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Novel targets for chronic inflammatory diseases: Focus on therapeutic drugs and natural compounds, volume II

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Novel targets for chronic inflammatory diseases: Focus on therapeutic drugs and natural compounds, volume II

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Table of contents

05 Advances of the small molecule drugs regulating fibroblast-like synovial proliferation for rheumatoid arthritis
Yitong Tong, Xinyu Li, Qichuan Deng, Jianyou Shi, Yibin Feng and Lan Bai

29 Ursolic acid reduces oxidative stress injury to ameliorate experimental autoimmune myocarditis by activating Nrf2/HO-1 signaling pathway
Yanan Fu, Tianshu Liu, Shukun He, Yichan Zhang, Yuting Tan, Ying Bai, Jiawei Shi, Wenhui Deng, Jiani Qiu, Zhen Wang, Yihan Chen, Qiaofeng Jin, Mingxing Xie and Jing Wang

42 Commentary: Identification of cuproptosis hub genes contributing to the immune microenvironment in ulcerative colitis using bioinformatic analysis and experimental verification
Shaopeng Sun and Bin Lv

45 Baricitinib treatment for refractory skin changes in POEMS syndrome: a case report
Jingjing Xie, Zhiling Li, Yubao Jiang, Dabin Tang, Xia Qiu, Ertao Jia and Jianyong Zhang

48 Tangeretin attenuates bleomycin-induced pulmonary fibrosis by inhibiting epithelial-mesenchymal transition via the PI3K/Akt pathway
Jiang Li, Qian Wei, Ke Song, Youxin Wang, Yuxin Yang, Miao Li, Jiaying Yu, Guangxu Su, Luyuan Peng, Bendong Fu and Pengfei Yi

60 Progression of the PI3K/Akt signaling pathway in chronic obstructive pulmonary disease
Yanhui Liu, Haobo Kong, Heping Cai, Guanru Chen, Huiying Chen and Wenyi Ruan

69 Role and mechanisms of SGLT-2 inhibitors in the treatment of diabetic kidney disease
Zhi-Cheng Dai, Jin-Xia Chen, Rong Zou, Xuan-Bing Liang, Ji-Xin Tang and Cui-Wei Yao

81 Research progress of NF-κB signaling pathway and thrombosis
Zilong Wang, Chucun Fang, Mengting Yao, Dongwen Wu, Maga Chen, Tianting Guo and Jianwen Mo

92 Whether MTHFD2 plays a new role: from anticancer targets to anti-inflammatory disease
Hui Tang and Ning Hou

96 β-sitosterol alleviates dextran sulfate sodium-induced experimental colitis via inhibition of NLRP3/Caspase-1/GSDMD-mediated pyroptosis
Di Zhang, Fei Ge, Jing Ji, Yu-Jing Li, Fu-Rong Zhang, Shu-Yan Wang, Shu-Jing Zhang, Dong-Mei Zhang and Meng Chen

108 Progress in studying the impact of hyperlipidemia and statins on rotator cuff injury and repair
Yinhua Qian, Haoqiang Huang, Renwen Wan, Yu Zhou, Xinting Feng, Feng Xu, Zhiwen Luo and Qing Wang

114 Multifaceted oncostatin M: novel roles and therapeutic potential of the oncostatin M signaling in rheumatoid arthritis
Liang Han, Jiahui Yan, Tingting Li, Weiji Lin, Yao Huang, Pan Shen, Xin Ba, Ying Huang, Kai Qin, Yinhong Geng, Huanhuan Wang, Kaifeng Zheng, Yafei Liu, Yu Wang, Zhe Chen and Shenghao Tu

126 GPR35 acts a dual role and therapeutic target in inflammation
Yetian Wu, Pei Zhang, Hongjie Fan, Caiying Zhang, Pengfei Yu, Xinmiao Liang and Yang Chen

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Advances of the small molecule drugs regulating fibroblast-like synovial proliferation for rheumatoid arthritis

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Rheumatoid arthritis (RA) is a type of chronic autoimmune and inflammatory disease. In the pathological process of RA, the alteration of fibroblast-like synoviocyte (FLS) and its related factors is the main influence in the clinic and fundamental research. In RA, FLS exhibits a uniquely aggressive phenotype, leading to synovial hyperplasia, destruction of the cartilage and bone, and a pro-inflammatory environment in the synovial tissue for perpetuation and progression. Evidently, it is a highly promising way to target the pathological function of FLS for new anti-RA drugs. Based on this, we summed up the pathological mechanism of RA-FLS and reviewed the recent progress of small molecule drugs, including the synthetic small molecule compounds and natural products targeting RA-FLS. In the end, there were some views for further action. Compared with MAPK and NF-κB signaling pathways, the JAK/STAT signaling pathway has great potential for research as targets. A small number of synthetic small molecule compounds have entered the clinic to treat RA and are often used in combination with other drugs. Meanwhile, most natural products are currently in the experimental stage, not the clinical trial stage, such as triptolide. There is an urgent need to unremittingly develop new agents for RA.

KEYWORDS

rheumatoid arthritis, fibroblast-like synoviocytes, signaling pathways, small molecule drugs, natural products

1 Introduction

Rheumatoid arthritis (RA) is a type of autoimmune joint disease. It often occurs in women and the elderly. RA might affect 0.5%–1% of the global population (Zhang et al., 2022). Among the multiple factors, genetic and autoimmune along with environmental factors might be the primary causes. It shows the clinical presentation of joint pain, thickening of the synovial membrane, pannus formation, and infiltration of various inflammatory cells in the joint space, leading to the damage of the cartilage as well as bone tissue, even remarkably joint deformity and dysfunction (Smolen et al., 2018). A lot of attention is paid to the treatment of RA because it has high morbidity, might lead to disability, and has poor prognosis (Davis et al., 2012; Almutairi et al., 2021). Currently, non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs

(DMARDs) (synthetic or biologic agents), and glucocorticoids (Lampropoulos et al., 2015; Zhang et al., 2022) are popular in the treatment of RA. With the use of NSAIDs, the risk of cardiovascular disease might occur as well as gastrointestinal side effects, so a comprehensive evaluation is needed (O'Shea et al., 2013). DMARDs such as methotrexate (MTX), while suppressing inflammation and joint destruction, might cause nausea, anorexia, stomatitis, alopecia, myelosuppression, and even liver and pulmonary toxicity in severe cases, which requires careful monitoring. In addition, there are also problems of high expense and gastrointestinal adverse effects for DMARDs (Zhang et al., 2019). Biologic disease-modifying anti-rheumatic drugs (bDMARDs) show therapeutic effects for RA, but there are some individual differences because of different genetic backgrounds and environmental stimuli (Lampropoulos et al., 2015), and they do not cure the disease (Yamada, 2023). There is an urgent need to continuously develop new anti-RA drugs.

The synovium is considered to be a structure of connective soft-tissue membrane located in the joint cavity and the fibrocartilage, around arthrosis to provide nutrition and lubrication (Jay et al., 2000). The fibroblast-like synoviocytes (FLSs) are highly specialized mesenchymal cells found in the synovial membrane. In normal physiological regulation, FLS produces joint lubricants, for example, hyaluronic acid which nourishes the cartilage surface and shapes the synovial extracellular matrix (ECM). However, in RA, FLS exhibits a distinctive aggressive phenotype, with this aggressive behavior toward the ECM further exacerbating joint damage (Nygaard and Firestein, 2020). For this reason, one potential strategy for treating RA is the creation of medicines that target FLS (Bartok and Firestein, 2010). It is important to note that several of their monomers appear to have a positive impact on preventing arthritic synovial hyperplasia. They are mainly related to the induction of apoptosis and the inhibition of FLS proliferation. In this review, taking the state of FLS as a starting point, we summarize and discuss the literature on the small molecule drugs of FLS from PubMed, Embase, and other databases in the recent 3 years until 28 February 2023. Specific keywords used are "RA," "FLS," "MAPK," "NF-κB," "JAK/STAT," "Wnt," and "signaling pathways." The small molecule drugs contain organic compounds with low molecular weights, typically ≤1000 Da. Also, these include both synthetic compounds and natural products derived mainly from plants and animals. Publications with incomplete data or conclusions and those not directly related to RA and small molecule compounds are excluded. Here, first, there is an introduction of the pathological mechanisms of RA-FLS. Second, according to the signaling pathways controlling the abnormal behavior of FLS, small molecule drugs of related pathways, especially drugs with high anti-RA-FLS potential, are analyzed in depth. Finally, we list our comments, which we hope will provide directions to developing targeted anti-rheumatic drugs for clinics.

2 FLS involved in the pathogenesis of RA

In RA, FLS proliferation releases several anti-inflammatory cytokines and growth factors, among which are tumor necrosis factor (TNF), interleukin (IL) (such as IL-6, IL-1β, and IL-17), chemokines, and inflammatory enzymes [such as nitric oxide synthase (NOS) and cyclooxygenase-2 (COX-2)]. Meanwhile, it provides the inflammatory microenvironment and potentially contributes to the initiation of chronic inflammation in the preliminary stage of RA. In addition, FLS produces large amounts of receptor activator of NF-κB ligand (RANKL), vascular endothelial growth factor (VEGF), matrix metalloproteinases (MMPs), and so on, which causes synovial hyperplasia and arthritic joint destruction (Wang et al., 2012). Worse still, the activated FLS migrates to the cartilage and bone. This migration occurs not only at local sites but also through the bloodstream into distant areas and joints, destroying the cartilage, activating osteoclasts, and enhancing joint destruction in RA (Neumann et al., 2010; Hu et al., 2019). Here, we review the pathological mechanisms of RA from the three perspectives shown in Figure 1: synovial hyperplasia, joint damage, and immune inflammation.

