Macrophages: versatile players in renal inflammation and fibrosis. *Nat Rev Nephrol.* (2019) 15:144–58. doi: 10.1038/s41581-019-0110-2
24. Tanita K, Fujimura T, Sato Y, Lyu C, Kambayashi Y, Ogata D, et al. Bexarotene reduces production of CCL22 from tumor-associated macrophages in cutaneous T-cell lymphoma. *Front Oncol.* (2019) 9:907. doi: 10.3389/fonc.2019.00907
25. Buscher K, Ehinger E, Gupta P, Pramod AB, Wolf D, Tweet G, et al. Natural variation of macrophage activation as disease-relevant phenotype predictive of inflammation and cancer survival. *Nat Commun.* (2017) 24:8:16041. doi: 10.1038/ncomms16041
26. Hume DA. Macrophages as APC and the dendritic cell myth. *J Immunol.* (2008) 181:5829–35. doi: 10.4049/jimmunol.181.9.5829
27. Satpathy AT, Wu X, Albring JC, Murphy KM. Re(de)fining the dendritic cell lineage. *Nat Immunol.* (2012) 13:1145–54. doi: 10.1038/ni.2467
28. Niida S, Kaku M, Amano H, Yoshida H, Kataoka H, Nishikawa S, et al. Vascular endothelial growth factor can substitute for macrophage colony- stimulating factor in the support of osteoclastic bone resorption. *J Exp Med.* (1999) 190:293–8. doi: 10.1084/jem.190.2.293
29. Pollard JW. Trophic macrophages in development and disease. *Nat Rev Immunol.* (2009) 9:259–70. doi: 10.1038/nri2528
30. Miller JC, Brown BD, Shay T, Gautier EL, Jojic V, Cohain A, et al. Deciphering the transcriptional network of the dendritic cell lineage. *Nat Immunol.* (2012) 13:888–99. doi: 10.1038/ni.2370
31. Fleetwood AJ, Lawrence T, Hamilton JA, Cook AD. Granulocyte-macrophage colony-stimulating factor (CSF) and macrophage CSF-dependent macrophage phenotypes display differences in cytokine profiles and transcription factor activities: implications

for CSF blockade in inflammation. *J Immunol.* (2007) 178:5245–52. doi: 10.4049/jimmunol.178.8.5245
32. Lacey DC, Achuthan A, Fleetwood AJ, Dinh H, Roiniotis J, Scholz GM, et al. Defining GM-CSF– and macrophage-CSF–dependent macrophage responses by *in vitro* models. *J Immunol.* (2012) 188:5752–65. doi: 10.4049/jimmunol.1103426
33. Rodriguez RM, Suarez-Alvarez B, Lavín JL, Ascensión AM, Gonzalez M, Lozano JJ, et al. Signal integration and transcriptional regulation of the inflammatory response mediated by the GM-/M-CSF signaling axis in human monocytes. *Cell Rep.* (2019) 29:860–72.e5. doi: 10.1016/j.celrep.2019.09.035
34. Sica A, Mantovani A. Macrophage plasticity and polarization: *in vivo* veritas. *J Clin Invest.* (2012) 122:787–95. doi: 10.1172/JCI59643
35. Little AC, Pathanjeli P, Wu Z, Bao L, Goo LE, Yates JA, et al. IL-4/IL-13 stimulated macrophages enhance breast cancer invasion via rho-GTPase regulation of synergistic VEGF/CCL-18 signaling. *Front Oncol.* (2019) 9:456. doi: 10.3389/fonc.2019.00456
36. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol.* (2008) 8:958–69. doi: 10.1038/nri2448
37. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol.* (2004) 25:677–86. doi: 10.1016/j.it.2004.09.015
38. Zizzo G, Hilliard BA, Monestier M, Cohen PL. Efficient clearance of early apoptotic cells by human macrophages requires M2c polarization and MerTK induction. *J Immunol.* (2012) 189:3508–20. doi: 10.4049/jimmunol.1200662
39. Lurier EB, Dalton D, Dampier W, Raman P, Nassiri S, Ferraro NM, et al. Transcriptome analysis of IL-10-stimulated (M2c) macrophages by next-generation sequencing. *Immunobiology.* (2017) 222:847–56. doi: 10.1016/j.imbio.2017.02.006
40. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, et al. Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity.* (2014) 41:14–20. doi: 10.1016/j.immuni.2014.06.008
41. Wang Y, Smith W, Hao D, He B, Kong L. M1 and M2 macrophage polarization and potentially therapeutic naturally occurring compounds. *Int Immunopharmacol.* (2019) 70:459–66. doi: 10.1016/j.intimp.2019.02.050
42. Batista-Gonzalez A, Vidal R, Criollo A, Carreño LJ. New insights on the role of lipid metabolism in the metabolic reprogramming of macrophages. *Front Immunol.* (2020) 10:2993. doi: 10.3389/fimmu.2019.02993
43. Van den Bossche J, O'Neill LA, Menon D. Macrophage immunometabolism: where are we (going)? *Trends Immunol.* (2017) 38:395–406. doi: 10.1016/j.it.2017.03.001
44. Munder M, Eichmann K, Modolell M. Alternative metabolic states in murine macrophages reflected by the nitric oxide synthase/arginase balance: competitive regulation by CD4+ T cells correlates with Th1/Th2 phenotype. *J Immunol.* (1998) 160:5347–54.
45. Mantovani A, Biswas SK, Galdiero MR, Sica A, Locati M. Macrophage plasticity and polarization in tissue repair and remodelling. *J Pathol.* (2013) 229:176–85. doi: 10.1002/path.4133
46. Mills CD, Ley K. M1 and M2 macrophages: the chicken and the egg of immunity. *J Innate Immun.* (2014) 6:716–26. doi: 10.1159/000364945
47. Corraliza IM, Soler G, Eichmann K, Modolell M. Arginase induction by suppressors of nitric oxide synthesis (IL-4, IL-10 and PGE2) in murine bone-marrow-derived macrophages. *Biochem Biophys Res Commun.* (1995) 206:667–73. doi: 10.1006/bbrc.1995.1094
48. Spiller KL, Wrona EA, Romero-Torres S, Pallotta I, Graney PL, Witherel CE, et al. Differential gene expression in human, murine, and cell line-derived macrophages upon polarization. *Exp Cell Res.* (2016) 347:1–13. doi: 10.1016/j.yexcr.2015.10.017
49. Rossi M, Korpak K, Doerfler A, Boudjeltia KZ. Deciphering the role of heme oxygenase-1 (Ho-1) expressing macrophages in renal ischemia-reperfusion injury. *Biomedicines.* (2021) 9:306. doi: 10.3390/biomedicines9030306
50. Palmer MB, Vichot AA, Cantley LG, Moeckel GW. Quantification and localization of M2 macrophages in human kidneys with acute tubular injury. *Int J Nephrol Renovasc Dis.* (2014) 7:415–9. doi: 10.2147/IJNRD.S66936
51. Hamann J, Koning N, Pouwels W, Ulfman LH, van Eijk M, Stacey M, et al. EMR1, the human homolog of F4/80, is an eosinophil-specific receptor. *Eur J Immunol.* (2007) 37:2797–802. doi: 10.1002/eji.200737553
52. Jablonski KA, Amici SA, Webb LM, Ruiz-Rosado JDD, Popovich PG, Partida-Sanchez S, et al. Novel markers to delineate murine M1 and M2 macrophages. *PLoS ONE.* (2015) 10:e0145342. doi: 10.1371/journal.pone.0145342
53. Orecchioni M, Ghosheh Y, Pramod AB, Ley K. Macrophage polarization: Different gene signatures in M1(Lps+) vs. classically and M2(LPS-) vs. Alternatively activated macrophages. *Front Immunol.* (2019) 10:1084. doi: 10.3389/fimmu.2019.01084
54. Amici SA, Young NA, Narvaez-Miranda J, Jablonski KA, Arcos J, Rosas L, et al. CD38 is robustly induced in human macrophages and monocytes in inflammatory conditions. *Front Immunol.* (2018) 9:1593. doi: 10.3389/fimmu.2018.01593
55. Zimmerman KA, Bentley MR, Lever JM, Li Z, Crossman DK, Song CJ, et al. Single-cell RNA sequencing identifies candidate renal resident macrophage gene expression signatures across species. *J Am Soc Nephrol.* (2019) 30:767–81. doi: 10.1681/ASN.2018090931
56. Lee SB, Kalluri R. Mechanistic connection between inflammation and fibrosis. *Kidney Int.* (2010) 119:S22–6. doi: 10.1038/ki.2010.418
57. Andrade-Oliveira V, Foresto-Neto O, Watanabe IKM, Zatz R, Câmara NOS. Inflammation in renal diseases: new and old players. *Front Pharmacol.* (2019) 0:1192. doi: 10.3389/fphar.2019.01192
58. Martin-Sanchez D, Ruiz-Andres O, Poveda J, Carrasco S, Cannata-Ortiz P, Sanchez-Niño MD, et al. Ferroptosis, but not necroptosis, is important in nephrotoxic folic acid-induced AKI. *J Am Soc Nephrol.* (2017) 28:218–29. doi: 10.1681/ASN.2015121376
59. Lee S, Huen S, Nishio H, Nishio S, Lee HK, Choi BS, et al. Distinct macrophage phenotypes contribute to kidney injury and repair. *J Am Soc Nephrol.* (2011) 22:317–26. doi: 10.1681/ASN.2009060615
60. Bonventre JV, Yang L. Cellular pathophysiology of ischemic acute kidney injury. *J Clin Invest.* (2011) 121:4210–21. doi: 10.1172/JCI45161
61. Sharfuddin AA, Molitoris BA. Pathophysiology of ischemic acute kidney injury. *Nat Rev Nephrol.* (2011) 7:189–200. doi: 10.1038/nrneph.2011.16
62. Kroner A, Greenhalgh AD, Zarruk JG, PassosdosSantos R, Gaestel M, David S. TNF and increased intracellular iron alter macrophage polarization to a detrimental M1 phenotype in the injured spinal cord. *Neuron.* (2014) 83:1098–116. doi: 10.1016/j.neuron.2014.07.027
63. Huen SC, Huynh L, Marlier A, Lee Y, Moeckel GW, Cantley LG. GM-CSF promotes macrophage alternative activation after renal ischemia/reperfusion injury. *J Am Soc Nephrol.* (2015) 26:1334–45. doi: 10.1681/ASN.2014060612
64. Ricardo SD, Van Goor H, Eddy AA. Macrophage diversity in renal injury and repair. *J Clin Invest.* (2008) 118:3522–30. doi: 10.1172/JCI36150
65. Chen W, Yuan H, Cao W, Wang T, Chen WW, Yu H, et al. Blocking interleukin-6 trans-signaling protects against renal fibrosis by suppressing STAT3 activation. *Theranostics.* (2019) 9:3980–91. doi: 10.7150/thno.32352
66. Wynn TA, Barron L. Macrophages: master regulators of inflammation and fibrosis. *Semin Liver Dis.* (2010) 30:245–57. doi: 10.1055/s-0030-1255354
67. Braga TT, Correa-Costa M, Azevedo H, Silva RC, Cruz MC, Almeida MES, et al. Early infiltration of p40IL12 + CCR7 + CD11b + cells is critical for fibrosis development. *Immun Inflamm Dis.* (2016) 4:300–14. doi: 10.1002/iid3.114
68. Han Y, Ma FY, Tesch GH, Manthey CL, Nikolic-Paterson DJ. Role of macrophages in the fibrotic phase of rat crescentic glomerulonephritis. *Am J Physiol Ren Physiol.* (2013) 304:1043–53. doi: 10.1152/ajprenal.00389.2012
69. Guiteras R, Flaquer M, Cruzado JM. Macrophage in chronic kidney disease. *Clin Kidney J.* (2016) 9:765–71. doi: 10.1093/ckj/sfw096
70. Cao Q, Wang Y, Zheng D, Sun Y, Wang C, Wang XM, et al. Failed renoprotection by alternatively activated bone marrow macrophages is due to a proliferation-dependent phenotype switch *in vivo*. *Kidney Int.* (2014) 85:794–806. doi: 10.1038/ki.2013.341
71. Engel DR, Krause TA, Snelgrove SL, Thiebes S, Hickey MJ, Boor P, et al. CX 3 CR1 reduces kidney fibrosis by inhibiting local proliferation of profibrotic macrophages. *J Immunol.* (2015) 194:1628–38. doi: 10.4049/jimmunol.1402149
72. Pan B, Liu G, Jiang Z, Zheng D. Regulation of renal fibrosis by macrophage polarization. *Cell Physiol Biochem.* (2015) 35:1062–9. doi: 10.1159/000373932

73. Cavalcante PAM, Alenina N, Budu A, Freitas-Lima LC, Alves-Silva T, Agudelo JSH, et al. Nephropathy in hypertensive animals is linked to M2 macrophages and increased expression of the YM1/Chi3l3 protein. *Mediators Inflamm.* (2019) 2019:9086758. doi: 10.1155/2019/9086758
74. Norlander AE, Madhur MS, Harrison DG. The immunology of hypertension. *J Exp Med.* (2018) 215:21–33. doi: 10.1084/jem.20171773
75. Wen Y, Crowley SD. Renal effects of cytokines in hypertension. In: *Advances in Experimental Medicine and Biology*. New York, NY: Springer (2019). p. 443–54. doi: 10.1007/978-981-13-8871-2_21
76. Orejudo M, Rodrigues-Diez RR, Rodrigues-Diez R, Garcia-Redondo A, Santos-Sánchez L, Rández-Garbayo J, et al. Interleukin 17A participates in renal inflammation associated to experimental and human hypertension. *Front Pharmacol.* (2019) 10:1015. doi: 10.3389/fphar.2019.01015
77. Orejudo M, García-Redondo AB, Rodrigues-Diez RR, Rodrigues-Díez R, Santos-Sanchez L, Tejera-Muñoz A, et al. Interleukin-17A induces vascular remodeling of small arteries and blood pressure elevation. *Clin Sci.* (2020) 134:513–27. doi: 10.1042/CS20190682
78. Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. *J Interface Cytokine Res.* (2009) 29:313–25. doi: 10.1089/jir.2008.0027
79. Mansour SG, Puthumana J, Coca SG, Gentry M, Parikh CR. Biomarkers for the detection of renal fibrosis and prediction of renal outcomes: a systematic review. *BMC Nephrol.* (2017) 18:72. doi: 10.1186/s12882-017-0490-0
80. Satirapoj B. Tubulointerstitial biomarkers for diabetic nephropathy. *J Diabetes Res.* (2018) 2018:2852398. doi: 10.1155/2018/2852398
81. Chow SL, Maisel AS, Anand I, Bozkurt B, De Boer RA, Felker GM, et al. Role of biomarkers for the prevention, assessment, and management of heart failure: a scientific statement from the American Heart Association. *Circulation.* (2017) 135:e1054–91. doi: 10.1161/CIR.0000000000000490
82. Schutyser E. Involvement of CC chemokine ligand 18 (CCL18) in normal and pathological processes. *J Leukoc Biol.* (2005) 78:14–26. doi: 10.1189/jlb.1204712
83. Chenivesse C, Tsicopoulos A. CCL18 – beyond chemotaxis. *Cytokine.* (2018) 109:52–6. doi: 10.1016/j.cyto.2018.01.023
84. Wang H, Liang X, Li M, Tao X, Tai S, Fan Z, et al. Chemokine (CC motif) ligand 18 upregulates Slug expression to promote stem-cell like features by activating the mammalian target of rapamycin pathway in oral squamous cell carcinoma. *Cancer Sci.* (2017) 108:1584–93. doi: 10.1111/cas.13289
85. Deegan PB, Moran MT, McFarlane I, Schofield JP, Boot RG, Aerts MF, et al. Clinical evaluation of chemokine and enzymatic biomarkers of Gaucher disease. *Blood Cells Mol. Dis.* (2005) 35:259–67. doi: 10.1016/j.bcmd.2005.05.005
86. Prasse A, Probst C, Bargagli E, Zissel G, Toews GB, Flaherty KR, et al. Serum CC-chemokine ligand 18 concentration predicts outcome in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med.* (2009) 179:717–23. doi: 10.1164/rccm.200808-1201OC
87. Kollert F, Binder M, Probst C, Uhl M, Zirlik A, Kayser G, et al. CCL18 - Potential biomarker of fibroinflammatory activity in chronic periaortitis. *J Rheumatol.* (2012) 39:1407–12. doi: 10.3899/jrheum.111143
88. Lin L, Chen YS, Yao YD, Chen JQ, Chen JN, Huang SY, et al. CCL18 from tumor-associated macrophages promotes angio-genesis in breast cancer. *Oncotarget.* (2015) 6:34758–73. doi: 10.18632/oncotarget.5325
89. Huang H, Li J, Hu WJ, Chen C, Luo HQ, Tang XD, et al. The serum level of CC chemokine ligand 18 correlates with the prognosis of non-small cell lung cancer. *Int J Biol Markers.* (2019) 34:156–62. doi: 10.1177/1724600819829758
90. Ma L, Wang H, Li Z, Geng X, Li M. Chemokine (C-C motif) ligand 18 is highly expressed in glioma tissues and promotes invasion of glioblastoma cells. *J Cancer Res Ther.* (2019) 15:358–64. doi: 10.4103/jcrt.JCRT-360-17
91. Urquidi V, Kim J, Chang M, Dai Y, Rosser CJ, Goodison S. CCL18 in a multiplex urine-based assay for the detection of bladder cancer. *PLoS ONE.* (2012) 7:e37797. doi: 10.1371/journal.pone.0037797
92. Su Y, Zhou Y, Sun Y, jue, Wang YL, Yin J, et al. Macrophage-derived CCL18 promotes osteosarcoma proliferation and migration by upregulating the expression of UCA1. *J Mol Med.* (2019) 97:49–61. doi: 10.1007/s00109-018-1711-0
93. Chen G, Liang YX, Zhu JG, Fu X, Chen YF, Mo RJ, et al. CC chemokine ligand 18 correlates with malignant progression of prostate cancer. *Biomed Res Int.* (2014) 2014:230183. doi: 10.1155/2014/230183
94. Korbecki J, Olbromski M, Dziegiel P. Ccl18 in the progression of cancer. *Int J Mol Sci.* (2020) 21:1–24. doi: 10.3390/ijms21217955
95. Brix SR, Stege G, Disteldorf E, Hoxha E, Krebs C, Krohn S, et al. CC chemokine ligand 18 in ANCA-associated crescentic GN. *J Am Soc Nephrol.* (2015) 26:2105–17. doi: 10.1681/ASN.2014040407
96. Bellón T, Martínez V, Lucendo B, Del Peso G, Castro MJ, Aroeira LS, et al. Alternative activation of macrophages in human peritoneum: implications for peritoneal fibrosis. *Nephrol Dial Transplant.* (2011) 26:2995–3005. doi: 10.1093/ndt/gfq771
97. Roos RS, Loetscher M, Legler DF, Clark-Lewis I, Baggiolini M, Moser B. Identification of CCR8, the receptor for the human CC chemokine I-309. *J Biol Chem.* (1997) 272:17251–4. doi: 10.1074/jbc.272.28.17251
98. Islam SA, Ling MF, Leung J, Shreffler WG, Luster AD. Identification of human CCR8 as a CCL18 receptor. *J Exp Med.* (2013) 210:1889–98. doi: 10.1084/jem.20130240
99. Tiffany HL, Lautens LL, Gao JL, Pease J, Locati M, Combadiere C, et al. Identification of CCR8: a human monocyte and thymus receptor for the CC chemokine I-309. *J Exp Med.* (1997) 186:165–70. doi: 10.1084/jem.186.1.165
100. Ebert LM, Meuter S, Moser B. Homing and function of human skin γδ T cells and NK cells: relevance for tumor surveillance. *J Immunol.* (2006) 176:4331–6. doi: 10.4049/jimmunol.176.7.4331
101. Segerer S, Cui Y, Eitner F, Goodpaster T, Hudkins KL, Mack M, et al. Expression of chemokines and chemokine receptors during human renal transplant rejection. *Am J Kidney Dis.* (2001) 37:518–31. doi: 10.1053/ajkd.2001.22076
102. Fu Q, Chang Y, Zhou L, An H, Zhu Y, Xu L, et al. Positive intratumoral chemokine (C-C motif) receptor 8 expression predicts high recurrence risk of post-operation clear-cell renal cell carcinoma patients. *Oncotarget.* (2016) 7:8413–21. doi: 10.18632/oncotarget.6761
103. Eruslanov E, Stoffs T, Kim WJ, Daurkin I, Gilbert SM, Su LM, et al. Expansion of CCR8+ inflammatory myeloid cells in cancer patients with urothelial and renal carcinomas. *Clin Cancer Res.* (2013) 19:1670–80. doi: 10.1158/1078-0432.CCR-12-2091
104. Basu S, Schaefer TM, Ghosh M, Fuller CL, Reinhart TA. Molecular cloning and sequencing of 25 different rhesus macaque chemokine cDNAS reveals evolutionary conservation among C. CC, CXC, and CX3C families of chemokines. *Cytokine.* (2002) 18:140–8. doi: 10.1006/cyto.2002.0875
105. Islam SA, Chang DS, Colvin RA, Byrne MH, McCully ML, Moser B, et al. Mouse CCL8, a CCR8 agonist, promotes atopic dermatitis by recruiting IL-5+TH2 cells. *Nat Immunol.* (2011) 12:167–77. doi: 10.1038/ni.1984
106. Korbecki J, Kojder K, Simińska D, Bohatyrewicz R, Gutowska I, Chlubek D, et al. Cc chemokines in a tumor: A Review of pro-cancer and anti-cancer properties of the ligands of receptors ccr1, ccr2, ccr3, and ccr4. *Int J Mol Sci.* (2020) 21:1–29. doi: 10.3390/ijms21218412
107. Droguett A, Valderrama G, Burgos ME, Carpio D, Saka C, Egido J, et al. Gremlin, a potential urinary biomarker of anca-associated crescentic glomerulonephritis. *Sci. Rep.* (2019) 9:6867. doi: 10.1038/s41598-019-43358-5
108. Montero RM, Bhangal G, Pusey CD, Frankel AH, Tam FWK. CCL18 synergises with high concentrations of glucose in stimulating fibronectin production in human renal tubuloepithelial cells. *BMC Nephrol.* (2016) 17:1–7. doi: 10.1186/s12882-016-0352-1$
109. Kashyap S, Osman M, Ferguson CM, Nath MC, Roy B, Lien KR, et al. Ccl2 deficiency protects against chronic renal injury in murine renovascular hypertension. *Sci Rep.* (2018) 8:8598. doi: 10.1038/s41598-018-26870-y
110. Cantero-Navarro E, Orejudo M, Rodrigues-Diez RR, Bellón T, Selgas R, Ruiz-Ortega M. CCL8/MCP2, un nuevo marcador en inflamación renal. *Hipertensión y Riesgo Vasc.* (2019) 36:43—6. Available online at: https://revistas.inia.es/doi.org/10.1094/PD-66-491
111. Orejudo M, Rodrigues-Diez RR, García-Redondo AB, Alique M, Egido J, Selgas R, et al. MCP-2/CCR8 axis is activated in experimental renal and vascular inflammation. *Nephrol Dial Transplant.* (2015) 30 (Suppl 3): iii393–419. doi: 10.1093/ndt/gfv188.44

112. Rodrigues-Díez R, Aroeira LS, Orejudo M, Bajo MA, Heffernan JJ, Rodrigues-Díez RR, et al. IL-17A is a novel player in dialysis-induced peritoneal damage. *Kidney Int.* (2014) 86:303–15. doi: 10.1038/ki.2014.33
113. Orejudo M, Rodrigues-Diez RR, García-Redondo AB, Alique M, Bellón T, Egido J, et al. Investigación del papel de la quimioquina CCL-8 en el daño renal y vascular asociado a inflamación. *Hipertensión y Riesgo Vasc.* (2015) 32:1–116.
114. Zwadlo G, Voegeli R, Oslhoff KS, Sorg C. A monoclonal antibody to a novel differentiation antigen on human macrophages associated with the down-regulatory phase of the inflammatory process. *Pathobiology.* (1987) 55:295–304. doi: 10.1159/000163432
115. Ehrchen J, Steinmüller L, Barczyk K, Tenbrock K, Nacken W, Eisenacher M, et al. Glucocorticoids induce differentiation of a specifically activated, anti-inflammatory subtype of human monocytes. *Blood.* (2007) 109:1265–74. doi: 10.1182/blood-2006-02-001115
116. Etzerodt A, Maniecki MB, Møller K, Møller HJ, Moestrup SK. Tumor necrosis factor α-converting enzyme (TACE/ADAM17) mediates ectodomain shedding of the scavenger receptor CD163. *J Leukoc Biol.* (2010) 88:1201–5. doi: 10.1189/jlb.0410235
117. Møller HJ. Soluble CD163. *Scand J Clin Lab Invest.* (2012) 72:1–13. doi: 10.3109/00365513.2011.626868
118. Aendekerk JP, Timmermans SAMEG, Busch MH, Potjewijd J, Heeringa P, Damoiseaux GMC, et al. Urinary soluble CD163 and disease activity in biopsy-proven ANCA-associated glomerulonephritis. *Clin J Am Soc Nephrol.* (2020) 15:1740–8. doi: 10.2215/CJN.07210520
119. Zhang T, Li H, Vanarsa K, Gidley G, Mok CC, Petri M, et al. Association of urine sCD163 with proliferative lupus nephritis, fibrinoid necrosis, cellular crescents and intrarenal M2 macrophages. *Front Immunol.* (2020) 11:671. doi: 10.3389/fimmu.2020.00671
120. Fabriek BO, Dijkstra CD, van den Berg TK. The macrophage scavenger receptor CD163. *Immunobiology.* (2005) 210:153–60. doi: 10.1016/j.imbio.2005.05.010
121. Etzerodt A, Moestrup SK. CD163 and inflammation: biological, diagnostic, therapeutic aspects. *Antioxidants Redox Signal.* (2013) 18:2352–63. doi: 10.1089/ars.2012.4834
122. Philippidis P, Mason JC, Evans BJ, Nadra I, Taylor KM, Haskard DO, et al. Hemoglobin scavenger receptor CD163 mediates interleukin-10 release and heme oxygenase-1 synthesis: antiinflammatory monocyte-macrophage responses *in vitro*, in resolving skin blisters *in vivo*, and after cardiopulmonary bypass surgery. *Circ Res.* (2004) 94:119–26. doi: 10.1161/01.RES.0000109414.78907.F9
123. Evans BJ, Haskard DO, Sempowksi G, Landis RC. Evolution of the macrophage CD163 phenotype and cytokine profiles in a human model of resolving inflammation. *Int J Inflam.* (2013) 2013:780502. doi: 10.1155/2013/780502
124. Rubio-Navarro A, Carril M, Padro D, Guerrero-Hue M, Tarín C, Samaniego R, et al. CD163-macrophages are involved in rhabdomyolysis-induced kidney injury and may be detected by MRI with targeted gold-coated iron oxide nanoparticles. *Theranostics.* (2016) 6:896–914. doi: 10.7150/thno.14915
125. Kishimoto D, Kirino Y, Tamura M, Takeno M, Kunishita Y, Takase-Minegishi K, et al. Dysregulated heme oxygenase-1 low M2-like macrophages augment lupus nephritis via Bach1 induced by type I interferons. *Arthritis Res Ther.* (2018) 20:64. doi: 10.1186/s13075-018-1568-1
126. Nakagawa M, Karim MR, Izawa T, Kuwamura M, Yamate J. Immunophenotypical characterization of M1/M2 macrophages and lymphocytes in cisplatin-induced rat progressive renal fibrosis. *Cells.* (2021) 10:257. doi: 10.3390/cells10020257
127. Alvarado-Vazquez PA, Bernal L, Paige CA, Grosick RL, Moracho Vilrriales C, Ferreira DW, et al. Macrophage-specific nanotechnology-driven CD163 overexpression in human macrophages results in an M2 phenotype under inflammatory conditions. *Immunobiology.* (2017) 222:900–912. doi: 10.1016/j.imbio.2017.05.011
128. Li J, Liu CH, Xu DL, Gao B. Significance of CD163-positive macrophages in proliferative glomerulonephritis. *Am J Med Sci.* (2015) 350:387–92. doi: 10.1097/MAJ.0000000000000569
129. Li J, Yu YF, Liu CH, Wang CM. Significance of M2 macrophages in glomerulonephritis with crescents. *Pathol Res Pract.* (2017) 213:1215–20. doi: 10.1016/j.prp.2017.04.011
130. Hu W, Lin J, Lian X, Yu F, Liu W, Wu Y, et al. M2a and M2b macrophages predominate in kidney tissues and M2 subpopulations were associated with the severity of disease of IgAN patients. *Clin Immunol.* (2019) 205:8–15. doi: 10.1016/j.clim.2019.05.005
131. Hou J, Zhang M, Ding Y, Wang X, Li T, Gao P, et al. Circulating CD14+CD163+CD206+ M2 monocytes are increased in patients with early stage of idiopathic membranous nephropathy. *Mediators Inflamm.* (2018) 21:2018:5270657. doi: 10.1155/2018/5270657
132. Olmes G, Büttner-Herold M, Ferrazzi F, Distel L, Amann K, Daniel C. CD163+ M2c-like macrophages predominate in renal biopsies from patients with lupus nephritis. *Arthritis Res. Ther.* (2016) 18:18:90. doi: 10.1186/s13075-016-0989-y
133. Yokoe Y, Tsuboi N, Imaizumi T, Kitagawa A, Karasawa M, Ozeki T, et al. Clinical impact of urinary CD11b and CD163 on the renal outcomes of anti-neutrophil cytoplasmic antibody-associated glomerulonephritis. *Nephrol Dial Transplant.* (2020) 8:gfaa097. doi: 10.1093/ndt/gfaa097
134. Nielsen AJ, Nielsen MC, Birn H, Ivarsen P, Møller HJ, Hviid CVB. Urine soluble CD163 (sCD163) as biomarker in glomerulonephritis: stability, reference interval and diagnostic performance. *Clin Chem Lab Med.* (2020) 19:cclm-2020-0466. doi: 10.1515/cclm-2020-0466
135. O'Reilly VP, Wong L, Kennedy C, Elliot LA, O'Meachair S, Coughlan AM, et al. Urinary soluble CD163 in active renal vasculitis. *J Am Soc Nephrol.* (2016) 27:2906–916. doi: 10.1681/ASN.2015050511
136. Hatemi G, Esatoglu SN, Yazici Y. Biomarkers in vasculitis. *Curr Opin Rheumatol.* (2018) 30:30–5. doi: 10.1097/BOR.0000000000000447
137. Endo N, Tsuboi N, Furuhashi K, Shi Y, Du Q, Abe T, et al. Urinary soluble CD163 level reflects glomerular inflammation in human lupus nephritis. *Nephrol Dial Transplant.* (2016) 31:2023–33. doi: 10.1093/ndt/gfw214
138. Mejia-Vilet JM, Zhang XL, Cruz C, Cano-Verduzco ML, Shapiro JP, Nagaraja HN, et al. Urinary soluble CD163: a novel noninvasive biomarker of activity for lupus nephritis. *J Am Soc Nephrol.* (2020) 31:1335–47. doi: 10.1681/ASN.2019121285
139. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest.* (2003) 112:1796–808. doi: 10.1172/jci19246
140. Axelsson J, Møller HJ, Witasp A, Qureshi AR, Carrero JJ, Heimbürger O, et al. Changes in fat mass correlate with changes in soluble sCD163, a marker of mature macrophages, in patients with CKD. *Am J Kidney Dis.* (2006) 48:916–25. doi: 10.1053/j.ajkd.2006.08.022
141. Levy AP, Purushothaman KR, Levy NS, Purushothaman M, Strauss M, Asleh R, et al. Downregulation of the hemoglobin scavenger receptor in individuals with diabetes and the Hp 2-2 genotype: implications for the response to intraplaque hemorrhage and plaque vulnerability. *Circ Res.* (2007) 101:106–10. doi: 10.1161/CIRCRESAHA.107.149435
142. Shakeri-Manesch S, Zeyda M, Huber J, Ludvik B, Prager G, Stulnig TM. Diminished upregulation of visceral adipose heme oxygenase-1 correlates with waist-to-hip ratio and insulin resistance. *Int J Obes.* (2009) 33:1257–64. doi: 10.1038/ijo.2009.160
143. Sporrer D, Weber M, Wanninger J, Weigert J, Neumeier M, Stögbauer F, et al. Adiponectin downregulates CD163 whose cellular and soluble forms are elevated in obesity. *Eur J Clin Invest.* (2009) 39:671–9. doi: 10.1111/j.1365-2362.2009.02170.x
144. Hiraoka A, Horiike N, Akbar SMF, Michitaka K, Matsuyama T, Onji M. Soluble CD163 in patients with liver diseases: very high levels of soluble CD163 in patients with fulminant hepatic failure. *J Gastroenterol.* (2005) 40:52–6. doi: 10.1007/s00535-004-1493-8
145. Møller HJ, Grønbæk H, Schiødt FV, Holland-Fischer P, Schilsky M, Munoz S, et al. Soluble CD163 from activated macrophages predicts mortality in acute liver failure. *J Hepatol.* (2007) 47:671–6. doi: 10.1016/j.jhep.2007.05.014
146. Holland-Fischer P, Grønbæk H, Sandahl TD, Moestrup SK, Riggio O, Ridola L, et al. Kupffer cells are activated in cirrhotic portal hypertension and not normalised by TIPS. *Gut.* (2011) 60:1389–93. doi: 10.1136/gut.2010.234542
147. Min D, Brooks B, Wong J, Aamidor S, Seehoo R, Sutanto S, et al. Monocyte CD163 is altered in association with diabetic