2.1 Synovial hyperplasia

The synovium of RA exhibits endothelial hyperplasia and transformation into pannus tissue that destroys the articular cartilage and bone, with occasional lymphatic-like aggregates. A large number of inflammatory cytokines (IL-1β, TNF-α, etc.) stimulate FLS to proliferate abnormally and exhibit anti-apoptosis. The imbalance between FLS anti-apoptotic and pro-apoptotic factors increases the number of FLS considerably, which directly leads to synovial hyperplasia. The FLS in the synovial lining layer is increased from the normal 1–3 to 10–15 cell layers (Neumann et al., 2010). The proliferated FLS develops into lymphoid-like structures, interacting with immune cells to form lymphoid organs and releasing pro-inflammatory factors and inflammatory mediators. Growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), and stimulatory cytokines in the synovial tissue, induce FLS proliferation through the activation of the signaling pathway. Along with the *in situ* proliferative capacity of FLS, the expression of anti-apoptotic molecules is also increased. The anti-apoptotic molecule FLICE inhibitory protein (FLIP) suppresses intracellular apoptosis-triggering cystatase-8, decreasing apoptosis and causing synovial proliferation (Bartok and Firestein, 2010).

2.2 Joint damage

Cartilage and bone destruction are hallmarks of RA. MMPs expressed by FLS degrade the chondral matrix, leading to impaired nutrient supply to the articular cartilage and tissue joint destruction.

2.2.1 Chondral matrix degradation

FLS mediates the overproduction of MMPs that interrupts the joint tissue, which contains a structure abundant in collagen and facilitates FLS infestation into the cartilage surface. Mediated by pro-inflammatory cytokines and toll-like receptors (TLRs), FLS upregulates the expression of MMPs, which activate osteoclasts and directly erode the bone, causing cartilage and bone destruction. Activated osteoclasts can reduce bone mass in the

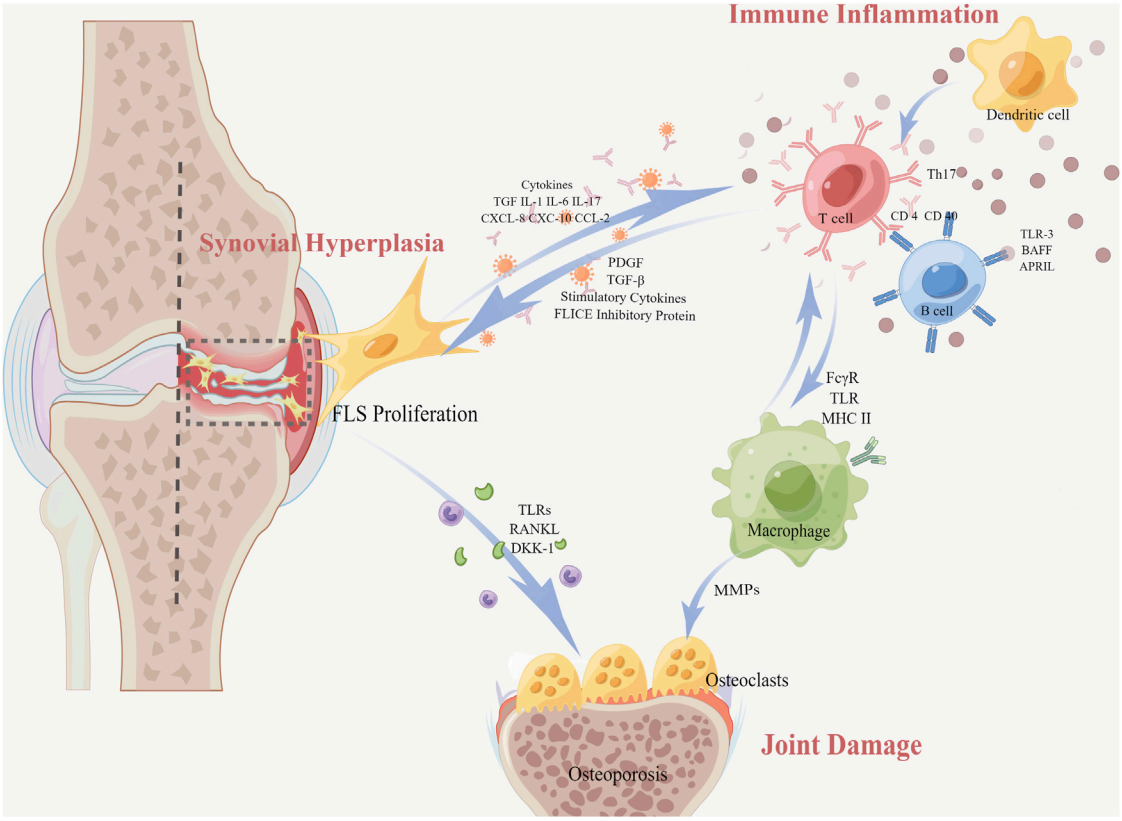

FIGURE 1
Pathological mechanisms of RA with FLS (In RA, the proliferation of FLS resulting from synovial hyperplasia releases various anti-inflammatory cytokines and growth factors. Meanwhile, the interaction between FLS and immune cells causes a transformation of regular FLS into an aggressive phenotype, resulting in an abnormal situation of T-cell and B-cell functions related to immune inflammation. Furthermore, FLS secretes pro-inflammatory cytokines into the joint space and invades the adjacent bone tissue through migration, inducing bone erosion and joint destruction. Macrophages also differentiate directly into mature osteoclasts).

periarticular bone early in the lesion, leading to osteoporosis. In addition, the extra expression of MMPs upregulates the levels of inflammatory factors and soluble mediators in the synovial tissue. Also, the factors are bound to receptors of MAPK, JAK/STAT, etc., signaling pathways, promoting and maintaining joint inflammation (Firestein, 2003).

2.2.2 Bone destruction

The migration of FLS is also the process of bone destruction. Due to the cytokines, FLS can migrate into the cartilage and bone, thus exacerbating cartilage destruction (Zeng et al., 2017). FLS produces RANKL in the cartilage or bone. Then, RANKL binds to the receptor activator of NF-κB (RANK) on osteoclast precursors, inducing osteoclast differentiation, activation, and production. A large number of osteoclasts erode the surface of the adjacent articular cartilage membrane and induce bone destruction. Not only that, RA-FLS hinders the recovery process of bone erosion by hindering osteoblast activation through the secretion of dickkopf-1 (DKK-1). DKK-1 is a crucial regulatory molecule within the Wnt pathway, acting as an inhibitor of osteoclast function (Miao et al., 2013). Under specific microenvironmental conditions, macrophages can also differentiate directly into mature osteoclasts. In addition, inflammatory macrophages are a consistent source of matrix metalloproteinases, such as MMP-1, MMP-3, MMP-7, MMP-10, MMP-12, MMP-14, and MMP-25, which participate in connective tissue transformation and joint surface erosion observed in RA.

2.3 Immune inflammation

FLS are known to contribute significantly to RA by secreting inflammatory chemokines that interact with synovial infiltrating cells. The chemokines secreted by FLS, including, CXC motif chemokine 8 (CXCL-8), CXCL-10, and CC motif chemokine ligand 2 (CCL2), can recruit a range of immune cells into the synovial tissue. Then, the inflammatory mediators, for example, IL, TNF-α, and TGF-β1, from these immune cells in turn stimulate FLS activation, resulting in a vicious circle. Macrophages are constantly affected by inflammatory stimuli and participate in the development of chronic synovitis, bone erosion, and cartilage erosion. Macrophages express a lot of molecules on their surface, such as Fc-gamma receptors (FcγRs), TLR, and the major histocompatibility complex class II (MHCII), which in turn, regulate their own activities, activate other cells in the local microenvironment, or attract immune cells outside the joint. TNF-α, IL-6, IL-1β, IL-23, and a wide range of CXCL and CCL chemokines promote and maintain

inflammation by recruiting and activating polymorphonuclear leukocytes, T cells, B cells, or monocytes.

2.3.1 FLS and B cells

There is a bidirectional signaling between FLS and B cells. On one hand, FLS affects the maturation and growth of B cells by secreting cytokines. The etiology of autoimmune disorders involves both humoral immunity and B lymphocytes as significant contributors. The preservation of the B-cell pool and humoral immunity depend on the B-cell-activating factor of the TNF family (BAFF, also known as BLYS) and a proliferation-inducing ligand (APRIL). Taking TLR-3 as an example, TLR-3 triggers not only B-cell-activating BAFF but also APRIL. Both of them participate in the stimulation of B cells, thus prolonging B-cell survival (Bombardieri et al., 2011; Leah, 2011). On the other hand, B cells in turn induce the FLS inflammatory phenotype. In the FLS co-culture experiments with age-associated B cells (ABCs), ABCs induce FLS phenotype excitation through TNF-α inducing the activation of ERK1/2 and JAK-STAT1 signaling pathways, consequently promoting the persistence of RA (Qin et al., 2022).

2.3.2 FLS and T cells

T-cell infiltration and excessive proliferation of FLS are significantly upregulated in RA patients. Both interact during RA inflammation to perpetuate inflammation. RA-FLS can present peptides of inflammatory antigens to antigen-specific T cells, contributing to the auto-reactive immune response in RA (Tran et al., 2007). Then, FLS expresses adhesion molecules, transmitting signals to CD4 T cells, such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular cell adhesion molecule-1 (ICAM-1). Finally, these adhesive molecules interact with integrins, for instance, lymphocyte function-associated antigen 1 (LFA-1), resulting in CD4 T-cell proliferation and IL-17 secretion and exacerbation of the inflammatory response (Mori et al., 2017). At the same time, macrophages express MHCII as antigen-presenting cells, thereby participating in the activation and recruitment of pathogenic T cells. So, there is also an interaction between T cells and FLS (Tran et al., 2008; Tu et al., 2022).

To sum up, FLS can secrete pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, and MMP, in the joint space of RA patients and invade the adjacent bone tissue through migration, inducing bone erosion and joint destruction. The interaction between FLS and immune cells causes a transformation of regular FLS into an aggressive phenotype, resulting in abnormal T- and B-cell functions. Also, our body gradually loses its normal immune regulatory and protective ability (Ding et al., 2023). It is evident that FLS is the central effector cell in the pathogenesis. Given that there is no effective treatment targeted at FLS, the inhibition FLS-mediated pro-inflammatory response and subsequent tissue destruction seems to be a feasible strategy for RA (Nygaard and Firestein, 2020). In the next part, we summarize the results in the recent 3 years of small molecule drugs targeted at FLS.

3 Small molecule drugs regulating FLS

In the previous sections, we have clarified that RA-FLS are activated by multiple cytokines involved in the activation of FLS. Targeted pathways of FLS might simultaneously block multiple signaling of cytokine receptors, inhibiting the activation, proliferation, and invasion of FLS and, thus, significantly controlling RA synovial inflammation and joint damage (Mavers et al., 2009; Wendling et al., 2010; Pan et al., 2016). Despite significant breakthroughs in RA therapy, many people with RA have persistent disease. The current RA therapy plans emphasize reducing T-cell and B-cell activity as well as cytokine signaling (Mahmoud et al., 2022). In RA, targeting signal transduction pathways is an emerging treatment option. According to the signaling pathway interacted with FLS, there are mainly MAPK, NF-κB, JAK/STAT, PI3K/Akt, and Wnt signaling pathways in Figure 2. So, we present the drugs' research progress which regulates FLS function on the signaling pathways, including the small molecule compounds and natural products. It is aimed to explore promising novel drug development directions and broaden the path of novel targeted FLS.

3.1 Small molecule drugs targeting MAPK regulating FLS

The MAPK signaling pathway is associated with various kinases, such as P38, c-Jun N-terminal kinase (JNK), and extracellular regulated protein kinases (ERKs), which are involved in the proliferation, apoptosis, and migration of FLS, with the addition of cytokine secretion (Harigai et al., 2004; Tang et al., 2019). ERK is involved in the secretion of certain cytokines and cell proliferation and differentiation through the regulation of B-cell lymphoma 2 (Bcl-2). JNK decreases proteoglycan synthesis and enhances MMP-13 synthesis, which are necessary for bone deterioration and joint inflammation. p38 is associated with the cytokine secretion of MMP. Through inhibiting p38, MMP reduces cartilage degradation and inhibits osteoclast formation. Additionally, the MAPK pathway contributes to the FLS's increase in TNF-α expression, amplifying inflammatory signals, inducing FLS proliferation, aggravating inflammation, and damaging joints (Zuo et al., 2015; Kadkhoda et al., 2016). An increasing number of studies have shown that the MAPK pathway is activated in immune and autoimmune response conditions, regulating the cell responses of division, differentiation, apoptosis, inflammation, and stress and also participating in the activation of FLS (Müller-Ladner et al., 2007; Bustamante et al., 2017). In addition, MAPK activates downstream transcription factors that promote synovial cell proliferation and chondrocyte apoptosis. It also leads to high expression of multiple MMPs in synovial cells and chondrocytes and overhydrolysis of the extracellular matrix, resulting in joint damage. Therefore, MAPK is one of the most studied targets to inhibit RA-FLS (Wang et al., 2010).

Here, we review the synthetic small molecule compounds and natural products in the recent 3 years targeted to MAPK for FLS in Table 1, and the natural products regulating MAPK are shown in Figure 3. It is important to note that the majority of drugs affected numerous signaling pathways and multiple targets. As an MAPK downstream effector, p38 is considered

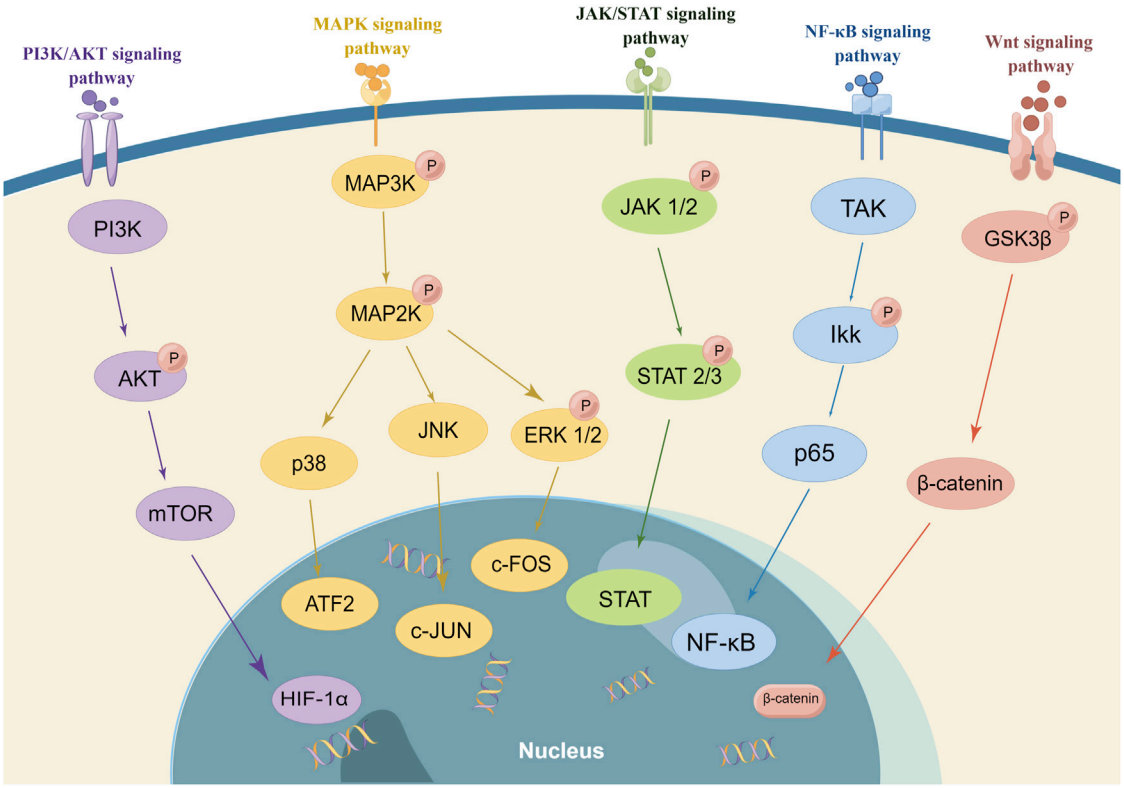

FIGURE 2
Signaling pathway regulating FLS (In RA, targeting signal transduction pathways is an emerging treatment option. The small molecule compounds and natural products interact with FLS in the different signaling pathways. There are mainly MAPK, NF-κB, JAK/STAT, PI3K/Akt, and Wnt signaling pathways. It is important to note that the majority of drugs affected numerous signaling pathways and multiple targets).

a possible target for RA, but only few p38 inhibitors have been tested in humans. Tacrolimus as a macrolide calcineurin inhibitor immunosuppressant drug decreased the production of angiopoietin-1 (Ang1), tyrosine-protein kinase receptor (Tie-2), and VEGF in human FLS by preventing the activation of the IL-1β-mediated JNK and p38 MAPK pathways. Sugiura et al.'s (2020) study was very interesting. They found that glycogen synthase kinase 3 (GSK-3) inhibitors significantly reduced synovial fibroblast migration after 72 h and decreased Akt phosphorylation [Ser (473)] after 48 h *in vitro*, which might have therapeutic efficacy targeting the invasion and migration of synovial fibroblasts. Also, 3′3-diindolylmethane exhibited the possibility of anti-RA-FLS activitiy *in vivo* and *in vitro* (Du et al., 2019). The small molecule compounds reported in recent years that could alter FLS *in vivo* and *in vitro* were elutriated extirpate, dasatinib, 4-phenylbutyric acid, and 3-(4-hydroxy-3-methoxy-phenyl)-1-3-[1]-phenyl-propenone. Unfortunately, these medications are still in the laboratory stage. Because of their poor performance, p38 inhibitors have limited efficacy in RA treatment. Also, blocking p38's downstream had a compensatory effect on other kinases, so alternative options for p38 have been progressively explored (Guma et al., 2012). Regulation of MAPK kinases upstream of p38, the human mitogen-activated protein kinase kinase (MKK), such as MKK6 and MKK1, could selectively block the production of MMPs and pro-inflammatory cytokines in FLS (Hammaker et al., 2012). In addition, ubiquitin D might be considered a possible therapeutic target for RA-FLS (Chen et al., 2023).

In natural products in Table 1 and Figure 3, alkaloids and flavonoids were more frequently reported and studied for their effects on the MAPK signaling pathway of FLS. Other categories, such as iridoids and saponins, were also found to have an impact. It is well known that flavonoids possess anti-oxidant and anti-inflammatory properties. Flavonoids can inhibit the inflammatory response and reduce the symptoms of inflammation while scavenging free radicals, reducing oxidative stress, and protecting cells from oxidative damage. Flavonoids usually inhibit FLS proliferation, migration, and invasion by inhibiting p38 and JNK. To our surprise, alkaloids also showed up significantly in the treatment of FLS. Preparations of berberine and paclitaxel were available for clinical use, but they have no indication for the treatment of RA.

Triptolide and tetrandrine from *Tripterygium wilfordii* Hook F. and *Stephania tetrandra* root, respectively, have anti-rheumatic effects in the classic sense. Tripterygium glycoside preparations have been clinically used for the treatment of RA. As the representative, we concentrate on triptolide, which has been studied more and has been proven to have multiple signaling pathways. The treatment with triptolide decreased the expression of phosphorylated JNK that TNF-α-produced, but it had no effect on the expression of phosphorylated p38 or

TABLE 1 Small molecule drugs targeting MAPK regulating FLS.