complications: possible protective role. *J Leukoc Biol.* (2016) 100:1375–83. doi: 10.1189/jlb.3a1015-461rr
148. Samuelsson M, Dereke J, Svensson MK, Landin-Olsson M, Hillman M. Soluble plasma proteins ST2 and CD163 as early biomarkers of nephropathy in Swedish patients with diabetes, 15-34 years of age: a prospective cohort study. *Diabetol Metab Syndr.* (2017) 25:9:41. doi: 10.1186/s13098-017-0240-2
149. Klessens CQF, Zandbergen M, Wolterbeek R, Bruijn JA, Rabelink TJ, Bajema IM, et al. Macrophages in diabetic nephropathy in patients with type 2 diabetes. *Nephrol Dial Transplant.* (2017) 32:1322–9. doi: 10.1093/ndt/gfw260
150. Zhang XL, Guo YF, Song ZX, Zhou M. Vitamin D prevents podocyte injury via regulation of macrophage M1/M2 phenotype in diabetic nephropathy rats. *Endocrinology.* (2014) 155:4939–50. doi: 10.1210/en.2014-1020
151. Barisione C, Garibaldi S, Furfaro AL, Nitti M, Palmieri D, Passalacqua M, et al. Moderate increase of indoxyl sulfate promotes monocyte transition into profibrotic macrophages. *PLoS ONE.* (2016) 11:e0149276.doi: 10.1371/journal.pone.0149276
152. Cleary CM, Moreno JA, Fernández B, Ortiz A, Parra EG, Gracia C, et al. Glomerular haematuria, renal interstitial haemorrhage and acute kidney injury. *Nephrol Dial Transplant.* (2010) 25:4103–6. doi: 10.1093/ndt/gfq493
153. Ballarín J, Arce Y, Torra Balcells R, Diaz Encarnación M, Manzarbeitia F, Ortiz A, et al. Acute renal failure associated to paroxysmal nocturnal haemoglobinuria leads to intratubular haemosiderin accumulation and CD163 expression. *Nephrol Dial Transplant.* (2011) 26:3408–11. doi: 10.1093/ndt/gfr391
154. García-Camín RM, Goma M, Osuna RG, Rubio-Navarro A, Buendía I, Ortiz A, et al. Molecular mediators of favism-induced acute kidney injury. *Clin Nephrol.* (2014) 81:203–9. doi: 10.5414/CN107520
155. Gutiérrez E, Egido J, Rubio-Navarro A, Buendía I, Blanco Colio LM, Toldos O, et al. Oxidative stress, macrophage infiltration and CD163 expression are determinants of long-term renal outcome in macrohematuria-induced acute kidney injury of igA nephropathy. *Nephron Clin Pract.* (2012) 121:c42–53. doi: 10.1159/000342385
156. Tang WW, Feng L, Vannice JL, Wilson CB. Interleukin-1 receptor antagonist ameliorates experimental anti-glomerular basement membrane antibody-associated glomerulonephritis. *J Clin Invest.* (1994) 93:273–9. doi: 10.1172/JCI116956
157. Chen A, Sheu LF, Chou WY, Tsai SC, Chang DM, Liang SC, et al. Interleukin-1 receptor antagonist modulates the progression of a spontaneously occurring IgA nephropathy in mice. *Am J Kidney Dis.* (1997) 30:693–702. doi: 10.1016/S0272-6386(97)90495-9
158. Timoshanko JR, Kitching AR, Iwakura Y, Holdsworth SR, Tipping PG. Contributions of IL-1β and IL-1α to crescentic glomerulonephritis in mice. *J Am Soc Nephrol.* (2004) 15:910–8. doi: 10.1097/01.ASN.0000115704.86897.F4
159. Yang B, Fu L, Privratsky JR, Lu X, Ren J, Mei C, et al. Interleukin-1 receptor activation aggravates autosomal dominant polycystic kidney disease by modulating regulated necrosis. *Am J Physiol Ren Physiol.* (2019) 317:F221–8. doi: 10.1152/ajprenal.00104.2019
160. Shahzad K, Bock F, Dong W, Wang H, Kopf S, Kohli S, et al. Nlrp3-inflammasome activation in non-myeloid-derived cells aggravates diabetic nephropathy. *Kidney Int.* (2015) 87:74–84. doi: 10.1038/ki.2014.271
161. Lei Y, Devarapu SK, Motrapu M, Cohen CD, Lindenmeyer MT, Moll S, et al. Interleukin-1β inhibition for chronic kidney disease in obese mice with type 2 diabetes. *Front Immunol.* (2019) 10:1223. doi: 10.3389/fimmu.2019.01223
162. Nowak KL, Chonchol M, Ikizler TA, Farmer-Bailey H, Salas N, Chaudhry R, et al. IL-1 inhibition and vascular function in CKD. *J Am Soc Nephrol.* (2017) 28:971–980. doi: 10.1681/ASN.2016040453
163. Ridker PM, MacFadyen JG, Glynn RJ, Koenig W, Libby P, Everett BM, et al. Inhibition of interleukin-1β by canakinumab and cardiovascular outcomes in patients with chronic kidney disease. *J Am Coll Cardiol.* (2018) 71:2405–14. doi: 10.1016/j.jacc.2018.03.490
164. Perez-Gomez M, Sanchez-Niño M, Sanz A, Martín-Cleary C, Ruiz-Ortega M, Egido J, et al. Horizon 2020 in diabetic kidney disease: the clinical trial pipeline for add-on therapies on top of renin angiotensin system blockade. *J Clin Med.* (2015) 4:1325–47. doi: 10.3390/jcm4061325
165. Therrien FJ, Agharazii M, Lebel M, Larivire R. Neutralization of tumor necrosis factor-alpha reduces renal fibrosis and hypertension in rats with renal failure. *Am J Nephrol.* (2012) 36:151–61. doi: 10.1159/000340033
166. Meldrum KK, Metcalfe P, Leslie JA, Misseri R, Hile KL, Meldrum DR. TNF-α neutralization decreases nuclear factor-κB activation and apoptosis during renal obstruction. *J Surg Res.* (2006) 131:182–8. doi: 10.1016/j.jss.2005.11.581
167. Gai Z, Kullak-Ublick GA. TNF-α deficiency prevents renal inflammation and oxidative stress in obese mice. *Kidney Blood Press Res.* (2017) 42:416–27. doi: 10.1159/000478869
168. Khan SB, Cook HT, Bhangal G, Smith J, Tam FWK, Pusey CD. Antibody blockade of TNF-α reduces inflammation and scarring in experimental crescentic glomerulonephritis. *Kidney Int.* (2005) 67:1812–20. doi: 10.1111/j.1523-1755.2005.00279.x
169. Awad AS, You H, Gao T, Cooper TK, Nedospasov SA, Vacher J, et al. Macrophage-derived tumor necrosis factor-α mediates diabetic renal injury. *Kidney Int.* (2015) 88:722–33. doi: 10.1038/ki.2015.162
170. Hueber AJ, Tunc A, Schett G, Manger B. Anti-tumour necrosis factor alpha therapy in patients with impaired renal function. *Ann Rheum Dis.* (2007) 66:981–2. doi: 10.1136/ard.2006.069211
171. Kim HW, Lee CK, Cha HS, Choe JY, Park EJ, Kim J. Effect of anti-tumor necrosis factor alpha treatment of rheumatoid arthritis and chronic kidney disease. *Rheumatol Int.* (2015) 35:727–34. doi: 10.1007/s00296-014-3146-4
172. Stokes MB, Foster K, Markowitz GS, Ebrahimi F, Hines W, Kaufman D, et al. Development of glomerulonephritis during anti-TNF-alpha; therapy for rheumatoid arthritis. *Nephrol Dial Transplant.* (2005) 20:1400–6. doi: 10.1093/ndt/gfh832
173. Ávila PJ, Gracia Iguacel C, Ortiz Arduán A, Martín-Cleary C, Acuña Ramos M, Sainz Prestel V, et al. Fracaso renal agudo en paciente tratado con antifactor de necrosis tumoral-alfa. *Nefrologia.* (2011) 31:484–8. doi: 10.3265/Nefrologia.pre2011.May.10978
174. Yarkan Tugsal H, Zengin B, Kenar G, Can G. Ünlü M, Önen F, et al. Infliximab-associated focal segmental glomerulosclerosis in a patient with ankylosing spondylitis. *Rheumatol Int.* (2019) 39:561–7. doi: 10.1007/s00296-019-04241-8
175. PremuŽić V, Padjen I, Cerovec M, Corić M, Jelaković B, Anić B. The association of TNF-alpha inhibitors and development of IgA nephropathy in patients with rheumatoid arthritis and diabetes. *Case Reports Nephrol.* (2020) 2020:1–8. doi: 10.1155/2020/9480860
176. Strobel T, Ahmed W, De la Sancha C, Bohm M, Fischer M. IgA nephropathy in the setting of anti-TNF-α therapy for inflammatory bowel disease. *ACG Case Reports J.* (2020) 7:e00462. doi: 10.14309/crj.0000000000000462
177. Su H, Lei CT, Zhang C. Interleukin-6 signaling pathway and its role in kidney disease: an update. *Front Immunol.* (2017) 8:405. doi: 10.3389/fimmu.2017.00405
178. Liang B, Gardner DB, Griswold DE, Bugelski PJ, Song XYR. Anti-interleukin-6 monoclonal antibody inhibits autoimmune responses in a murine model of systemic lupus erythematosus. *Immunology.* (2006) 119:296–305. doi: 10.1111/j.1365-2567.2006.02433.x
179. Kiberd BA. Interleukin-6 receptor blockage ameliorates murine lupus nephritis. *J Am Soc Nephrol.* (1993) 4:58–61.
180. Cash H, Relle M, Menke J, Brochhausen C, Jones SA, Topley N, et al. Interleukin 6 (IL-6) deficiency delays lupus nephritis in MRL-Fas lpr mice: the IL-6 pathway as a new therapeutic target in treatment of autoimmune kidney disease in systemic lupus erythematosus. *J Rheumatol.* (2010) 37:60–70. doi: 10.3899/jrheum.090194
181. Luig M, Kluger MA, Goerke B, Meyer M, Nosko A, Yan I, et al. Inflammation-induced IL-6 functions as a natural brake on macrophages and limits GN. *J Am Soc Nephrol.* (2015) 26:1597–607. doi: 10.1681/ASN.2014060620
182. Yang J, Chen J, Yan J, Zhang L, Chen G, He L, et al. Effect of interleukin 6 deficiency on renal interstitial fibrosis. *PLoS ONE.* (2012) 7:e52415. doi: 10.1371/journal.pone.0052415
183. Jones SA, Fraser DJ, Fielding CA, Jones GW. Interleukin-6 in renal disease and therapy. *Nephrol Dial Transplant.* (2015) 30:564–74. doi: 10.1093/ndt/gfu233

184. Kang S, Tanaka T, Narazaki M, Kishimoto T. Targeting interleukin-6 signaling in clinic. *Immunity.* (2019) 50:1007–23. doi: 10.1016/j.immuni.2019.03.026
185. Rosas IO, Bräu N, Waters M, Go RC, Hunter BD, Bhagani S, et al. Tocilizumab in hospitalized patients with severe covid-19 pneumonia. *N Engl J Med.* (2021) 25:NEJMoa2028700. doi: 10.1056/NEJMoa2028700
186. Haller H, Bertram A, Nadrowitz F, Menne J. Monocyte chemoattractant protein-1 and the kidney. *Curr Opin Nephrol Hypertens.* (2016) 25:42–9. doi: 10.1097/MNH.0000000000000186
187. Hasegawa H, Kohno M, Sasaki M, Inoue A, Ito MR, Terada M, et al. Antagonist of monocyte chemoattractant protein 1 ameliorates the initiation and progression of lupus nephritis and renal vasculitis in MRL/lpr mice. *Arthritis Rheum.* (2003) 48:2555–66. doi: 10.1002/art.11231
188. De Lema GP, Maier H, Franz TJ, Escribese M, Chilla S, Segerer S, et al. Chemokine receptor Ccr2 deficiency reduces renal disease and prolongs survival in MRL/lpr lupus-prone mice. *J Am Soc Nephrol.* (2005) 16:3592–601. doi: 10.1681/ASN.2005040426
189. Wada T, Yokoyama H, Furuichi K, Kobayashi K, Harada K, Naruto M, et al. Intervention of crescentic glomerulonephritis by antibodies to monocyte chemotactic and activating factor (MCAF/MCP-1). *FASEB J.* (1996) 10:1418–25. doi: 10.1096/fasebj.10.12.8903512
190. Lloyd CM, Minto AW, Dorf ME, Proudfoot A, Wells TNC, Salant DJ, et al. RANTES and monocyte chemoattractant protein-1 (MCP-1) play an important role in the inflammatory phase of crescentic nephritis, but only MCP-1 is involved in crescent formation and interstitial fibrosis. *J Exp Med.* (1997) 185:1371–80. doi: 10.1084/jem.185.7.1371
191. Kanamori H, Matsubara T, Mima A, Sumi E, Nagai K, Takahashi T, et al. Inhibition of MCP-1/CCR2 pathway ameliorates the development of diabetic nephropathy. *Biochem Biophys Res Commun.* (2007) 360:772–7. doi: 10.1016/j.bbrc.2007.06.148
192. Chow FY, Nikolic-Paterson DJ, Ozols E, Atkins RC, Rollin BJ, Tesch GH. Monocyte chemoattractant protein-1 promotes the development of diabetic renal injury in streptozotocin-treated mice. *Kidney Int.* (2006) 69:73–80. doi: 10.1038/sj.ki.5000014
193. Ninichuk V, Clauss S, Kulkarni O, Schmid H, Segerer S, Radomska E, et al. Late onset of Ccl2 blockade with the Spiegelmer mNOX-E36-3′PEG prevents glomerulosclerosis and improves glomerular filtration rate in db/db mice. *Am J Pathol.* (2008) 172:628–37. doi: 10.2353/ajpath.2008.070601
194. Boels MGS, Koudijs A, Avramut MC, Sol WMPJ, Wang G, van Oeveren-Rietdijk AM, et al. Systemic monocyte chemotactic protein-1 inhibition modifies renal macrophages and restores glomerular endothelial glycocalyx and barrier function in diabetic nephropathy. *Am J Pathol.* (2017) 187:2430–40. doi: 10.1016/j.ajpath.2017.07.020
195. Clauss S, Gross O, Kulkarni O, Avila-Ferrufino A, Radomska E, Segerer S, et al. Ccl2/Mcp-I blockade reduces glomerular and interstitial macrophages but does not ameliorate renal pathology in co//agen4A3-deficient mice with autosomal recessive alport nephropathy. *J Pathol.* (2009) 218:40–7. doi: 10.1002/path.2505
196. De Zeeuw D, Bekker P, Henkel E, Hasslacher C, Gouni-Berthold I, Mehling H, et al. The effect of CCR2 inhibitor CCX140-B on residual albuminuria in patients with type 2 diabetes and nephropathy: a randomised trial. *Lancet Diabetes Endocrinol.* (2015) 3:687–96. doi: 10.1016/S2213-8587(15)00261-2
197. Menne J, Eulberg D, Beyer D, Baumann M, Saudek F, Valkusz Z, et al. C-C motif-ligand 2 inhibition with emapticap pegol (NOX-E36) in type 2 diabetic patients with albuminuria. *Nephrol Dial Transplant.* (2017) 32:307–15. doi: 10.1093/ndt/gfv459
198. Fernandez-Fernandez B, Sarafidis P, Kanbay M, Navarro-González JF, Soler MJ, Górriz JL, et al. SGLT2 inhibitors for non-diabetic kidney disease: drugs to treat CKD that also improve glycaemia. *Clin Kidney J.* (2020) 13:728–33. doi: 10.1093/ckj/sfaa198
199. Engel JE, Chade AR. Macrophage polarization in chronic kidney disease: a balancing act between renal recovery and decline? *Am J Physiol Ren Physiol.* (2019) 317:F1409–13. doi: 10.1152/ajprenal.00380.2019
200. Wang Y, Wang YP, Zheng G, Lee VWS, Ouyang L, Chang DHH, et al. *Ex vivo* programmed macrophages ameliorate experimental chronic inflammatory renal disease. *Kidney Int.* (2007) 72:290–9. doi: 10.1038/sj.ki.5002275
201. Cao Q, Wang Y, Zheng D, Sun Y, Wang Y, Lee VWS, et al. IL-10/TGF-β-modified macrophages induce regulatory T cells and protect against adriamycin nephrosis. *J Am Soc Nephrol.* (2010) 21:933–42. doi: 10.1681/ASN.2009060592
202. Zheng D, Wang Y, Cao Q, Lee VWS, Zheng G, Sun Y, et al. Transfused macrophages ameliorate pancreatic and renal injury in murine diabetes mellitus. *Nephron Exp Nephrol.* (2011) 118:e87–99. doi: 10.1159/000321034
203. Du Q, Tsuboi N, Shi Y, Ito S, Sugiyama Y, Furuhashi K, et al. Transfusion of CD206+ M2 macrophages ameliorates antibody-mediated glomerulonephritis in mice. *Am J Pathol.* (2016) 186:3176–88. doi: 10.1016/j.ajpath.2016.08.012
204. Taguchi K, Okada A, Hamamoto S, Unno R, Moritoki Y, Ando R, et al. M1/M2-macrophage phenotypes regulate renal calcium oxalate crystal development. *Sci Rep.* (2016) 6:35167. doi: 10.1038/srep35167
205. Mao R, Wang C, Zhang F, Zhao M, Liu S, Liao G, et al. Peritoneal M2 macrophage transplantation as a potential cell therapy for enhancing renal repair in acute kidney injury. *J Cell Mol Med.* (2020) 24:3314–27. doi: 10.1111/jcmm.15005
206. Engel JE, Williams E, Williams ML, Bidwell GL, Chade AR. Targeted VEGF (Vascular Endothelial Growth Factor) therapy induces long-term renal recovery in chronic kidney disease via macrophage polarization. *Hypertension.* (2019) 74:1113–23. doi: 10.1161/HYPERTENSIONAHA.119.13469
207. Yokoo T, Ohashi T, Utsunomiya Y, Kojima H, Imasawa T, Kogure T, et al. Prophylaxis of antibody-induced acute glomerulonephritis with genetically modified bone marrow-derived vehicle cells. *Hum Gene Ther.* (1999) 10:2673–8. doi: 10.1089/10430349950016717
208. Yamagishi H, Yokoo T, Imasawa T, Mitarai T, Kawamura T, Utsunomiya Y. Genetically modified bone marrow-derived vehicle cells site specifically deliver an anti-inflammatory cytokine to inflamed interstitium of obstructive nephropathy. *J Immunol.* (2001) 166:609–16. doi: 10.4049/jimmunol.166.1.609
209. Kluth DC, Ainslie CV, Pearce WP, Finlay S, Clarke D, Anegon I, et al. Macrophages transfected with adenovirus to express IL-4 reduce inflammation in experimental glomerulonephritis. *J Immunol.* (2001) 166:4728–36. doi: 10.4049/jimmunol.166.7.4728
210. Wilson HM, Stewart KN, Brown PAJ, Anegon I, Chettibi S, Rees AJ, et al. Bone-marrow-derived macrophages genetically modified to produce IL-10 reduce injury in experimental glomerulonephritis. *Mol Ther.* (2002) 6:710–7. doi: 10.1006/mthe.2002.0802
211. Wilson HM, Chettibi S, Jobin C, Walbaum D, Rees AJ, Kluth DC. Inhibition of macrophage nuclear factor-κB leads to a dominant anti-inflammatory phenotype that attenuates glomerular inflammation *in vivo*. *Am J Pathol.* (2005) 167:27–37. doi: 10.1016/S0002-9440(10)62950-1
212. Ferenbach DA, Ramdas V, Spencer N, Marson L, Anegon I, Hughes J, et al. Macrophages expressing heme oxygenase-1 improve renal function in ischemia/reperfusion injury. *Mol Ther.* (2010) 18:1706–13. doi: 10.1038/mt.2010.100
213. Ren GL, Zhu J, Li J, Meng XM. Noncoding RNAs in acute kidney injury. *J Cell Physiol.* (2019) 234:2266–76. doi: 10.1002/jcp.27203
214. Essandoh K, Li Y, Huo J, Fan GC. MiRNA-mediated macrophage polarization and its potential role in the regulation of inflammatory response. *Shock.* (2016) 46:122–31. doi: 10.1097/SHK.0000000000000604
215. Graff JW, Dickson AM, Clay G, McCaffrey AP, Wilson ME. Identifying functional microRNAs in macrophages with polarized phenotypes. *J Biol Chem.* (2012) 287:21816–25. doi: 10.1074/jbc.M111.327031
216. Zhang Y, Zhang M, Zhong M, Suo Q, Lv K. Expression profiles of miRNAs in polarized macrophages. *Int J Mol Med.* (2013) 31:797–802. doi: 10.3892/ijmm.2013.1260
217. Cobos Jiménez V, Bradley EJ, Willemsen AM, van Kampen AHC, Baas F, Kootstra NA. Next-generation sequencing of microRNAs uncovers expression signatures in polarized macrophages. *Physiol Genomics.* (2014) 46:91–103. doi: 10.1152/physiolgenomics.00140.2013
218. Fierro-Fernández M, Miguel V, Márquez-Expósito L, Nuevo-Tapioles C, Herrero JI, Blanco-Ruiz E, et al. MiR-9-5p protects from kidney fibrosis by metabolic reprogramming. *FASEB J.* (2020) 34:410–31. doi: 10.1096/fj.201901599RR
219. Sheedy FJ. Turning 21: induction of miR-21 as a key switch in the inflammatory response. *Front Immunol.* (2015) 6:19. doi: 10.3389/fimmu.2015.00019

220. Jia Y, Zheng Z, Xue M, Zhang S, Hu F, Li Y, et al. Extracellular vesicles from albumin-induced tubular epithelial cells promote the m1 macrophage phenotype by targeting klotho. *Mol Ther.* (2019) 27:1452–66. doi: 10.1016/j.ymthe.2019.05.019
221. Lv LL, Feng Y, Wu M, Wang B, Li ZL, Zhong X, et al. Exosomal miRNA-19b-3p of tubular epithelial cells promotes M1 macrophage activation in kidney injury. *Cell Death Differ.* (2020) 27:210–26. doi: 10.1038/s41418-019-0349-y
222. Luly FR, Lévêque M, Licursi V, Cimino G, Martin-Chouly C, Théret N, et al. MiR-146a is over-expressed and controls IL-6 production in cystic fibrosis macrophages. *Sci Rep.* (2019) 9:16259. doi: 10.1038/s41598-019-52770-w
223. Zheng CZ, Shu YB, Luo YL, Luo J. The role of miR-146a in modulating TRAF6-induced inflammation during lupus nephritis. *Eur Rev Med Pharmacol Sci.* (2017) 21:1041–8.
224. Ichii O, Otsuka S, Sasaki N, Namiki Y, Hashimoto Y, Kon Y. Altered expression of microRNA miR-146a correlates with the development of chronic renal inflammation. *Kidney Int.* (2012) 81:280–92. doi: 10.1038/ki.2011.345
225. Bhatt K, Lanting LL, Jia Y, Yadav S, Reddy MA, Magilnick N, et al. Anti-inflammatory role of microrna-146a in the pathogenesis of diabetic nephropathy. *J Am Soc Nephrol.* (2016) 27:2277–88. doi: 10.1681/ASN.2015010111
226. Chau BN, Xin C, Hartner J, Ren S, Castano AP, Linn G, et al. MicroRNA-21 promotes fibrosis of the kidney by silencing metabolic pathways. *Sci Transl Med.* (2012) 4:121ra18. doi: 10.1126/scitranslmed.3003205
227. Gomez IG, MacKenna DA, Johnson BG, Kaimal V, Roach AM, Ren S, et al. Anti-microRNA-21 oligonucleotides prevent Alport nephropathy progression by stimulating metabolic pathways. *J Clin Invest.* (2015) 125:141–56. doi: 10.1172/JCI75852
228. Lakhia R, Hajarnis S, Williams D, Aboudehen K, Yheskel M, Xing C, et al. MicroRNA-21 aggravates cyst growth in a model of polycystic kidney disease. *J Am Soc Nephrol.* (2016) 27:2319–30. doi: 10.1681/ASN.2015060634
229. Song N, Zhang T, Xu XL, Lu Z, Yu X, Fang Y, et al. miR-21 protects against ischemia/reperfusion-induced acute kidney injury by preventing epithelial cell apoptosis and inhibiting dendritic cell maturation. *Front Physiol.* (2018) 9:790. doi: 10.3389/fphys.2018.00790
230. Rayego-Mateos S, Morgado-Pascual JL, Opazo-Ríos L, Guerrero-Hue M, García-Caballero C, Vázquez-Carballo C, et al. Pathogenic pathways and therapeutic approaches targeting inflammation in diabetic nephropathy. *Int J Mol Sci.* (2020) 21:3798. doi: 10.3390/ijms21113798
231. Reynolds G, Haniffa M. Human and mouse mononuclear phagocyte networks: a tale of two species? *Front Immunol.* (2015) 6:330. doi: 10.3389/fimmu.2015.00330
232. Li Y, Oosting M, Deelen P, Ricaño-Ponce I, Smeekens S, Jaeger M, et al. Inter-individual variability and genetic influences on cytokine responses to bacteria and fungi. *Nat Med.* (2016) 22:952–60. doi: 10.1038/nm.4139
233. Das A, Yang CS, Arifuzzaman S, Kim S, Kim SY, Jung KH, et al. High-resolution mapping and dynamics of the transcriptome, transcription factors, and transcription co-factor networks in classically and alternatively activated macrophages. *Front Immunol.* (2018) 9:22. doi: 10.3389/fimmu.2018.00022
234. Emam M, Cánovas A, Islas-Trejo AD, Fonseca PAS, Medrano JF, Mallard B. Transcriptomic profiles of monocyte-derived macrophages in response to *Escherichia coli* is associated with the host genetics. *Sci Rep*. (2020) 10:271. doi: 10.1038/s41598-019-57089-0

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REVIEW
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The Role of Macrophages in Kidney Fibrosis

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The phenotypic heterogeneity and functional diversity of macrophages confer on them complexed roles in the development and progression of kidney diseases. After kidney injury, bone marrow-derived monocytes are rapidly recruited to the glomerulus and tubulointerstitium. They are activated and differentiated on site into pro-inflammatory M1 macrophages, which initiate Th1-type adaptive immune responses and damage normal tissues. In contrast, anti-inflammatory M2 macrophages induce Th2-type immune responses, secrete large amounts of TGF-β and anti-inflammatory cytokines, transform into αSMA+ myofibroblasts in injured kidney, inhibit immune responses, and promote wound healing and tissue fibrosis. Previous studies on the role of macrophages in kidney fibrosis were mainly focused on inflammation-associated injury and injury repair. Apart from macrophage-secreted profibrotic cytokines, such as TGF-β, evidence for a direct contribution of macrophages to kidney fibrosis is lacking. However, under inflammatory conditions, Wnt ligands are derived mainly from macrophages and Wnt signaling is central in the network of multiple profibrotic pathways. Largely underinvestigated are the direct contribution of macrophages to profibrotic signaling pathways, macrophage phenotypic heterogeneity and functional diversity in relation to kidney fibrosis, and on their cross-talk with other cells in profibrotic signaling networks that cause fibrosis. Here we aim to provide an overview on the roles of macrophage phenotypic and functional diversity in their contribution to pro-fibrotic signaling pathways, and on the therapeutic potential of targeting macrophages for the treatment of kidney fibrosis.

Keywords: macrophages, fibrosis, signaling pathways, TGF-β, Wnt

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INTRODUCTION

Kidney fibrosis is an inevitable outcome of all progressive chronic kidney diseases (CKD), including hypertensive, diabetic, and vascular nephropathy. Chronic inflammation is a direct cause of kidney injury. Chronic inflammation leads to excessive kidney repair and consequent kidney fibrosis and thereby failure of kidney function. Macrophages have long been known to be master players in inflammatory kidney diseases and to be associated with the development of kidney fibrosis in CKD. However, evidence for a direct contribution of macrophages to kidney fibrosis is lacking. Here we summarize the biological and pathological functions of macrophages polarized during the course

of disease progression and their role in the development of kidney fibrosis in CKD, in particular, their contribution to profibrotic signaling networks.

MACROPHAGE AS A MASTER PLAYER IN KIDNEY FIBROSIS

Macrophages are an important part of the mononuclear phagocyte system comprising monocytes, macrophages, and dendritic cells (Viehmann et al., 2018). Mouse F4/80 or human epidermal growth factor module-containing mucin-like receptor 1 (EMR1) are considered to be signature markers of macrophage (Khazen et al., 2005). Macrophages are primarily responsible for pathogen clearance and the repair of injured tissues (Rosenberger and Finlay, 2003; Das et al., 2015). They are multifunctional cells with great phenotypic plasticity serving at the frontier of innate immune defenses. Kidney macrophages include long-lived tissue-resident macrophages and macrophages derived from circulating monocytes of bone marrow origin (Tang et al., 2019). With functional diversity depending on the local microenvironment, macrophages play a critical role in inflammatory kidney disease (Wang and Harris, 2011).

Kidney fibrosis develops in a milieu of inflammatory cell infiltration, mesenchymal cell proliferation and activation, and progressive deposition of extracellular matrix (ECM), leading to scar formation (fibrosis) that destroys the parenchymal structure of kidney and causes progressive loss of kidney function. Observations from human CKD and experimental CKD models have shown that tubulointerstitial fibrosis is an essential feature of chronic kidney failure, and the degree of macrophage infiltration is directly associated with the severity of fibrosis (Yu et al., 2010). Accumulation of kidney macrophages correlates with severity of kidney injury and kidney fibrosis in human and experimental diabetic nephropathy (Chow et al., 2004) and also in other classically non-inflammatory kidney diseases. The infiltration of monocytes expressing chemokine (C-C motif) receptor 2 (CCR2) leads to kidney inflammation and fibrosis in murine chronic obstructive nephropathy (Braga et al., 2018). Kidney macrophage numbers and chemokine (C-C motif) ligand 2 (CCL2) levels correlate significantly with the progression of interstitial fibrosis in human CKD (Eardley et al., 2008). Moreover, selective depletion of macrophages reduces kidney fibrosis (Furuta et al., 1993). These studies support a role for macrophages in genesis and progression of kidney inflammation and fibrosis.

In CKD, macrophages polarize to various phenotypes in response to complex microenvironmental stimuli in diseased kidneys. Macrophages of different phenotypes secrete a variety of growth factors, cytokines, proteins, and enzymes which contribute to or mitigate fibrosis (Eddy and Neilson, 2006). Macrophages produce profibrotic mediators including TGF-β, Wingless and Int-1 (Wnt), platelet-derived growth factor (PDGF), tumor necrosis factor α (TNF-α), hepatocyte growth factor (HGF), connective tissue growth factor (CTGF), angiotensin converting enzyme (ACE), angiotensin I (Ang I) and II (Ang II), plasminogen activators, plasminogen activator inhibitor-1 (PAI-1), tissue inhibitor of metalloproteinases (TIMP), collagen, fibronectin, thrombospondin, coagulation factors, reactive oxygen species, and endothelin. They can also produce mediators that protect against kidney fibrosis including collagenases, matrix metalloproteinase 12 (MMP-12), nitric oxide, and bone morphogenic protein-7 (BMP-7) (Eddy, 2011). Macrophages of various phenotypes are therefore responsible for several key processes in progressive fibro-inflammatory kidney disease, including initiation of inflammatory damage, resolution of inflammation, phagocytotic clearance of debris after inflammation, tissue repair, remodeling of fibrotic tissue, and excessive repair leading to irreversible kidney fibrosis. Thus, macrophages play very complex roles in kidney fibrosis (Ricardo et al., 2008; Shen et al., 2014; **Table 1**).

MACROPHAGE CONTRIBUTIONS TO KIDNEY FIBROSIS VIA INFLAMMATION

Inflammation, starting from recruitment and activation of macrophages, is considered to be a key factor behind fibrotic diseases (Cao et al., 2015). Macrophages are rapidly recruited to the glomerulus or tubulointerstitium to initiate innate immune responses and play important defensive as well as destructive roles in kidney injury. Ongoing kidney damage can cause continuing macrophage infiltration in a vicious cycle that leads to destruction of the normal kidney tissue structure and irreversible tissue fibrosis. Although it is widely believed that glomerular and interstitial macrophages are closely associated with development of kidney fibrosis, they also play beneficial roles in stromal remodeling during tissue repair (Ricardo et al., 2008; Alikhan and Ricardo, 2013). It is important to understand the complex roles of macrophages in kidney inflammation and fibrosis.

Inflammatory Role of M1 Macrophages

The ability of macrophages to play complex roles in kidney diseases is explained by their phenotypic heterogeneity and functional diversity (Anders and Ryu, 2011). Macrophages are activated and differentiated under specific microenvironmental conditions into two broad phenotypes, namely classically activated macrophages (CAM or M1) and alternatively activated macrophages (AAM or M2) (**Figure 1**). However, the concept of M1 and M2 macrophage phenotypes was mostly derived from *in vitro* observations of cultured macrophages. Such distinct M1 and M2 macrophage phenotypes are not consistent with *in vivo* observations, where M1 and M2 markers can co-exist on same macrophage (Wang et al., 2014). We use the terms of M1 and M2 macrophage phenotypes in this review for the convenience in citing respective studies and for description of functionally different macrophages. The existence of such heterogeneous phenotypes is explained by the cellular plasticity of circulating monocytes and macrophages in response to different stimuli. There is compelling evidence that the major factor determining kidney injury versus tissue restoration is the activation state of macrophages within local tissues rather than the degree of macrophage infiltration (Ricardo et al., 2008).

Circulating monocytes are recruited by cytokines and chemoattractants within the pathogenic microenvironment of

TABLE 1 | Macrophage phenotypes, stimuli, Secreted products, and functions.

Macrophage phenotypes	Stimuli	Secreted products	Phenotypic function
M1	LPS (Kalish et al., 2015), TNF-α (Venturin et al., 2016), IFN-γ (Lee et al., 2011), S100A9 (Tang et al., 2019), IL-1α (Tang et al., 2019)	IL-1 (Tang et al., 2019), IL-1β (Wong et al., 2018), IL-6 (Tang et al., 2019), IL-8 (Kadowaki et al., 2009), IL-12 (Tang et al., 2019), IL-17A (Wong et al., 2018), IL-23 (Tang et al., 2019), TNF-α (Inoue, 2017), iNOS (Inoue, 2017), MMP-12 (Tang et al., 2019), CCL-2 (Wong et al., 2018), CCL-3 (Meng et al., 2015), CCL-5 (Meng et al., 2015), CXCL1 (Meng et al., 2015), CXCL2 (Meng et al., 2015), CXCL10 (Meng et al., 2015), ICAM-1 (Wong et al., 2018), Wnt5a (Blumenthal et al., 2006) RAS (Ulrich et al., 2011)	Pro-inflammatory and TH1-like immune response (Tang et al., 2019)
M2a	IL-4 (Zhang et al., 2017; Tang et al., 2019), IL-13 (Zhang et al., 2017; Tang et al., 2019)	Mannose and scavenger receptor (Anders and Ryu, 2011), decoy IL-1R11 (Anders and Ryu, 2011), FIZZ1 (Anders and Ryu, 2011), YM-1 (Anders and Ryu, 2011), IL-10 (Lu et al., 2013), TGF-β (Lu et al., 2013), Wnt1, Wnt3a (Cosin-Roger et al., 2019), Wnt7b (Lin et al., 2010) CCL13 (Meng et al., 2015), CCL14 (Meng et al., 2015), CCL17 (Meng et al., 2015), CCL18 (Meng et al., 2015), CCL22 (Meng et al., 2015), CCL23, CCL24 (Meng et al., 2015), CCL26 (Meng et al., 2015), MMP-9 (Meng et al., 2015), MMP-12 (Meng et al., 2015), IGF-1 (Meng et al., 2015), arginase 1 (Tseng et al., 2020), Fibronectin (Tseng et al., 2020)	Anti-inflammatory TH2-like immune response (Tang et al., 2019), wound healing and tissue fibrosis (Tang et al., 2019), inhibition of T-cell proliferation (Lu et al., 2013)
M2b	Immune complexes (IgG4) (Bianchini et al., 2019; Tang et al., 2019), TLR/IL-1R ligand (Tang et al., 2019), IL-1R (Lisi et al., 2014), IgG Fc receptor ligands (Lisi et al., 2014), CD40 (Lisi et al., 2014), IL-6 (Philipp et al., 2018)	IL-1 (Anders and Ryu, 2011), IL-6 (Anders and Ryu, 2011), TNF-α (Anders and Ryu, 2011), MHCIIhi (Anders and Ryu, 2011), IL-10hi (Anders and Ryu, 2011), IL-12lo (Anders and Ryu, 2011), IL-1β (Wang et al., 2019), MCP-1 (Chen et al., 2013), iNOS (Chen et al., 2013)	Immunoregulation (Tang et al., 2019), Th2 activation (Meng et al., 2015)
M2c	IL-10 (Tang et al., 2019), TGF-β (Tang et al., 2019), glucocorticoids (Tang et al., 2019)	IL-10 (Anders and Ryu, 2011), TGF-β (Anders and Ryu, 2011), mannose receptor (Anders and Ryu, 2011), B7-H4 (Lu et al., 2013), arginase 1 (Meng et al., 2015)	Immunosuppression (Tang et al., 2019), matrix remodeling and tissue repair (Tang et al., 2019), inhibition of T-cell proliferation (Lu et al., 2013), induction of Tregs (Lu et al., 2013)

PAMPs, pathogen-associated molecular patterns; DAMPs, danger- associated molecular patterns; FGF2, basic fibroblast growth factor; LPS, lipopolysaccharide; TLR, toll-like receptors; TNF, tumor necrosis factor; iNOS, inducible nitric oxide synthase; MINCLE, macrophage-inducible C-type lectin; Arg1, arginase-1; MCHII, major histocompatibility complex (MHC) class II; MR, mannose receptor; IGF-1, insulin like growth factor; IRF, interferon-related factor; IL-1R, IL-1 receptor; TGF-β, transforming growth factor-β; Wnt, Wingless and Int-1; RAS, renin angiotensin system.

diseased kidneys. They adhere to activated endothelial surfaces, infiltrate into interstitial and/or glomerular compartments, and differentiate into pro-inflammatory M1 macrophages (Tang et al., 2019). M1 macrophages can be polarized by pathogen-related molecular patterns (PAMPs) such as lipopolysaccharides (LPS), alarmins such as S100A9 and IL-1α, and pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α) (Kalish et al., 2015; Venturin et al., 2016). Polarized M1 macrophages highly express major histocompatibility complex (MHC) class II and co-stimulating molecule CD86 and initiate Th1 type adaptive immune responses, resulting in cytotoxicity and more effective killing of bacteria, intracellular pathogens and tumor cells (Lv et al., 2017; Tang et al., 2019). Concurrently, M1 macrophages secrete a series of pro-inflammatory factors (including IL-1, IL-6, IL-12, TNF-α), chemokines (such as IL-8), activated oxygen species, and nitric oxide (NO) which promote inflammation and damage of normal tissues (Inoue, 2017; Tang et al., 2019).

In the early stage of kidney ischemia-reperfusion injury (IRI) in rats, macrophages are M1 in phenotype and highly express iNOS (Huen and Cantley, 2015). Depletion of macrophages at this stage by liposome clodronate significantly attenuated kidney injury, accompanied with decreased expression of inflammatory and profibrotic cytokines (Ko et al., 2008). Similarly, miR-30c-5p agomir which directly inhibits Interferon regulatory factor 1 (IRF1) reduced kidney ischemic injury by reducing M1 macrophages and increasing of M2 macrophages, and by reducing inflammatory cytokine TNF-α and increasing anti-inflammatory cytokines IL-4 and IL-10 (Zhang et al., 2019; Guo et al., 2020). In contrast, transfusion of IFN- induced M1 macrophages following acute kidney IRI increased tubulointerstitial fibrosis and functional impairment (Lee et al., 2011).

Apart from inflammatory cytokines, activated macrophages also secrete matrix metalloproteinases (MMPs), including abundant MMP- 1, −3, −7, −9, −10, −12, −14, and −25 with less abundant MMP-2, 3, 8, 10, 11, 12 (Huang et al., 2012). Those MMPs contribute not only to degradation of extracellular matrix, but also to inflammatory injury in kidney (Kunugi et al., 2011). Macrophage-derived MMP-9 has been shown to contribute to kidney fibrosis through induction

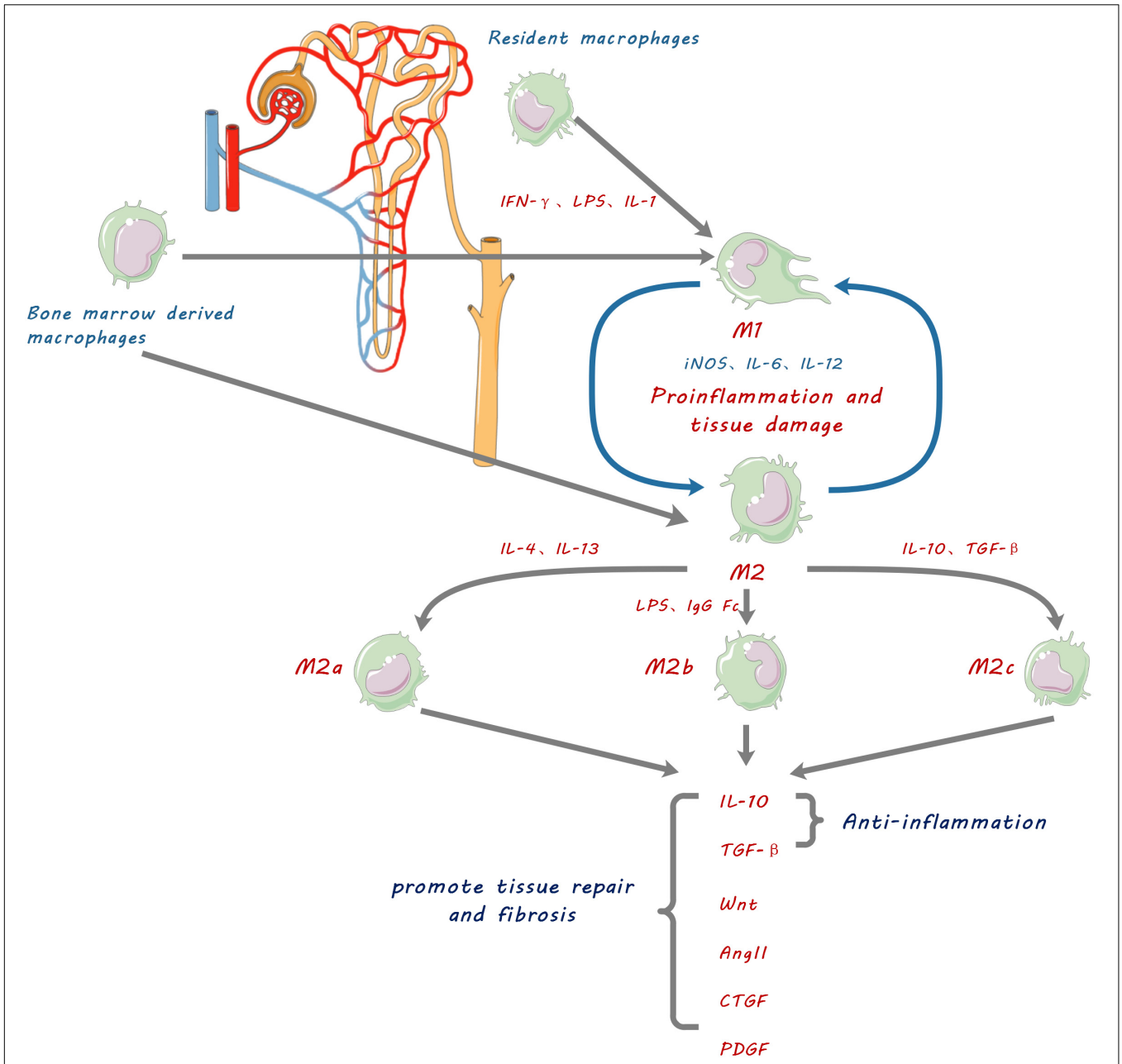

FIGURE 1 | Schematic diagram showing macrophage contribution to kidney fibrosis. Kidney resident macrophages and infiltrating bone marrow-derived macrophages are stimulated by inflammatory factors such as IFN-γ, lipopolysaccharide (LPS), and IL-1, to be polarized into pro-inflammatory M1 macrophages which promote inflammation and tissue damage by releasing IL-6, 12 and inducible nitric oxide synthase (iNOS). They can also be polarized into three functionally different phenotypes of anti-inflammatory and reparative M2 macrophages by anti-inflammatory cytokines IL-4, IL-10, IL-13, and TGF-β. M2 macrophages resolve inflammation, promote tissue repair and cause fibrosis by secretion of anti-inflammatory cytokines and tissue repair mediators including IL-10, transforming growth factor.(TGF-β), Wingless and Int-1 (Wnts), Angiotensin II (Ang II), connective tissue growth factor (CTGF), and platelet-derived growth factor (PDGF).

of profibrotic changes in tubular epithelial cells (Tan et al., 2010) and recruitment of macrophages via proteolytic activation of osteopontin (Tan et al., 2013). More importantly, MMP-mediated proteolytic releasing and activation of TGF-β bound to extracellular matrix (Karsdal et al., 2002) may directly contribute to kidney fibrosis and indirectly through induction of profibrotic M2 macrophages.

In addition to promotion of inflammation and tissue damage, pro-inflammatory M1 macrophages were found also to be capable of switching to anti-inflammatory and reparative M2 macrophages (Arnold et al., 2007). Thus, the classification of M1 and M2 macrophage phenotypes may well represent but oversimplify the plastic functional status of macrophages at different stages of disease progression.

Anti-inflammatory and Pro-fibrotic Roles of M2 Macrophages

Alternatively activated macrophages, M2 macrophages, can be defined from *in vitro* experiments into three functional subtypes according to their activation stimuli and functions: M2a, M2b, and M2c (Tang et al., 2019; **Figure 1**). M2a macrophages are typically induced by IL-4 and IL-13 (Zhang et al., 2017); M2b macrophages are induced by immune complexes, LPS, IgG Fc receptor ligands, and CD40 (Lisi et al., 2014); M2c macrophages are induced by IL-10 and TGF-β or glucocorticoids (Kim et al., 2015). Those phenotypic definitions of anti-inflammatory M2 macrophages are used for the convenience in description of their respective functions.

The subtypes of M2 macrophages are thought to suppress immune responses and promote tissue repair, but with different and sometimes controversial functions (Mantovani et al., 2004). M2a macrophages, highly express the marker arginase 1 (Arg-1), produce a large amounts of anti-inflammatory IL-10 and IL-1 receptor antagonist (IL-1ra), and inhibit secretion of pro-inflammatory cytokines (IL-12, IL-1, TNF-α) and production of NO, thereby exerting anti-inflammatory and immunosuppressive functions. M2b macrophages specifically up-regulate IL-10 and down-regulate IL-12, and induce T cells to secrete IL-4, which in turn promotes B cells to produce antibodies, and induce anti-inflammatory Th2 immune responses. M2c macrophages secrete large amounts of IL-10 and TGF-β, suppress inflammatory immune responses, and promote wound healing and tissue fibrosis (Tang et al., 2017, 2019). Supporting evidence includes that reduced infiltration of macrophages (mainly M2) in murine models of kidney disease can prevent progressive interstitial collagen deposition and inhibit kidney fibrosis (Kim et al., 2015). Furthermore, the adoptive transfer of M2c macrophages rather than M1 macrophages reversed the beneficial effects of macrophage depletion in kidney fibrosis (Tang et al., 2019). In the unilateral ureteral obstruction (UUO) model, depletion of macrophages from day 4 significantly reduced kidney fibrosis, while the adoptive transfer of M2 macrophages promoted the accumulation of αSMA+ cells and kidney fibrosis (Shen et al., 2014). In a rat model of anti-glomerular basement membrane disease, inhibition of M2 macrophage infiltration by inhibitor of the macrophage-specific c-fms receptor at days 14–35 resulted in a significant reduction in both glomerular sclerosis and interstitial fibrosis (Han et al., 2013). Consistent with findings from experimental animal models, the number of M2 macrophages expressing CD206 and/or CD163 is associated with kidney interstitial fibrosis and tubular atrophy in human kidney diseases such as diabetic nephropathy, IgA nephropathy, and in kidney transplants (Wu et al., 2020). Together these findings indicate that M2 macrophage polarization and infiltration can promote kidney fibrosis and progression of kidney disease. However, in acute or non-persistent kidney injuries such as acute tubular necrosis (ATN), M2 macrophages were mainly anti-inflammatory and promoted epithelial healing and rapid regeneration of intact tubules (Anders and Ryu, 2011).

M2 macrophages were thought to promote kidney fibrosis via secretion of TGF-β1 which is well-known to cause fibrosis; larger quantities of TGF-β1 were detected in M2 macrophages than in myofibroblasts in the UUO model (Shen et al., 2014). However, macrophage-specific deletion of TGF-β1 failed to prevent renal fibrosis after severe ischemia-reperfusion or obstructive injury (Huen et al., 2013). In contrast, selective deletion of TGF-β receptor II (TβRII) in monocytes/macrophages promoted kidney fibrosis by enhancing renal macrophage infiltration (Chung et al., 2018). These controversial findings suggested that it would be too simplistic to conclude or disprove profibrotic roles of macrophage TGF-β1 by selective depletion of either TGF-β1 or its receptor (TβRII) alone, given that TGF-β1 is also the most potent anti-inflammatory factor secreted by M2 macrophages (Ricardo et al., 2008), and inflammation is unarguably the initial cause of kidney fibrosis (Tang et al., 2019). We found that by alteration of TGF-β1 signaling in bone marrow-derived macrophages via shifting β-catenin binding from TCF to Foxo1 using β-catenin/TCF inhibitor ICG-001, the anti-inflammatory function of TGF-β1 was enhanced by increased production of anti-inflammatory IL-10 and reduced production of IL-6 and TNF-α in the bone marrow-derived macrophages. Concurrently the pro-fibrotic effect of TGF-β1 was abolished by significant reduction of GFP (+) F4/80 (+) α-SMA (+) bone marrow-derived macrophages undergoing macrophage-myofibroblast transformation (MMT) (Wang et al., 2017) and thereby kidney fibrosis was reduced in the murine model of unilateral ureteral obstruction (UUO) (Yang et al., 2019).

In addition to TGF-β1, M2 macrophage polarization is also tightly regulated by the Wnt pathway. Wnt5a can enhance TGF-β-induced macrophage M2 polarization and the expression of Yes-associated protein (Yap)/transcriptional coactivator with PDZ-binding motif (Taz) to promote kidney fibrosis (Feng et al., 2018a). The Wnt ligand Wnt3a induces the polarization of M2 macrophages by enhancing IL-4 or TGF-β1 (Feng et al., 2018b). Conditional deletion of Wnt3a in bone marrow cells lessens the accumulation of macrophages and the polarization of M2, and reduces kidney fibrosis in the murine UUO model (Feng et al., 2018b).

Bone Marrow Macrophage Contribution to Kidney Fibrosis

Bone marrow-derived monocytes are recruited to the kidney after injury. They constitute a large proportion of interstitial infiltrating macrophages (Tang et al., 2019) and play a major role in progression of kidney fibrosis as they polarize to macrophages of various phenotypes (Conway et al., 2020). Bone marrow-derived macrophages can differentiate into α-SMA+ myofibroblasts in injured kidney, via MMT (Ikezumi et al., 2015; Wang et al., 2017). Flow cytometric analysis found that most CD45+ leukocytes isolated from obstructed kidneys expressed both collagen I and α-SMA (Chen et al., 2011). The CD45+ cells in these fibrotic kidneys are infiltrating monocytes derived from bone marrow. They have undergone MMT and transdifferentiated into collagen-producing myofibroblasts within the microenvironment of the damaged kidney, driven by TGF-β1 (Nikolic-Paterson et al., 2014) secreted by M2 macrophages (Shen et al., 2014). *In vitro* TGF-β1 drove

transdifferentiation of cultured macrophages into collagen-secreting α-SMA+ myofibroblasts (Pilling and Gomer, 2012). Cells expressing macrophage marker CD68 and myofibroblast marker α-SMA+ have been identified in the kidney of patients with active fibrosis (Meng et al., 2016b). Nikolic-Paterson et al. (2014) found evidence of MMT in human kidney disease with active fibrosis using confocal microscopy, and showed that the severity of kidney fibrosis correlated with the number of MMT cells co-expressing α-SMA and CD68. In addition to TGF-β1, chemokine receptor CXCR6 contributes to recruitment of bone marrow-derived fibroblast precursors (Xia et al., 2014), while IL-4 and IL-13 activated Jak3/STAT6 signaling stimulates bone marrow–derived fibroblast MMT in the UUO model of kidney fibrosis (Yan et al., 2015; Liang et al., 2017).

The contribution of bone marrow-derived macrophages to kidney fibrosis is also supported by the observation that down-regulation of CCR2 expression reduced recruitment and activation of myeloid derived macrophages and alleviated kidney fibrosis in UUO model (Jiang et al., 2019). Production of chemokine CXCL16 by kidney tubular epithelial cells is necessary for recruitment of myeloid derived CD45+ col I+ α-SMA+ cells and development of kidney fibrosis in UUO model (Chen et al., 2011; Nikolic-Paterson et al., 2014).

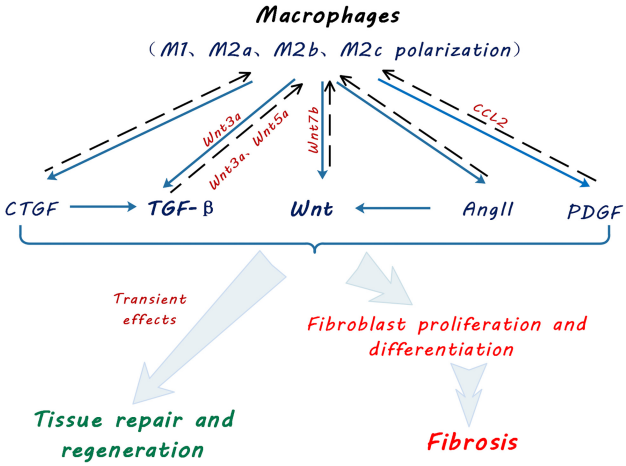

FIGURE 2 | Schematic diagram showing macrophage contribution to profibrotic signaling pathways. Macrophages promote tissue repair, regeneration and fibroblast activation and myofibroblast proliferation via multiple signaling pathways by secretion of transforming growth factor beta (TGF-β), Wingless and Int-1 (Wnts), angiotensin II (Ang II), connective tissue growth factor (CTGF) and platelet-derived growth factor (PDGF). Solid arrows indicate secretion of cytokines and growth factors by macrophages. Broken line arrows indicate autocrine effects of macrophage-secreted cytokines and growth factors on macrophage functional polarization. Light blue arrows indicate effects of macrophage secreted cytokines and growth factors on kidney tissue repair and fibrosis.

MACROPHAGE CONTRIBUTION TO PROFIBROTIC SIGNALING PATHWAYS

Kidney fibrosis is the direct result of activation of fibroblasts and accumulation of myofibroblasts, driven by multiple profibrotic signaling pathways (Kuppe et al., 2021). Profibrotic changes in other cells, including mesenchymal transition of tubular epithelial cells (EMT) (Zheng et al., 2009; Tan et al., 2010; Qiao et al., 2018; Yang et al., 2020; Rao et al., 2021) and endothelial cells (EndoMT) (Zeisberg et al., 2008; Li et al., 2010; LeBleu et al., 2013; Zhao et al., 2016), also contribute to the activation of fibroblasts and kidney fibrosis, but may not directly transform into myofibroblasts (Kuppe et al., 2021).

Wnt/β-CATENIN SIGNALING PATHWAY

The Wnt/β-catenin signaling pathway is activated in various kidney diseases, contributing to the development and progression of kidney fibrosis (Zhou et al., 2020). Wnt/β-catenin signaling is an evolutionarily conserved pathway involved in embryonic development, tissue homeostasis, and organ injury repair (Ng et al., 2019; Perugorria et al., 2019). Wnt ligands are a large family of secreted glycoproteins and fundamentally indispensable for transduction of the Wnt signaling pathway (Nie et al., 2020).

Wnt/β-catenin signaling in kidney disease is versatile; transient activation of Wnt/β-catenin signaling induces repair and regeneration during acute kidney injury, but sustained (uncontrolled) Wnt/β-catenin activation promotes kidney fibrosis (Schunk et al., 2021). Lin et al. (2010) found that Wnt7b secreted by macrophages facilitates kidney regeneration through directing epithelial cell-cycle progression and basement membrane repair; kidney injury repair was substantially retarded after macrophage specific deletion of Wnt7b.

In kidney, Wnt5a promotes fibrosis by stimulating Yap/Taz-mediated macrophage polarization in both UUO and IRI models (Feng et al., 2018a). Wnt3a can also promote M2 macrophage polarization induced by IL-4 or TGF-β1, following Wnt/β-catenin signaling activation, and in turn accelerate macrophage proliferation and accumulation, giving rise to kidney fibrosis (Cosin-Roger et al., 2019; **Figure 2**).

Studies of fibrosis in other organs also demonstrated macrophage contribution to the Wnt/β-catenin pathway. After myocardial infarction in mice, macrophages within the area of infarction exhibited an increase in expression of non-canonical Wnt ligands Wnt5a and Wnt11 (Palevski et al., 2017). The activated Wnt/β-catenin signal promoted cardiac fibrosis by inducing the transition of endothelial cells and epicardial cells to a mesenchymal state, fibroblast differentiation into myofibroblasts and collagen production (Palevski et al., 2017). In a murine model of intestinal fibrosis, CD16+ macrophages expressed high levels of Wnt6, inducing intestinal fibrosis (Salvador et al., 2018). M2 macrophage release of Wnt7a promoted myofibroblast differentiation of lung resident mesenchymal stem cells, leading to lung fibrosis (Hou et al., 2018).

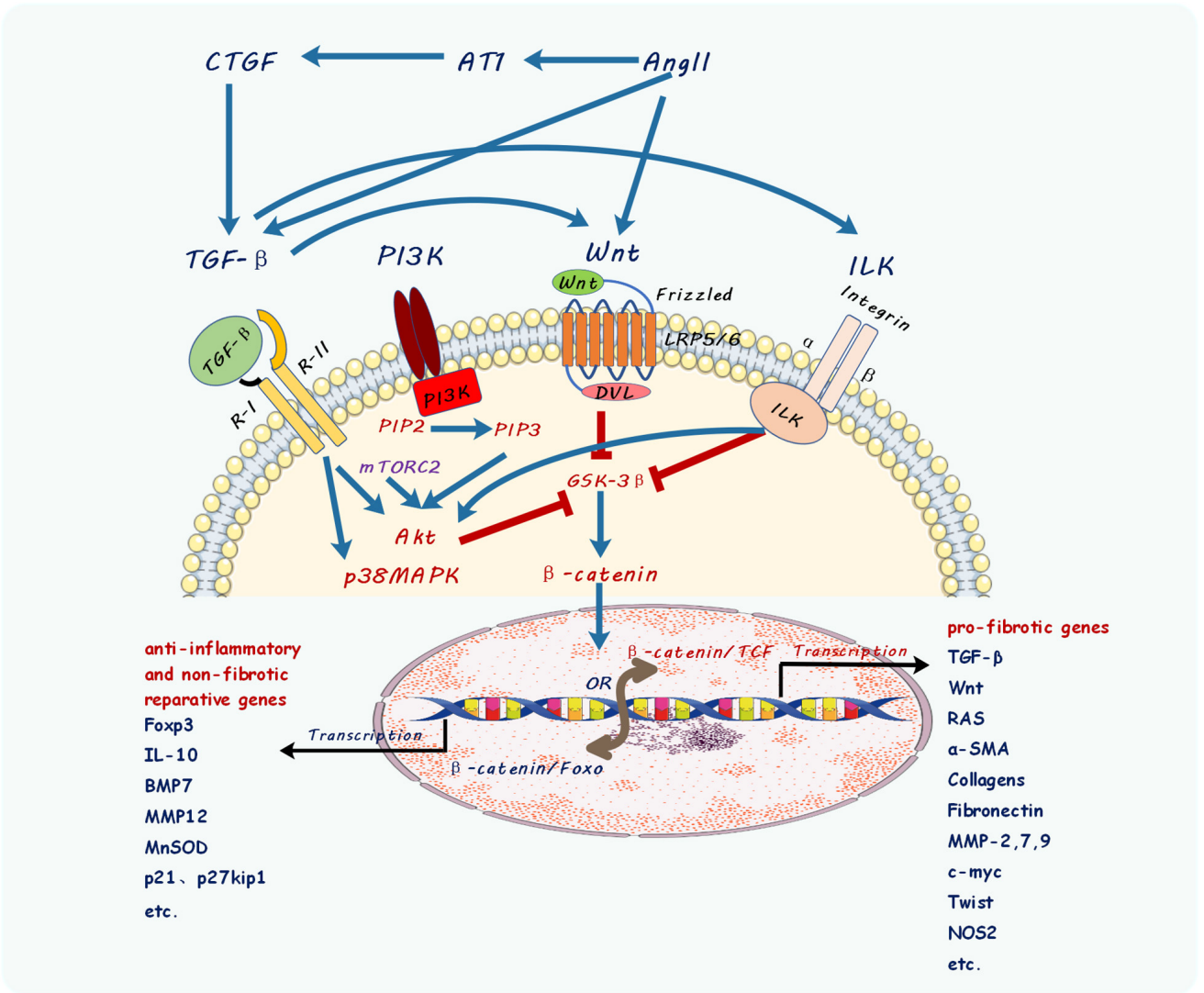

FIGURE 3 | Schematic diagram showing cross-talk between different signaling pathways including those of transforming growth factor beta (TGF-β), Wingless and Int-1 (Wnts), Renin Angiotensin System (RAS), Integrin linked kinases (ILK), connective tissue growth factor (CTGF), and PI3K–mTOR. Multiple signaling pathways cross-talk and converge at β-catenin nuclear translocation and binding with different transcription factors to activate different target genes in macrophage and kidney cells described. PI3K, Phosphatidylinositol 3 kinase; Akt, Ak strain protein kinase B; mTOR, mammalian target of rapamycin; PIP2, phosphatidylinositol (4,5)-trisphosphate; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; mTORC2, mTOR complex 2 including mTOR, Rictor, GβL, Sin1, PRR5/Protor-1, and DEPTOR; Ang II, Angiotensin II; AT1, Angiotensin receptor 1.

TGF-β SIGNALING PATHWAY

TGF-β is a well-known inducer of kidney fibrosis. While secretion of anti-inflammatory TGF-β by M2 macrophages contributes to resolution of inflammation, it also mediates kidney injury repair and causes kidney fibrosis when in excess. The mechanism by which macrophages promote kidney fibrosis through the TGF-β signaling has been extensively investigated. M1 macrophages can be reprogrammed into alternately activated M2 macrophages by anti-inflammatory cytokine stimulation (IL-10 or colony-stimulating factor 1) or upon their phagocytotic ingestion of apoptotic cells. M2 macrophages promote and coordinate the regeneration of kidney tubular cells and maintain the integrity of the kidney tubules after injury (Rogers et al., 2014). During tissue repair, M2b and M2c macrophages are mainly responsible for immunosuppression, matrix remodeling and wound healing once tissue damage has been resolved (Tang et al., 2019). In contrast, uncontrolled kidney inflammation triggers M2a

macrophage polarization in the injured kidney through IL-4 and IL-13, promoting increased TGF-β1 production and kidney fibrosis (Pan et al., 2015). M2 macrophages exert anti-inflammatory effects and promote kidney fibrosis through tissue repair by producing a large amount of TGF-β1 in the UUO model (Eddy, 2005).

RENIN-ANGIOTENSIN SYSTEM (RAS), PDGF AND CTGF SIGNALING PATHWAYS

In addition to Wnt and TGF-β, macrophages are also identified as a source of components of the renin-angiotensin system (RAS), including renin, angiotensin converting enzyme (ACE), Ang I and Ang II, AT1 and AT2 receptors (Okamura et al., 1999). The RAS is known to cause kidney fibrosis through Wnt/β-catenin signaling (Miao et al., 2019; **Figure 2**). Other pro-fibrotic mediators such as PDGF and CTGF were also found to be produced by macrophages (Cicha et al., 2005; Eitner et al., 2008).

INTEGRIN/ILK AND NOTCH SIGNALING PATHWAYS

Apart from direct secretion of pro-fibrotic mediators, macrophages produce matrix metalloproteinases (MMP), which not only contribute to tissue remodeling after injury, but also activate other pro-fibrotic signaling pathways such as Integrin/ILK (Zheng et al., 2009, 2016; Tan et al., 2010) and Notch (Zhao et al., 2016).

ACTIVATION AND PROLIFERATION OF MYOFIBROBLASTS BY CROSSTALK BETWEEN PROFIBROTIC SIGNALING PATHWAYS

Activation and proliferation of myofibroblasts is a central and complex event in development of kidney fibrosis. It involves multiple signaling pathways activated by profibrotic mediators from the fibro-inflammatory microenvironment of the injured kidney. Macrophages are unarguably a major source of those mediators (**Table 1**). A profibrotic signaling network including TGF-β/Smad, Wnt/β-catenin, the renin- angiotensin system (RAS) and Integrin /ILK pathways cross-talk and synchronize to promote kidney fibrosis (**Figure 3**).

Wnt/β-catenin signaling is a key player in kidney fibrosis contributing to activation of fibroblasts into myofibroblasts and consequent excessive extracellular matrix production. Upon binding of Wnt ligands to its receptor Frizzled (Fz) and transmembrane receptor LRP5/6, dishevelled (dvl) protein in the cytoplasm is phosphorylated and activated to bind to Axin to antagonize GSK3β, which prevents β-catenin signaling by degradation of cytosolic β-catenin via phosphorylation and ubiquitination machinery (Tan et al., 2014; Wang Y. et al., 2018). Inhibition of GSK3β by Wnt ligands results in β-catenin nucleus translocation followed by transcriptional activation of Wnt target genes when β-catenin complexes TCF/LEF, the transcription binding partners of β-catenin (Zhou et al., 2012; Guo et al., 2019). This canonical Wnt/β-catenin signaling pathway activates a transcriptome of profibrotic inducers such as Snail/Slug, and fibrotic genes such as α smooth muscle actin (α-SMA), collagen, fibronectin and other extracellular matrix genes involved in fibroblast activation and extracellular matrix production. Importantly, Wnt/β-catenin signaling is not acting alone during the development of kidney fibrosis.

TGF-β released by M2 macrophage is also one of the most important contributors to kidney fibrosis. TGF-β signals through both Smad-dependent and Smad-independent pathways. TGF-β binds to TGF-β receptor II which sequentially complexes with TGF-β receptor I. TGF-β receptor II binding to receptor I then leads to receptor I phosphorylation of Smad2/3 which translocate into the nucleus with co-Smad4 to activate profibrotic gene transcription in kidney myfibroblasts (Meng et al., 2016a). In addition to Smad-dependent signaling in activating profibrotic genes in myofibroblasts, TGF-β also promotes β-catenin nuclear translocation through phosphorylation of β-catenin Tyr-654 and dephosphorylation of β-catenin Ser-37 and Thr-41. Furthermore, Smad-independent activation of Akt and p38 MAP kinase (Wang et al., 2011; Zhou et al., 2012; Tan et al., 2014) also subsequently inhibit GSK3β, thereby promoting β-catenin nuclear translocation and activation of Wnt/β-catenin signaling.

The renin-angiotensin system (RAS) is also known to cause hypertension and fibrosis in CKD (Floege, 2015). Macrophage secretion of RAS components [renin, angiotensin converting enzyme (ACE), Ang I and Ang II] promote synthesis and release of profibrotic factors TGF-β, CTGF, PDGF, ET1 (Wang M. et al., 2018; Zhou et al., 2020) and is a direct target of the Wnt/β-catenin pathway, causing kidney injury, and fibrosis. Reciprocally, blockade of Wnt/β-catenin by inhibition of β-catenin/TCF signaling also blocks RAS and consequent hypertension and kidney fibrosis in CKD (Floege, 2015).

Integrin/ILK are known to contribute to both glomerular and interstitial fibrosis in diseased kidneys (Liu, 2010; Zheng et al., 2016). The underlying mechanism for ILK in causing fibrotic signaling involves its direct or indirect (via activation of Akt) inhibition of GSK3β in facilitating β-catenin nuclear translocation and activation of Wnt/β-catenin signaling (Liu, 2010). We found that proximal tubular cell upregulation of ILK via the compensatory increase of α3 integrin worsened kidney fibrosis in the UUO model in proximal tubular specific E-cadherin knockout mice (Zheng et al., 2016). Importantly, ILK is downstream of TGF-β mediation of both glomerular and tubulointerstitial fibrosis in kidneys (Li et al., 2009; Kang et al., 2010). Our study demonstrated that autophagy links TGF-β/Smad signaling with β-catenin through the pY654-beta-catenin/p-Smad2/ILK pathway (Pang et al., 2016).

mTOR activation has been identified in macrophages and myofibroblasts in kidney fibrosis (Chen et al., 2012). mTORC1 activation in podocytes led to the development of glomerular crescents contributing to fibrosis of glomeruli

in both experimental and human glomerulonephritis (Mao et al., 2014). mTORC2 is activated by TGF-β to transduce profibrotic signaling through mTOR activation of PI3K-Akt (Li et al., 2015) which subsequently inactivates GSK3β to facilitate β-catenin nuclear translocation and thereby activate β-catenin/TCF in the Wnt/β-catenin pathway. Macrophage polarization has been shown to be controlled by the PI3K-Akt-mTOR pathway; increased mTORC1 activity promoted M1 macrophage polarization and reduced M2 macrophage polarization (Weichhart et al., 2015). mTOR activation was observed in myofibroblasts and macrophages and inhibition of mTOR pathway by rapamycin ameliorated kidney fibrosis (Chen et al., 2012). Both TGF-β and ILK activate PI3K-Akt and thus cross-talk with mTOR, whereas mTORC2 activation of PI3K-Akt also links with the Wnt/β-catenin pathway via PI3K-Akt inhibition of GSK3β (Ching and Hansel, 2010).

Together multiple signaling pathways (TGF-β, Wnt, ILK, RAS, mTOR, etc.) interact via activation of β-catenin in the initiation and progression of kidney fibrosis. The functional status of β-catenin determines the activity of these signaling pathways and the progression or regression of kidney fibrosis. Studies from us and others demonstrated the key role for β-catenin/TCF in mediating profibrotic signaling of multiple pathways (Liu, 2010; Qiao et al., 2018). Importantly, we found that shifting β-catenin binding from TCF toward Foxo in both macrophages and kidney tubular cells by inhibition of β-catenin/TCF redirected TGF-β signaling from pro-fibrotic to anti-inflammatory, protected against kidney fibrosis and promoted epithelial repair in UUO and IRI models (Qiao et al., 2018; Rao et al., 2019, 2021; Yang et al., 2019).

TARGETING MACROPHAGES AS A TREATMENT FOR KIDNEY FIBROSIS

Anti-inflammatory and reparative properties of macrophages (Lin et al., 2010; Urbina and Singla, 2014; Ratnayake et al., 2021) argue for their therapeutic application. We have shown that *ex vivo* programmed M2 macrophages protect against inflammation and kidney injury in experimental models of inflammatory renal disease (Wang et al., 2007; Cao et al., 2010, 2011). Jung et al. (2012) found that infusion of IL-10 overexpressing macrophages protected ischemia injury in an IRI model. Adoptive transfer of genetically modified macrophages expressing heme-oxygenase-1 (HO-1) protected kidney function in mice with IRI (Ferenbach et al., 2010). Netrin-1-induced M2 macrophages suppressed inflammation and protected against kidney injury in IRI mice (Ranganathan et al., 2013).