Name	Source	Targets/ signaling pathways	Estimate	References
The synthetic small molecule compounds				
GSK-3 inhibitors (6-bromoindirubin-3′-oxime and thiadiazolidinone-8)	Serine/threonine protein kinase	JNK, p38, NF-κB	Experimental: NF-κB ↓	Kwon et al. (2014); Sugiura et al. (2020)
			The phosphorylated JNK, c-Jun, ATF-2, p38 ↓	
			IL-6 ↓	
			IL-10 ↑	
Tacrolimus	Macrolide antibiotics from *Streptomyces*	JNK, p38	Clinical: showed higher retention rates combined with bDMARDs	Choe et al. (2012); Kaneko et al. (2021); Terabe et al. (2023)
			Adverse events stable in long-term observation	
			Effective with acceptable safety	
			Experimental: the expressions of Ang-1, Tie-2, VEGF ↓	
3′3-Diindolylmethane	The main product of indole-3-carbinol oligomerization catalyzed by acid	p38, JNK, Akt, mTOR	Experimental: proliferation, migration, and invasion of RA-FLS *in vitro* ↓	Du et al. (2019)
			MMP-2, MMP-3, MMP-8, and MMP-9 ↓ p-p38, JNK ↓	
			Akt, mTOR ↓	
			Pro-inflammatory cytokines and arthritis severity in mice ↓	
Telotristat etiprate	A tryptophan hydroxylase inhibitor	MAPK	Experimental: migration and invasion of RA-FLS *in vitro* ↓	Zhang et al. (2023)
			Targeting LGALS3	
Dasatinib	A Src kinase inhibitor	MAPK, STATs	Experimental: Src, Fyn, MAPK, STATs ↓	Yalcin Kehribar et al. (2021); Min et al. (2023)
			MMP-1, MMP-3, MMP-13 in FLS ↓	
4-Phenylbutyric acid	An HDAC inhibitor	MAPK, NF-κB	Experimental: p-MAPK, p-NF-κB ↓	Choi et al. (2021)
			MMP-1, MMP-3, COX-2 ↓	
			Endoplasmic reticulum stress ↓	
3-(4-Hydroxy-3-methoxy-phenyl)-1-3-[1]-phenyl-propenone	A benzylideneacetophenone derivative	MAPK	Experimental: IL-8, IL-6, PGE (2) ↓	Sur et al. (2020)
			Reducing the inflammation in the knee joints in C/K-arthritic rats	
The natural products				
Fangchinoline	A bisbenzylisoquinoline alkaloid from *Stephania tetrandra*	MAPK, NF-κB	Experimental: inflammatory cytokine secretion and ROS in human FLS ↓	Villa et al. (2020)
			Phosphorylation of the MAPK and NF-κB pathway in human FLS ↓	
Berberine	An alkaloid from *Coptis chinensis*	PI3K/Akt, Wnt, RAS/MAPK/FOXO/ HIF-1	Clinical: no indication for treatment of RA	Wang et al. (2019); Shen et al. (2020); Sujitha et al. (2020); Li et al. (2023); Li et al. (2023)
			Experimental: LRP5 protein ↓	
			β-Catenin transcription ↓ p38/ ERK ↓	
			Proliferation and adhesion of FLS ↓	
			MMP-1, MMP-3, RANKL, TNF-α ↓	

(Continued on following page)

TABLE 1 (*Continued*) Small molecule drugs targeting MAPK regulating FLS.

Name	Source	Targets/ signaling pathways	Estimate	References
Paclitaxel	An alkaloid from *Taxus chinensis*	MAPK, Akt/mTOR	Clinical: no indication for treatment of RA	Chen et al. (2021)
			Experimental: FLS migration dose dependently ↓	
			IL-6, IL-8, RANKL ↓	
			MMP-8, MMP-9 gene transcription ↓ p-ERK1/2 ↓	
			p-JNK ↓	
			Akt, p70S6K, 4EBP1, HIF-1α ↓	
Peimine	A steroidal alkaloid from *Fritillaria*	ERK, JNK, p38	Experimental: TNF-α induced destructive behaviors in MAPK for FLS↓	Zhou et al. (2022)
			RANKL-induced osteoclast formation ↓	
			Bone-resorption function ↓	
Tetrandrine	An alkaloid from *Stephania tetrandra* root	NF-κB, Ca_2 (+), PI3K/Akt, MAPK	Experimental: Rac1, Cdc42, RhoA ↓	Lv et al. (2015); Zhong et al. (2019)
			MMP-2/9, F-actin, FAK↓	
			RANKL-induced osteoclastogenesis ↓	
Dehydroevodiamine	A quinazoline alkaloid from Evodiae Fructus	MAPK	Experimental: pro-inflammatory factors in AIA rats ↓	Dai et al. (2022)
			MMP-1, MMP-3 ↓ p-p38, p-JNK, and p-ERK ↓	
Tomatidine	A steroidal alkaloid from the Solanaceae family	MAPK, NF-κB	Experimental: proliferation and migration of FLS ↓	Yu et al. (2021)
			Synovial inflammation and joint destruction in CIA rats ↑	
			IL-1β, IL-6, TNF-α ↓	
			MMP-9, RANKL ↓	
Benzoylaconitine	An alkaloid from *Aconitum*	MAPK, Akt, NF-κB	Experimental: IL-6, IL-8 ↓	Yu et al. (2020)
			MAPK, p-Akt ↓	
			Degradation of IκB α↓ p-p65 and nuclear transposition ↓	
Kaempferol	A flavonoid from *Kaempferol galanga* L.	ERK-1/2, p38, JNK, NF-κB	Experimental: MAPK activation ↓, instead of altering TNF-α receptor activation	Yoon et al. (2013); Pan et al. (2018)
			Phosphorylation of ERK-1/2, p38, JNK ↓	
			NF-κB ↓	
Orientin	A flavonoid from *P. orientale*	p38, ERK	Experimental: viability, migration as well as invasion of FLS ↓	Ji and Xu (2022)
			TNFα-induced inflammatory makers ↓	

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TABLE 1 (*Continued*) Small molecule drugs targeting MAPK regulating FLS.

Name	Source	Targets/ signaling pathways	Estimate	References
Apigenin-4′-O-alpha-L-rhamnoside	A flavonoid from apigenin derivative	MAPK	Experimental: migration of FLS ↓	Cao et al. (2022)
			MMP-1, MMP3, RANKL, TNF-α ↓	
			MAPK1, HRAS, ATF-2, p38, JNK ↓	
Naringin	A flavonoid from citrus fruits	PI3K/Akt, ERK	Experimental: inflammation, MMPs ↓	Aihaiti et al. (2021)
			Apoptosis of FLS↑ the activation of caspase-3 ↑	
			Bax/Bcl-2 ↑ p- Akt, p-ERK ↓	
Liquiritin	A flavonoid from the roots of *Glycyrrhiza uralensis*	JNK, P38	Experimental: FLS proliferation ↓	Zhai et al. (2019)
			DNA fragmentation in the nucleus ↑	
			Altering the potential of the mitochondrial membrane	
			Bcl-2/Bax ratio ↓	
			VEGF ↓ p-JNK, p-p38 ↓	
Neohesperidin	A flavanone glycoside from citrus fruits	MAPK	Experimental: IL-1β, IL-6, IL-8, TNF-α, MMP-3, MMP-9 and MMP-13 in FLSs ↓	Wang et al. (2021)
			MAPK ↓	
			ROS induced by TNF-α↓	
Ononin	An isoflavone glycoside from the fruit of *Cnidium monnieri* (L.) cusson	NF-κB, MAPK	Experimental: TNF-α mediated cells viability of FLS and MH7A ↓	Meng et al. (2021)
			Cell apoptosis↑	
			IL-1β, IL-6 ↓	
Cyanidin	An anthocyanidin from grapes, bilberry, blackberry, etc.	p38, STAT-3	Experimental: IL-17A induced the migration of monocytes from AA rats ↓	Samarpita and Rasool (2021); Samarpita et al. (2020)
			HSP27, CCR7, CXCR4 ↓	
			RANKL ↓	
			OPG ↑ p38 MAPK ↓	
Cyanidin-3-glucoside	An anthocyanin from berries	p38, ERK and JNK, NF-κB	Experimental: TNF-α, IL-1β, IL-6 ↓ p65 ↓	Sun and Li (2018)
			Phosphorylation of IκBα, p38, ERK, JNK ↓	
Paris saponin VII Chonglou	A steroidal saponin from *Trillium tschonoskii* Maxim.	JNK, p38	Experimental: FLS invasion via managing the mitochondrial apoptosis, MAPK pathway	Meng et al. (2021)
			Improving histopathological changes	
			TNF-α, IL-1β, IL-6 ↓	
			Modulating the expressions of apoptosis proteins in AIA rats	

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TABLE 1 (*Continued*) Small molecule drugs targeting MAPK regulating FLS.