However, the phenotypic instability of those M2 macrophages remains as a challenge (Cao et al., 2014). To overcome the hurdle of phenotypic instability, adenovirus vector NGAL (Neutrophil gelatinase-associated lipocalin-2) was used to stabilize phenotype of injected M2 macrophages which reduced inflammation and fibrosis in UUO model. While protection by anti-inflammatory M2 macrophages has been reported increasingly, the profibrotic effects of M2 macrophages remain largely unaddressed as another hurdle for their therapeutic application; M2 macrophages secrete large amounts of TGF-β which not only suppresses inflammation but also promotes kidney fibrosis (Kim et al., 2015).

Depletion of inflammatory M1 macrophages does not protect against kidney fibrosis, while depletion of anti-inflammatory and reparative M2 macrophages can reduce kidney fibrosis (Shen et al., 2014). Thus, although inflammation is an important driver of fibrosis, other non-inflammatory profibrotic pathways are activated by anti-inflammatory and tissue reparative cytokines from M2 macrophages such as TGF-β, Wnt, Ang II, CTGF, and PDGF. Moreover, the results of these macrophage depletion studies are consistent with the fact that M1 and M2 macrophages represent different and sometimes co-existing functional phenotypes of the same population. They polarize across their life span according to stimuli within the microenvironment in which they reside during the progression kidney diseases.

Opposing roles of phenotypically distinct macrophages suggested that targeting macrophages of different phenotypes may not be practical in developing therapeutic treatment for fibrotic diseases (Cao et al., 2014). More importantly, precise targeting of functionally different macrophages with opposing roles requires a better understanding of downstream signaling events and the diverse functions of multi-functional cytokines, such as TGF-β1 (Qiao et al., 2018), which although profibrotic contributes to suppression of inflammation and to tissue repair in kidney (Tang et al., 2019).

Instead of targeting specific functional phenotypes of macrophages, targeting a central factor in multiple profibrotic signaling pathways in macrophages is likely to be a more effective strategy for treating kidney diseases. Indeed, we found in the UUO model that inhibition of β-catenin/TCF promotes β-catenin/Foxo in the Wnt and TGF-β signaling pathways of bone marrow-derived macrophages (Yang et al., 2019). Importantly, redirection of β-catenin binding from TCF to Foxo resulted in reduction of inflammatory cytokines produced by bone marrow-derived macrophages, altered the fate of MMT macrophages and protected against kidney fibrosis (Yang et al., 2019).

CONCLUSION

Macrophages are master regulators of inflammation and kidney fibrosis. Monocytes and macrophages are recruited and activated in response to chemoattractants and stimuli released after kidney injury. Macrophage plasticity adds complexity to their central roles in kidney fibrosis. After kidney injury, macrophages polarize into various phenotypes in response to alteration of the microenvironment in kidney disease. M1 pro-inflammatory macrophages clear infection but also cause kidney injury; M2 anti-inflammatory macrophages contribute to resolution of inflammation and kidney repair yet cause kidney fibrosis (Tang et al., 2019). Functionally distinct macrophage phenotypes contribute to the fibro-inflammatory microenvironment by

abundant secretion of inflammatory and anti-inflammatory cytokines, mediators of tissue repair including TGF-β, Wnt ligands, PDGF, CTGF as well as all components of RAS. Those tissue repair mediators are also key inducers of kidney fibrosis when secreted in excess and maintained at higher levels in the chronic inflammatory milieu of kidney disease. Profibrotic mediators activate a profibrotic signaling network by cross-talking among multiple signaling pathways including TGF-β, Wnts, RAS, intergin/ILK, mTOR. Importantly, multiple pro-fibrotic signaling pathways all converge at activation of β-catenin/TCF, making β-catenin/TCF a key target for prevention of kidney fibrosis. Switching β-catenin/TCF to β-catenin/Foxo redirects signaling from profibrotic to anti-inflammatory and protects against kidney fibrosis. Targeting macrophages has long been proposed as a treatment for fibro-inflammatory kidney diseases. However, the phenotypic plasticity and conflicting roles of M2 macrophages are major hurdles for their therapeutic application. Recently we have identified the β-catenin/TCF/Foxo axis as a key determinant of the signaling direction of multiple profibrotic pathways. Thus, targeting macrophage signaling pathway via the β-catenin/TCF/Foxo axis may provide a new promising strategy for the treatment of kidney fibrosis in chronic kidney diseases.

AUTHOR CONTRIBUTIONS

JXu, JXie, DH, and GZ contributed to conception and design of the study. XW wrote the first draft of the manuscript. GZ, JC, JXu, DH, and JXie wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

REFERENCES

Alikhan, M., and Ricardo, S. (2013). Mononuclear phagocyte system in kidney disease and repair. *Nephrology* 18, 81–91. doi: 10.1111/nep.12014

Anders, H. J., and Ryu, M. (2011). Renal microenvironments and macrophage phenotypes determine progression or resolution of renal inflammation and fibrosis. *Kidney Int.* 80, 915–925. doi: 10.1038/ki.2011.217

Arnold, L., Henry, A., Poron, F., Baba-Amer, Y., van Rooijen, N., Plonquet, A., et al. (2007). Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. *J. Exp. Med.* 204, 1057–1069. doi: 10.1084/jem.20070075

Bianchini, R., Roth-Walter, F., Ohradanova-Repic, A., Flicker, S., Hufnagl, K., Fischer, M. B., et al. (2019). IgG4 drives M2a macrophages to a regulatory M2b-like phenotype: potential implication in immune tolerance. *Allergy* 74, 483–494. doi: 10.1111/all.13635

Blumenthal, A., Ehlers, S., Lauber, J., Buer, J., Lange, C., Goldmann, T., et al. (2006). The wingless homolog WNT5A and its receptor Frizzled-5 regulate inflammatory responses of human mononuclear cells induced by microbial stimulation. *Blood* 108, 965–973. doi: 10.1182/blood-2005-12-5046

Braga, T., Correa-Costa, M., Silva, R., Cruz, M., Hiyane, M., da Silva, J., et al. (2018). CCR2 contributes to the recruitment of monocytes and leads to kidney inflammation and fibrosis development. *Inflammopharmacology* 26, 403–411. doi: 10.1007/s10787-017-0317-4

Cao, Q., Harris, D., and Wang, Y. (2015). Macrophages in kidney injury, inflammation, and fibrosis. *Physiology (Bethesda, Md)* 30, 183–194. doi: 10.1152/physiol.00046.2014

Cao, Q., Wang, C., Zheng, D., Wang, Y., Lee, V. W., Wang, Y. M., et al. (2011). IL-25 induces M2 macrophages and reduces renal injury in proteinuric kidney disease. *J. Am. Soc. Nephrol.* 22, 1229–1239. doi: 10.1681/asn.2010070693

Cao, Q., Wang, Y., Zheng, D., Sun, Y., Wang, C., Wang, X. M., et al. (2014). Failed renoprotection by alternatively activated bone marrow macrophages is due to a proliferation-dependent phenotype switch in vivo. *Kidney Int.* 85, 794–806. doi: 10.1038/ki.2013.341

Cao, Q., Wang, Y., Zheng, D., Sun, Y., Wang, Y., Lee, V. W., et al. (2010). IL-10/TGF-beta-modified macrophages induce regulatory T cells and protect against adriamycin nephrosis. *J. Am. Soc. Nephrol.* 21, 933–942. doi: 10.1681/asn.2009060592

Chen, G., Chen, H., Wang, C., Peng, Y., Sun, L., Liu, H., et al. (2012). Rapamycin ameliorates kidney fibrosis by inhibiting the activation of mTOR signaling in interstitial macrophages and myofibroblasts. *PLoS One* 7:e33626. doi: 10.1371/journal.pone.0033626

Chen, G., Lin, S., Chen, J., He, L., Dong, F., Xu, J., et al. (2011). CXCL16 recruits bone marrow-derived fibroblast precursors in renal fibrosis. *J. Am. Soc. Nephrol. JASN* 22, 1876–1886. doi: 10.1681/asn.2010080881

Chen, X., Wen, Z., Xu, W., and Xiong, S. (2013). Granulin exacerbates lupus nephritis via enhancing macrophage M2b polarization. PLoS One 2013, 8:e65542.

Ching, C. B., and Hansel, D. E. (2010). Expanding therapeutic targets in bladder cancer: the PI3K/Akt/mTOR pathway. *Laboratory Investigat.* 90, 1406–1414. doi: 10.1038/labinvest.2010.133

Chow, F., Ozols, E., Nikolic-Paterson, D., Atkins, R., and Tesch, G. (2004). Macrophages in mouse type 2 diabetic nephropathy: correlation with diabetic state and progressive renal injury. *Kidney Int.* 65, 116–128. doi: 10.1111/j.1523-1755.2004.00367.x

Chung, S., Overstreet, J. M., Li, Y., Wang, Y., Niu, A., Wang, S., et al. (2018). TGF-β promotes fibrosis after severe acute kidney injury by enhancing renal macrophage infiltration. *JCI Insight* 3:e123563.

Cicha, I., Yilmaz, A., Klein, M., Raithel, D., Brigstock, D. R., Daniel, W. G., et al. (2005). Connective tissue growth factor is overexpressed in complicated atherosclerotic plaques and induces mononuclear cell chemotaxis in vitro. *Arterioscler. Thromb. Vasc. Biol.* 25, 1008–1013. doi: 10.1161/01.atv.0000162173.27682.7b

Conway, B. R., O'Sullivan, E. D., Cairns, C., O'Sullivan, J., Simpson, D. J., Salzano, A., et al. (2020). Kidney single-cell atlas reveals myeloid heterogeneity in progression and regression of kidney disease. *J. Am. Soc. Nephrol.* 31, 2833–2854. doi: 10.1681/asn.2020060806

Cosin-Roger, J., Ortiz-Masià, M., and Barrachina, M. (2019). Macrophages as an emerging source of Wnt ligands: relevance in mucosal integrity. *Front. Immunol.* 10:2297.

Das, A., Sinha, M., Datta, S., Abas, M., Chaffee, S., Sen, C. K., et al. (2015). Monocyte and macrophage plasticity in tissue repair and regeneration. *Am. J. Pathol.* 185, 2596–2606. doi: 10.1016/j.ajpath.2015.06.001

Eardley, K. S., Kubal, C., Zehnder, D., Quinkler, M., Lepenies, J., Savage, C. O., et al. (2008). The role of capillary density, macrophage infiltration and interstitial scarring in the pathogenesis of human chronic kidney disease. *Kidney Int.* 74, 495–504.

Eddy, A. (2005). Progression in chronic kidney disease. *Adv. Chronic Kidney Dis.* 12, 353–365. doi: 10.1053/j.ackd.2005.07.011

Eddy, A., and Neilson, E. (2006). Chronic kidney disease progression. *J. Am. Soc. Nephrol. JASN* 17, 2964–2966.

Eddy, A. A. (2011). Overview of the cellular and molecular basis of kidney fibrosis. *Kidney Int. Suppl.* 2014, 2–8. doi: 10.1038/kisup.2014.2

Eitner, F., Bücher, E., van Roeyen, C., Kunter, U., Rong, S., Seikrit, C., et al. (2008). PDGF-C is a proinflammatory cytokine that mediates renal interstitial fibrosis. *J. Am. Soc. Nephrol.* 19, 281–289. doi: 10.1681/asn.2007030290

Feng, Y., Liang, Y., Zhu, X., Wang, M., Gui, Y., Lu, Q., et al. (2018a). The signaling protein Wnt5a promotes TGFβ1-mediated macrophage polarization and kidney fibrosis by inducing the transcriptional regulators Yap/Taz. *J. Biol. Chem.* 293, 19290–19302. doi: 10.1074/jbc.ra118.005457

Feng, Y., Ren, J., Gui, Y., Wei, W., Shu, B., Lu, Q., et al. (2018b). βWnt/-catenin-promoted macrophage alternative activation contributes to kidney fibrosis. *J. Am. Soc. Nephrol. JASN* 29, 182–193. doi: 10.1681/asn.2017040391

Ferenbach, D. A., Ramdas, V., Spencer, N., Marson, L., Anegon, I., Hughes, J., et al. (2010). Macrophages expressing heme oxygenase-1 improve renal function in ischemia/reperfusion injury. *Mol. Ther.* 18, 1706–1713. doi: 10.1038/mt.2010.100

Floege, J. (2015). Antagonism of canonical Wnt/beta-catenin signaling: taking RAS blockade to the next level? *J. Am. Soc. Nephrol.* 26, 3–5. doi: 10.1681/asn.2014060567

Furuta, T., Saito, T., Ootaka, T., Soma, J., Obara, K., Abe, K., et al. (1993). The role of macrophages in diabetic glomerulosclerosis. *Am. J. Kidney Dis. Offi. J. Natl. Kidney Found.* 21, 480–485. doi: 10.1016/s0272-6386(12)80393-3

Guo, Q., Zhong, W., Duan, A., Sun, G., Cui, W., Zhuang, X., et al. (2019). Protective or deleterious role of Wnt/beta-catenin signaling in diabetic nephropathy: an unresolved issue. *Pharmacol. Res.* 144, 151–157. doi: 10.1016/j.phrs.2019.03.022

Guo, Q., Zhu, X., Wei, R., Zhao, L., Zhang, Z., Yin, X., et al. (2020). miR-130b-3p regulates M1 macrophage polarization via targeting IRF. *J. Cell. Physiol.* 236, 2008–2022. doi: 10.1002/jcp.29987

Han, Y., Ma, F., Tesch, G., Manthey, C., and Nikolic-Paterson, D. (2013). Role of macrophages in the fibrotic phase of rat crescentic glomerulonephritis. *Am. J. Physiol. Renal. Physiol.* 304, F1043–F1053.

Hou, J., Shi, J., Chen, L., Lv, Z., Chen, X., Cao, H., et al. (2018). M2 macrophages promote myofibroblast differentiation of LR-MSCs and are associated with pulmonary fibrogenesis. *Cell Commun. Sign. CCS* 16:89.

Huang, W.-C., Sala-Newby, G. B., Susana, A., Johnson, J. L., and Newby, A. C. (2012). Classical macrophage activation up-regulates several matrix metalloproteinases through mitogen activated protein kinases and nuclear factor-κB. *PLoS One* 7:e42507. doi: 10.1371/journal.pone.0042507

Huen, S., and Cantley, L. (2015). Macrophage-mediated injury and repair after ischemic kidney injury. *Pediatr. Nephrol.* 30, 199–209. doi: 10.1007/s00467-013-2726-y

Huen, S. C., Moeckel, G. W., and Cantley, L. G. (2013). Macrophage-specific deletion of transforming growth factor-β1 does not prevent renal fibrosis after severe ischemia-reperfusion or obstructive injury. *Am. J. Physiol. Renal. Physiol.* 305, F477–F484.

Ikezumi, Y., Suzuki, T., Yamada, T., Hasegawa, H., Kaneko, U., Hara, M., et al. (2015). Alternatively activated macrophages in the pathogenesis of chronic kidney allograft injury. *Pediatr. Nephrol.* 30, 1007–1017. doi: 10.1007/s00467-014-3023-0

Inoue, T. (2017). M1 macrophage triggered by mincle leads to a deterioration of acute kidney injury. *Kidney Int.* 91, 526–529. doi: 10.1016/j.kint.2016.11.026

Jiang, Y., Wang, Y., Ma, P., An, D., Zhao, J., Liang, S., et al. (2019). Myeloid-specific targeting of Notch ameliorates murine renal fibrosis via reduced infiltration and activation of bone marrow-derived macrophage. *Protein Cell* 10, 196–210. doi: 10.1007/s13238-018-0527-6

Jung, M., Sola, A., Hughes, J., Kluth, D. C., Vinuesa, E., Viñas, J. L., et al. (2012). Infusion of IL-10-expressing cells protects against renal ischemia through induction of lipocalin-2. *Kidney Int.* 81, 969–982. doi: 10.1038/ki.2011.446

Kadowaki, T., Yasui, Y., Takahashi, Y., Kohchi, C., Soma, G., and Inagawa, H. (2009). Comparative immunological analysis of innate immunity activation after oral administration of wheat fermented extract to teleost fish. *Anticancer Res.* 29, 4871–4877.

Kalish, S., Lyamina, S., Usanova, E., Manukhina, E., Larionov, N., and Malyshev, I. (2015). Macrophages reprogrammed in vitro towards the M1 phenotype and activated with LPS extend lifespan of mice with ehrlich ascites carcinoma. *Med. Sci. Monitor Basic Res.* 21, 226–234. doi: 10.12659/msmbr.895563

Kang, Y. S., Li, Y., Dai, C., Kiss, L. P., Wu, C., and Liu, Y. (2010). Inhibition of integrin-linked kinase blocks podocyte epithelial–mesenchymal transition and ameliorates proteinuria. *Kidney Int.* 78, 363–373.

Karsdal, M. A., Larsen, L., Engsig, M. T., Lou, H., Ferreras, M., Lochter, A., et al. (2002). Matrix metalloproteinase-dependent activation of latent transforming growth factor-beta controls the conversion of osteoblasts into osteocytes by blocking osteoblast apoptosis. *J. Biol. Chem.* 277, 44061–44067. doi: 10.1074/jbc.m207205200

Khazen, W., M'Bika, J. P., Tomkiewicz, C., Benelli, C., Chany, C., Achour, A., et al. (2005). Expression of macrophage-selective markers in human and rodent adipocytes. *FEBS Lett.* 579, 5631–5634. doi: 10.1016/j.febslet.2005.09.032

Kim, M. G., Kim, S. C., Ko, Y. S., Lee, H. Y., Jo, S. K., and Cho, W. (2015). The role of M2 macrophages in the progression of chronic kidney disease following acute kidney injury. *PLoS One* 10:e0143961. doi: 10.1371/journal.pone.0143961

Ko, G., Boo, C., Jo, S., Cho, W., and Kim, H. (2008). Macrophages contribute to the development of renal fibrosis following ischaemia/reperfusion-induced acute kidney injury. *Nephrol. Dialy. Trans. Offi. Publ. Eur. Dialy. Trans. Assoc. Eur. Renal Assoc.* 23, 842–852. doi: 10.1093/ndt/gfm694

Kunugi, S., Shimizu, A., Kuwahara, N., Du, X., Takahashi, M., Terasaki, Y., et al. (2011). Inhibition of matrix metalloproteinases reduces ischemia-reperfusion acute kidney injury. *Laboratory Investigat.* 91, 170–180. doi: 10.1038/labinvest.2010.174

Kuppe, C., Ibrahim, M. M., Kranz, J., Zhang, X., Ziegler, S., Perales-Patón, J., et al. (2021). Decoding myofibroblast origins in human kidney fibrosis. *Nature* 589, 281–286.

LeBleu, V. S., Taduri, G., O'Connell, J., Teng, Y., Cooke, V. G., Woda, C., et al. (2013). Origin and function of myofibroblasts in kidney fibrosis. *Nat. Med.* 19, 1047–1053.

Lee, S., Huen, S., Nishio, H., Nishio, S., Lee, H., Choi, B., et al. (2011). Distinct macrophage phenotypes contribute to kidney injury and repair. *J. Am. Soc. Nephrol. JASN* 22, 317–326. doi: 10.1681/asn.2009060615

Li, J., Qu, X., Yao, J., Caruana, G., Ricardo, S. D., Yamamoto, Y., et al. (2010). Blockade of endothelial-mesenchymal transition by a smad3 inhibitor delays the early development of streptozotocin-induced diabetic nephropathy. *Diabetes* 59, 2612–2624. doi: 10.2337/db09-1631

Li, J., Ren, J., Liu, X., Jiang, L., He, W., Yuan, W., et al. (2015). Rictor/mTORC2 signaling mediates TGFβ1-induced fibroblast activation and kidney fibrosis. *Kidney Int.* 88, 515–527. doi: 10.1038/ki.2015.119

Li, Y., Tan, X., Dai, C., Stolz, D. B., Wang, D., and Liu, Y. (2009). Inhibition of integrin-linked kinase attenuates renal interstitial fibrosis. *J. Am. Soc. Nephrol.* 20, 1907–1918. doi: 10.1681/asn.2008090930

Liang, H., Zhang, Z., Yan, J., Wang, Y., Hu, Z., Mitch, W. E., et al. (2017). The IL-4 receptor α has a critical role in bone marrow-derived fibroblast activation and renal fibrosis. *Kidney Int.* 92, 1433–1443. doi: 10.1016/j.kint.2017.04.021

Lin, S., Li, B., Rao, S., Yeo, E., Hudson, T., Nowlin, B., et al. (2010). Macrophage Wnt7b is critical for kidney repair and regeneration. *Proc. Natl. Acad. Sci. U.S.A.* 107, 4194–4199. doi: 10.1073/pnas.0912228107

Lisi, L., Stigliano, E., Lauriola, L., Navarra, P., and Dello Russo, C. (2014). Proinflammatory-activated glioma cells induce a switch in microglial polarization and activation status, from a predominant M2b phenotype to a mixture of M1 and M2a/B polarized cells. *ASN Neuro* 6, 171–183.

Liu, Y. (2010). New insights into epithelial-mesenchymal transition in kidney fibrosis. *J. Am. Soc. Nephrol. JASN* 21, 212–222. doi: 10.1681/asn.2008121226

Lu, J., Cao, Q., Zheng, D., Sun, Y., Wang, C., Yu, X., et al. (2013). Discrete functions of M2a and M2c macrophage subsets determine their relative efficacy in treating chronic kidney disease. *Kidney Int.* 84, 745–755. doi: 10.1038/ki.2013.135

Lv, L., Tang, P., Li, C., You, Y., Li, J., Huang, X., et al. (2017). The pattern recognition receptor, Mincle, is essential for maintaining the M1 macrophage phenotype in acute renal inflammation. *Kidney Int.* 91, 587–602. doi: 10.1016/j.kint.2016.10.020

Mantovani, A., Sica, A., Sozzani, S., Allavena, P., Vecchi, A., and Locati, M. (2004). The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol.* 25, 677–686. doi: 10.1016/j.it.2004.09.015

Mao, J., Zeng, Z., Xu, Z., Li, J., Jiang, L., Fang, Y., et al. (2014). Mammalian target of rapamycin complex 1 activation in podocytes promotes cellular crescent formation. *Am. J. Physiol. Renal Physiol.* 307, F1023–F1032.

Meng, X., Wang, S., Huang, X., Yang, C., Xiao, J., Zhang, Y., et al. (2016b). Inflammatory macrophages can transdifferentiate into myofibroblasts during renal fibrosis. *Cell Death Dis.* 7:e2495. doi: 10.1038/cddis.2016.402

Meng, X. M., Nikolic-Paterson, D. J., and Lan, H. Y. (2016a). TGF-β: the master regulator of fibrosis. *Nat. Rev. Nephrol.* 12, 325–338. doi: 10.1038/nrneph.2016.48

Meng, X. M., Tang, P. M., Li, J., and Lan, H. Y. (2015). Macrophage phenotype in kidney injury and repair. *Kidney Dis.* 1, 138–146. doi: 10.1159/000431214

Miao, J., Liu, J., Niu, J., Zhang, Y., Shen, W., Luo, C., et al. (2019). Wnt/β-catenin/RAS signaling mediates age-related renal fibrosis and is associated with mitochondrial dysfunction. *Aging Cell* 18: e13004-e.

Ng, L., Kaur, P., Bunnag, N., Suresh, J., Sung, I., Tan, Q., et al. (2019). WNT signaling in disease. *Cells* 8:826.

Nie, X., Liu, H., Liu, L., Wang, Y. D., and Chen, W. D. (2020). Emerging roles of Wnt ligands in human colorectal cancer. *Front. Oncol.* 10:1341.

Nikolic-Paterson, D., Wang, S., and Lan, H. (2014). Macrophages promote renal fibrosis through direct and indirect mechanisms. *Kidney Int. Suppl.* 4, 34–38. doi: 10.1038/kisup.2014.7

Okamura, A., Rakugi, H., Ohishi, M., Yanagitani, Y., Takiuchi, S., Moriguchi, K., et al. (1999). Upregulation of renin-angiotensin system during differentiation of monocytes to macrophages. *J. Hypertens* 17, 537–545. doi: 10.1097/00004872-199917040-00012

Palevski, D., Levin-Kotler, L. P., Kain, D., Naftali-Shani, N., Landa, N., Ben-Mordechai, T., et al. (2017). Loss of macrophage Wnt secretion improves remodeling and function after myocardial infarction in mice. *J. Am. Heart Assoc.* 6:e004387.

Pan, B., Liu, G., Jiang, Z., and Zheng, D. (2015). Regulation of renal fibrosis by macrophage polarization. *Cell. Physiol. Biochem. Int. J. Exp. Cell. Physiol. Biochem. Pharmacol.* 35, 1062–1069. doi: 10.1159/000373932

Pang, M., Wang, H., Rao, P., Zhao, Y., Xie, J., Cao, Q., et al. (2016). Autophagy links beta-catenin and smad signaling to promote epithelial-mesenchymal transition via upregulation of integrin linked kinase. *Int. J. Biochem. Cell. Biol.* 76, 123–134. doi: 10.1016/j.biocel.2016.05.010

Perugorria, M., Olaizola, P., Labiano, I., Esparza-Baquer, A., Marzioni, M., Marin, J., et al. (2019). Wnt-β-catenin signalling in liver development, health and disease. *Nat. Rev. Gastroenterol. Hepatol.* 16, 121–136. doi: 10.1111/j.1365-2184.2012.00806.x

Philipp, D., Suhr, L., Wahlers, T., Choi, Y.-H., and Paunel-Görgülü, A. (2018). Preconditioning of bone marrow-derived mesenchymal stem cells highly strengthens their potential to promote IL-6-dependent M2b polarization. *Stem. Cell Res. Ther.* 9:286.

Pilling, D., and Gomer, R. (2012). Differentiation of circulating monocytes into fibroblast-like cells. *Methods Mol. Biol.* 904, 191–206. doi: 10.1007/978-1-61779-943-3_16

Qiao, X., Rao, P., Zhang, Y., Liu, L., Pang, M., Wang, H., et al. (2018). Redirecting TGF-beta Signaling through the beta-catenin/foxo complex prevents kidney fibrosis. *J. Am. Soc. Nephrol.* 29, 557–570. doi: 10.1681/asn.2016121362

Ranganathan, P. V., Jayakumar, C., and Ramesh, G. (2013). Netrin-1-treated macrophages protect the kidney against ischemia-reperfusion injury and suppress inflammation by inducing M2 polarization. *Am. J. Physiol. Renal Physiol.* 304, F948–F957.

Rao, P., Pang, M., Qiao, X., Yu, H., Wang, H., Yang, Y., et al. (2019). Promotion of beta-catenin/Foxo1 signaling ameliorates renal interstitial fibrosis. *Lab Invest* 99, 1689–1701. doi: 10.1038/s41374-019-0276-z

Rao, P., Qiao, X., Hua, W., Hu, M., Tahan, M., Chen, T., et al. (2021). Promotion of β-catenin/Foxo signaling mediates epithelial repair in kidney injury. *Am. J. Pathol.* 191, 993–1009. doi: 10.1016/j.ajpath.2021.03.005

Ratnayake, D., Nguyen, P. D., Rossello, F. J., Wimmer, V. C., Tan, J. L., Galvis, L. A., et al. (2021). Macrophages provide a transient muscle stem cell niche via NAMPT secretion. *Nature* 591, 281–287. doi: 10.1038/s41586-021-03199-7

Ricardo, S., van Goor, H., and Eddy, A. (2008). Macrophage diversity in renal injury and repair. *J. Clin. Investigat.* 118, 3522–3530. doi: 10.1172/jci36150

Rogers, N., Ferenbach, D., Isenberg, J., Thomson, A., and Hughes, J. (2014). Dendritic cells and macrophages in the kidney: a spectrum of good and evil. *Nat. Rev. Nephrol.* 10, 625–643. doi: 10.1038/nrneph.2014.170

Rosenberger, C. M., and Finlay, B. B. (2003). Phagocyte sabotage: disruption of macrophage signalling by bacterial pathogens. *Nat. Rev. Mol. Cell Biol.* 4, 385–396. doi: 10.1038/nrm1104

Salvador, P., Macías-Ceja, D., Gisbert-Ferrándiz, L., Hernández, C., Bernardo, D., Alós, R., et al. (2018). CD16+ macrophages mediate fibrosis in inflammatory bowel disease. *J. Crohn's Colitis* 12, 589–599. doi: 10.1093/ecco-jcc/jjx185

Schunk, S. J., Floege, J., Fliser, D., and Speer, T. (2021). WNT–β-catenin signalling a versatile player in kidney injury and repair. *Nat. Rev. Nephrol.* 17, 172–184. doi: 10.1038/s41581-020-00343-w

Shen, B., Liu, X., Fan, Y., and Qiu, J. (2014). Macrophages regulate renal fibrosis through modulating TGFβ superfamily signaling. *Inflammation* 37, 2076–2084. doi: 10.1007/s10753-014-9941-y

Tan, R., Zhou, D., Zhou, L., and Liu, Y. (2014). Wnt/β-catenin signaling and kidney fibrosis. *Kidney Int. Suppl.* 4, 84–90. doi: 10.1038/kisup.2014.16

Tan, T. K., Zheng, G., Hsu, T. T., Lee, S. R., Zhang, J., Zhao, Y., et al. (2013). Matrix metalloproteinase-9 of tubular and macrophage origin contributes to the pathogenesis of renal fibrosis via macrophage recruitment through osteopontin cleavage. *Lab Invest* 93, 434–449. doi: 10.1038/labinvest.2013.3

Tan, T. K., Zheng, G., Hsu, T. T., Wang, Y., Lee, V. W., Tian, X., et al. (2010). Macrophage matrix metalloproteinase-9 mediates epithelial-mesenchymal transition in vitro in murine renal tubular cells. *Am. J. Pathol.* 176, 1256–1270. doi: 10.2353/ajpath.2010.090188

Tang, L., Zhang, H., Wang, C., Li, H., Zhang, Q., and Bai, J. (2017). M2A and M2C macrophage subsets ameliorate inflammation and fibroproliferation in acute lung injury through interleukin 10 pathway. *Shock* 48, 119–129. doi: 10.1097/shk.0000000000000820

Tang, P. M., Nikolic-Paterson, D. J., and Lan, H. Y. (2019). Macrophages: versatile players in renal inflammation and fibrosis. *Nat. Rev. Nephrol.* 15, 144–158. doi: 10.1038/s41581-019-0110-2

Tseng, W., Tsai, M., Chen, N., and Tarng, D. (2020). Trichostatin a alleviates renal interstitial fibrosis through modulation of the M2 macrophage subpopulation. *Int. J. Mol. Sci.* 21:5966. doi: 10.3390/ijms21175966

Ulrich, C., Seibert, E., Heine, G. H., Fliser, D., and Girndt, M. (2011). Monocyte angiotensin converting enzyme expression may be associated with atherosclerosis rather than arteriosclerosis in hemodialysis patients. *Clin. J. Am. Soc. Nephrol.* 6, 505–511. doi: 10.2215/cjn.06870810

Urbina, P., and Singla, D. K. (2014). BMP-7 attenuates adverse cardiac remodeling mediated through M2 macrophages in prediabetic cardiomyopathy. *Am. J. Physiol. Heart Circ. Physiol.* 307, H762–H772.

Venturin, G., Chiku, V., Silva, K., de Almeida, B., and de Lima, V. (2016). M1 polarization and the effect of PGE on TNF-α production by lymph node cells from dogs with visceral leishmaniasis. *Parasite Immunol.* 38, 698–704. doi: 10.1111/pim.12353

Viehmann, S. F., Böhner, A. M. C., Kurts, C., and Brähler, S. (2018). The multifaceted role of the renal mononuclear phagocyte system. *Cell Immunol.* 330, 97–104. doi: 10.1016/j.cellimm.2018.04.009

Wang, D., Dai, C., Li, Y., and Liu, Y. (2011). Canonical Wnt/β-catenin signaling mediates transforming growth factor-β1-driven podocyte injury and proteinuria. *Kidney Int.* 80, 1159–1169. doi: 10.1038/ki.2011.255

Wang, H., Melton, D. W., Porter, L., Sarwar, Z. U., McManus, L. M., and Shireman, P. K. (2014). Altered macrophage phenotype transition impairs skeletal muscle regeneration. *Am. J. Pathol.* 184, 1167–1184. doi: 10.1016/j.ajpath.2013.12.020

Wang, L. X., Zhang, S. X., Wu, H. J., Rong, X. L., and Guo, J. (2019). M2b macrophage polarization and its roles in diseases. *J. Leukocyte Biol.* 106, 345–358. doi: 10.1002/jlb.3ru1018-378rr

Wang, M., Chen, D., Chen, L., Cao, G., Zhao, H., Liu, D., et al. (2018). Novel inhibitors of the cellular renin-angiotensin system components, poricoic acids, target Smad3 phosphorylation and Wnt/β-catenin pathway against renal fibrosis. *Br. J. Pharmacol.* 175, 2689–2708. doi: 10.1111/bph.14333

Wang, Y., and Harris, D. C. (2011). Macrophages in renal disease. *J. Am. Soc. Nephrol.* 22, 21–27. doi: 10.1681/asn.2010030269

Wang, Y., Jiang, H., Pan, J., Huang, X., Wang, Y., Huang, H., et al. (2017). Macrophage-to-myofibroblast transition contributes to interstitial fibrosis in chronic renal allograft injury. *J. Am. Soc. Nephrol. JASN* 28, 2053–2067. doi: 10.1681/asn.2016050573

Wang, Y., Wang, Y. P., Zheng, G., Lee, V. W., Ouyang, L., Chang, D. H., et al. (2007). Ex vivo programmed macrophages ameliorate experimental chronic inflammatory renal disease. *Kidney Int.* 72, 290–299. doi: 10.1038/sj.ki.5002275

Wang, Y., Zhou, C., and Liu, Y. (2018). Wnt signaling in kidney development and disease. *Prog. Mol. Biol. Trans. Sci.* 153, 181–207. doi: 10.1016/bs.pmbts.2017.11.019

Weichhart, T., Hengstschläger, M., and Linke, M. (2015). Regulation of innate immune cell function by mTOR. *Nat. Rev. Immunol.* 15, 599–614. doi: 10.1038/nri3901

Wong, D. W. L., Yiu, W. H., Chan, K. W., Li, Y., Li, B., Lok, S. W. Y., et al. (2018). Activated renal tubular Wnt/β-catenin signaling triggers renal inflammation during overload proteinuria. *Kidney Int.* 93, 1367–1383. doi: 10.1016/j.kint.2017.12.017

Wu, S., Li, M., Xu, F., Li, G., Han, B., He, X., et al. (2020). Fibrinogen-like protein 2 deficiency aggravates renal fibrosis by facilitating macrophage polarization. *Biomed. Pharmacother.* 130:110468. doi: 10.1016/j.biopha.2020.110468

Xia, Y., Yan, J., Jin, X., Entman, M. L., and Wang, Y. (2014). The chemokine receptor CXCR6 contributes to recruitment of bone marrow-derived fibroblast precursors in renal fibrosis. *Kidney Int.* 86, 327–337. doi: 10.1038/ki.2014.64

Yan, J., Zhang, Z., Yang, J., Mitch, W. E., and Wang, Y. (2015). JAK3/STAT6 stimulates bone marrow-derived fibroblast activation in renal fibrosis. *J. Am. Soc. Nephrol. JASN* 26, 3060–3071. doi: 10.1681/asn.2014070717

Yang, Y., Feng, X., Liu, X., Wang, Y., Hu, M., Cao, Q., et al. (2019). Fate alteration of bone marrow-derived macrophages ameliorates kidney fibrosis in murine model of unilateral ureteral obstruction. *Nephrol. Dialy. Trans. Offi. Publi. Eur. Dialy. Trans. Assoc. Eur. Renal Assoc.* 34, 1657–1668. doi: 10.1093/ndt/gfy381

Yang, Y., Nankivell, B. J., Hua, W., Rao, P., Ren, X., Yu, H., et al. (2020). Renal tubular cell binding of beta-catenin to TCF1 versus FoxO1 is associated with chronic interstitial fibrosis in transplanted kidneys. *Am. J. Trans.* 21, 727–739. doi: 10.1111/ajt.16287

Yu, F., Wu, L., Tan, Y., Li, L., Wang, C., Wang, W., et al. (2010). Tubulointerstitial lesions of patients with lupus nephritis classified by the 2003 international society of nephrology and renal pathology society system. *Kidney Int.* 77, 820–829. doi: 10.1038/ki.2010.13

Zeisberg, E. M., Potenta, S. E., Sugimoto, H., Zeisberg, M., and Kalluri, R. (2008). Fibroblasts in kidney fibrosis emerge via endothelial-to-mesenchymal transition. *J. Am. Soc. Nephrol.* 19, 2282–2287. doi: 10.1681/asn.2008050513

Zhang, C., Yu, S., Zheng, B., Liu, D., Wan, F., Ma, Y., et al. (2019). miR-30c-5p reduces renal ischemia-reperfusion involving macrophage. *Med. Sci. Monitor Int. Med. J. Exp. Clin. Res.* 25, 4362–4369. doi: 10.12659/msm.914579

Zhang, M., Wang, X., Wang, Y., Niu, A., Wang, S., Zou, C., et al. (2017). IL-4/IL-13-mediated polarization of renal macrophages/dendritic cells to an M2a phenotype is essential for recovery from acute kidney injury. *Kidney Int.* 91, 375–386. doi: 10.1016/j.kint.2016.08.020

Zhao, Y., Qiao, X., Wang, L., Tan, T. K., Zhao, H., Zhang, Y., et al. (2016). Matrix metalloproteinase 9 induces endothelial-mesenchymal transition via Notch activation in human kidney glomerular endothelial cells. *BMC Cell Biol.* 17:21.

Zheng, G., Lyons, J. G., Tan, T. K., Wang, Y., Hsu, T. T., Min, D., et al. (2009). Disruption of E-cadherin by matrix metalloproteinase directly mediates epithelial-mesenchymal transition downstream of transforming growth factor-beta1 in renal tubular epithelial cells. *Am. J. Pathol.* 175, 580–591. doi: 10.2353/ajpath.2009.080983

Zheng, G., Zhang, J., Zhao, H., Wang, H., Pang, M., Qiao, X., et al. (2016). alpha3 integrin of cell-cell contact mediates kidney fibrosis by integrin-linked kinase in proximal tubular E-cadherin deficient mice. *Am. J. Pathol.* 186, 1847–1860. doi: 10.1016/j.ajpath.2016.03.015

Zhou, B., Liu, Y., Kahn, M., Ann, D. K., Han, A., Wang, H., et al. (2012). Interactions between beta-catenin and transforming growth factor-beta signaling pathways mediate epithelial-mesenchymal transition and are dependent on the transcriptional co-activator cAMP-response element-binding protein (CREB)-binding protein (CBP). *J. Biol. Chem.* 287, 7026–7038. doi: 10.1074/jbc.m111.276311

Zhou, G., Li, J., Zeng, T., Yang, P., and Li, A. (2020). The regulation effect of WNT-RAS signaling in hypothalamic paraventricular nucleus on renal fibrosis. *J. Nephrol.* 33, 289–297. doi: 10.1007/s40620-019-00637-8

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The Advances of Single-Cell RNA-Seq in Kidney Immunology

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Kidney diseases are highly prevalent and treatment is costly. Immune cells play important roles in kidney diseases; however, it has been challenging to investigate the contribution of each cell type in kidney pathophysiology. Recently, the development of single-cell sequencing technology has allowed the extensive study of immune cells in blood, secondary lymphoid tissues, kidney biopsy and urine samples, helping researchers generate a comprehensive immune cell atlas for various kidney diseases. Here, we discuss several recent studies using scRNA-seq technology to explore the immune-related kidney diseases, including lupus nephritis, diabetic kidney disease, IgA nephropathy, and anti-neutrophil cytoplasmic antibody-associated glomerulonephritis. Application of scRNA-seq successfully defined the transcriptome profiles of resident and infiltrating immune cells, as well as the intracellular communication networks between immune and adjacent cells. In addition, the discovery of similar immune cells in blood and urine suggests the possibility of examining kidney immunity without biopsy. In conclusion, these immune cell atlases will increase our understanding of kidney immunology and contribute to novel therapeutics for patients with kidney diseases.

Keywords: kidney, immune system, single-cell RNA sequencing, lupus nephritis, diabetic kidney disease, IgA nephropathy, anti-neutrophil cytoplasmic antibody-associated glomerulonephritis

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BACKGROUND

The kidneys are vital organs serving critical functions in the human body, including clearance of waste from blood, maintenance of the salt/water balance, and regulation of blood pressure. In addition to these functions, the kidneys also maintain the homeostasis of the immune system through filtration and excretion of bacterial toxins, circulating cytokines, and inflammatory molecules (Kurts et al., 2013; Donnan et al., 2021). Although considerable progress has been achieved in elucidating the contribution of immune cells in kidney diseases and in translating these results from the bench to the bedside, determining how these immune cells orchestrate kidney immunology in health and disease remains a challenge due to the relatively small number of immune cells and the highly complex cell composition of kidneys. Hence, our understanding of kidney immunology in humans remains incomplete.

In health, the major kidney resident immune cells are dendritic cells (DCs) and macrophages (Cao et al., 2011; Kurts et al., 2013, 2020). A small number of lymphocytes are also present in healthy kidneys (Turner et al., 2018). Therefore, the kidneys, similar to the spleen, can maintain the peripheral tolerance through the kidney resident antigen-presenting cells (APCs). This becomes more obvious in end-stage renal disease (ESRD), where both kidney function and the immune system are severely compromised. Retention of excessive toxins and cytokines in ESRD activates innate immune cells and increases the production of cytokines and proinflammatory molecules,

which further causes kidney damage (Betjes, 2013). Kidney-resident DCs, derived from common DC precursors (CDPs) and monocytes, are located in the tubulointerstitium and absent in the glomeruli (Teteris et al., 2011). Resident DCs in the kidney appear to be crucial for maintaining peripheral tolerance via direct interaction with filtered antigens in the tubular lumen regions and for presenting these antigens to T cells in lymph nodes, which may be more efficient in kidneys than in other organs. In ischemia-reperfusion (IR) and unilateral ureter obstruction (UUO) mouse models, resident DCs promoted kidney injury by producing proinflammatory cytokines. In contrast to DCs, kidney-resident macrophages are scattered in the renal cortex and infiltrate into the tubulointerstitium during tissue damage. Some evidence suggests that kidney-resident macrophages play protective roles during acute and chronic kidney diseases. Studies found that the lack of resident kidney $CD45^{+}$ Ly6G-$F4/80^{high}CD11b^{int}$ macrophages, which express an inhibitory immune checkpoint molecule, V-domain Ig suppressor of T cell activation (VISTA), delays tissue repair in several animal models (Park et al., 2020). $CD11b^{int}F4/80^{bright}$ kidney resident macrophages were found to be protective in the ischemic kidney by promoting proangiogenic environments (Puranik et al., 2018). In contrast, other studies argue that activation of macrophages aggravates kidney injury through the production of proinflammatory cytokines (Yang et al., 1998; Isbel et al., 2001; Eardley et al., 2008). Thus, kidney-resident macrophages seem to have diverse effects depending on cell identity, the kidney injury type and the disease stage.

On one hand, kidneys can be the direct target of the immune system, by which lymphocytes and/or antibodies interact with kidney cells and lead to kidney damage. Tubulointerstitium DCs might capture glomerular antigens, which in turn induce infiltrating T cells to produce proinflammatory cytokines. Infiltrating monocytes also contribute to local inflammation and tissue injury, which have been shown to cause tubular atrophy and interstitial scarring. On the other hand, kidneys can be an indirect victim of immune system dysregulation, such as the immune complexes deposition in glomeruli that promote local inflammation. Immune complexes can be a result of either an immune response to infection or a systemic autoimmune disease condition (e.g., SLE). In both cases, activation of innate and adaptive immune cells, as well as the complement system, play important roles in various kidney diseases.

Although a number of previous studies have confirmed the contribution of immune cells in kidney diseases (Kurts et al., 2013; Donnan et al., 2021), each of these studies focus on fewer than three immune cell populations. To date, therefore, our knowledge and understanding of kidney immunology as a whole is still incomplete. Here, we discuss the recent progress and findings in kidney diseases uncovered by scRNA-seq technology. We briefly introduce the general principles and current methodologies of scRNA-seq as well as the experimental design for basic and clinical research related to the kidney field. We then discuss recent findings focusing on several immune-related kidney diseases, including LN, DKD, IgAN, and ANCA-GN, highlighting the changes in immune cell populations and the possible immune mechanisms revealed by scRNA-seq technology. Finally, we discuss how the generation of immune cell atlases might help transform these results into immunological therapies in the future.

COMPARISON OF SINGLE-CELL RNA-SEQ METHODS

scRNA-seq technology has been rapidly developed in recent decades (**Figure 1**; Tang et al., 2009; Islam et al., 2011; Ramskold et al., 2012; Macosko et al., 2015; Gierahn et al., 2017), enabling the characterization of transcriptome profiles in heterogeneous cell populations at the single-cell resolution (Wagner et al., 2016). To date, several scRNA-seq methods are commercially available; each with distinct strengths and limitations with respect to experiment throughput, detection sensitivity, gene coverage, and cost per cell (**Table 1**). General workflow of scRNA-seq includes single-cell preparation, cell capture and lysis, mRNA capture and reverse transcription, cDNA amplification, library construction, next-generation sequencing, and computational analysis. According to the sequencing length, single-cell RNA-seq can be divided into full-length, 3′ and 5′ end sequencing methods. Full-length sequencing captures the whole mRNA sequences and has a deeper sequencing depth, which make it very useful in studying genes related to alternative splicing or at low levels. Both 3′ and 5′ end sequencing has higher experimental throughput and lower cost per cell. Currently, 3′ end sequencing is more frequently used for whole transcriptomic assays, while the 5′ end is used to detect the clonal diversity of T and B cells trough TCR or BCR V(D)J sequencing (Papalexi and Satija, 2018). With the rapid development and commercialization of these scRNA-seq methods, an increasing number of open-source computational tools, from data quality control, batch effect correction, dimension reduction analysis, to data visualization, have been developed, which further made scRNA-seq technology applicable to most researchers. To date, several scRNA-seq methods have been used to investigate more than 10,000 cells simultaneously, allowing us to identify both rare and novel cell populations, investigate the transcriptomic changes in each cell and analyze the intercellular communication networks. As mentioned above, single-cell preparation is the first step of all scRNA-seq technologies which require fresh tissue samples and successful dissociation of tissue to generate good quality data and cells should be smaller than the droplets or microwells. To overcome these technical barriers, researchers developed the single-nucleus RNA-sequencing (snRNA-seq) method (Krishnaswami et al., 2016; Lacar et al., 2016; Habib et al., 2017), where nuclear, not cytoplasmic RNA is sequenced. Although the RNA-splicing information is lost and the detection sensitivity is reduced because of the increased level of pre-mRNA, snRNA-seq is particularly helpful in studying the dissociation-resistant or frozen samples, and cells with diameters over 50 μm. Therefore, researchers should carefully select scRNA-seq methods for their studies based on the tissue origin, sample size, sample storage method, and target cell abundance. It is highly recommended that target cells should be enriched by FACS when their population is less than 5% of the total.

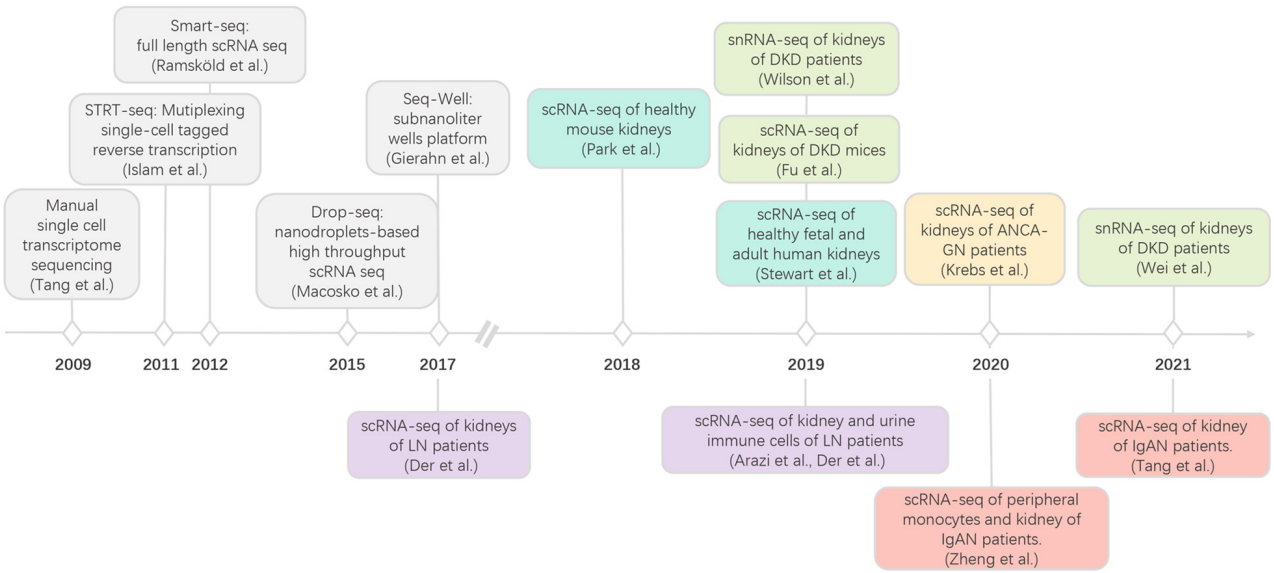

FIGURE 1 | Development of scRNA-seq and its applications in kidney immunology. LN, lupus nephritis; diabetic kidney disease; IgAN, IgA nephropathy; ANCA-GN anti-neutrophil cytoplasmic antibody-associated glomerulonephritis.

TABLE 1 | List of scRNA-seq methods.

	SMART-seq/C1	SMART-seq2	CEL-seq2/C1	10× Genomics chromium	BD rhapsody
Single cell preparation and capture	Fluidigm C1 platform	FACS	C1 platform	10× Genomics chromium platform	BD rhapsody platform
Sample preparation	Complicated	Complicated	Complicated	Easy	Easy
Quality check	Microscope	No	Microscope	No	BD rhapsody platform
UMI-based	No	No	Yes	Yes	Yes
cDNA coverage	Full-length	Full-length	3′ counting	5′ or 3′ counting	5′ or 3′ counting
Amplification method	TS-based PCR	TS-based PCR	*In vitro* transcription	TS-based PCR	TS-based PCR
Sample multiplexing	No	No	No	Yes	Yes
Cell number limitation	5–10, 10–17, or 17–25 μm	Less than 50 μm	Less than 50 μm	Less than 50 μm	Less than 50 μm
Required cell numbers per run	>10,000	No limitation	>10,000	>10,000	>20,000
Long term storage	No	Yes	No	No	Yes
Throughput capability	Limited by number of machines	Limited by operator efficiency	Limited by number of machines	Up to 8 samples per chip	Limited by operator efficiency
Cost	High	High	Medium	Low	Low

A study showed that snRNA-seq performed well on inflamed fibrotic tissue and could capture more cell types including glomerular podocytes, mesangial cells, endothelial cells, and juxtaglomerular cells with reduced dissociation-induced bias and transcriptional stress responses compared to scRNA-seq (Wu et al., 2019).

In addition to aforementioned scRNA-seq methods, which provide robust information of different cell populations but lose the spatial characteristics of samples, high-throughput spatial transcriptomics techniques were developed and most of them recorded the spatial information by labeling mRNA transcripts with location barcodes. Following detachment and deep sequencing, spatial transcriptomics techniques align transcripts to their capture spots of origin based on their location barcode (Longo et al., 2021). Spatial transcriptomics techniques were powerful in resolving the dynamic change within the niches under specific biological context, and efforts were made to increase the resolution and integrated the scRNA seq with spatial transcriptomes.

In most cases, scRNA-seq was employed *in vivo* experiment but its application *in vitro* also helps people to resolve complex kidney pathogenic mechanism, especially when studying cells underwent asynchronous differentiation or continuous transition. Kidney organoids which were generated from human

TABLE 2 | Summary of scRNA-seq in kidney diseases.

Author information	Arazi et al., 2019	Der et al., 2019	Fu et al., 2019	Wilson et al., 2019	Zheng et al., 2020	Tang et al., 2021	Krebs et al., 2020
Disease type	LN	LN	DKD	DKD	IgAN	IgAN	ANCA-GN
scRNA-seq method	Modified CEL-Seq2	Fluidigm C1	Fluidigm C1	10× chromium	Modified STRT-seq	Singleron Matrix	10× chromium CITE-seq
Species	Human	Human	Mouse	Human	Human	Human	Human and mouse
Tissue origin	Kidney biopsy, blood	Kidney and skin biopsy, blood	Kidney tissue	Kidney biopsy, blood	Kidney biopsy, blood	Kidney biopsy, blood	Kidney biopsy, blood
Human sample number	10 control	3 control	3 control	3 non-diabetic	5 control	1 control (GSE131685)	3 control
	24 LN	21 LN kidney biopsy, 17 skin biopsy	3 STZ-diabetes	3 Diabetes	5 IgAN	4 IgAN	3 ANCA-GN
Cell number for analysis	2,736	4,019	829	23,980	8,880	20,570	5,905
Features of the study	21 leukocyte clusters; local activation of B cells; renal differentiation of monocytes; type 1 interferon response genes in most cells; broad expression of CXCR4 and CX3CR1	Enriched type I interferon response genes; association of high IFN response and fibrotic genes in tubular cells with non-responders	Increased number of immune cells in DKD glomeruli; macrophages are major immune cell type; higher number of M1 macrophages than M2	4 clusters of immune cells, including T, B, monocytes, and plasma cells; increased number of leukocytes; increased expression of TNFRSF21 in monocytes	Increased interactions between mesangial cells, macrophages, and T cells; increased Notch, glycolysis, fatty acid, and amino-acid metabolism genes in macrophages; increased expression of CCL2 and CX3CR1 in macrophages	Detected macrophages, monocytes, and DCs but not T cells in IgAN kidney; decreased expression of GPX3, FAM49B, and FCGBP in macrophages; increased expression of CCL2 and CXCL1; increased expression of Notch, FGF2 and PDGFD in mesangial cells	12 clusters of renal T cells; $CCR6^{high}CCR7^{-}CD69^{+}$ $CD4^{+}$ T_{RM} cells display Th17 signature in ANCA-GN; pathogen infection induces $T_{RM}17$ cells in kidney; increased level of IL-17A from $T_{RM}17$ cells

pluripotent stem cells had self-organizing 3D structure and mimicked the functions of human kidney. Kidney organoids emerged as human-based models to study multiple kidney cells as a whole *in vitro* and serve to biomarker discovery and drug invention (Bonventre, 2018). By performing scRNA-seq of kidney organoids, Digby et al. (2020) found that kidney organoid demonstrated phenotype of acute kidney injury (AKI) with higher expression of KIM1 and increased cell damage. The main injured cell types of cisplatin was change as concentration altered with interstitial cells at high dose (50 μM) and proximal tubule cell at low repeated dose. Their study validates the use of kidney organoids to model AKI *in vitro* and provided a suitable stimulation protocol to study AKI. Macrophage-myofibroblast transition (MMT) is a newly discovered process and promote kidney fibrosis in a TGF-β/Smad3-dependent pattern (Meng et al., 2016; Wang et al., 2016). By performing scRNA-seq to study sorted TGF-β1-treated $Smad3^{+/+}$ and $Smad3^{-/-}$ bone marrow-derived macrophages (BMDMs), Tang et al. (2018) uncovered that the proto-oncogene Src is a direct target gene of Smad3 and serves as a centric hub in the gene regulatory network during MMT. They also found that neural transcription factor Pou4f1 was predicted as the only transcription factor involved in MMT, and later experiments validated that Pou4f1 promoted MMT-mediated fibrosis via a fibrogenic gene network including Fn1, Pdgfrb, Itgb3 et al. (Tang et al., 2020).

GENERATION OF A KIDNEY CELL ATLAS THROUGH scRNA-SEQ

Recently, a number of studies have been published using scRNA-seq technology in kidney research, from those seeking to discover rare and novel kidney cell types to elucidating cell type-specific contributions to kidney diseases and understanding intracellular communication networks (**Table 2**). A groundbreaking progress is the generation of healthy kidney cell atlas, which can serve as a reference transcriptomic map for the future studies (Park et al., 2018). Park et al. (2018) applied scRNA-seq to healthy mouse kidneys and provided a molecular definition of kidney cells. They found five clusters of immune cells in the kidney including macrophages, neutrophils, natural killer cells, B lymphocytes and T lymphocytes. Among them, T lymphocytes had the largest number. They also discovered a novel collecting duct cell population, which expresses both intercalated and principal cell markers. They found that a transition between cells of this subpopulation. When compared with the transcriptional profiles of kidney cells from a mouse fibrosis model, these novel cells

discovered by Park et al. (2018) exhibited a shift in the transition balance toward principal cells. Since intercalated cells play critical roles in proton secretion, a decreased number of intercalated cells may lead to metabolic acidosis in CKD patients.

To define the heterogeneity among epithelial, myeloid, and lymphoid cells, Stewart et al. (2019) performed scRNA-seq using healthy fetal and adult kidney samples. They discovered that several immune cell populations in fetal kidneys appear at the different time points. Certain types of DCs and macrophages were found to be present at the earliest development stage, while NK cells, T cells, and monocytes appeared at 9 weeks of gestation, and B cells were found to present at 12 weeks. These data reveal the temporal development of immune cells at single-cell resolution in human fetal kidney. Using mature kidney samples, this group identified a higher number of immune cells, including resident macrophages, pDCs, neutrophils, mast cells, T, B, NK, and NKT cells, compared to that of fetal kidney samples. In the lymphoid compartment of fetal kidney, B cells did not express class-switching genes, and CD8 + T cells expressed the low effector gene GZMH, while in healthy mature kidneys, B cell clusters expressed IgM, IgG, and IgA, and polarization of CD4 + T cells was not found through an analysis of cytokine and transcription factor expression. Within the NK clusters, there were cells expressing both γ- and δ-T cell receptors as well as markers of mucosal-associated invariant T (MAIT) cells. They also demonstrated that CD8 + T and NK cells in fetal kidneys show decreased enrichment with genes involved in "T cell receptor signaling" and "NK cell-mediated immunity," respectively, compared to those cells in mature kidneys. Interestingly, Stewart et al. (2019) also discovered that the transcriptome profiles of macrophages in both fetal and mature kidneys resembled those of anti-inflammatory M2 macrophages. Compared with the monocytes in fetal kidney, monocyte-derived macrophages displayed higher levels of phagocytosis genes and defense genes to bacteria in mature kidney. Taken together, these studies provide a novel immune cell landscape in mouse and human kidneys, which can be used as a reference dataset and will facilitate the future study of pathogenic mechanisms.

scRNA-SEQ IN LUPUS NEPHRITIS

Lupus nephritis (LN) is caused by systemic lupus erythematosus (SLE), an autoimmune disorder in which the immune system targets the body's own cells (Davidson and Aranow, 2010; Tsokos, 2011). LN occurs when lupus autoantibodies attack the kidneys, which leads to hematuria, proteinuria, impaired kidney function, and even kidney failure. Considerable evidence from pathological and bulk RNA-seq analyses of LN kidney samples suggests that the infiltration of lymphocytes is closely associated with reduced kidney function as well as a poor patient prognosis. One early study, in which scRNA-seq was performed with kidney and skin samples from patients with LN and with skin samples from healthy donors, generated data on 899 cells (Der et al., 2017). This study demonstrated a correlation between type I interferon signaling in renal tubular cells and skin keratinocytes in patients with active lupus nephritis, suggesting the potential of evaluating LN activity with skin biopsy samples. Unfortunately, the immune cells identified in this study were very limited due to the small number of sequenced cells, although some T cells and myeloid cells were observed in patients with LN.

Later, using strategies to enrich immune cells before sequencing, two studies reported detailed immune cell landscapes of the kidneys in LN patients (Arazi et al., 2019; Der et al., 2019). Both studies suggested that an abundant number of immune cells are present in the kidneys of patients with LN, including inflammatory and phagocytic macrophages, DCs, NK cells, B cells, and a group of memory T cells. The infiltrating myeloid and lymphoid cells detected by scRNA-seq were confirmed using immunohistochemical and immunofluorescent methods. Interestingly, a subset consisting of B and plasma cells, which are the major sources of autoantibodies, was found to express type I interferon signature genes in LN kidneys, implying that these cells play important roles in LN pathogenesis. In normal kidney tissue, there are two predominant immune cell populations: a population of myeloid cells that differs from the blood myeloid cell population and a population of effector memory CD4 + T cells. Notably, with scRNA-seq, no B cells were found in healthy kidneys, which was confirmed by the flow cytometry assay. These results identify the cell populations that contribute to immune-mediated kidney injury in lupus nephritis and provide novel insights into B cells and plasma cells, showing that they may contribute to LN pathogenesis through clonal expansion at the site of injury; however, further studies are needed to investigate the detailed mechanism.

In addition, Arazi et al. (2019) demonstrated the possibility of performing scRNA-seq with immune cells in urine samples, which provides a non-invasive way to study immune cells in kidneys. Previously, Park et al. (2018) mapped the disease-associated genes, which were identified from the GWAS and other genetic studies, to their scRNA-seq dataset of mouse kidneys and generated a cell type-specific expression profile of these disease-associated genes. Similar to this approach, the Accelerating Medicines Partnership Network integrated risk genes from GWAS of SLE patients with the scRNA-seq data generated from patients with LN (Der et al., 2017; Arazi et al., 2019). Therefore, this approach, the integration of scRNA-seq data and genetic data, provides additional information to understand the cell type-specific contribution to kidney disease pathogenesis.

scRNA-SEQ IN DIABETIC KIDNEY DISEASE

Diabetic kidney disease (DKD) is one of the most common microvascular complications of diabetic mellitus and a leading cause of end-stage renal disease (ESRD) worldwide (Reidy et al., 2014; Thomas et al., 2015). Although numerous studies have indicated that immune cells play essential roles in DKD pathogenesis, the detailed mechanism remains unelucidated (Wada and Makino, 2016; Flyvbjerg, 2017; Tang and Yiu, 2020). In a recent study, scRNA-seq was performed using DKD and

normal kidney tissues, and showed that several types of immune cells are increased in the glomeruli of diabetic mice (Fu et al., 2019). The major immune cells detected were macrophages with highly expressed C1qa, Cd74, and Adgre1. Macrophages have been previously classified into two subtypes: M1 and M2 macrophages, which are associated with tissue damage and repair, respectively. Studies have demonstrated that the increased ratio of M1/M2 macrophages in kidneys strongly correlates with the urine albumin level and renal fibrosis in DKD (You et al., 2013). Fu et al. (2019) investigated macrophage clusters in DKD kidney samples based on 57 and 33 marker genes of M1 and M2, respectively. As expected, they found a higher number of M1 macrophages than M2 macrophages in the DKD samples. This result is consistent with previous studies showing that inflammatory macrophages are the major immune cells in the glomeruli of DKD. In addition, only a small number of other immune cells, such as neutrophils and B cells, were observed in the DKD kidney samples. Thus, this study suggests that dysregulation of the M1/M2 macrophage balance may contribute to tissue injury in DKD kidneys.

In contrast to Fu et al. (2019) study, Wilson et al. (2019) performed snRNA-seq and observed an approximate 7 fold increase in leukocyte number in diabetic patients; however, they failed to detect a significant number of resident macrophages in the diabetic samples. This discrepancy between two studies may be due to a limited sequencing cell number or different sequencing methods. Intriguingly, Wilson et al. (2019) found that infiltrating monocytes expressed the IFN gamma (IFNGR1 and IFNGR2) downstream signaling genes, such as HLA class II genes (HLA-DRB1, HLA-DRB5, HLA-DQA1), and TNFRSF1B, which are implicated as biomarkers for DKD (Benjafield et al., 2001; Robson et al., 2018; Xu et al., 2018). When comparing diabetic samples with 2 public datasets of PBMCs, Wilson et al. (2019) observed increased expression of TNFRSF21, one of the kidney risk inflammatory signature (KRIS) genes, in infiltrating CD14 + monocyte populations in diabetic samples. Interestingly, TNFRSF21 is also one of the few KRIS urinary markers that was correlated with enhanced urinary excretion and ESRD. Taken together, these studies indicate that macrophages/monocytes play important roles in DKD pathogenesis.

Using the snRNA-seq dataset generated by Wilson et al. (2019), Wei et al. (2021) found that DKD samples with high interstitial fibrosis and tubular atrophy displayed a higher cell number of B cells, suggesting that B cells may also play roles in DKD. Although a number of studies have confirmed the contribution of B cells in the pathogenesis of LN, recurrent focal segmental glomerulosclerosis, and membranous nephropathy (Fornoni et al., 2011; Gregersen and Jayne, 2012; Fervenza et al., 2019), the roles of B cells in DKD remains unclear. Studies have shown that the peripheral $CD19^+$ $CD38^+$ B cells was increased in diabetic patients (Smith et al., 2017). Notably, the number of $CD19^+$ $CD38^+$ B cells was found to be closely correlated with 24 h proteinuria level and was reduced after treatment. Taken together, these studies provide a landscape of immune cells in DKD and potential targets for DKD diagnosis and treatment.

scRNA-SEQ IN IgA NEPHROPATHY

IgA nephropathy (IgAN) is a common primary glomerulonephritis worldwide. Although this disease was initially described five decades ago, there are no specific or effective treatments to date. Approximately one-third of IgAN patients will progress to ESRD within 30 years after biopsy-based diagnosis (Roberts, 2014; Lai et al., 2016; Rodrigues et al., 2017). Recently, a "four-hit" model was proposed to explain the pathogenesis of IgAN: Unknown upstream factors cause the synthesis of galactose-deficient (gd)-IgA1 and the formation of gd-IgA-IgG immune complexes; deposition of immune complexes causes mesangial cell proliferation and secretion of inflammatory molecules and extracellular matrix (ECM) components; these molecules eventually lead to local activation of the immune system, glomerular sclerosis, mesangial expansion, and interstitial fibrosis (Lai, 2012; Wyatt and Julian, 2013; Roberts, 2014; Soares and Roberts, 2018). However, studies have shown that deposition of gd-IgA1-IgG immune complexes alone in the mesangium is not sufficient to induce severe kidney injury, suggesting that the accompanying inflammation contributes to IgAN pathogenesis, although the detailed mechanisms remain unclear.

To study circulating and resident immune cells, as well as other kidney cells, in IgAN, Zheng et al. (2020) applied a modified STRT-seq method and generated a single-cell transcriptome atlas of mesangial cells, epithelial cells, and circulating and resident immune cells. They applied the stepwise isolation method and captured major cell types in kidney cortex, generating a dataset with more than 3,000 genes and 30,000 transcripts per cell. Interestingly, they uncovered that mesangial cell of IgAN patients expressed higher levels of JCHAIN, a gene related to the dimerization and transportation of IgA molecules, which is predominately expressed in B cells. The increased expression level of this gene in mesangial cells may explain the mesangium-specific deposition of IgA immune complexes. This group also validated this result at the protein level; however, the detailed mechanism remains to be further investigated. In addition, Zheng et al. (2020) found that mesangial cells of IgAN patients expressed several inflammatory and ECM genes. In normal adult kidneys, mesangial cells are thought to clear immunoglobulins and ECM components; therefore, mesangial cell proliferation and ECM accumulation are tightly regulated (Mene et al., 1989). Under IgAN conditions, however, mesangial cells may have impaired functions in production and clearance of specific cellular components, leading to progressive kidney injury.

In another scRNA-seq study, Tang et al. (2021) discovered that mesangial cells of IgAN patients displayed the increased expression of MALAT1, GADD45B, SOX4, and EDIL3, which are also related to cell proliferation and matrix accumulation. The JCHAIN gene discovered by Zheng et al. (2020), however, did not appear in Tang et al. (2021) analysis results. This discrepancy might be due to the different sample dissociation methods (stepwise isolation vs. the GEXSCOPE tissue dissociation method) or the different sequencing platforms (modified STRT-seq vs. Singleron Biotechnologies

platform). Nonetheless, both studies showed that mesangial cell proliferation and matrix accumulation play an essential role in IgAN pathogenesis.

It has been reported that the infiltration and accumulation of immune cells, especially macrophages/monocytes, in IgAN kidney tissue are associated with proteinuria and kidney damage in IgAN. However, the detailed mechanism of macrophage/monocyte recruitment remains unclear. Both studies discovered increased numbers of macrophages, monocytes, and DCs in the kidney of IgAN patients. Zheng et al. (2020) revealed that PLGRKT and CCL2, two cytokines that can recruit macrophages/monocytes, were highly expressed in mesangial cells of IgAN patients. Tang et al. (2021) found that three genes GPX3, FAM49B, and FCGBP, which are related to the mitochondrial function, ROS production, and EMT, respectively, were decreased in macrophages of IgAN. Taken together, these results provide new evidence showing how macrophages/monocytes contribute to IgAN pathogenesis. Furthermore, Zheng et al. (2020) found that the expression of effector T cell marker genes and cytotoxicity genes was significantly reduced, while the expression of T cell exhaustion genes was upregulated in CD8 + T cells of IgAN patients. These findings suggest a close association between CD8 + T cell dysfunction and IgAN pathogenesis; however, the detailed mechanism needs to be further investigated.

scRNA-SEQ IN ANTI-NEUTROPHIL CYTOPLASMIC ANTIBODY-ASSOCIATED GLOMERULONEPHRITIS

Anti-neutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis (ANCA-GN) is characterized by an autoimmune response to ANCAs and distinct glomerular lesions (Berden et al., 2010). Previous studies have indicated that distinct HLA class II haplotypes and $CD4^+$ T cells are strongly associated with ANCA-GN (Spencer et al., 1992; Chanouzas et al., 2015). Substantial infiltrating $CD4^+$ T cells was found in kidney biopsy samples of patients with crescentic GN. In addition, $CD4^+$ T cells react to self-antigens in the active disease stage (Krebs et al., 2017). Experiments with rodent crescentic GN models suggested that $CD4^+$ T cells, particularly Th17 cells, promote GN disease progression through the production of related cytokines, such as IL-17A, IL-17F, and IFN-γ. However, the contribution of other T cells to ANCA-GN pathogenesis remains unclear.