Name	Source	Targets/ signaling pathways	Estimate	References
Gintonin	A ginseng-derived exogenous ligand of lysophosphatidic acid	MAPK, NF-κB	Experimental: iNOS, IL-6, TNF-α, COX-2↓	Kim et al. (2021); Kim et al. (2021)
			NF-κB/p65 into the nucleus ↓	
Triptolide	An epoxide diterpene lactone from *Tripterygium wilfordii* Hook F.	JNK, MAPK8, PI3K/Akt	Experimental: p-JNK ↓	Yang et al. (2016); Xie et al. (2019); Song et al. (2020)
			The polymerization of F-actin ↓	
			The activation of MMP-9 ↓	
			Activating autophagy	
Geniposide	An iridoid glycoside from *Gardenia jasminoides* Ellis fruit	JNK, ERK1/2 and p38; PI3K; Akt	Experimental: proliferation of FLS ↓	Li et al. (2018); Bu et al. (2022)
			IFN-γ, IL-17 ↓	
			IL-4, TGFβ1↑ p-JNK, p-ERK1/2, p-p38 ↓	
			p-PI3K, p-Akt ↑	
Gentiopicroside	A secoiridoid glycoside from *Gentiana macrophylla* Pall.	CD147, p38, NF-κB	Experimental: proliferation of FLS ↓	Jia et al. (2022)
			MMP secretion↓	
			Regulating the CD147/p38/NF-κB pathway, p38, IkκB α, and p65 ↓	
18β-Glycyrrhetinic acid	A triterpene glycoside from *Glycyrrhiza*	MAPK, NF-κB	Experimental: IL-1β, IL-6, COX-2 in MH7A ↓	Feng et al. (2021)
			Cell viability	
			Cell apoptosis and G1 phase cell cycle arrest *in vitro* ↑	
			FOXO3 ↑	
			Liver damage caused by collagen or MTX *in vivo*↓	
			Inflammation and proliferation in FLS ↓	
Pristimerin	A triterpenoid from Celastraceae and Hippocrateaceae families	MAPK/Erk1/2, PI3K/Akt	Experimental: viability and migration of FLS ↓	Lv et al. (2022)
			TNF-α, NO, p-Akt, p-ERK ↓	
Echinocystic acid	A pentacyclic triterpene from *Gleditsia sinensis*	MAPK, NF-κB	Experimental: arthritis symptoms in SKG mice ↓	Cheng et al. (2022)
			TNF-α, IL -6, IL-1β ↓	
			P-STAT3 ↓	
			MAPK, NF-κB	
Osthole	A coumarin from *Cnidium monnieri* and *Angelica pubescens*	NF-κB, MAPK	Experimental: IL-1β, TNF-α, IL-6 ↓	Xu et al. (2018); Lin et al. (2023)
			Proliferation and migration ↓	
			TGM2/Myc/WTAP-positive feedback circuit ↓	
Imperatorin	A coumarin from Umbelliferae	p38, ERK NF-κB	Experimental: proliferation and migration of FLS ↓	Lin et al. (2022)
			TNF-α, IL-6, and IL-8 ↓ p38, ERK ↓	
			p-IκBα ↓	

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TABLE 1 (*Continued*) **Small molecule drugs targeting MAPK regulating FLS.**

Name	Source	Targets/ signaling pathways	Estimate	References
Tanshinone IIA	A diterpene quinone from *Salvia miltiorrhiza* Bunge	MAPK, Akt/mTOR, HIF-1, and NF-κB	Experimental: FLS proliferation, migration, infiltration time, and dose dependently ↓	Du et al. (2020)
			MMPs, pro-inflammatory factors ↓	
Piceatannol	A derivative of resveratrol	MAPK, NF-κB	Experimental: Bax, cleaved caspase-3 ↑	Gao et al. (2022)
			PGE2, IL-6, IL-1β↓	
			COX-2 ↓	
			MMP-3, MMP-13 ↓	
			MAPK, NF-κB ↓	

↓: suppress, downregulate, inhibit, block, prevent, reduce, decrease; ↑: promote, upregulate, active, increase. mTOR, mammalian target of rapamycin; NFATc1, c-Fos and nuclear factor of activated T cells c1; ATF2, activating transcription factor-2; PGE2, prostaglandin E2; ROS, reactive oxygen species; HIF1, hypoxia-inducible factor 1; CIA, collagen-induced arthritis; IκB, inhibitor of κB; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated X; AA, adjuvant-induced arthritic; OPG, osteoprotegerin; MEKK, mitogen-activated protein kinase kinase; IKK, IκB kinase; TGM2, transglutaminase 2.

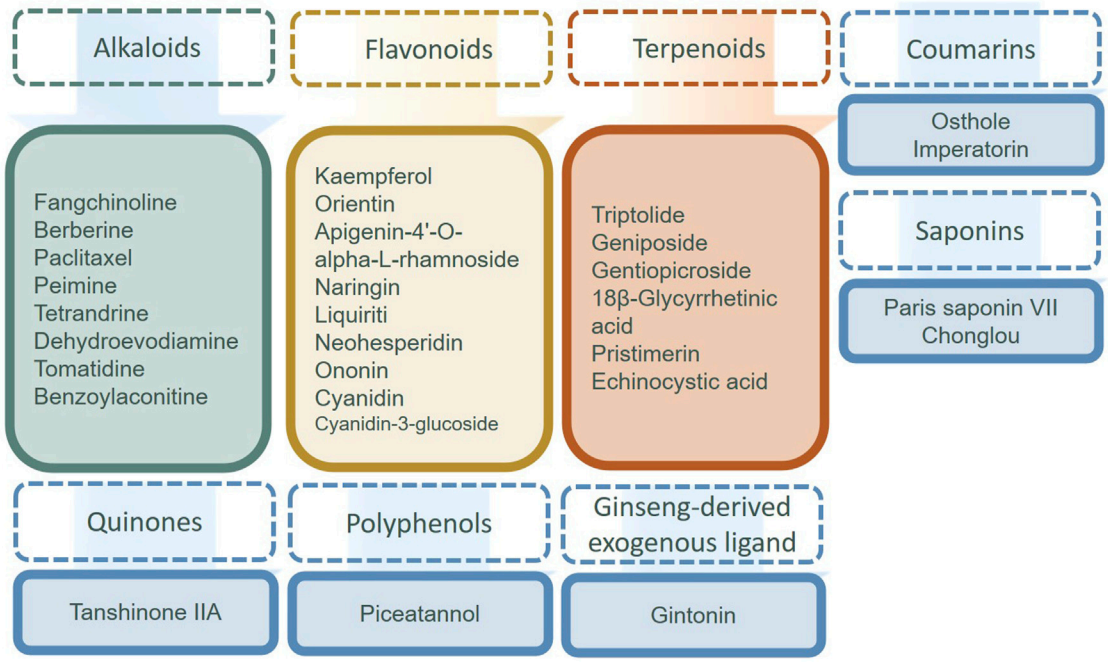

FIGURE 3
Natural products targeting MAPK regulating FLS.

ERK (Yang et al., 2016) and reduced FLS migration and invasion by targeting the JNK/MAPK signaling pathway (Tang et al., 2020). Triptolide dramatically increased the p-Akt/Akt ratio, and inhibiting the PI3K/Akt signaling pathway in MH7A cells caused autophagy to be triggered, indicating that triptolide repressed autophagy via activating p-Akt/Akt (Xie et al., 2019). Other natural products, such as Paris saponin VII/ Chonglou, geniposide, and gentiopicroside, shown in Table 1, also have the potential to regulate FLS against RA. However, it is currently in the experimental stage.

3.2 Small molecule drugs targeting NF-κB regulating FLS

As a major signaling transcription factor, NF-κB contributes to synovial inflammation, proliferation, and decay in bones in RA and regulates inflammatory gene expression and cell proliferation. Both innate and adaptive immune cells include NF-B, which is a key mediator of the stimulation of pro-inflammatory genes (Liu et al., 2017). In a normal situation, NF-κB is bound to its repressor protein IκB and not activated. The nuclear-localization sequence (NLS) that

TABLE 2 Small molecule drugs targeting NF-κB regulating FLS.

Name	Source	Targets/signaling pathways	Estimate	References
The synthetic small molecule compounds				
TAK-242	A TLR 4 antagonist	TLR4, TLR3; NF-κB	Experimental: TLR4, TLR3 ↓	Samarpita et al. (2020)
			The migration of NF-κB to the nucleus	
			IL-8, IL-1, MMP-7 ↓	
CKD-506	A HDAC inhibitor	NF-κB	Experimental: MMP-1, MMP-3, IL-6, IL-8 ↓	Park et al. (2020)
			The proliferation of Teff ↓	
			Exerting a synergistic effect with MTX	
Oxymatrine hydrazone	Synthesized from oxidized bitter ginseng	MEK/1/2, NF-κB	Experimental: IL-1β, IL-6, IL-8 ↓	Zhang et al. (2021)
			MMP-1, MMP-13 ↓	
			MEK/1/2 and p65 phosphorylation ↓	
Paeoniflorin-6′-O-benzene sulfonate (CP-25)	A paeoniflorin derivative	NF-κB, PI3K, GRK2	Experimental: the protein membrane expression and combination↓	Wang et al. (2020); Wang et al. (2023)
Edaravone	Synthetic: 3-methyl-1-phenyl-2-pyrazolin-5-one	NF-κB, MAPK	Clinical: no indication for the treatment of RA	Zhang et al. (2020); Liu et al. (2023)
			Experimental: altering the antioxidant factors, inflammatory mediators, and pro-inflammatory cytokines [NF-κB, COX-2, and PGE (2)]	
			The level of cytokines and OPN, RANKL, and macrophage M-CSF ↓	
Roflumilast	An inhibitor of phosphodiesterase-4	NF-κB	Clinical: no indication for the treatment of RA	Zhong et al. (2021)
			Experimental: ROS and MDA in MH7A cells ↓	
			IL-6, IL-8, TNF-α↓	
			CCL5, CXCL9, CXCL10 ↓	
			MMP-1, MMP-13 ↓	
Sorafenib	A kinase inhibitor	NF-κB, c-Jun	Clinical: no indication for treatment of RA	Wang et al. (2020)
			Experimental: apoptosis in AA FLSs ↓	
			Fas, caspase-3, Mcl-1 ↑	
			NF-κB, C-Jun ↓	
Dexmedetomidine	A specific and selective alpha-2 adrenoceptor agonist	NF-κB	Clinical: no indication for treatment of RA	Ji et al. (2020)
			Experimental: IL-1β, IL-6, IL-17A, TNF-α, and P-P65↓	
			NLRC5 ↓	
Alogliptin	An important selective dipeptidyl peptidase-4 inhibitor	NF-κB	Clinical: no indication for the treatment of RA	Guo et al. (2020)
			Experimental: MMP-3, MMP-13, IL-6, IL-8, and TNF-α p- Jun, p-IκBβ, nuclear translocation of NF-κB p65 ↓	

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TABLE 2 (*Continued*) Small molecule drugs targeting NF-κB regulating FLS.

Name	Source	Targets/signaling pathways	Estimate	References
The natural products				
Diosmetin	A flavonoid from Rutaceae	NF-κB	Experimental: proliferation of MH7A cells ↓	Chen et al. (2020)
			IL-1β, IL-6, IL-8, MMP-1 ↓	
			and NF-κB pathways activation ↓	
Mangiferin	A flavonoid of the bisphenirone family from mango leaves	ERK2, p38, NF-κB	Experimental: MAPKs (ERK2 and p38), NF-κB ↓	Luczkiewicz et al. (2014); Wang et al. (2021)
Icariin	A flavonoid glycoside from Epimedii Herba	NF-κB	Experimental: TRIB1 ↑ by promoting Nrf2 expression regulating the TRIB1/TLR2/NF-κB pathway	Wu et al. (2022)
Isoginkgetin	A biflavonoid from the leaves of the *Ginkgo biloba* tree	IκBβ, p65	Experimental: IL-1β, IL-6, IL-8 ↓	Shao et al. (2022)
			Migration and invasion of FLS↓ p-IκBα, p-p65, MMP9↓	
Tectoridin	An isoflavone from dry rhizome of iris	TLR4/NLRP3/NF-κB MAPK	Experimental: proliferation of FLS ↓	Huang et al. (2022); Niu et al. (2022)
			Cleaved caspase-3, Bax ↑	
			Bcl-2 ↓	
			Pro-inflammatory cytokines ↓	
			TLR4/NLRP3/NF-κB ↓	
			ERK, JNK, p38 ↓	
Celastrol	A quinone-methylated triterpenoid from *Tripterygium wilfordii*	NF-κB, Notch1, ERK, PI3K/Akt/mTOR	Experimental: NF-κB pathway ↓	Gan et al. (2015); Yu et al. (2015); Doss et al. (2016); Fang et al. (2017); An et al. (2020); Yang et al. (2022)
			NLRP3 inflammasome activation↓	
			ROS ↓	
			Changing some chemokine genes expression (CCL2, CXCL10, CXCL12, CCR2 and CXCR4)	
			SYK-MEK-ERK-NF-κB signaling cascade↓	
			Autophagy ↑	
			PI3K/Akt/mTOR↓	
Aucubin	A monoterpenoid from asterids	NF-κB	Experimental: inflammatory factors ↓	Zhang et al. (2022)
			Bone metabolism factors ↓ p-Iκκ α/β, p-IκBα, p-p65 ↓	
Heilaohuacid G	A triterpenoid from *Kadsura coccinea*/heilaohu	NF-κB	Experimental: apoptosis and inflammatory reactions of FLS↓	Yang et al. (2021); Yang et al. (2022)
Sinomenine	An alkaloid from *Sinomenium acutum*	NF-κB	Experimental: adenosine receptor ↑	Zhou et al. (2017); Yi et al. (2021); Chen et al. (2011); Li et al. (2013); Zhou et al. (2015); Yao et al. (2017)
			NF-κB activation via α7nAChR↓	
			Selective mPGES-1 expression ↓	
			TLR4/MyD88/NF-κB signaling cascade↓	

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TABLE 2 (*Continued*) Small molecule drugs targeting NF-κB regulating FLS.

Name	Source	Targets/signaling pathways	Estimate	References
Magnoflorine	An alkaloid from *Clematis manshurica* Rupr.	PI3K/Akt/NF-κB, Keap1-Nrf2/HO-1	Experimental: proliferation, migration, invasion, and reactive oxygen species levels of MH7A cells ↓	Shen et al. (2022)
			Bax ↑	
			Bcl-2↓ iNOS, COX-2, IL-6, IL-8, MMPs ↓	
			PI3K/Akt/NF-κB ↓	
			Keap1-Nrf2/HO-1 ↑	
Curcumin	A polyphenol from turmeric, *curcuma longa*	NF-κB, AP-1, and p38	Experimental: function of pro-inflammatory mediators↓	Buhrmann et al. (2010); Shang et al. (2016); Mohammadian Haftcheshmeh et al. (2021); Xu et al. (2022)
			Osteoclastogenic potential	
			ERK1/2, p38, JNK ↓	
			RANK, c-Fos, NFATc1 levels↓	
Punicalagin	A polyphenol from pomegranate juice	NF-κB	Experimental: IL-1beta, IL-6, IL-8 and IL-17A ↓	Huang et al. (2021)
			MMP-1 and MMP-13 ↓	
			Proliferation and migration of RA FLSs ↓ phosphorylation of IKK and IκBα ↓	
Corilagin	A tannic acid from *Geranium wilfordii* Maxim.	NF-κB p65, ERK, p38, JNK, IκBα	Experimental: Bcl-2, IL-6, IL-8, MMP-1, MMP-2, MMP-3, MMP-9, COX-2, iNOS ↓	Shen et al. (2022)
			Bax ↑	
			P-p65/p65, P-IκBα/IκBα, P-ERK/ERK, P-JNK/JNK, and P-p38/p38 ↓	
			NF-κB p65 nuclear translocation ↓	
			Proliferation, migration, and invasion of FLS ↓	
Eugenol	A phenylpropanoid from a variety of aromatic herbal plants such as clove and tulsi	NF-κB	Experimental: proliferation, migration, invasion, angiogenesis, and inflammatory response of FLS ↓	Wang et al. (2022)
			NF-κB, COX-2 ↓	
Resveratrol	A phenol from grape	SIRT1, NF-κB	Experimental: SIRT1 and downstream paths ↑	Wang et al. (2020); Sheng et al. (2022)
			The striking interplay between the SIRT1 and NF-κB	
Plumbagin	A naphthoquinone from *Plumbago zeylanica* L.	p65	Experimental: viability of human FLS	Shu et al. (2022)
			Inflammatory cytokines, MMPs ↓	
			IκB, NF-κB, p65 into the nucleus↓	
Emodin	An anthraquinone from rhubarb, buckthorn, etc.	MAPK, NF-κB	Experimental: proliferation of the MH7A cell ↓	Cao et al. (2022)
			MAPK, PTGS2 ↓	
			CASP3↑	
Aucubin	An iridoid glycoside from *Eucommia ulmoides* Oliv.	NF-κB	Experimental: migration and invasion of human FLS ↓	Zhang et al. (2022)
			NF-κB -p65 activity of MC3T3-E1 cells ↓ p-Iκκα β, p-Iκβ, and p-p65 proteins ↓	

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TABLE 2 (*Continued*) Small molecule drugs targeting NF-κB regulating FLS.

Name	Source	Targets/signaling pathways	Estimate	References
Cantleyoside	An iridoid glycoside from *Pterocephalus hookeri* (C. B. Clarke) Hoeck	AMPK/Sirt 1/NF-κB	Experimental: proliferation of human FLS ↓	Bai et al. (2022)
			NO, TNF-α, IL-1β/6, MCP-1 and MMP-1/3/9 ↓	
			OCR, ECAR and real-time ATP generation rate p-NF-κB and translocation ↓	

↓: suppress, downregulate, inhibit, block, prevent, reduce, decrease; ↑: promote, upregulate, active, increase. HDAC, histone deacetylase; PGE (2), prostaglandin E (2); GRK2, G protein-coupled receptor kinase 2; M-CSF, macrophage colony stimulating factor; MDA, malondialdehyde; TRIB1, Tribbles pseudokinase 1; NFATc1, nuclear factor of activated T cells; NLRP3, NOD-like receptor protein 3; HO-1, heme oxygenase; SIRT1, silent information regulator 1; MCP-1, monocyte chemotactic protein-1; OPN, osteopontin; ATP, adenosine triphosphate; α7nAChR, α7 nicotinic acetylcholine receptor; mPGES-1, microsomal prostaglandin E synthase 1; AP-1, activated protein-1.

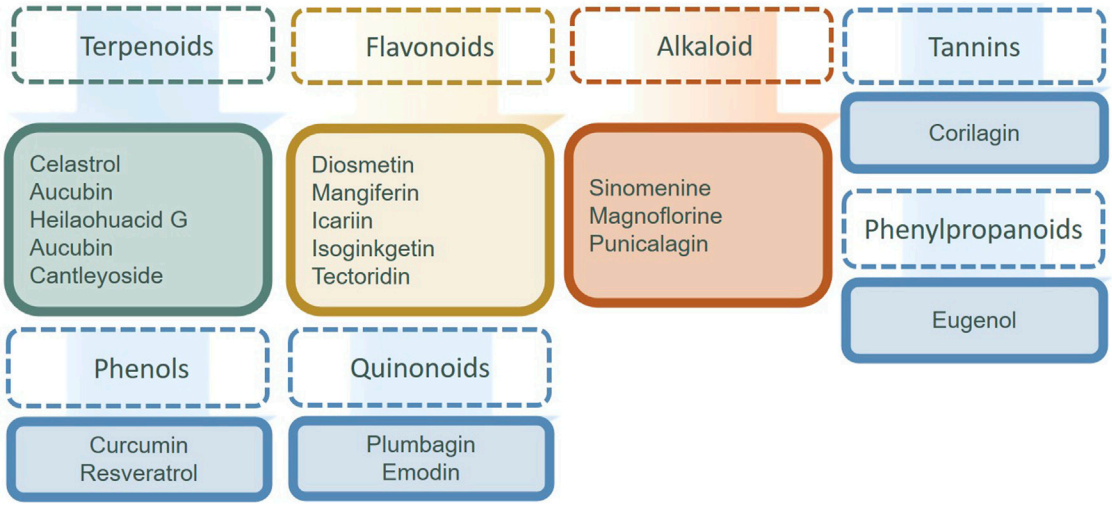

FIGURE 4
Natural products targeting NF-κB regulating FLS.

belongs to NF-κB is covered by the IκB unable to undergo nuclear translocation. However, in RA due to the activators (TNF-α, IL-17, etc.), IκB is phosphorylated, ubiquitinated by IκB kinase, and eventually degraded by the enzyme, releasing NF-κB. Following that, NF-κB p65 enters the nucleus and combines with target genes (Aupperle et al., 1999). The production of inflammatory mediators such as TNF-α, COX-2, and IL-1β increases as a result of this nuclear translocation in the synovium. Those activated sustaining states lead to massive abnormal activation of FLS (Saravanan et al., 2014). NF-κB p65 regulates apoptosis and inhibits protein expression, which has an antagonistic effect on apoptosis in FLS (Kadkhoda et al., 2016), leading to synovial hyperplasia and aggravating joint destruction (Yin et al., 2015). In addition, p38 mediates IκB phosphorylation, which is involved in regulating NF-κB activation (Carter et al., 1999; Kaminska, 2005).