Therefore, Krebs et al. (2020) investigated T cells in ANCA-GN kidneys using scRNA-seq. Compared with healthy kidney samples, the authors found that samples of patients with ANCA-GN displayed a significantly increased number of $CD4^+$ tissue resident memory T (T_{RM}) cells. In addition, they discovered that a higher number of kidney resident $CD69^+$ cells is negatively associated with kidney function in patients with active ANCA-GN. Interestingly, major subsets of $CD4^+$ $CD69^+$ T_{RM} cells exhibit transcriptomic profiles similar to that of Th1 or Th17 cells. They also displayed increased expression level of genes involved in cell proliferation, activation, and cytokine signaling. Therefore, $CD4^+$ T_{RM} cells induced by pathogens may play an important role in aggravating ANCA-GN, although the detailed mechanism remains to be elucidated.

FUTURE PROSPECT

scRNA-seq methods have broadly expand our knowledge of kidney immunity and become more and more powerful and indispensable tools to study kidney diseases nowadays. Using scRNA-seq, people could detect gene expression alterations of specific cell clusters and distinguish universal and unique regulation pattern. scRNA-seq technologies not only help people to uncover mechanism under specific pathology process, but also offer useful information in the disease diagnosis. The diagnosis of many kidney diseases rely largely on invasive biopsy and histologic report. As mentioned before, Arazi et al. (2019) showed that the scRNA-seq profile of immune cells in urine samples was highly correlated. It is possible that scRNA-seq could be considered as a substitution of kidney biopsy as it becomes more economic in the future.

CONCLUSION

scRNA-seq data generated in different laboratories on the basis of many kinds of human tissues will contribute to the Human Cell Atlas, an international database with a comprehensive and systematic reference map of human cells in health and disease. Eventually, this database will facilitate research by providing a reference transcriptome atlas at single-cell resolution. Currently, single-cell technology is increasing our understanding of kidney immunology at a revolutionary speed. It helps researchers understand cell heterogeneity, gene regulation, and cell-cell communication in kidney diseases, which will benefit disease diagnosis, therapeutic target identification, and off-target effect improvement. The studies discussed here applied single-cell technology to a wide range of immune-related kidney diseases using renal biopsy samples, cells in urine, and/or blood samples. scRNA-seq has led to the discovery of novel immune cell populations, gene regulation, and signaling pathways in immune-related kidney diseases. These findings will improve our understanding of kidney immunology in healthy individuals and patients with disease.

AUTHOR CONTRIBUTIONS

HZ, XY, SL, and YZ wrote the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

Arazi, A., Rao, D. A., Berthier, C. C., Davidson, A., Liu, Y., Hoover, P. J., et al. (2019). The immune cell landscape in kidneys of patients with lupus nephritis. *Nat. Immunol.* 20, 902–914. doi: 10.1038/s41590-019-0398-x

Benjafield, A. V., Glenn, C. L., Wang, X. L., Colagiuri, S., and Morris, B. J. (2001). TNFRSF1B in genetic predisposition to clinical neuropathy and effect on HDL cholesterol and glycosylated hemoglobin in type 2 diabetes. *Diabetes Care* 24, 753–757. doi: 10.2337/diacare.24.4.753

Berden, A. E., Ferrario, F., Hagen, E. C., Jayne, D. R., Jennette, J. C., Joh, K., et al. (2010). Histopathologic classification of ANCA-associated glomerulonephritis. *J. Am. Soc. Nephrol.* 21, 1628–1636. doi: 10.1681/ASN.2010050477

Betjes, M. G. (2013). Immune cell dysfunction and inflammation in end-stage renal disease. *Nat. Rev. Nephrol.* 9, 255–265. doi: 10.1038/nrneph.2013.44

Bonventre, J. V. (2018). Kidney organoids-a new tool for kidney therapeutic development. *Kidney Int.* 94, 1040–1042. doi: 10.1016/j.kint.2018.07.029

Cao, Q., Zheng, D., Wang, Y. P., and Harris, D. C. (2011). Macrophages and dendritic cells for treating kidney disease. *Nephron. Exp. Nephrol.* 117, e47–e52. doi: 10.1159/000320595

Chanouzas, D., Dyall, L., Dale, J., Moss, P., Morgan, M., and Harper, L. (2015). CD4+CD28- T-cell expansions in ANCA-associated vasculitis and association with arterial stiffness: baseline data from a randomised controlled trial. *Lancet* 385(Suppl. 1):S30. doi: 10.1016/S0140-6736(15)60345-2

Davidson, A., and Aranow, C. (2010). Lupus nephritis: lessons from murine models. *Nat. Rev. Rheumatol.* 6, 13–20. doi: 10.1038/nrrheum.2009.240

Der, E., Ranabothu, S., Suryawanshi, H., Akat, K. M., Clancy, R., Morozov, P., et al. (2017). Single cell RNA sequencing to dissect the molecular heterogeneity in lupus nephritis. *JCI Insight* 2017:2. doi: 10.1172/jci.insight.93009

Der, E., Suryawanshi, H., Morozov, P., Kustagi, M., Goilav, B., Ranabothu, S., et al. (2019). Tubular cell and keratinocyte single-cell transcriptomics applied to lupus nephritis reveal type I IFN and fibrosis relevant pathways. *Nat. Immunol.* 20, 915–927. doi: 10.1038/s41590-019-0386-1

Digby, J. L. M., Vanichapol, T., Przepiorski, A., Davidson, A. J., and Sander, V. (2020). Evaluation of cisplatin-induced injury in human kidney organoids. *Am. J. Physiol. Renal. Physiol.* 318, F971–F978. doi: 10.1152/ajprenal.00597.2019

Donnan, M. D., Kenig-Kozlovsky, Y., and Quaggin, S. E. (2021). The lymphatics in kidney health and disease. *Nat. Rev. Nephrol.* 17, 655–675. doi: 10.1038/s41581-021-00438-y

Eardley, K. S., Kubal, C., Zehnder, D., Quinkler, M., Lepenies, J., Savage, C. O., et al. (2008). The role of capillary density, macrophage infiltration and interstitial scarring in the pathogenesis of human chronic kidney disease. *Kidney Int* 74, 495–504. doi: 10.1038/ki.2008.183

Fervenza, F. C., Appel, G. B., Barbour, S. J., Rovin, B. H., Lafayette, R. A., Aslam, N., et al. (2019). Rituximab or Cyclosporine in the Treatment of Membranous Nephropathy. *N. Engl. J. Med.* 381, 36–46. doi: 10.1056/NEJMoa1814427

Flyvbjerg, A. (2017). The role of the complement system in diabetic nephropathy. *Nat. Rev. Nephrol.* 13, 311–318. doi: 10.1038/nrneph.2017.31

Fornoni, A., Sageshima, J., Wei, C., Merscher-Gomez, S., Aguillon-Prada, R., Jauregui, A. N., et al. (2011). Rituximab targets podocytes in recurrent focal segmental glomerulosclerosis. *Sci. Transl. Med.* 3:85ra46. doi: 10.1126/scitranslmed.3002231

Fu, J., Akat, K. M., Sun, Z., Zhang, W., Schlondorff, D., Liu, Z., et al. (2019). Single-Cell RNA Profiling of Glomerular Cells Shows Dynamic Changes in Experimental Diabetic Kidney Disease. *J. Am. Soc. Nephrol.* 30, 533–545. doi: 10.1681/asn.2018090896

Gierahn, T. M., Wadsworth, M. H. II, Hughes, T. K., Bryson, B. D., Butler, A., Satija, R., et al. (2017). Seq-Well: portable, low-cost RNA sequencing of single cells at high throughput. *Nat. Methods* 14, 395–398. doi: 10.1038/nmeth.4179

Gregersen, J. W., and Jayne, D. R. (2012). B-cell depletion in the treatment of lupus nephritis. *Nat. Rev. Nephrol.* 8, 505–514. doi: 10.1038/nrneph.2012.141

Habib, N., Avraham-Davidi, I., Basu, A., Burks, T., Shekhar, K., Hofree, M., et al. (2017). Massively parallel single-nucleus RNA-seq with DroNc-seq. *Nat. Methods* 14, 955–958. doi: 10.1038/nmeth.4407

Isbel, N. M., Nikolic-Paterson, D. J., Hill, P. A., Dowling, J., and Atkins, R. C. (2001). Local macrophage proliferation correlates with increased renal M-CSF expression in human glomerulonephritis. *Nephrol. Dial. Transplant* 16, 1638–1647. doi: 10.1093/ndt/16.8.1638

Islam, S., Kjallquist, U., Moliner, A., Zajac, P., Fan, J. B., Lonnerberg, P., et al. (2011). Characterization of the single-cell transcriptional landscape by highly multiplex RNA-seq. *Genome Res.* 21, 1160–1167. doi: 10.1101/gr.110882.110

Krebs, C. F., Reimers, D., Zhao, Y., Paust, H. J., Bartsch, P., Nunez, S., et al. (2020). Pathogen-induced tissue-resident memory TH17 (TRM17) cells amplify autoimmune kidney disease. *Sci. Immunol.* 2020:5. doi: 10.1126/sciimmunol.aba4163

Krebs, C. F., Schmidt, T., Riedel, J. H., and Panzer, U. (2017). T helper type 17 cells in immune-mediated glomerular disease. *Nat. Rev. Nephrol.* 13, 647–659. doi: 10.1038/nrneph.2017.112

Krishnaswami, S. R., Grindberg, R. V., Novotny, M., Venepally, P., Lacar, B., Bhutani, K., et al. (2016). Using single nuclei for RNA-seq to capture the transcriptome of postmortem neurons. *Nat. Protoc.* 11, 499–524. doi: 10.1038/nprot.2016.015

Kurts, C., Ginhoux, F., and Panzer, U. (2020). Kidney dendritic cells: fundamental biology and functional roles in health and disease. *Nat. Rev. Nephrol.* 16, 391–407. doi: 10.1038/s41581-020-0272-y

Kurts, C., Panzer, U., Anders, H. J., and Rees, A. J. (2013). The immune system and kidney disease: basic concepts and clinical implications. *Nat. Rev. Immunol.* 13, 738–753. doi: 10.1038/nri3523

Lacar, B., Linker, S. B., Jaeger, B. N., Krishnaswami, S. R., Barron, J. J., Kelder, M. J. E., et al. (2016). Nuclear RNA-seq of single neurons reveals molecular signatures of activation. *Nat. Commun.* 7:11022. doi: 10.1038/ncomms12020

Lai, K. N. (2012). Pathogenesis of IgA nephropathy. *Nat. Rev. Nephrol.* 8, 275–283. doi: 10.1038/nrneph.2012.58

Lai, K. N., Tang, S. C., Schena, F. P., Novak, J., Tomino, Y., Fogo, A. B., et al. (2016). IgA nephropathy. *Nat. Rev. Dis. Primers* 2:16001. doi: 10.1038/nrdp.2016.1

Longo, S. K., Guo, M. G., Ji, A. L., and Khavari, P. A. (2021). Integrating single-cell and spatial transcriptomics to elucidate intercellular tissue dynamics. *Nat. Rev. Genet.* 22, 627–644. doi: 10.1038/s41576-021-00370-8

Macosko, E. Z., Basu, A., Satija, R., Nemesh, J., Shekhar, K., Goldman, M., et al. (2015). Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets. *Cell* 161, 1202–1214. doi: 10.1016/j.cell.2015.05.002

Mene, P., Simonson, M. S., and Dunn, M. J. (1989). Physiology of the mesangial cell. *Physiol. Rev.* 69, 1347–1424. doi: 10.1152/physrev.1989.69.4.1347

Meng, X. M., Wang, S., Huang, X. R., Yang, C., Xiao, J., Zhang, Y., et al. (2016). Inflammatory macrophages can transdifferentiate into myofibroblasts during renal fibrosis. *Cell Death Dis.* 7:e2495. doi: 10.1038/cddis.2016.402

Papalexi, E., and Satija, R. (2018). Single-cell RNA sequencing to explore immune cell heterogeneity. *Nat. Rev. Immunol.* 18, 35–45. doi: 10.1038/nri.2017.76

Park, J., Shrestha, R., Qiu, C., Kondo, A., Huang, S., Werth, M., et al. (2018). Single-cell transcriptomics of the mouse kidney reveals potential cellular targets of kidney disease. *Science* 360, 758–763. doi: 10.1126/science.aar2131

Park, J. G., Lee, C. R., Kim, M. G., Kim, G., Shin, H. M., Jeon, Y. H., et al. (2020). Kidney residency of VISTA-positive macrophages accelerates repair from ischemic injury. *Kidney Int.* 97, 980–994. doi: 10.1016/j.kint.2019.11.025

Puranik, A. S., Leaf, I. A., Jensen, M. A., Hedayat, A. F., Saad, A., Kim, K. W., et al. (2018). Kidney-resident macrophages promote a proangiogenic environment in the normal and chronically ischemic mouse kidney. *Sci. Rep.* 8:13948. doi: 10.1038/s41598-018-31887-4

Ramskold, D., Luo, S., Wang, Y. C., Li, R., Deng, Q., Faridani, O. R., et al. (2012). Full-length mRNA-Seq from single-cell levels of RNA and individual circulating tumor cells. *Nat. Biotechnol.* 30, 777–782. doi: 10.1038/nbt.2282

Reidy, K., Kang, H. M., Hostetter, T., and Susztak, K. (2014). Molecular mechanisms of diabetic kidney disease. *J. Clin. Invest.* 124, 2333–2340. doi: 10.1172/JCI72271

Roberts, I. S. (2014). Pathology of IgA nephropathy. *Nat. Rev. Nephrol.* 10, 445–454. doi: 10.1038/nrneph.2014.92

Robson, K. J., Ooi, J. D., Holdsworth, S. R., Rossjohn, J., and Kitching, A. R. (2018). HLA and kidney disease: from associations to mechanisms. *Nat. Rev. Nephrol.* 14, 636–655. doi: 10.1038/s41581-018-0057-8

Rodrigues, J. C., Haas, M., and Reich, H. N. (2017). IgA Nephropathy. *Clin. J. Am. Soc. Nephrol.* 12, 677–686. doi: 10.2215/CJN.07420716

Smith, M. J., Simmons, K. M., and Cambier, J. C. (2017). B cells in type 1 diabetes mellitus and diabetic kidney disease. *Nat. Rev. Nephrol.* 13, 712–720. doi: 10.1038/nrneph.2017.138

Soares, M. F. S., and Roberts, I. S. D. (2018). Histologic Classification of IgA Nephropathy: Past, Present, and Future. *Semin. Nephrol.* 38, 477–484. doi: 10.1016/j.semnephrol.2018.05.017

Spencer, S. J., Burns, A., Gaskin, G., Pusey, C. D., and Rees, A. J. (1992). HLA class II specificities in vasculitis with antibodies to neutrophil cytoplasmic antigens. *Kidney Int.* 41, 1059–1063. doi: 10.1038/ki.1992.161

Stewart, B. J., Ferdinand, J. R., Young, M. D., Mitchell, T. J., Loudon, K. W., Riding, A. M., et al. (2019). Spatiotemporal immune zonation of the human kidney. *Science* 365, 1461–1466. doi: 10.1126/science.aat5031

Tang, F., Barbacioru, C., Wang, Y., Nordman, E., Lee, C., Xu, N., et al. (2009). mRNA-Seq whole-transcriptome analysis of a single cell. *Nat. Methods* 6, 377–382. doi: 10.1038/nmeth.1315

Tang, P. M., Zhang, Y. Y., Xiao, J., Tang, P. C., Chung, J. Y., Li, J., et al. (2020). Neural transcription factor Pou4f1 promotes renal fibrosis via macrophage-myofibroblast transition. *Proc. Natl. Acad. Sci. U S A* 117, 20741–20752. doi: 10.1073/pnas.1917663117

Tang, P. M., Zhou, S., Li, C. J., Liao, J., Xiao, J., Wang, Q. M., et al. (2018). The proto-oncogene tyrosine protein kinase Src is essential for macrophage-myofibroblast transition during renal scarring. *Kidney Int* 93, 173–187. doi: 10.1016/j.kint.2017.07.026

Tang, R., Meng, T., Lin, W., Shen, C., Ooi, J. D., Eggenhuizen, P. J., et al. (2021). A Partial Picture of the Single-Cell Transcriptomics of Human IgA Nephropathy. *Front. Immunol.* 12:645988.

Tang, S. C. W., and Yiu, W. H. (2020). Innate immunity in diabetic kidney disease. *Nat. Rev. Nephrol.* 16, 206–222. doi: 10.1038/s41581-019-0234-4

Teteris, S. A., Engel, D. R., and Kurts, C. (2011). Homeostatic and pathogenic role of renal dendritic cells. *Kidney Int.* 80, 139–145. doi: 10.1038/ki.2011.129

Thomas, M. C., Brownlee, M., Susztak, K., Sharma, K., Jandeleit-Dahm, K. A., Zoungas, S., et al. (2015). Diabetic kidney disease. *Nat. Rev. Dis. Primers* 1:15018. doi: 10.1038/nrdp.2015.70

Tsokos, G. C. (2011). Systemic lupus erythematosus. *N. Engl. J. Med.* 365, 2110–2121. doi: 10.1056/NEJMra1100359

Turner, J. E., Becker, M., Mittrucker, H. W., and Panzer, U. (2018). Tissue-Resident Lymphocytes in the Kidney. *J. Am. Soc. Nephrol.* 29, 389–399. doi: 10.1681/asn.2017060599

Wada, J., and Makino, H. (2016). Innate immunity in diabetes and diabetic nephropathy. *Nat. Rev. Nephrol.* 12, 13–26. doi: 10.1038/nrneph.2015.175

Wagner, A., Regev, A., and Yosef, N. (2016). Revealing the vectors of cellular identity with single-cell genomics. *Nat. Biotechnol.* 34, 1145–1160. doi: 10.1038/nbt.3711

Wang, S., Meng, X. M., Ng, Y. Y., Ma, F. Y., Zhou, S., Zhang, Y., et al. (2016). TGF-β/Smad3 signalling regulates the transition of bone marrow-derived macrophages into myofibroblasts during tissue fibrosis. *Oncotarget* 7, 8809–8822. doi: 10.18632/oncotarget.6604

Wei, Y., Gao, X., Li, A., Liang, M., and Jiang, Z. (2021). Single-Nucleus Transcriptomic Analysis Reveals Important Cell Cross-Talk in Diabetic Kidney Disease. *Front. Med.* 8:657956. doi: 10.3389/fmed.2021.657956

Wilson, P. C., Wu, H., Kirita, Y., Uchimura, K., Ledru, N., Rennke, H. G., et al. (2019). The single-cell transcriptomic landscape of early human diabetic nephropathy. *Proc. Natl. Acad. Sci. U S A* 116, 19619–19625. doi: 10.1073/pnas.1908706116

Wu, H., Kirita, Y., Donnelly, E. L., and Humphreys, B. D. (2019). Advantages of Single-Nucleus over Single-Cell RNA Sequencing of Adult Kidney: Rare Cell Types and Novel Cell States Revealed in Fibrosis. *J. Am. Soc. Nephrol.* 30, 23–32. doi: 10.1681/ASN.2018090912

Wyatt, R. J., and Julian, B. A. (2013). IgA nephropathy. *N. Engl. J. Med.* 368, 2402–2414. doi: 10.1056/NEJMra1206793

Xu, X., Eales, J. M., Akbarov, A., Guo, H., Becker, L., Talavera, D., et al. (2018). Molecular insights into genome-wide association studies of chronic kidney disease-defining traits. *Nat. Commun.* 9:4800. doi: 10.1038/s41467-018-07260-4

Yang, N., Wu, L. L., Nikolic-Paterson, D. J., Ng, Y. Y., Yang, W. C., Mu, W., et al. (1998). Local macrophage and myofibroblast proliferation in progressive renal injury in the rat remnant kidney. *Nephrol. Dial. Transplant.* 13, 1967–1974. doi: 10.1093/ndt/13.8.1967

You, H., Gao, T., Cooper, T. K., Brian Reeves, W., and Awad, A. S. (2013). Macrophages directly mediate diabetic renal injury. *Am. J. Physiol. Renal. Physiol.* 305, F1719–F1727. doi: 10.1152/ajprenal.00141.2013

Zheng, Y., Lu, P., Deng, Y., Wen, L., Wang, Y., Ma, X., et al. (2020). Single-Cell Transcriptomics Reveal Immune Mechanisms of the Onset and Progression of IgA Nephropathy. *Cell Rep.* 33:108525. doi: 10.1016/j.celrep.2020.108525xs

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Crescentic Glomerulonephritis: Pathogenesis and Therapeutic Potential of Human Amniotic Stem Cells

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Chronic kidney disease (CKD) leads to significant morbidity and mortality worldwide. Glomerulonephritis (GN) is the second leading cause of CKD resulting in end stage renal failure. The most severe and rapidly progressive type of GN is characterized by glomerular crescent formation. The current therapies for crescentic GN, which consist of broad immunosuppressive drugs, are partially effective, non-specific, toxic and cause many serious side effects including infections, cancer, and cardiovascular problems. Therefore, new and safer therapies are needed. Human amniotic epithelial cells (hAECs) are a type of stem cell which are isolated from the placenta after birth. They represent an attractive and novel therapeutic option for the treatment of various inflammatory conditions owing to their unique and selective immunosuppressive ability, as well as their excellent safety profile and clinical applicability. In this review, we will discuss the immunopathogenesis of crescentic GN, issues with currently available treatments and how hAECs offer potential to become a new and harmless treatment option for this condition.

Keywords: chronic kidney disease, crescentic glomerulonephritis, human amniotic stem cells, inflammation, immunity

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INTRODUCTION

The immune system and the kidneys are closely linked, and chronic kidney disease (CKD) often results from various auto(immune) disorders. CKD is due to slowly progressive, chronic deterioration of kidney function. Crescentic glomerulonephritis (GN) is a chronic immune-mediated disease which causes severe glomerular inflammation and injury, and often leads to irreversible kidney failure. It is a common cause of morbidity and mortality worldwide. GN is a major contributor to the escalating health burden associated with CKD.

Almost all current therapeutic concepts in autoimmune diseases are based on the systemic suppression of immune functions and are not curative. Currently used immunosuppressive therapies for crescentic GN are only partially effective, toxic and provide broad, non-specific immunosuppression, thus producing significant adverse effects. These including mainly severe infections, but also cancer and cardiovascular events. It is these treatment-caused side effects which cause the majority of patient deaths. Therefore, new and safer therapies are much needed.

Human amniotic epithelial cells (hAECs) are a type of stem cell which are isolated from the human placenta after birth. They are an attractive and novel therapeutic option for the treatment of crescentic GN due to their (i) ethical, non-invasive, and speedy isolation from the amniotic membrane of the placenta which results in an abundance of readily available cells, (ii) potent immunosuppressive capacity, (iii) low immunogenicity (ability to activate the immune system) and (iv) their ability to fight microbes and cancer and protect against cardiovascular disease. hAECs have attenuated various inflammatory diseases in mouse models, without being rejected, producing tumors or causing any major side effects. They are currently being tested in several clinical trials to treat different conditions including lung and liver disease and stroke. The purpose of this review is to discuss and link some of the major immune and inflammatory mechanisms to the progression of crescentic GN, discuss the common therapies and their limitations, and outline the therapeutic potential of hAECs in safely reducing glomerular injury.

CRESCENTIC GLOMERULONEPHRITIS

The term glomerulonephritis (GN) refers to immune-mediated inflammation of the renal glomeruli. GN is diagnosed based on clinical presentation, etiology, histopathology or pathogenesis. Most patients present with hematuria, proteinuria, and impaired glomerular filtration rate (Chadban and Atkins, 2005). This pathological condition can be acute or chronic (developing over several months to years) based on the timing of clinical presentation (Mejia-Vilet and Parikh, 2019). Acute GN that develops into rapidly progressive disease most often results from conditions that involve an abnormal immune reaction. Sometimes, acute GN does not resolve, and instead becomes long lasting (chronic) (Vinen and Oliveira, 2003).

Crescentic GN is a severe form of glomerulonephritis characterized by the destruction of the renal glomeruli that often lead to end-stage renal disease over a relatively short period of time (days, weeks, or months) (Jennette and Thomas, 2001; Jennette, 2003). It is characterized morphologically by extensive crescent formation, defined as two or more cell layers in Bowman's or urinary space (Jennette and Thomas, 2001; Parmar and Bashir, 2019). Crescents are formed by infiltrating and proliferating immune and local cells, along with deposited fibrin. This occurs after the disruption of the glomerular structure, which allow for circulating cells, inflammatory cytokines, and blood proteins to pass through the blood vessel wall into the Bowman space. The major components in the glomerular crescent are procoagulant factors, macrophages, T cells, fibroblasts, and parietal and visceral epithelial cells (Karras, 2018; Tsui et al., 2018).

On the basis of immunopathological findings, crescentic GN can be classified into three major categories: anti-glomerular basement membrane (GBM) antibody disease (Goodpasture's syndrome), immune complex GN (e.g., lupus nephritis), and pauci-immune GN which is often associated with anti-neutrophil cytoplasmic autoantibodies (ANCA) and is thus also known as ANCA-associated vasculitis (AAV) (Jennette and Thomas, 2001; Parmar and Bashir, 2019). Anti-GBM autoantibodies are highly specific for Goodpasture's disease, in which they are generally directed against the non-collagenous (NC1) domain of the alpha 3 chain of type IV collagen [α3(IV)NC1] (Gulati and McAdoo, 2018). These immunoglobulins cause glomerular capillary wall damage by local complement activation and neutrophils. T-cells also play a distinct pathogenic role in driving cell-mediated destruction of the glomeruli in this disease (Dean et al., 2005; McAdoo and Pusey, 2017). Environmental factors, like smoking, hydrocarbons and exposure to high oxygen, are thought to increase the likelihood of developing anti-GBM (McAdoo and Pusey, 2017. Anti-GBM GN accounts for around 10–15% of all cases of crescentic GN (McAdoo and Pusey, 2017). Immune complex GN, which comprises 25–30% of all cases crescentic GN, is characterized by a granular pattern of immune complex deposition in glomeruli (Naik and Shawar, 2020). AAV is associated with ANCA specific for neutrophil proteins, predominantly myeloperoxidase (MPO) or proteinase 3 (PR3) (Dey et al., 2016). This is the most common form of crescentic GN, contributing to about 65-70% of all cases (Naik and Shawar, 2020), and it refers to a necrotizing/crescentic GN with few or no immune deposits in glomeruli, as detected by immunofluorescence (Syed et al., 2015).

Pre-clinical Models of Crescentic Glomerulonephritis

Experimental evidence for the pathogenesis of crescentic GN comes mainly from pre-clinical models, including lupus nephritis, experimental autoimmune GN (EAG; model of Goodpasture's disease), MPO-ANCA vasculitis and nephrotoxic nephritis (NTN).

Nephrotoxic Nephritis

A large body of evidence about the immunopathogenesis of crescentic GN has come from one of the most widely used and best-characterized models, called nephrotoxic nephritis (NTN), also known as autologous anti-GBM globulin GN. In this model, rodents such as rats, mice and rabbits are passively injected with foreign polyclonal antibodies (globulins) targeting the mouse GBM (Odobasic et al., 2014). It comprises of two distinct phases. The first phase is called the heterologous phase and is associated with transient glomerular injury and inflammation due to the binding of injected foreign antibodies in a linear fashion to the GBM, before the development of an adaptive immune response (Odobasic et al., 2014). This phase is characterized by a neutrophil influx which peaks around 2 h after the injection of transferred antibodies, and proteinuria, peaking within the first 24 h (Schrijver et al., 1990; Tipping et al., 1994; Odobasic et al., 2014). Neutrophils contribute to glomerular damage by producing several inflammatory mediators including reactive oxygen species (ROS), proteases (Johnson et al., 1988; Couser, 1998), and MPO (Odobasic et al., 2007). Accumulation of neutrophils in inflamed glomeruli is dependent on intraglomerular expression of adhesion molecules, P-selectin, and intercellular adhesion molecule-1 (ICAM-1) (Tipping et al., 1994; Nomura et al., 1996; Saleem et al., 1998). The pathogenic role of neutrophils in glomerulonephritis is based

on the production of several inflammatory mediators including ROS and protease such as elastase, proteinases and cathepsin G (Johnson et al., 1988; Couser, 1998).

The second phase is called the autologous phase and tends to begin about 6 to 7 days later. It is caused by an adaptive immune response to the foreign anti-GBM globulin (Odobasic et al., 2014; Ougaard et al., 2018). This phase is characterized by more severe injury and crescent formation due to the presence of cellular effectors including CD4 + T cells, macrophages, neutrophils and fibrin (Huang X.-R. et al., 1997; Huang X. et al., 1997).

In mice, an accelerated model of NTN can be induced by pre-immunizing mice with normal sheep globulin (NSG) (Ougaard et al., 2018). In accelerated NTN, the use of adjuvants which contain dead mycobacteria are included in the pre-immunization to improve the immune response (Ougaard et al., 2018). This pre-immunization produces a strong T and B cell response to sheep globulin and gives rise to an immediate autologous phase once sheep anti-GBM antibodies are administered (Odobasic et al., 2014). For animal models without pre-immunization, injury is less acute compared to the pre-immunized accelerated model and termed as a non-accelerated model (Odobasic et al., 2014).

Experimental Models of MPO-AAV, Lupus Nephritis, and Goodpasture's

Other models of crescentic GN exist and they have been also widely used to reveal pathogenetic mechanisms of disease. MPO-AAV can be induced in mice by immunizing animals with MPO which results in the development of active anti-MPO autoimmunity, followed by neutrophil lodgment in glomeruli and deposition of the autoantigen for subsequent recognition by MPO-specific T cells (Ruth et al., 2006; Odobasic et al., 2019). Alternatively, glomerular injury can be initiated by passive transfer of anti-MPO antibodies (Xiao et al., 2002; Ooi et al., 2014). Experimental autoimmune GN (EAG), a model of Goodpasture's disease, can be induced in mice by repeated immunization with α3(IV)NC1 (Ooi et al., 2009). Several models of lupus nephritis exist in which susceptible mice such as MRL/lpr and NZB/NZWF1 spontaneously develop disease (Richard and Gilkeson, 2018). Although none of these models fully recapitulate human disease, they all (including NTN) closely resemble crescentic GN seen in patients, both immunologically and pathologically, and are therefore invaluable pre-clinical tools to study disease pathogenesis and test new potential therapies.

Mechanisms of Pathogenesis of Crescentic Glomerulonephritis

Multiple immune mechanisms contribute to the pathogenesis of crescentic GN. A summary of the main immune pathways which positively or negatively regulate the development of crescentic GN is shown in **Figure 1A**.

The Role of Innate Immunity – Neutrophils and Macrophages

Innate and adaptive immune system activation are a common underlying mechanism for several forms of crescentic GN (Imig and Ryan, 2013). Studies in the NTN model using neutrophil depletion have shown that these cells contribute to glomerular injury during the early autologous phase of disease (Disteldorf et al., 2015). Macrophages are the dominant effector cells present in the kidney which mediates glomerular crescent formation and kidney damage in all models of crescentic GN (Lan et al., 1997; Odobasic et al., 2014; Rousselle et al., 2017). Their accumulation within glomeruli has been blocked by administration of polyclonal sheep anti-rabbit macrophage serum in models of anti-GBM disease, leading to prevention of the development of GN (Holdsworth et al., 1981). Moreover, a micro-encapsulated approach in which macrophages are depleted by clodronate has resulted in reducing renal damage in anti-GBM GN (D'Souza et al., 1999). Further studies have shown that macrophages can induce glomerular injury by several mechanisms including deposition of fibrin (which impairs glomerular filtration) and production of various proinflammatory mediators including ROS, reactive nitrogen species such as nitric oxide (NO) and cytokines such as IL-1, TNF-α, and macrophage migration inhibitory factor (MIF) (Imig and Ryan, 2013).

The Role of Adaptive Immunity – T Cells and B Cells

Numerous studies in various models of crescentic GN have shown that CD4 + T cells play a critical role in this disease (Tipping et al., 1998; Dean et al., 2005; Ruth et al., 2006). CD4 T helper cells (Th) can be divided into several subsets, with the major and best-understood ones being Th1, Th17 and Th2, mainly characterized by the expression of IFNγ, IL-17A, and IL-4, respectively. Several studies have indicated that crescentic anti-GBM globulin GN is driven by Th1 cells, while Th2 cells reduce the severity of disease. For instance, GN induced in mice with a predominant Th1 response (C57BL/6) shows severe crescentic formation with prominent glomerular T cell and macrophage infiltration and fibrin deposition which are associated with elevated IFN-γ and weak IL-4 production (Huang X.-R. et al., 1997). On the other hand, in Th2-prone (BALB/c) mice, crescent formation as well as glomerular T cell and macrophage influx were relatively low (Huang X.-R. et al., 1997). In addition, mice lacking endogenous IFN-γ developed less severe GN than genetically normal C57BL/6 mice, whereas IL-4-deficient mice developed more severe crescentic GN associated with increased accumulation of T cells and macrophages in glomeruli (Kitching et al., 1998, 1999). While IFNγ is protective in EAG (Kitching et al., 2004), the Th1 pathway has also been shown to promote glomerular crescent formation and kidney injury in experimental lupus nephritis and MPO-AAV (Richards et al., 2001; Summers et al., 2011).

Moreover, it has become clear that the Th17 pathway is important in the development of crescentic GN. In experimental MPO-AAV, mice lacking IL-17A are protected from early glomerular injury (Gan et al., 2010). Similarly, several lines of evidence, coming from using IL-17A-deficient mice or infusion of Th17 cells, have shown that the Th17 pathway promotes early glomerular injury in NTN (Paust et al., 2009; Summers et al., 2009; Odobasic et al., 2011), in line with results from experimental autoimmune uveitis (Luger et al., 2008). On the contrary, the Th1 pathway acts later in the disease process and produces more severe GN (Odobasic et al., 2011). Interestingly, local IL-17A production is protective in crescentic

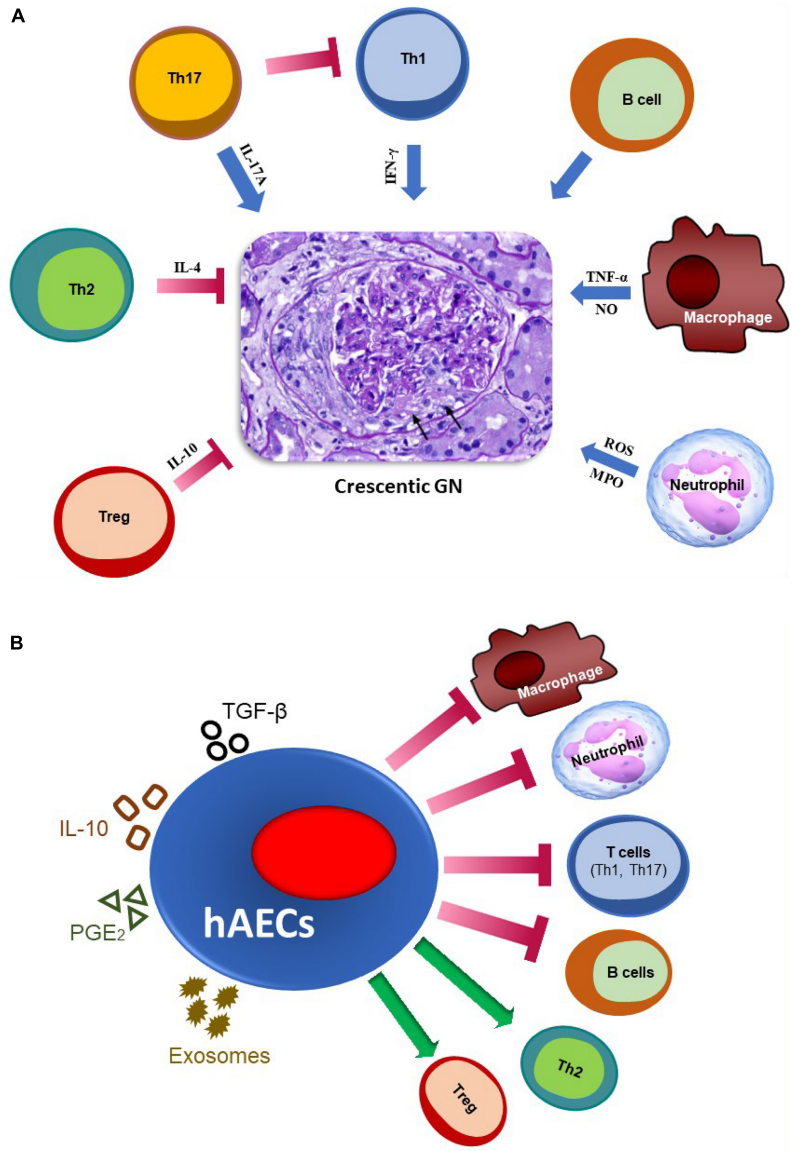

FIGURE 1 | The major immune pathways involved in the pathogenesis of crescentic GN and mechanisms by which hAECs may attenuate glomerular injury. **(A)** Effector immune mechanisms in crescentic GN. Macrophages contribute by secreting nitric oxide (NO) and TNF-α, while neutrophils contribute by producing reactive oxygen species (ROS) and releasing myeloperoxidase (MPO) enzyme. IFN-γ produced by Th1 cells mediates crescent formation. IL-17A (a Th17 cytokine) promotes early stage of kidney injury, but attenuates established disease by inhibiting Th1 responses. B cells, which act as antigen-presenting cells and produce autoantibodies, also promote glomerular injury. On the contrary, IL-4 producing Th2 and IL-10-producing regulatory T cells (Tregs) play a protective role in this disease. Arrows indicate glomerular crescent formation. **(B)** Potential mechanisms by which hAECs may reduce inflammation and damage in glomeruli. hAECs suppress activation and/or infiltration of pro-inflammatory T cells (Th1 and Th17), B cells, macrophages and neutrophils, while promoting inhibitory cells such as Tregs and Th2. They exert their effects by producing anti-inflammatory mediators such as TGF-β, IL-10 and PGE2, and releasing exosomes, to restrict kidney injury.

GN (Hamour et al., 2015), and in later stages of the disease (which is mediated by Th1), IL-17A can attenuate injury by systemically inhibiting Th1 responses (Odobasic et al., 2011).

Tregs are a specialized inhibitory subset of CD4 + T cells characterized by expression of CD25 and Foxp3. They play a key role as negative regulators of pathogenic immunity in crescentic GN. Studies using Foxp3-GFP reporter mice explored the functional role of T-regulatory cell in inhibiting anti-GBM nephritis (Ooi et al., 2011). Foxp3 protected against renal damage in anti-GBM GN induced in antigen-primed Foxp3-transgenic mice, via boost of Treg numbers and activity, and inhibition of Th immune responses at the systemic level and at sites of tissue injury (Yang et al., 2017). Similarly, Tregs are protective in other models of crescentic GN including MPO-AAV and lupus nephritis (Humrich et al., 2010; Tan et al., 2013; Odobasic et al., 2019). Further studies have shown that Tregs attenuate crescentic GN by releasing a potent anti-inflammatory cytokine, IL-10 (Ostmann et al., 2013).

In addition to T cells, B cells and autoantibodies have also been demonstrated to play a major role in the development of crescentic GN. B cells and/or antibodies against various autoantigens including MPO in AAV, α3(IV)NC1 in Goodpasture's and nuclear antigens in lupus, are pathogenic in experimental crescentic GN (Xiao et al., 2002; Dean et al., 2005; Richard and Gilkeson, 2018). In contrast, evidence coming from μ-chain knockout mice, which lack mature B cells and cannot produce immunoglobulin, has shown that glomerular antibody deposition is not essential for crescent formation in response to the planted glomerular antigen in the NTN model (Li et al., 1997). B cells are well known to promote immune-mediated injury by several mechanisms including acting as antigen-presenting cells to activate T cells and differentiating into autoantibody-producing plasma cells.

Pathogenic Mechanisms in Human Crescentic Glomerulonephritis

In human studies, strong evidence from patients' kidney biopises, and blood samples has accumulated to support the role of immune cells and antibody in cresentic GN, with findings similar to those in animal models. While neutrophils are prominent in cresentic GN (Kaplan, 2013), a considerable body of evidence has emerged to show that CD4 + T cells and macrophages are critically involved in all patterns of human cresentic glomerulonephritis (Stachura et al., 1984; Bolton et al., 1987; Nolasco et al., 1987; Müller et al., 1988; Cunningham et al., 1999). Although many limitations have constrained the assessment of nephritogenic responses in human GN, a number of studies have indicated that cresentic glomerulonephritis is a manifestation of a Th1 predominant delayed type hypersensitivity (DTH)-mediated immune response associated with predominant infiltration of macrophages and deposition of fibrin (Kitching et al., 2000). Furthermore, patients with crescentic GN have increased serum levels of IL-17A in comparison to healthy individuals, suggesting a role for the Th17 pathway in disease pathogenesis (Lu et al., 2017; Herrnstadt and Steinmetz, 2020). Human studies in AAV, SLE, and Goodpasture's syndrome have revealed that dysregulation of Treg homeostasis and function is also associated with the development of crescentic GN (Herrnstadt and Steinmetz, 2020). Finally, B cells and autoantibodies against various endogenous targets in crescentic GN are pathogenic in human disease (Falk and Jennette, 1988; McAdoo and Pusey, 2017).

Current Therapies for Crescentic Glomerulonephritis

In general, crescentic GN therapy is often classified into two phases. First is the activation of remission phase during the acute period, followed by the subsequent phase of maintenance therapy to control the underlying long-term immunopathology (Parmar and Bashir, 2017). The induction treatment is mainly composed of high-dose corticosteroids and intravenous (i.v.) pulse cyclophosphamide with the purpose of blocking the active inflammation and reducing the cellular and humoral immune response (Jennette, 2003; Moroni and Ponticelli, 2014). In addition, therapeutic plasma apheresis (plasma exchange) is a form of treatment that is frequently used to treat almost all types of crescentic GN by removing pathogenic autoantibodies (Flossmann et al., 2011; Kallenberg, 2014; Prendecki and Pusey, 2019).

Corticosteroids, synthetic drugs which closely resemble cortisol hormone, have been used for decades to modulate inflammation therapeutically by decreasing the movement of neutrophils to the inflammatory sites and inducing a transient lymphocytopenia (Olnes et al., 2016). This medication inhibits the expression and action of most proinflammatory cytokines, adhesion molecules, and suppresses MHC expression (Ponticelli and Locatelli, 2018). Cyclophosphamide, a cytostatic drug, is one of the oldest anti-cancer drugs and is widely used in therapy of crescentic GN by reducing the activity of the immune system (Jhaveri et al., 2013). Cyclophosphamide therapy significantly reduces total neutrophil, macrophage and lymphocyte counts (Jhaveri et al., 2013; Ménétrier-Caux et al., 2019).

Combination therapy with pulse cyclophosphamide plus pulse methyl prednisolone therapy significantly reduce proteinuria, serum creatinine, the level cellular crescents in crescentic GN (Tumlin et al., 2003), and present a greater reduction in the risk for ESRD.

However, induction therapy (corticosteroids with cyclophosphamide) is only partially effective, non-specific and produces many toxicities and serious side effects. Therefore, there is a major unmet need for safer, effective therapies. For example, in AAV, this treatment induces remission in 70–90% of patients, but the incidence of dialysis or death at 5 years is still high (∼30%), with the majority of early deaths caused by drug-related side effects, mainly infections caused by broad immunosuppression (Flossmann et al., 2011; Kallenberg, 2014; King and Harper, 2017). In lupus nephritis, remission is achieved in 50–60% of patients at best (Menez et al., 2018), relapse occurs in up to 25% of cases (Menez et al., 2018; Anders et al., 2020) and despite optimal care, many patients (up to 20%) develop kidney failure which requires renal transplantation or dialysis for survival (Menez et al., 2018; Anders et al., 2020). The unwanted adverse effects caused by these drugs include predominantly severe infections, as well as increased risk of malignancy, bone disease, dysglycemia, obesity, hypertension, mental problems, gastrointestinal bleeding, cataracts, and long-term risks of developing cardiovascular disease (King and Harper, 2017; Jefferson, 2018). More recently, rituximab, a pan B cell depleting anti-CD20 monoclonal antibody, has been approved for use instead of cyclophosphamide as a second line of treatment, particularly in AAV. However, although it is not inferior to cyclophosphamide in reducing disease, it induces similar rates of adverse effects, mainly infections, most likely due to hypogammaglobulinaemia and late onset neutropenia (Jones et al., 2015; Santos et al., 2020).

Adalimumab, an anti-TNFα monoclonal antibody, has been used as an adjunct therapy to standard immunosuppression. It can be an effective therapy for the induction of remission in AAV and may permit reduced prednisolone dosing, thus decreasing therapy-related toxicity (Laurino et al., 2010). However, other anti-TNFα agents, infliximab and etanercept,

failed to demonstrate a benefit for remission maintenance in patients with AAV (Booth et al., 2004; Wegener's Granulomatosis Etanercept Trial (WGET) Research Group, 2005).

Mycophenolate mofetil (MMF), is a salt form of the immunosuppressive drug mycophenolic acid. It inhibits the releasing of proinflammatory cytokines, nitric oxide, and LDH in macrophages and suppresses proliferation and infiltration of both T and B lymphocytes (Allison and Eugui, 2000). Recent studies, indicate that MMF may be as or even more effective in reducing proteinuria and hematuria and less toxic compared with cyclophosphamide in LN (Hu et al., 2002).

STEM CELL-BASED THERAPY FOR AUTOIMMUNE AND INFLAMMATORY DISEASES

Stem cell-based therapy is an attractive approach to ameliorate a broad range of human diseases and injuries, and it has been proven to be safe and effective in a wide range of immune-mediated diseases (Jin et al., 2014). Several types of stem cells, including mesenchymal, induced pluripotent and embryonic (Ryu et al., 2020), have reduced organ injury in models of inmune diseases and their potential side effects and efficiency have been assessed in clinical trials (Mûzes and Sipos, 2019).

Mesenchymal, Embryonic, and Induced Pluripotent Stem Cells

Mesenchymal stem cells (MSCs) are multipotent adult stem cells that exist in various locations including umbilical cord, bone marrow and adipose tissue (Murray and Péault, 2015). MSCs have been successfully applied in treating a vast array of inflammatory and autoimmune conditions such as graft-versus-host disease (GVHD), multiple sclerosis (MS), type 1 diabetes (T1D), inflammatory bowel diseases (IBD), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), lung fibrosis, liver, and pancreatic fibrosis (Ryu et al., 2020). These cells modulate the immune reaction directly through regulating various immune cells including dendritic cells (DCs), macrophages, B cells, T cells and neutrophils, and by producing suppressive cytokines such as IL-10, TGF-β, and IL-35, as well as expresssing inhibitory ligands and receptors (e.g., PD-L1 and PD-1) (Jiang and Xu, 2020). MSCs can attenuate the development of crescentic GN (Thakkar et al., 2017) by decreasing neutrophil and macrophage recruitment to the kidney and promoting the phenotypic switching of renal macrophages to immunoregulatory cells (Furuhashi et al., 2013). In addition, the beneficial effects of MSCs in GN appear to be mediated by modification of the Th1/Th2 and/or Th17/Treg balance and inhibition of B-cell activation, as well as stimulation of IL-10, IL-4, foxp3, prostaglandin E2 (PGE2), and TGF-β production (Ma et al., 2013; Suzuki et al., 2013). Hence, MSCs have demonstrated capacity to attenuate experimental crescentic GN, but they also have a few disadvantages and carry some risks. For example, some methods of MSC isolation are invasive (e.g., obtaining MSCs from the bone marrow or adipose tissue). MSCs also require long-term culture (weeks) to expand adequate cell numbers for infusion. This increases the cost and risk of *in vitro* mal-transformation. It is also not optimal for the treatment of diseases such as crescentic GN in which early treatment is required to stop the progression of rapidly progressing deterioration of kidney function.

Embryonic and induced pluripotent stem cells (ESCs and iPSCs) are pluripotent stem cells. ESCs are isolated from the inner cell mass of blastocysts, while iPSCs are produced from adult somatic cells that are genetically reprogrammed to an ESC-like state by transcription factors (Ryu et al., 2020). It has been reported that the ESC-loaded gelatin microcryogels on rats slowed down the progression of CKD and alleviated glomerular injury (Geng et al., 2016). Likewise, administration of iPSCs in models of CKD preserved residual renal function by decreasing macrophage infiltration, upregulating TGF-β, inhibiting apoptosis and regulating cell proliferation and death signaling (Caldas et al., 2017; Sheu et al., 2020). Thus, ESCs and iPSCs may be able to reduce glomerular damage and retard the progression of CKD, but they also have some disadvantages. For instance, isolation of ESCs poses obvious ethical concerns, while iPSCs carry the risk of tumor development (Caldas et al., 2017).

Human Amniotic Epithelial Cells

One particular stem cell type, hAECs, have attracted much attention in the recent years as an ideal therapuetic option for the treatment of autoimmune and inflammatory disorders due to their immunosuppresive ability and their superior safety and clinical applicability over other stem cell types. hAECs are a heterogeneous epithelial population that originates from the lining of the inner membrane of the placenta which provides an abundant cellular source for stem cell-based therapy (Miki et al., 2005). In comparison to other sources of stem cells, gestational tissue gives great advantages including easy collection without the need for invasive methods (Qiu et al., 2020). Large numbers of hAECs can be isolated from the placenta after birth, thus bypassing ethical barriers and resulting in an abundance of immediately available, primary (non-cultured) cells to be used therapeutically. These epithelial stem cells are pluripotent and have the capability of self-renewal and differentiating into all three germ layers, including the ectoderm, mesoderm, and endoderm (Miki et al., 2005). hAECs have immense potential to safely reduce the burden of many serious diseases and injuries to different organs, including the kidney, due to their unique properties (Ren et al., 2020). They possess some degree of plasticity, immune privilege, non-tumorigenicity, anti-infection/cancer properties, and lack of ethical concerns and paracrine properties that are essential to their potential therapeutic applications in immune-mediated diseases (Qiu et al., 2020).

Low Immunogenic Profile

Human Leukocyte antigens (HLA), encoded by the major histocompatibility complex (MHC) gene complex in humans, are the major molecules that initiate graft rejection (Mahdi, 2013).

However, low HLA class-I (HLA-A, HLA-B, and HLA-C) expression and HLA class-II (HLA-DR) on hAEC surface have been identified, resulting in a low immunogenic profile upon transplantation (Hori et al., 2006). hAECs express non-classical HLA-G, which is thought to protect the fetal semi-allograft from maternal immune system rejection (Lefebvre et al., 2000). Expression of HLA-G confers a degree of immune privilege by suppressing natural killer cells, inducing apoptosis of activated CD8 + T cells and inhibiting CD4 + T cell proliferation (Banas et al., 2008).

Low Risk of Tumor Formation

Tumorigenicity is a common obstacle for cell-based therapies, since some cells may cause formation of tumors due to being immortal. Unlike ESCs and iPSCs (Ben-David and Benvenisty, 2011), hAECs do not express telomerase reverse transcriptase (Miki et al., 2005), which is a catalytic subunit of the telomerase enzyme playing a central role in tumorigenesis (Daniel et al., 2012). Therefore, hAECs do not promote tumor formation after transfer into recipients.

Immunosuppressive Capacity

Consistent with the role of the placenta to protect the fetus from being attacked by the maternal immune system during pregnancy, hAECs are immunosupressive. Similar to MSCs, the beneficial effects of hAECs are primarily mediated via their paracrine actions and not by their differentiation into target cells (Tögel et al., 2005; Wang et al., 2014). hAECs have blocked the immue system by suppressing effector T cells, switching macrophage polarization from M1 to anti-inflammatory M2 phenotype, and inhibiting neutrophils (Li et al., 2005; Tan J.L. et al., 2018). In addition, several studies in animal models have shown that they can exert immunomodulatory effects by inducing other immunosupressive cells, in particular Tregs and Bregs (Manuelpillai et al., 2010; Liu et al., 2012; Tan et al., 2014, 2015; Evans et al., 2018; Li et al., 2018; Tan B. et al., 2018). Moreover, *in vitro* studies showed similar inhibitory effects of hAECs on human cells. For instance, hAECs suppressed human CD4 + T cell proliferation, induced a Th2 cytokine profile, suppressed production of Th1 and Th17 cytokines and promoted differentiation of naïve CD4 + T cells into Tregs (Wolbank et al., 2007; Motedayyen et al., 2018).

Human amniotic epithelial cells have been reported to inhibit immunity by secreting various immunosuppresssive mediators. For example, they produce prostaglandin E_2 (PGE_2), which has several immunosuppressive properties such as inhibition of T cell proliferation. hAECs also secrete TGFb, a T cell growth inhibitor and a powerful immunosuppressive molecule (Liu et al., 2012). In addition, the suppressive activity of hAECs has been demonstrated through an increased secretion of the anti-inflammatory cytokine IL-10 (Charles-Henri and Ekaterine, 2020). IL-10 inhibits proinflammatory cytokine production, as well as Th1 and macrophage activation (Howes et al., 2014).

Anti-infection and Anti-cancer Properties

Unlike the current therapies for crescentic GN, hAECs have anti-infection properties and protect against cancer development. Human β-defensins, small proteins which promote microbial death, are primarily expressed by epithelial and immune cells at mucosal surfaces (Dorin et al., 2015). These natural anti-microbial molecules have been reported to be secreted by human placenta cells including hAECs and by the fetal membrane during human pregnancy in order to protect the uterus from infection (King et al., 2007; Nemr et al., 2017). hAECs also produce type I interferons (IFNs) in response to viruses *in vitro* (Uchide et al., 2002; Nemr et al., 2017). This group of proteins, which is made up mainly of IFNα and IFNβ, initiate intracellular anti-microbial systems and influence innate and adaptive immune responses (Ivashkiv and Donlin, 2014). They are secreted by infected cells and are important for host protection against viruses through the induction of anti-viral effector molecules (Uchide and Toyoda, 2007). Moreover, hAECs supress cancers directly by inducing apoptosis and reducing motility of malignant cells (Niknejad et al., 2014). They also suppress tumor development indirectly by promoting anti-tumor cytotoxic T cell immunity *in vivo*, as shown in a mouse model of colon adenocarcinoma (Tabatabaei et al., 2018).

Protection From Cardiovascular Disease

In contrast to the current drugs used to treat crescentic GN, hAECs also protect against cardiovascular disease. They have decreased areas of myocardial infarction in athymic nude rats (Fang et al., 2012). In addition, administration of hAECs has reduced brain injury in a murine and non-human primate model of ischemic stroke (Evans et al., 2018).

Human Amniotic Epithelial Cells in Preclinical Studies

Human Amniotic Epithelial Cells in Autoimmune Diseases

Administration of hAECs has ameliorated immune-mediated organ damage in models of several autoimmune diseases. hAEC infusion in experimental autoimmune thyroiditis (EAT) and SLE maintained organ function, minimized inflammation and modified the immune balance (Tan B. et al., 2018). In EAT, hAECs reduced disease severity by inhibiting infiltration of inflammatory cells in thyroid glands, as well as suppressing Th17 responses. Moreover, hAECs improved the local cytokine environment in both EAT and SLE mice, by suppressing the levels of IFN-γ and enhancing TGF-β (Tan B. et al., 2018). In SLE mice specifically, hAEC administration promoted Tregs and decreased the levels of pathogenic autoantibodies (Tan B. et al., 2018).

Human amniotic epithelial cell also potently attenuated disease severity in experimental autoimmune encephalomyelitis (EAE), and a mouse model of multiple sclerosis (MS) (Liu et al., 2012). T cell and macrophage infiltration were significantly reduced by hAEC treatment. It was reported that hAECs utilized PGE2 and TGF-β for their immunosuppressive effects (Liu et al., 2012). In another relapsing model of MS, hAECs significantly ameliorated disease progression, while promoting Tregs and augmenting Th2 responses (McDonald et al., 2015).

In an autoimmune uveitis (EAU) rat model, hAECs treatment ameliorated the pathological progression of disease and maintained the retinal structural organization (Li et al., 2018).

Infiltration of macrophages and T cells was suppressed after hAEC administration. The stem cells regulated the balance of T cell subsets by decreasing Th17 cells and boosting IL-10-producing Tregs in the spleen and lymph nodes. Furthermore, hAEC treatment changed the ocular chemokine and cytokine environment in EAU rats, indicated by decreased levels of monocyte chemoattractant protein-1, IL-17 and IFN-γ levels, and enhancement of IL-10 (Li et al., 2018).

The immunomodulatory effect of hAECs has also been investigated in mice with autoimmune ovarian disease (AOD) (Zhang Q. et al., 2019). The outcomes showed that hAEC injection improved ovarian function. This was associated with a significant increase in the number of Tregs in the spleen of AOD mice (Zhang Q. et al., 2019).

Human Amniotic Epithelial Cells in Other Inflammatory Diseases

Transplantation of both hAECs and their soluble factors have shown beneficial effects in animal models of hepatic fibrosis. hAECs given to mice with induced liver fibrosis reduced hepatocyte apoptosis and decreased hepatic inflammation and fibrosis (Manuelpillai et al., 2010). This study showed that intact cells expressing human-specific markers, inner mitochondrial membrane protein and HLA-G were found in mouse liver 2 weeks following hAEC injection, without evidence of host rejection of the transplanted cells (Manuelpillai et al., 2010). In another study using the same model, hAECs significantly decreased liver fibrosis, in line with reduced hepatic levels of the pro-fibrogenic cytokine TGF-β1, increased expression of the anti-inflammatory mediator IL-10 and decreased hepatic T cell infiltration (Manuelpillai et al., 2012). Furthermore, hAECs administration reduced hepatic macrophage numbers and induced an anti-inflammatory M2 macrophage phenotype (Manuelpillai et al., 2012).

In a rat model of ischemic stroke, hAECs were administered by intracerebral injection, after which they reduced the infarct volume and cerebral apoptosis (Liu et al., 2008). A further study in mice found that hAECs injected 1.5 h after stroke migrated to the ischemic brain and spleen, and limited functional deficit, infarct volume and brain inflammation (Evans et al., 2018). In a rat model of intracerebral hemorrhage, Liang et al. reported that hAECs reduced the levels of proinflammatory cytokines TNF-α and IL-1β in microglia culture medium (Liang et al., 2014). Similarly, in preterm fetal sheep models of brain injury, inflammation was reduced in fetuses that received hAECs (Yawno et al., 2013).

Many studies have explored the immunomodulatory effect of hAECs on lung fibrosis. A study using the bleomycin-induced model of pulmonary fibrosis showed that hAECs can modulate the host inflammatory response, decrease fibrosis and preserve lung function (Murphy et al., 2011). hAECs reduced expression of the pro-inflammatory cytokines TNF-α, IFN-γ and IL-6, and reduced inflammatory cell infiltration (Murphy et al., 2011). A further study using the same model demonstrated that hAEC administration significantly decreased macrophage recruitment into the lung and promoted the majority of alveolar macrophages toward the M2 phenotype (Tan et al., 2014). Moreover, hAECs treatment increased Treg numbers in the injured lungs. It was found that hAECs require Tregs to polarize macrophages toward an M2 phenotype and that hAECs promote Treg cells via TGF-β (Tan et al., 2015).

A recent study in a mouse model of renal ischemia-reperfusion injury, which leads to acute kidney injury, has shown that systematically administered hAECs effectively regulated the kidney immune response (Ren et al., 2020). hAEC infusion attenuated tubular cell death and endothelial necrosis and increased cell proliferation in the injured kidney. The stem cells reprogramed macrophages to shift from a pro-inflammatory M1 to anti-inflammatory M2 phenotype. In addition, hAECs enhanced levels of IL-4 and IL-13 and decreased levels of TNFα and IFNγ, which in turn helped to minimize the inflammatory response.

Secretome Derived From Human Amniotic Epithelial Cells

The secreted factors (also named secretome) exist in the medium where the stem cells are cultured. Numerous studies on stem cell-derived secreted factors showed that these mediators alone, without the stem cell itself, affect the maturation, migration, polarization and function of immune cells, and thus influence the strength and duration of immune responses. The hAEC secretome contains metabolites, lipids, free nucleic acids, cytokines, growth factors and extracellular matrix proteins. These are all known to play crucial roles in cell-cell communication, acting proximally as well as systemically.

The application of cell-free therapy confers some advantages over stem-cell based applications. Usage of soluble factors bypasses a number of safety concerns potentially linked to the administration of living cell populations including post-transfer mal-transformation, embolism, and transmission of infections. In addition, hAEC secretome may be evaluated for safety, dosage and potency in an approach similar to that used for traditional medications (Vizoso et al., 2017). hAEC-conditioned media (CM) can be also manufactured, packaged, and transported more easily than hAECs themselves (Kay et al., 2017). Therefore, stem cell-derived secretomes have a promising prospect to be used as pharmaceuticals for immune diseases. hAEC CM has shown beneficial effects in reducing a range of conditions by modulating immune responses including liver fibrosis, AOD, inflammatory bowel disease and diabetic wound healing (Hodge et al., 2014; Kuk et al., 2018; Zheng et al., 2018; Zhang Q. et al., 2019). In general, to prepare hAEC CM, hAECs are cultured in chemically defined, serum-free ultraculture medium for 4 days at 37°C in a humidified chamber containing 5% CO_2, after which conditioned media is harvested and secretome obtained by serial centrifugation (Alhomrani et al., 2017).

Human amniotic epithelial cells also mediate their effects by secreting exosomes. Exosomes are nano-sized biovesicles secreted by various cell types including stem cells under both normal and pathophysiological conditions. They are characterized by a diameter of 50–100 nm and a density of 1.09–1.18 g/mL (Zhang Y. et al., 2019). Exosome cargo is diverse

TABLE 1 | Completed and active clinical trials utilizing hAECs to treat immune-related diseases*.

Category	Registration number	Disease	Phase	Age	Country
Neurology	ACTRN12618000076279	Ischemic stroke	1	18–85 years	Australia
Ophthalmology	NCT00344708	Corneal epithelial dystrophy	N/A	18–88 years	United States
Pneumology	ACTRN12614000174684	Bronchopulmonary dysplasia	1	36 weeks	Australia
Pneumology	ACTRN12618000920291	Bronchopulmonary dysplasia, extremely preterm birth	1	14–18 days	Australia
Orthopedics	NCT03031509	Non-union fracture	1	18–80 years	China
Gynecology	NCT03207412	Premature ovarian failure	N/A	18–40 years	China
Others	ACTRN12616000437460	Cirrhosis, liver fibrosis	1	18–70 years	Australia
Others	ACTRN12618001883202	Crohn's disease, perianal fistulas	N/A	18–80 years	Australia

These data were collected from the clinical trial database (ClinicalTrials.gov and anzctr.org.au).

and a pool of exosomes can demonstrate all cellular elements including protein, nucleic acids and lipids (Spada, 2020). They represent a novel manner of intercellular communication, which may play a central role in many cellular processes such as the immune response, signal transduction and antigen presentation (Zhang Y. et al., 2019). Exosomes derived from hAECs produce potent immunomodulatory, anti-fibrotic and pro-regenerative effects and have been successfully used as a cell-free therapy in inflammatory conditions (Alhomrani et al., 2017; Tan J.L. et al., 2018). hAEC-derived exosomes can suppress many immune cells such as T cells, macrophages, and neutrophils *in vitro*. In addition, they have been highly effective in delivering protection from organ damage in a range of disease models including pulmonary and liver fibrosis, as well as acute kidney injury (Alhomrani et al., 2017; Tan J.L. et al., 2018; Ren et al., 2020). Thus, hAEC-derived soluble factors and exosomes may represent a potential cell-free therapy in crescentic GN.

Human Amniotic Epithelial Cells in Clinical Trials

Based on their immunomodulatory properties, the amniotic membrane and hAECs have been safely used for several years as a therapy for wounds and ocular injuries (Parmar et al., 2006; Jirsova and Jones, 2017). hAECs have also entered clinical trials as a treatment for several conditions including liver fibrosis, stroke and lung injury in premature babies (**Table 1**; Lim et al., 2017, 2018; Phan et al., 2018; Malhotra et al., 2020). So far, these trials have shown that hAECs are very safe and well-tolerated in humans.

Human Amniotic Epithelial Cells as a Potential Therapy for Crescentic Glomerulonephritis

Overall, the studies described above show that hAECs can inhibit organ damage in various autoimmune and inflammatory diseases, without producing major side effects. They exert their effects by inhibiting various pathogenic immune cells including neutrophils, macrophages, effector T cells and B cells, as well as by promoting inhibitory immune subsets such as Tregs and Th2 cells. All these types of immune cells are also involved in the pathogenesis of crescentic GN, thus hAECs may inhibit glomerular injury through similar mechanisms. A summary of the pathways through which hAECs could potentially attenuate crescentic GN is given in **Figure 1B**.

CONCLUSION

In summary, there is a requirement for alternative, safer treatments for crescentic GN as the only effective therapies currently available are broadly immunosuppressive drugs which cause many serious side effects (mainly infections, cancer and cardiovascular problems) and patient deaths. hAECs, as well as hAEC-CM and hAEC-derived exosomes, exert a protective effect in models of various immune-driven conditions, with minimal side effects. They mediate their effects through multiple immunomodulatory and anti-inflammatory mechanisms, and their safety has been proven in clinical trials. Thus, hAEC-based therapy offers promise to be a safe, feasible and effective treatment for crescentic GN. This is due to their (i) ethical and speedy isolation from the placenta which results in an abundance of readily available cells, (ii) unique and selective immunosuppressive capacity, (iii) low immunogenicity, and (iv) ability to fight microbes and cancer and protect against cardiovascular conditions. If proven to be effective in pre-clinical models of crescentic GN, hAECs have the potential to change clinical practice in this disease and provide immense advantages to patients by alleviating their risk of death and complications from unwanted symptoms caused by the existing treatments.

AUTHOR CONTRIBUTIONS

AA, JO, and DO wrote the article and agreed with the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

Alhomrani, M., Correia, J., Zavou, M., Leaw, B., Kuk, N., Xu, R., et al. (2017). The human amnion epithelial cell secretome decreases hepatic fibrosis in mice with chronic liver fibrosis. *Front. Pharmacol.* 8:748. doi: 10.3389/fphar.2017.00748

Allison, A. C., and Eugui, E. M. (2000). Mycophenolate mofetil and its mechanisms of action. *Immunopharmacology* 47, 85–118.

Anders, H. J., Saxena, R., Zhao, M. H., Parodis, I., Salmon, J. E., and Mohan, C. (2020). Lupus nephritis. *Nat. Rev. Dis. Primers* 6:7.

Banas, R. A., Trumpower, C., Bentlejewski, C., Marshall, V., Sing, G., and Zeevi, A. (2008). Immunogenicity and immunomodulatory effects of amnion-derived multipotent progenitor cells. *Hum. Immunol.* 69, 321–328. doi: 10.1016/j.humimm.2008.04.007

Ben-David, U., and Benvenisty, N. (2011). The tumorigenicity of human embryonic and induced pluripotent stem cells. *Nat. Rev. Cancer* 11, 268–277.

Bolton, W. K., Innes, D. J. Jr., Sturgill, B. C., and Kaiser, D. L. (1987). T-cells and macrophages in rapidly progressive glomerulonephritis: clinicopathologic correlations. *Kidney Int.* 32, 869–876.

Booth, A., Harper, L., Hammad, T., Bacon, P., Griffith, M., Levy, J., et al. (2004). Prospective study of TNFα blockade with infliximab in anti-neutrophil cytoplasmic antibody-associated systemic vasculitis. *J. Am. Soc. Nephrol.* 15, 717–721. doi: 10.1097/01.asn.0000114554.67106.28

Caldas, H. C., Lojudice, F. H., Dias, C., Fernandes-Charpiot, I. M. M., Baptista, M., Kawasaki-Oyama, R. S., et al. (2017). Induced pluripotent stem cells reduce progression of experimental chronic kidney disease but develop wilms'. *Tumors. Stem Cells Int.* 2017:7428316. doi: 10.1155/2017/7428316

Chadban, S. J., and Atkins, R. C. (2005). Glomerulonephritis. *Lancet* 365, 1797–1806.

Charles-Henri, W., and Ekaterine, B. (2020). Immunomodulatory properties of amniotic membrane derivatives and their potential in regenerative medicine. *Curr. Diab. Rep.* 20:31.

Couser, W. G. (1998). Pathogenesis of glomerular damage in glomerulonephritis. *Nephrol. Dial. Transpl.* 13(Suppl._1), 10–15.

Cunningham, M. A., Huang, X. R., Dowling, J. P., Tipping, P. G., and Holdsworth, S. R. (1999). Prominence of cell-mediated immunity effectors in "pauci-immune" glomerulonephritis. *J. Am. Soc. Nephrol.* 10, 499–506. doi: 10.1681/ASN.V103499

Daniel, M., Peek, G. W., and Tollefsbol, T. O. (2012). Regulation of the human catalytic subunit of telomerase (hTERT). *Gene* 498, 135–146.

Dean, E. G., Wilson, G. R., Li, M., Edgtton, K. L., O'Sullivan, K., Hudson, B. G., et al. (2005). Experimental autoimmune Goodpasture's disease: a pathogenetic role for both effector cells and antibody in injury. *Kidney Int.* 67, 566–575. doi: 10.1111/j.1523-1755.2005.67113.x

Dey, B., Dange, P., Ganesh, R. N., Parameswaran, S., and Sivan Pillai, P. P. (2016). Immune-complex deposits in anti-neutrophil cytoplasmic antibody associated crescentic glomerulonephritis; a report of two cases. *Immunopathol. Persa* 3:e09.

Disteldorf, E. M., Krebs, C. F., Paust, H. J., Turner, J. E., Nouailles, G., Tittel, A., et al. (2015). CXCL5 drives neutrophil recruitment in TH17-mediated GN. *J. Am. Soc. Nephrol.* 26, 55–66. doi: 10.1681/ASN.2013101061

Dorin, J. R., McHugh, B. J., Cox, S. L., and Davidson, D. J. (2015). Mammalian antimicrobial peptides; defensins and cathelicidins. *Mol. Med. Microbiol.* 1, 539–565.

D'Souza, M. J., Oettinger, C. W., Shah, A., Tipping, P. G., Huang, X. R., and Milton, G. V. (1999). Macrophage depletion by albumin microencapsulated clodronate: attenuation of cytokine release in macrophage-dependent glomerulonephritis. *Drug Dev. Ind. Pharm.* 25, 591–596. doi: 10.1081/ddc-100102213

Evans, M. A., Lim, R., Kim, H. A., Chu, H. X., Gardiner-Mann, C. V., Taylor, K. W., et al. (2018). Acute or delayed systemic administration of human amnion epithelial cells improves outcomes in experimental stroke. *Stroke* 49, 700–709.