The small molecule drugs and natural products targeted at NF-κB in recent 3 years are summarized in Table 2, and the classification of the natural products is in Figure 3. There have been many studies on small molecule compounds that modulate FLS in the NF-κB signaling pathway, such as TAK-242 (Samarpita et al., 2020), CKD-506 (Park et al., 2020), and synthetic derivatives from natural products that also showed the activity of inhibiting proliferation. For example, oxymatrine hydrazone synthesized from oxidized bitter ginseng induced apoptosis and prevented TNF-α-mediated enhanced viability of RA-FLS (Zhang et al., 2021). Paeoniflorin-6′-O-benzene sulfonate (CP-25), a paeoniflorin derivative, had the ability to decrease membrane expression and the combination of these proteins (Wang et al., 2020; Wang et al., 2023). Edaravone, roflumilast, sorafenib, dexmedetomidine, and alogliptin have been used clinically, without the indication for the treatment of RA. The existing experiments showed that they have the anti-proliferation ability of FLS and were worthy of inclusion in the secondary development of drugs. In the natural products in Figure 4, flavonoids still predominated, such as diosmetin, icariin, isoginkgetin, and tectoridin. In a similar situation with the MAPK inhibitions for RA-FLS, these natural products were in the experimental stage. In addition, some inhibitors modulated both NF-κB and MAPK pathways to regulate FLS activity, such as tectoridin and corilagin.

TABLE 3 Small molecule drugs targeting JAK/STAT regulating FLS.

Name	Source	Targets/signaling pathways	Estimate	References
The synthetic small molecule compounds				
Peficitinib	A JAK inhibitor	JAK1, JAK2, JAK3, and Tyk2; STAT3	Clinical: phase II and III clinical trials and extension studies completed	Emori et al. (2020); Gutierrez-Urena et al. (2020); Kitanaga et al. (2020)
			Showed efficacy, safety, and tolerability in monotherapy or csDMARDs	
			Experimental: STAT3 phosphorylation by diversified cytokine concentration-dependently ↓	
			Growth factor-A, MMPs, IL-6, TNFSF11 ↓	
Filgotinib	A selective JAK1 inhibitor	JAK1	Clinical: under clinical trial pending approval for use in RA	Shimizu et al. (2023); Westhovens (2023)
			Dose-related effect was not observed for safety excepting for herpes zoster and the increases of lipids and creatine phosphokinase	
Takinib	A selective TAK1 inhibitor	TAK1, TAK3, JNK, NF-κB	Clinical: JAK-STAT pathways in RA patients ↓	Palmroth et al. (2021); Panipinto et al. (2021); Mardani et al. (2023)
			One case of liver failure	
			Experimental: p-TAK1, no effect for the TAK1 downstream factors ↑	
Baricitinib	A JAK 1 and 2 inhibitor	STAT1, JAK	Clinical: monocyte frequency and p-STAT1 in circulating monocytes served as potential early response markers to baricitinib treatment	Tucci et al. (2022); Weston et al. (2022); Taylor et al. (2023)
			Low-risk-related AESI	
			Low incidence with the dermatologic indications	
			Experimental: OSM-induced JAK signaling ↓	
			IL-6, MCP-1, IP-10 expression in the following stages ↓	
Upadacitinib	A selective JAK 1 inhibitor	JAK 1	Clinical: combination with MTX	Panchal et al. (2023); Taldaev et al. (2021)
			Maximum adverse events were reported at 12 mg twice daily	
Tofacitinib	A JAK/STAT inhibitor	STAT6/miR-425-5p/IGF1	Clinical: treatment of RA	Di Benedetto et al. (2021); Palmroth et al. (2021); Panipinto et al. (2021); Liu et al. (2022); Vomero et al. (2022); Ruscitti et al. (2022)
			Beneficial for RA patients who don't respond to TNF-inhibitors or methotrexate	
			Modulate autophagy of FLS	
			Experimental: pro-inflammatory cytokines ↓ collagen I and α-SMA of RA-FLS ↓	
Momelotinib	A competitive JAK1/JAK2 inhibitor	IL-6/JAK1/STAT3	Clinical: no indication for treatment of RA.	Srivastava et al. (2022)
			Experimental: proliferative, migratory of FLS↓	
			PRMT, survivin, HIF-1α ↓	
			JAK1 and STAT3 by IL-6/sIL-6R activation↓	
			SOCS3 ↑	

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TABLE 3 (*Continued*) Small molecule drugs targeting JAK/STAT regulating FLS.

Name	Source	Targets/signaling pathways	Estimate	References
The natural products				
Matrine	An alkaloid from genus *Sophora*	JAK/STAT; PI3K/Akt/mTOR; TGF-β/Smad; Wnt	Experimental: Bcl-2 ↓	Yang et al. (2017); Ao et al. (2022); Lin et al. (2022)
			Bax, caspase-3↑	
			JAK2, STAT1, STAT3 phosphorylation ↓	
Vitexin	An apigenin flavone glycoside from passion flower, bamboo leaves, and pearl millet	JAK/STAT	Experimental: inflammatory enzyme markers ↓ iNOS ↓	Zhang et al. (2022)
			JAK/STAT expressions ↓	
			SOCS↑	
Isobavachalcone	A chalcone from *Psoralea corylifolia* Linn.	PI3K/Akt, JAK/STAT	Experimental: proliferation, migration, and invasion and promoted apoptosis of MH7A cells ↓ p-PI3K, p-STAT3, p-JAK1 SOCS3, p- Akt ↓	Wang et al. (2022)

↓: suppress, downregulate, inhibit, block, prevent, reduce, decrease; ↑: promote, upregulate, active, increase. csDMARDs, conventional synthetic disease-modifying anti-rheumatic drugs; TNFSF11, TNF Superfamily Member 11; AESI, adverse events of special interest; OSM, oncostatin M; α-SMA, smooth muscle alpha-actin; SOCS, suppressor of cytokine signaling; TAK, TGF β-activated kinase.

3.3 Small molecule drugs targeting JAK/STAT regulating FLS

JAK/STAT signaling has been instrumental in regulating immune and inflammatory responses. The JAK/STAT pathway can be segmented into three components: receptor-associated tyrosine kinase, JAK tyrosine kinase, and STAT transcription factor. The JAK kinase activates JAK upon receptor binding, leading to JAK-mediated phosphorylation of STAT. Among the STAT family, STAT1 and STAT3 serve as the primary activators (Kim et al., 2011). The expression and activity of STAT1 are elevated in the initial synovial tissue of RA, while STAT3 facilitates the survival of synovial fibroblasts. Elevated STAT3 expression contributes to the inhibition of programmed cell death-induced anti-apoptotic molecule expression, blocks apoptosis in RA-FLS, and promotes RA synovial thickening (Yang et al., 2017). The JAK/STAT pathway is also involved in regulating the response of RA-FLS to pro-inflammatory cytokines and plays an essential role in the pro-inflammatory response and invasive behavior of FLS (Diller et al., 2019).

Inhibitors of JAKs could block the activation of STATs in RA-LS in the synthesis of various drugs and in the study of natural products. We included the synthetic small molecule compounds and natural products in the last 3 years in Table 3. Tofacitinib is a Food and Drug Administration (FDA)- and European Medicines Agency (EMA)-approved JAK inhibitor that effectively treats RA (Vomero et al., 2022). The synthetic small molecule compounds of peficitinib, fingolitinib, takinib, tolvamycin, baricitinib, and abatinib all demonstrated monotherapy effectiveness in clinical trials in RA. The synthetic JAK inhibitors appeared to be an important treatment choice for difficult-to-treat RA patients and researchers (Kubo et al., 2023). Momelotinib had no indication for the treatment of RA in the clinic, but could inhibit the proliferation and migration of FLS (Srivastava et al., 2022). On the contrary, there are few research reports on the natural products in the JAK/STAT signal pathway.

3.4 Small molecule drugs targeting PI3k/Akt regulating FLS

The PI3K/Akt signaling pathway is involved in regulating cell growth, proliferation, differentiation, and survival and is associated with the production of pro-inflammatory cytokines, degrading enzymes of the extracellular matrix, and other factors in FLS. The activation of PI3K induces the phosphorylation of Akt and p-Akt. As a downstream effector, it can be involved in FLS invasion by regulating the transcriptional levels of MMPs. The Akt phosphorylation also activates downstream mTOR complex 1 (mTORC1). mTORC1 translates mRNA into proteins to regulate the cell activities of metabolism, growth, and differentiation and is involved in RA-FLS proliferation and survival (Wendel et al., 2004; Malemud, 2013).