Falk, R., and Jennette, J. (1988). Anti-neutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis. *N. Engl. J. Med.* 318, 1651–1657.

Fang, C.-H., Jin, J., Joe, J.-H., Song, Y.-S., So, B.-I., Lim, S. M., et al. (2012). In vivo differentiation of human amniotic epithelial cells into cardiomyocyte-like cells and cell transplantation effect on myocardial infarction in rats: comparison with cord blood and adipose tissue-derived mesenchymal stem cells. *Cell Transplant.* 21, 1687–1696. doi: 10.3727/096368912X653039

Flossmann, O., Berden, A., de Groot, K., Hagen, C., Harper, L., Heijl, C., et al. (2011). Long-term patient survival in ANCA-associated vasculitis. *Ann. Rheum. Dis.* 70, 488–494.

Furuhashi, K., Tsuboi, N., Shimizu, A., Katsuno, T., Kim, H., Saka, Y., et al. (2013). Serum-starved adipose-derived stromal cells ameliorate crescentic GN by promoting immunoregulatory macrophages. *J. Am. Soc. Nephrol.* 24, 587–603. doi: 10.1681/ASN.2012030264

Gan, P. Y., Steinmetz, O. M., Tan, D. S., O'Sullivan, K. M., Ooi, J. D., Iwakura, Y., et al. (2010). Th17 cells promote autoimmune anti-myeloperoxidase glomerulonephritis. *J. Am. Soc. Nephrol.* 21, 925–931.

Geng, X. D., Zheng, W., Wu, C. M., Wang, S. Q., Hong, Q., Cai, G. Y., et al. (2016). Embryonic stem cells-loaded gelatin microcryogels slow progression of chronic kidney disease. *Chin. Med. J.* 129, 392–398. doi: 10.4103/0366-6999.176088

Gulati, K., and McAdoo, S. P. (2018). Anti-glomerular basement membrane disease. *Rheum Dis. Clin. North Am.* 44, 651–673.

Hamour, S., Gan, P. Y., Pepper, R., Florez Barros, F., Wang, H. H., O'Sullivan, K., et al. (2015). Local IL-17 production exerts a protective role in murine experimental glomerulonephritis. *PLoS One* 10:e0136238. doi: 10.1371/journal.pone.0136238

Herrnstadt, G., and Steinmetz, O. (2020). The role of Treg subtypes in glomerulonephritis. *Cell Tissue Res.* doi: 10.1007/s00441-020-03359-7 Online ahead of print

Hodge, A., Lourensz, D., Vaghjiani, V., Nguyen, H., Tchongue, J., Wang, B., et al. (2014). Soluble factors derived from human amniotic epithelial cells suppress collagen production in human hepatic stellate cells. *Cytotherapy* 16, 1132–1144. doi: 10.1016/j.jcyt.2014.01.005

Holdsworth, S. R., Neale, T. J., and Wilson, C. B. (1981). Abrogation of macrophage-dependent injury in experimental glomerulonephritis in the rabbit: use of an antimacrophage serum. *J. Clin. Invest.* 68, 686–698. doi: 10.1172/jci110304

Hori, J., Wang, M., Kamiya, K., Takahashi, H., and Sakuragawa, N. (2006). Immunological characteristics of amniotic epithelium. *Cornea* 25, S53–S58.

Howes, A., Stimpson, P., Redford, P., Gabrysova, L., and O'Garra, A. (2014). "Interleukin-10: cytokines in anti-inflammation and tolerance," in *Cytokine Frontiers*, eds T. Yoshimoto and T. Yoshimoto (Tokyo: Springer), 327–352.

Hu, W., Liu, Z., Chen, H., Tang, Z., Wang, Q., Shen, K., et al. (2002). Mycophenolate mofetil vs cyclophosphamide therapy for patients with diffuse proliferative lupus nephritis. *Chin. Med. J.* 115, 705–709.

Huang, X., Tipping, P. G., Apostolopoulos, J., Oettinger, C., D'souza, M., Milton, G., et al. (1997). Mechanisms of T cell-induced glomerular injury in anti-glomeruler basement membrane (GBM) glomerulonephritis in rats. *Clin. Exp. Immunol.* 109, 134–142.

Huang, X.-R., Tipping, P. G., Shuo, L., and Holdsworth, S. R. (1997). Th1 responsiveness to nephritogenic antigens determines susceptibility to crescentic glomerulonephritis in mice. *Kidney Int.* 51, 94–103.

Humrich, J. Y., Morbach, H., Undeutsch, R., Enghard, P., Rosenberger, S., Weigert, O., et al. (2010). Homeostatic imbalance of regulatory and effector T cells due to IL-2 deprivation amplifies murine lupus. *Proc. Natl. Acad. Sci. U.S.A.* 107, 204–209. doi: 10.1073/pnas.0903158107

Imig, J. D., and Ryan, M. J. (2013). Immune and inflammatory role in renal disease. *Compr. Physiol.* 3, 957–976.

Ivashkiv, L. B., and Donlin, L. T. (2014). Regulation of type I interferon responses. *Nat. Rev. Immunol.* 14, 36–49.

Jefferson, J. A. (2018). Complications of immunosuppression in glomerular disease. *Clin. J. Am. Soc. Nephrol.* 13, 1264–1275.

Jennette, J. C. (2003). Rapidly progressive crescentic glomerulonephritis. *Kidney Int.* 63, 1164–1177.

Jennette, J. C., and Thomas, D. (2001). Crescentic glomerulonephritis. *Nephrol. Dial. Transplant.* 16(Suppl._6), 80–82.

Jhaveri, K. D., Shah, H. H., Calderon, K., Campenot, E. S., and Radhakrishnan, J. (2013). Glomerular diseases seen with cancer and chemotherapy: a narrative review. *Kidney Int.* 84, 34–44. doi: 10.1038/ki.2012.484

Jiang, W., and Xu, J. (2020). Immune modulation by mesenchymal stem cells. *Cell Prolif.* 53:e12712.

Jin, M., Xie, Y., Li, Q., and Chen, X. (2014). Stem cell-based cell therapy for glomerulonephritis. *BioMed. Res. Int.* 2014:124730.

Jirsova, K., and Jones, G. L. (2017). Amniotic membrane in ophthalmology: properties, preparation, storage and indications for grafting—a review. *Cell Tissue Bank.* 18, 193–204. doi: 10.1007/s10561-017-9618-5

Johnson, R., Couser, W. G., Alpers, C. E., Vissers, M., Schulze, M., and Klebanoff, S. J. (1988). The human neutrophil serine proteinases, elastase and cathepsin G, can mediate glomerular injury in vivo. *J. Exp. Med.* 168, 1169–1174. doi: 10.1084/jem.168.3.1169

Jones, R. B., Furuta, S., Tervaert, J. W., Hauser, T., Luqmani, R., Morgan, M. D., et al. (2015). Rituximab versus cyclophosphamide in ANCA-associated renal vasculitis: 2-year results of a randomised trial. *Ann. Rheum. Dis.* 74, 1178–1182. doi: 10.1136/annrheumdis-2014-206404

Kallenberg, C. G. (2014). Key advances in the clinical approach to ANCA-associated vasculitis. *Nat. Rev. Rheumatol.* 10, 484–493.

Kaplan, M. J. (2013). Role of neutrophils in systemic autoimmune diseases. *Arthritis Res. Ther.* 15:219.

Karras, A. (2018). Microscopic polyangiitis: new insights into pathogenesis, clinical features and therapy. *Semin. Respir. Crit. Care Med.* 39, 459–464.

Kay, A. G., Long, G., Tyler, G., Stefan, A., Broadfoot, S. J., Piccinini, A. M., et al. (2017). Mesenchymal stem cell-conditioned medium reduces disease severity and immune responses in inflammatory arthritis. *Sci. Rep.* 7:18019. doi: 10.1038/s41598-017-18144-w

King, A., Paltoo, A., Kelly, R., Sallenave, J.-M., Bocking, A., and Challis, J. (2007). Expression of natural antimicrobials by human placenta and fetal membranes. *Placenta* 28, 161–169.

King, C., and Harper, L. (2017). Avoidance of harm from treatment for ANCA-associated vasculitis. *Curr. Treatm. Opt. Rheumatol.* 3, 230–243. doi: 10.1007/s40674-017-0082-y

Kitching, A. R., Holdsworth, S. R., and Tipping, P. G. (1999). IFN-γ mediates crescent formation and cell-mediated immune injury in murine glomerulonephritis. *J. Am. Soc. Nephrol.* 10, 752–759. doi: 10.1681/ASN.V104752

Kitching, A. R., Holdsworth, S. R., and Tipping, P. G. (2000). Crescentic glomerulonephritis-a manifestation of a nephritogenic Thl response? *Histol. Histopathol.* 15, 993–1003. doi: 10.14670/HH-15.993

Kitching, A. R., Tipping, P. G., Mutch, D. A., Huang, X. R., and Holdsworth, S. R. (1998). Interleukin-4 deficiency enhances Th1 responses and crescentic glomerulonephritis in mice. *Kidney Int.* 53, 112–118.

Kitching, A. R., Turner, A. L., Semple, T., Li, M., Edgtton, K. L., Wilson, G. R., et al. (2004). Experimental autoimmune anti-glomerular basement membrane glomerulonephritis: a protective role for IFN-gamma. *J. Am. Soc. Nephrol.* 15, 1764–1774.

Kuk, N., Correia, J., Alhomrani, M., Lim, R., Sievert, W., Hodge, A., et al. (2018). DOP060 Human amnion epithelial cells and their conditioned media reduces intestinal inflammation and fibrosis in a murine model of chronic colitis. *J. Crohns. Colitis* 12(Suppl._1), S072–S.

Lan, H., Nikolic-Paterson, D., Mu, W., and Atkins, R. (1997). Local macrophage proliferation in the pathogenesis of glomerular crescent formation in rat anti-glomerular basement membrane (GBM) glomerulonephritis. *Clin. Exp. Immunol.* 110, 233–240.

Laurino, S., Chaudhry, A., Booth, A., Conte, G., and Jayne, D. (2010). Prospective study of TNFα blockade with adalimumab in ANCA-associated systemic vasculitis with renal involvement. *Nephrol. Dial. Transplant.* 25, 3307–3314.

Lefebvre, S., Adrian, F., Moreau, P., Gourand, L., Dausset, J., Berrih-Aknin, S., et al. (2000). Modulation of HLA-G expression in human thymic and amniotic epithelial cells. *Hum. Immunol.* 61, 1095–1101. doi: 10.1016/s0198-8859(00)00192-0

Li, H., Niederkorn, J. Y., Neelam, S., Mayhew, E., Word, R. A., McCulley, J. P., et al. (2005). Immunosuppressive factors secreted by human amniotic epithelial cells. *Invest. Ophthalmol. Vis. Sci.* 46, 900–907.

Li, J., Qiu, C., Zhang, Z., Yuan, W., Ge, Z., Tan, B., et al. (2018). Subretinal transplantation of human amniotic epithelial cells in the treatment of autoimmune uveitis in rats. *Cell Transplant.* 27, 1504–1514. doi: 10.1177/0963689718796196

Li, S., Holdsworth, S. R., and Tipping, P. G. (1997). Antibody independent crescentic glomerulonephritis in μ chain deficient mice. *Kidney Int.* 51, 672–678. doi: 10.1038/ki.1997.97

Liang, H., Guan, D., Gao, A., Yin, Y., Jing, M., Yang, L., et al. (2014). Human amniotic epithelial stem cells inhibit microglia activation through downregulation of tumor necrosis factor-α, interleukin-1β and matrix metalloproteinase-12 in vitro and in a rat model of intracerebral hemorrhage. *Cytotherapy* 16, 523–534. doi: 10.1016/j.jcyt.2013.11.007

Lim, R., Hodge, A., Moore, G., Wallace, E. M., and Sievert, W. (2017). A pilot study evaluating the safety of intravenously administered human amnion epithelial cells for the treatment of hepatic fibrosis. *Front. Pharmacol.* 8:549. doi: 10.3389/fphar.2017.00549

Lim, R., Malhotra, A., Tan, J., Chan, S. T., Lau, S., Zhu, D., et al. (2018). First-in-human administration of allogeneic amnion cells in premature infants with bronchopulmonary dysplasia: a safety study. *Stem Cells Transl. Med.* 7, 628–635. doi: 10.1002/sctm.18-0079

Liu, T., Wu, J., Huang, Q., Hou, Y., Jiang, Z., Zang, S., et al. (2008). Human amniotic epithelial cells ameliorate behavioral dysfunction and reduce infarct size in the rat middle cerebral artery occlusion model. *Shock* 29, 603–611. doi: 10.1097/SHK.0b013e318157e845

Liu, Y. H., Vaghjiani, V., Tee, J. Y., To, K., Cui, P., Oh, D. Y., et al. (2012). Amniotic epithelial cells from the human placenta potently suppress a mouse model of multiple sclerosis. *PLoS One* 7:e35758. doi: 10.1371/journal.pone.0035758

Lu, G., Zhang, X., Shen, L., Qiao, Q., Li, Y., Sun, J., et al. (2017). CCL20 secreted from IgA1-stimulated human mesangial cells recruits inflammatory Th17 cells in IgA nephropathy. *PLoS One* 12:e0178352. doi: 10.1371/journal.pone.0178352

Luger, D., Silver, P. B., Tang, J., Cua, D., Chen, Z., Iwakura, Y., et al. (2008). Either a Th17 or a Th1 effector response can drive autoimmunity: conditions of disease induction affect dominant effector category. *J. Exp. Med.* 205, 799–810.

Ma, X., Che, N., Gu, Z., Huang, J., Wang, D., Liang, J., et al. (2013). Allogenic mesenchymal stem cell transplantation ameliorates nephritis in lupus mice via inhibition of B-cell activation. *Cell Transplant.* 22, 2279–2290. doi: 10.3727/096368912X658692

Mahdi, B. M. (2013). A glow of HLA typing in organ transplantation. *Clin. Transl. Med.* 2:6.

Malhotra, A., Lim, R., Mockler, J. C., and Wallace, E. M. (2020). Two-year outcomes of infants enrolled in the first-in-human study of amnion cells for bronchopulmonary dysplasia. *Stem Cells Transl. Med.* 9, 289–294. doi: 10.1002/sctm.19-0251

Manuelpillai, U., Lourensz, D., Vaghjiani, V., Tchongue, J., Lacey, D., Tee, J.-Y., et al. (2012). Human amniotic epithelial cell transplantation induces markers of alternative macrophage activation and reduces established hepatic fibrosis. *PLoS One* 7:e38631. doi: 10.1371/journal.pone.0038631

Manuelpillai, U., Tchongue, J., Lourensz, D., Vaghjiani, V., Samuel, C. S., Liu, A., et al. (2010). Transplantation of human amnion epithelial cells reduces hepatic fibrosis in immunocompetent CCl4-treated mice. *Cell Transplant.* 19, 1157–1168. doi: 10.3727/096368910X504496

McAdoo, S. P., and Pusey, C. D. (2017). Anti-glomerular basement membrane disease. *Clin. J. Am. Soc. Nephrol.* 12, 1162–1172.

McDonald, C. A., Payne, N. L., Sun, G., Moussa, L., Siatskas, C., Lim, R., et al. (2015). Immunosuppressive potential of human amnion epithelial cells in the treatment of experimental autoimmune encephalomyelitis. *J. Neuroinflammation* 12:112. doi: 10.1186/s12974-015-0322-8

Mejia-Vilet, J., and Parikh, S. (2019). "Overview of the current approach to glomerular disease classification," in *Glomerulonephritis*, eds H. Trachtman, L. Herlitz, E. Lerma and J. Hogan (Cham: Springer), 59–85.

Ménétrier-Caux, C., Ray-Coquard, I., Blay, J. Y., and Caux, C. (2019). Lymphopenia in cancer patients and its effects on response to immunotherapy: an opportunity for combination with cytokines? *J. Immunother. Cancer.* 7:85. doi: 10.1186/s40425-019-0549-5

Menez, S. P., El Essawy, B., and Atta, M. G. (2018). Lupus nephritis: current treatment paradigm and unmet needs. *Rev. Recent. Clin. Trials.* 13, 105–113.

Miki, T., Lehmann, T., Cai, H., Stolz, D. B., and Strom, S. C. (2005). Stem cell characteristics of amniotic epithelial cells. *Stem Cells* 23, 1549–1559.

Moroni, G., and Ponticelli, C. (2014). Rapidly progressive crescentic glomerulonephritis: early treatment is a must. *Autoimmun. Rev.* 13, 723–729.

Motedayyen, H., Zarnani, A. H., Tajik, N., Ghotloo, S., and Rezaei, A. (2018). Immunomodulatory effects of human amniotic epithelial cells on naive CD4(+)

T cells from women with unexplained recurrent spontaneous abortion. *Placenta* 71, 31–40.

Müller, G. A., Müller, C. A., Markovic-Lipkovski, J., Kilper, R. B., and Risler, T. (1988). Renal, major histocompatibility complex antigens and cellular components in rapidly progressive glomerulonephritis identified by monoclonal antibodies. *Nephron* 49, 132–139. doi: 10.1159/000185039

Murphy, S., Lim, R., Dickinson, H., Acharya, R., Rosli, S., Jenkin, G., et al. (2011). Human amnion epithelial cells prevent bleomycin-induced lung injury and preserve lung function. *Cell Transplant.* 20, 909–924. doi: 10.3727/096368910X543385

Murray, I. R., and Péault, B. (2015). Q&A: mesenchymal stem cells - where do they come from and is it important? *BMC Biol.* 13:99.

Mûzes, G., and Sipos, F. (2019). Issues and opportunities of stem cell therapy in autoimmune diseases. *World J. Stem Cells.* 11, 212–221.

Naik, R. H., and Shawar, S. H. (2020). *Rapidly Progressive Glomerulonephritis*. Treasure Island, FL: StatPearls Publishing.

Nemr, W., Bashandy, M., Araby, E., and Khamiss, O. (2017). Molecular displaying of differential immunoresponse to various infections of amniotic epithelia. *Am. J. Reprod. Immunol.* 77:e12662. doi: 10.1111/aji.12662

Niknejad, H., Khayat-Khoei, M., Peirovi, H., and Abolghasemi, H. (2014). Human amniotic epithelial cells induce apoptosis of cancer cells: a new anti-tumor therapeutic strategy. *Cytotherapy* 16, 33–40. doi: 10.1016/j.jcyt.2013.07.005

Nolasco, E. F., Cameron, J. S., Hartley, B., Coelho, A., Hildreth, G., and Reuben, R. (1987). Intraglomerular T cells and monocytes in nephritis: study with monoclonal antibodies. *Kidney Int.* 31, 1160–1166.

Nomura, S., Sasaki, T., Kitano, Y., Osawa, G., Niederstadt, C., Lerche, L., et al. (1996). The critical role of intercellular adhesion molecule-1 in masugi nephritis in rats. *Nephron* 73, 264–272. doi: 10.1159/000189050

Odobasic, D., Gan, P. Y., Summers, S. A., Semple, T. J., Muljadi, R. C., Iwakura, Y., et al. (2011). Interleukin-17A promotes early but attenuates established disease in crescentic glomerulonephritis in mice. *Am. J. Pathol.* 179, 1188–1198. doi: 10.1016/j.ajpath.2011.05.039

Odobasic, D., Ghali, J. R., O'Sullivan, K. M., Holdsworth, S. R., and Kitching, A. R. (2014). Glomerulonephritis induced by heterologous anti-GBM globulin as a planted foreign antigen. *Curr. Protoc. Immunol.* 106, 15.26.1–15.26.20. doi: 10.1002/0471142735.im1526s106

Odobasic, D., Kitching, A. R., Semple, T. J., and Holdsworth, S. R. (2007). Endogenous myeloperoxidase promotes neutrophil-mediated renal injury, but attenuates T cell immunity inducing crescentic glomerulonephritis. *J. Am. Soc. Nephrol.* 18, 760–770. doi: 10.1681/ASN.2006040375

Odobasic, D., Oudin, V., Ito, K., Gan, P. Y., Kitching, A. R., and Holdsworth, S. R. (2019). Tolerogenic dendritic cells attenuate experimental autoimmune antimyeloperoxidase glomerulonephritis. *J. Am. Soc. Nephrol.* 30, 2140–2157. doi: 10.1681/ASN.2019030236

Olnes, M. J., Kotliarov, Y., Biancotto, A., Cheung, F., Chen, J., Shi, R., et al. (2016). Effects of systemically administered hydrocortisone on the human immunome. *Sci. Rep.* 6:23002.

Ooi, J. D., Gan, P. Y., Chen, T., Eggenhuizen, P. J., Chang, J., Alikhan, M. A., et al. (2014). FcgammaRIIB regulates T-cell autoreactivity, ANCA production, and neutrophil activation to suppress anti-myeloperoxidase glomerulonephritis. *Kidney Int.* 86, 1140–1149. doi: 10.1038/ki.2014.189

Ooi, J. D., Phoon, R. K., Holdsworth, S. R., and Kitching, A. R. (2009). IL-23, not IL-12, directs autoimmunity to the Goodpasture antigen. *J. Am. Soc. Nephrol.* 20, 980–989.

Ooi, J. D., Snelgrove, S. L., Engel, D. R., Hochheiser, K., Ludwig-Portugall, I., Nozaki, Y., et al. (2011). Endogenous foxp3+ T-regulatory cells suppress anti-glomerular basement membrane nephritis. *Kidney Int.* 79, 977–986. doi: 10.1038/ki.2010.541

Ostmann, A., Paust, H. J., Panzer, U., Wegscheid, C., Kapffer, S., Huber, S., et al. (2013). Regulatory T cell-derived IL-10 ameliorates crescentic GN. *J. Am. Soc. Nephrol.* 24, 930–942. doi: 10.1681/ASN.2012070684

Ougaard, M. K. E., Kvist, P. H., Jensen, H. E., Hess, C., Rune, I., and Søndergaard, H. (2018). Murine nephrotoxic nephritis as a model of chronic kidney disease. *Int. J. Nephrol.* 2018:8424502.

Parmar, D. N., Alizadeh, H., Awwad, S. T., Li, H., Neelam, S., Bowman, R. W., et al. (2006). Ocular surface restoration using non-surgical transplantation of tissue-cultured human amniotic epithelial cells. *Am. J. Ophthalmol.* 141, 299.–307. doi: 10.1016/j.ajo.2005.09.008

Parmar, M. S., and Bashir, K. (2017). *Crescentric Glomerulonephritis*. Treasure Island, FL: StatPearls Publishing.

Parmar, M. S., and Bashir, K. (2019). *Crescentric Glomerulonephritis*. Treasure Island, FL: StatPearls Publishing.

Paust, H.-J., Turner, J.-E., Steinmetz, O. M., Peters, A., Heymann, F., Hölscher, C., et al. (2009). The IL-23/Th17 axis contributes to renal injury in experimental glomerulonephritis. *J. Am. Soc. Nephrol.* 20, 969–979.

Phan, T. G., Ma, H., Lim, R., Sobey, C. G., and Wallace, E. M. (2018). Phase 1 trial of amnion cell therapy for ischemic stroke. *Front. Neurol.* 9:198. doi: 10.3389/fneur.2018.00198

Ponticelli, C., and Locatelli, F. (2018). Glucocorticoids in the treatment of glomerular diseases: pitfalls and pearls. *Clin. J. Am. Soc. Nephrol.* 13, 815–822. doi: 10.2215/CJN.12991117

Prendecki, M., and Pusey, C. (2019). Plasma exchange in anti-glomerular basement membrane disease. *Presse Med.* 48, 328–337.

Qiu, C., Ge, Z., Cui, W., Yu, L., and Li, J. (2020). Human amniotic epithelial stem cells: a promising seed cell for clinical applications. *Int. J. Mol. Sci.* 21:7730. doi: 10.3390/ijms21207730

Ren, Y., Chen, Y., Zheng, X., Wang, H., Kang, X., Tang, J., et al. (2020). Human amniotic epithelial cells ameliorate kidney damage in ischemia-reperfusion mouse model of acute kidney injury. *Stem Cell Res. Therapy* 11, 410. doi: 10.1186/s13287-020-01917-y

Richard, M. L., and Gilkeson, G. (2018). Mouse models of lupus: what they tell us and what they don't. *Lupus Sci. Med.* 5:e000199. doi: 10.1136/lupus-2016-000199

Richards, H. B., Satoh, M., Jennette, J. C., Croker, B. P., Yoshida, H., and Reeves, W. H. (2001). Interferon-gamma is required for lupus nephritis in mice treated with the hydrocarbon oil pristane. *Kidney Int.* 60, 2173–2180. doi: 10.1046/j.1523-1755.2001.00045.x

Rousselle, A., Kettritz, R., and Schreiber, A. (2017). Monocytes promote crescent formation in anti-myeloperoxidase antibody-induced glomerulonephritis. *Am. J. Pathol.* 187, 1908–1915.

Ruth, A., Kitching, A., Kwan, R., Odobasic, D., Ooi, J., Timoshanko, J., et al. (2006). Anti-neutrophil cytoplasmic antibodies and effector CD4+ cells play nonredundant roles in anti-myeloperoxidase crescentic glomerulonephritis. *J. Am. Soc. Nephrol.* 17, 1940–1949. doi: 10.1681/ASN.2006020108

Ryu, J. S., Jeong, E. J., Kim, J. Y., Park, S. J., Ju, W. S., Kim, C. H., et al. (2020). Application of mesenchymal stem cells in inflammatory and fibrotic diseases. *Int. J. Mol. Sci.* 21:8366.

Saleem, S., Dai, Z., Coelho, S. N., Konieczny, B. T., Assmann, K. J., Baddoura, F. K., et al. (1998). IL-4 is an endogenous inhibitor of neutrophil influx and subsequent pathology in acute antibody-mediated inflammation. *J. Immunol.* 160, 979–984.

Santos, J. E., Fiel, D., Santos, R., Vicente, R., Aguiar, R., Santos, I., et al. (2020). Rituximab use in adult glomerulopathies and its rationale. *J. Bras. Nephrol.* 42, 77–93. doi: 10.1590/2175-8239-JBN-2018-0254

Schrijver, G., Bogman, M. J. J., Assmann, K. J., de Waal, R. M., Robben, H. C., van Gasteren, H., et al. (1990). Anti-GBM nephritis in the mouse: role of granulocytes in the heterologous phase. *Kidney Inter.* 38, 86–95.

Sheu, J. J., Sung, P. H., Wallace, C. G., Yang, C. C., Chen, K. H., Shao, P. L., et al. (2020). Intravenous administration of iPS-MSC(SPIONs) mobilized into CKD parenchyma and effectively preserved residual renal function in CKD rat. *J. Cell Mol. Med.* 24, 3593–3610. doi: 10.1111/jcmm.15050

Spada, S. (2020). Methods to purify DNA from extracellular vesicles: focus on exosomes. *Methods Enzymol.* 645, 109–118. doi: 10.1016/bs.mie.2020.09.004

Stachura, I., Si, L., and Whiteside, T. L. (1984). Mononuclear-cell subsets in human idiopathic crescentic glomerulonephritis (ICGN): analysis in tissue sections with monoclonal antibodies. *J. Clin. Immunol.* 4, 202–208. doi: 10.1007/BF00914967

Summers, S. A., Steinmetz, O. M., Gan, P. Y., Ooi, J. D., Odobasic, D., Kitching, A. R., et al. (2011). Toll-like receptor 2 induces Th17 myeloperoxidase autoimmunity while Toll-like receptor 9 drives Th1 autoimmunity in murine vasculitis. *Arthritis Rheum.* 63, 1124–1135. doi: 10.1002/art.30208

Summers, S. A., Steinmetz, O. M., Li, M., Kausman, J. Y., Semple, T., Edgtton, K. L., et al. (2009). Th1 and Th17 cells induce proliferative glomerulonephritis. *J. Am. Soc. Nephrol.* 20, 2518–2524.

Suzuki, T., Iyoda, M., Shibata, T., Ohtaki, H., Matsumoto, K., Shindo-Hirai, Y., et al. (2013). Therapeutic effects of human mesenchymal stem cells in Wistar-Kyoto rats with anti-glomerular basement membrane glomerulonephritis. *PLoS One* 8:e67475. doi: 10.1371/journal.pone.0067475

Syed, R., Rehman, A., Valecha, G., and El-Sayegh, S. (2015). Pauci-immune crescentic glomerulonephritis: an ANCA-associated vasculitis. *BioMed Res. Int.* 2015:402826.

Tabatabaei, M., Mosaffa, N., Ghods, R., Nikoo, S., Kazemnejad, S., Khanmohammadi, M., et al. (2018). Vaccination with human amniotic epithelial cells confer effective protection in a murine model of colon adenocarcinoma. *Int. J. Cancer* 142, 1453–1466. doi: 10.1002/ijc.31159

Tan, B., Yuan, W., Li, J., Yang, P., Ge, Z., Liu, J., et al. (2018). Therapeutic effect of human amniotic epithelial cells in murine models of Hashimoto's thyroiditis and Systemic lupus erythematosus. *Cytotherapy* 20, 1247–1258. doi: 10.1016/j.jcyt.2018.04.001

Tan, D. S., Gan, P. Y., O'Sullivan, K. M., Hammett, M. V., Summers, S. A., Ooi, J. D., et al. (2013). Thymic deletion and regulatory T cells prevent antimyeloperoxidase GN. *J. Am. Soc. Nephrol.* 24, 573–585. doi: 10.1681/ASN.2012090898

Tan, J. L., Chan, S. T., Lo, C. Y., Deane, J. A., McDonald, C. A., Bernard, C. C., et al. (2015). Amnion cell-mediated immune modulation following bleomycin challenge: controlling the regulatory T cell response. *Stem Cell Res. Ther.* 6:8. doi: 10.1186/scrt542

Tan, J. L., Chan, S. T., Wallace, E. M., and Lim, R. (2014). Human amnion epithelial cells mediate lung repair by directly modulating macrophage recruitment and polarization. *Cell Transplant.* 23, 319–328. doi: 10.3727/096368912X661409

Tan, J. L., Lau, S. N., Leaw, B., Nguyen, H. P. T., Salamonsen, L. A., Saad, M. I., et al. (2018). Amnion epithelial cell-derived exosomes restrict lung injury and enhance endogenous lung repair. *Stem Cells Transl. Med.* 7, 180–196. doi: 10.1002/sctm.17-0185

Thakkar, U. G., Vanikar, A. V., and Trivedi, H. L. (2017). Stem cell therapy: an emerging modality in glomerular diseases. *Cytotherapy* 19, 333–348. doi: 10.1016/j.jcyt.2016.11.003

Tipping, P. G., Huang, X. R., Berndt, M. C., and Holdsworth, S. R. (1994). A role for P selectin in complement-independent neutrophil-mediated glomerular injury. *Kidney Inter.* 46, 79–88. doi: 10.1038/ki.1994.246

Tipping, P. G., Huang, X. R., Qi, M., Van, G. Y., and Tang, W. W. (1998). Crescentic glomerulonephritis in CD4-and CD8-deficient mice. Requirement for CD4 but not CD8 cells. *Am. J. Pathol.* 152:1541.

Tögel, F., Hu, Z., Weiss, K., Isaac, J., Lange, C., and Westenfelder, C. (2005). Administered mesenchymal stem cells protect against ischemic acute renal failure through differentiation-independent mechanisms. *Am. J. Physiol. Renal. Physiol.* 289, F31–F42. doi: 10.1152/ajprenal.00007.2005

Tsui, C., Dokouhaki, P., and Prasad, B. (2018). Fibrillary glomerulonephritis with crescentic and necrotizing glomerulonephritis and concurrent thrombotic microangiopathy. *Case Rep. Nephrol. Dial.* 8, 182–191. doi: 10.1159/000492529

Tumlin, J. A., Lohavichan, V., and Hennigar, R. (2003). Crescentic, proliferative IgA nephropathy: clinical and histological response to methylprednisolone and intravenous cyclophosphamide. *Nephrol. Dial. Transplant.* 18, 1321–1329.

Uchide, N., Ohyama, K., Yuan, B., Sano, T., Bessho, T., and Yamakawa, T. (2002). Differential mRNA expression of inflammatory cytokines in cultured human fetal membrane cells responding to influenza virus infection. *Biol. Pharm. Bull.* 25, 239–243. doi: 10.1248/bpb.25.239

Uchide, N., and Toyoda, H. (2007). Current status of monocyte differentiation-inducing (MDI) factors derived from human fetal membrane chorion cells undergoing apoptosis after influenza virus infection. *Gene Regul. Syst. Biol.* 1, 295–302. doi: 10.4137/grsb.s374

Vinen, C., and Oliveira, D. (2003). Acute glomerulonephritis. *Postgrad. Med. J.* 79, 206–213.

Vizoso, F. J., Eiro, N., Cid, S., Schneider, J., and Perez-Fernandez, R. (2017). Mesenchymal stem cell secretome: toward cell-free therapeutic strategies in regenerative medicine. *Int. J. Mol. Sci.* 18:1852.

Wang, Y., Chen, X., Cao, W., and Shi, Y. (2014). Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. *Nat. Immunol.* 15, 1009–1016.

Wegener's Granulomatosis Etanercept Trial (WGET) Research Group (2005). Etanercept plus standard therapy for Wegener's granulomatosis. *N. Engl. J. Med.* 352, 351–361. doi: 10.1056/NEJMoa041884

Wolbank, S., Peterbauer, A., Fahrner, M., Hennerbichler, S., van Griensven, M., Stadler, G., et al. (2007). Dose-dependent immunomodulatory effect of human stem cells from amniotic membrane: a comparison with human mesenchymal stem cells from adipose tissue. *Tissue Eng.* 13, 1173–1183. doi: 10.1089/ten.2006.0313

Xiao, H., Heeringa, P., Hu, P., Liu, Z., Zhao, M., Aratani, Y., et al. (2002). Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. *J. Clin. Invest.* 110, 955–963.

Yang, C., Huang, X.-R., Fung, E., Liu, H.-F., and Lan, H.-Y. (2017). The regulatory T-cell transcription factor Foxp3 protects against crescentic glomerulonephritis. *Sci. Rep.* 7:1481. doi: 10.1038/s41598-017-01515-8

Yawno, T., Schuilwerve, J., Moss, T. J., Vosdoganes, P., Westover, A. J., Afandi, E., et al. (2013). Human amnion epithelial cells reduce fetal brain injury in response to intrauterine inflammation. *Dev. Neurosci.* 35, 272–282.

Zhang, Q., Huang, Y., Sun, J., Gu, T., Shao, X., and Lai, D. (2019). Immunomodulatory effect of human amniotic epithelial cells on restoration of ovarian function in mice with autoimmune ovarian disease. *Acta Biochim. Biophys. Sin.* 51, 845–855. doi: 10.1093/abbs/gmz065

Zhang, Y., Liu, Y., Liu, H., and Tang, W. (2019). Exosomes: biogenesis, biologic function and clinical potential. *Cell Biosci.* 9:19.

Zheng, Y., Zheng, S., Fan, X., Li, L., Xiao, Y., Luo, P., et al. (2018). Amniotic epithelial cells accelerate diabetic wound healing by modulating inflammation and promoting neovascularization. *Stem Cells Int.* 2018:1082076. doi: 10.1155/2018/1082076

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