Table 4 is a summary of the synthetic small molecules and natural drugs that have been developed recently that target PI3k/Akt. Metformin, a drug used to treat type 2 diabetes, has been shown to have a protective effect against the development of RA (Liang et al., 2023), and RA-FLS proliferation is inhibited by metformin in a dose- and time-dependent manner (Chen et al., 2019). The natural products targeted at PI3k/Akt regulating FLS came from a variety of sources. Against the development of inflammatory arthritis, ginger is a preventive substance. There was evidence that ginger helped reduce RA-related joint pain (Al-Nahain et al., 2014). The active ingredients of ginger, 6-shogaol, and 8-shogaol reduced the production of TNF-α, IL-1β, IL-6, etc., prevented migration, invasion, and population growth, and ameliorated joint destruction in mice (N. Li et al., 2023; Jo et al., 2022).

3.5 Wnt signaling pathway and relevant drugs regulating FLS

The Wnt signaling cascade participates in regulating the growth, differentiation, production, and apoptosis of osteoblasts. The conventional Wnt/β-catenin cascade, Wnt/Ca_2^+ signaling cascade, and Wnt/JNK signaling cascade coordinate with each other to

TABLE 4 Small molecule drugs targeting PI3k/Akt regulating FLS.

Name	Source	Targets/signaling pathways	Estimate	References
The synthetic small molecule compounds				
Metformin	The biguanide hypoglycemic agents	IGF-IR/PI3K/Akt/m-TOR	Clinical: preventing RA	Liang et al. (2023); Chen et al. (2019); Gharib et al. (2021)
			Inflammation, disease severity, and quality of life with high safety ↑	
			Experimental: G2/M cell cycle phase arrest ↓	
			mTOR phosphorylation ↓	
			Adjusting the p70s6k and 4EBP1 phosphorylation	
The natural products				
Baicalein	A flavone from *Scutellaria baicalensis*	PI3K/Akt/mTOR	Experimental: apoptotic proteins ↑	Zhang et al. (2022)
			EMT-related proteins ↓	
			Cell apoptosis ↑	
			Cell migration phosphorylation ↓	
			The phosphorylation of PI3K, Akt, and mTOR dose dependently ↓	
Nobiletin	A polymethoxylated flavonoid from citrus peels	PI3K/Akt/HIF-1α	Experimental: enhanced the performance in synovial tissue combined with MTX	Liu et al. (2022)
			P-gp expression ↓	
			Contribute to MTX resistance	
Artemisitene	A derivatives of artemisinin from *Artemisia annua* L.	METTL3/ICAM2/PI3K/Akt/p300	Experimental: progression of FLS↓	Chen et al. (2022)
			N6-methyladenosine modification of ICAM2 mRNA ↓	
Shikonin	A naphthoquinone pigment from the root of *Lithospermum erythrorhizon*	PI3K- Akt -mTOR, MAPK	Experimental: migration, adhesion, and invasion of MH7A cells↓	Lian-Hua et al. (2020); Li et al. (2021)
			The phosphorylation levels of Akt, JNK, p38, ERK ↓	
Cinnamaldehyde	An aldehyde from the bark of *Cinnamomum cassia*	PI3K/Akt	Experimental: proliferation and metastasis ↓	Li and Wang (2020)
Daphnetin	A coumarin derivative from *Daphne odora*	PI3K/Akt/mTOR	Experimental: inflammatory response ↓	Deng et al. (2020)
			Cytokine expression ↓	
			IL-10 ↑	
6-Shogaol	An alkylphenol from ginger	PI3K/AKT/NF-κB	Experimental: proliferation, migration, and invasion of FLS and MH7A cells ↓	Li et al. (2023)
			IL-1β, IL-6, IL-8↓	
			MMP-2, MMP-9 ↓	
			PPAR-γ ↑	
8-Shogaol		TAK1, Akt, MAPK	Experimental: TAK1 activity selectively ↓	Jo et al. (2022)
			IKK, Akt, MAPK ↓	
			Reversing pathologies of joint structure	

↓: suppress, downregulate, inhibit, block, prevent, reduce, decrease; ↑: promote, upregulate, active, increase. METTL3, methyltransferase-like 3.

regulate the dynamic balance between osteoclasts and osteoblasts. Once the balance is disturbed, it might lead to bone erosion and bone destruction (Walsh et al., 2009; De, 2011; Deal, 2012). Studies had shown that the growth Wnt3a/5a proteins could activate the Wnt signaling cascade as well as downstream genes, thus increasing fibronectin expression and promoting FLS function. The

aforementioned processes also promoted the proliferation of RA synovial tissue without pro-inflammatory factors (Kim et al., 2010; Rabelo Fde et al., 2010; Maeda et al., 2013). Researchers (Cici et al., 2019) suggested that the inflammatory activation of the Wnt pathway might inhibit T-cell function and exacerbate the immune response [181]. In the recent 3 years, we inquired natural products, including paeoniflorin (Yang et al., 2022), 7-hydroxycoumarin (Umbelliferone) (Cai et al., 2022; Cai et al., 2022), and penta-acetyl geniposide (Cai et al., 2021).

4 Conclusion

In this review, we summarized as much as possible the involvement of FLS, covering the RA-FLS pathogenesis, synthetic small molecular compounds, and natural products targeting primary signaling pathways in the last 3 years. Natural products comprise a range of substances derived from diverse natural sources, such as plants, animals, and microorganism. These sources provided valuable resources for the design and development of drugs. From the results, the content of this paper could be continuously extended in the following aspects. 1) For the synthetic small molecule compounds, the popular targeting signaling pathways are still MAPK and NF-κB in the current research stage. We cannot ignore that JAK/STAT has great potential for research studies, due to the fact that several drugs have appeared in the clinic. Moreover, modulation of Wnt signaling might not only repair articular bone damage but also inhibit the production of pro-inflammatory cytokines, showing a new strategy for RA treatment (Miao et al., 2013; Liu et al., 2019). Typically, these signaling pathways interacted with each other. A small molecule could act through multiple pathways. 2) For the natural products, there was great potential. Researchers have tried to explore drugs targeted to activate FLS to treat RA using traditional human experience and herbs. For example, triptolide has been a hot area of research for several years. Most of the results are currently in the experimental stage, not the clinical trial stage. Fortunately, the source plants of these natural products have been used for RA in clinical studies. 3) The natural products derived from herbal medicine that can regulate RA-FLS abnormalities are mainly alkaloids, flavonoids, saponins, phenols, and quinones (Smolen et al., 2018). 4) In addition, we have found many reports on the mechanisms of herbal extract, Chinese herbal compound prescription, and traditional Chinese patent medicines in RA that were worthy of further research.

Author contributions

YT was responsible for writing and drawing by Figdraw. XL drafted the original framework and figures. QD collected and sorted materials. JS and YF provided guidance. LB reviewed writing and drawing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Aihaiti, Y., Song Cai, Y., Tuerhong, X., Ni Yang, Y., Ma, Y., Shi Zheng, H., et al. (2021). Therapeutic effects of naringin in rheumatoid arthritis: Network pharmacology and experimental validation. *Front. Pharmacol.* 12, 672054. doi:10.3389/fphar.2021.672054

Al-Nahain, A., Jahan, R., and Rahmatullah, M. (2014). Zingiber officinale: A potential plant against rheumatoid arthritis. *Arthritis* 2014, 159089–159098. doi:10.1155/2014/159089

Almutairi, K., Nossent, J., Preen, D., Keen, H., and Inderjeeth, C. (2021). The global prevalence of rheumatoid arthritis: A meta-analysis based on a systematic review. *Rheumatol. Int.* 41 (5), 863–877. doi:10.1007/s00296-020-04731-0

An, L., Li, Z., Shi, L., Wang, L., Wang, Y., Jin, L., et al. (2020). Inflammation-targeted celastrol nanodrug attenuates collagen-induced arthritis through NF-κB and Notch1 pathways. *Nano Lett.* 20 (10), 7728–7736. doi:10.1021/acs.nanolett.0c03279

Ao, L., Gao, H., Jia, L., Liu, S., Guo, J., Liu, B., et al. (2022). Matrine inhibits synovial angiogenesis in collagen-induced arthritis rats by regulating HIF-VEGF-Ang and inhibiting the PI3K/Akt signaling pathway. *Mol. Immunol.* 141, 13–20. doi:10.1016/j.molimm.2021.11.002

Aupperle, K. R., Bennett, B. L., Boyle, D. L., Tak, P. P., Manning, A. M., and Firestein, G. S. (1999). NF-kappa B regulation by I kappa B kinase in primary fibroblast-like synoviocytes. *J. Immunol.* 163 (1), 427–433. doi:10.4049/jimmunol.163.1.427

Bai, J., Xie, N., Hou, Y., Chen, X., Hu, Y., Zhang, Y., et al. (2022). The enhanced mitochondrial dysfunction by cantleyoside confines inflammatory response and promotes apoptosis of human HFLS-RA cell line via AMPK/Sirt 1/NF-κB pathway activation. *Biomed. Pharmacother.* 149, 112847. doi:10.1016/j.biopha.2022.112847

Bartok, B., and Firestein, G. S. (2010). Fibroblast-like synoviocytes: Key effector cells in rheumatoid arthritis. *Immunol. Rev.* 233 (1), 233–255. doi:10.1111/j.0105-2896.2009.00859.x

Bombardieri, M., Kam, N. W., Brentano, F., Choi, K., Filer, A., Kyburz, D., et al. (2011). A BAFF/APRIL-dependent TLR3-stimulated pathway enhances the capacity of rheumatoid synovial fibroblasts to induce AID expression and Ig class-switching in B cells. *Ann. Rheum. Dis.* 70 (10), 1857–1865. doi:10.1136/ard.2011.150219

Bu, Y., Wu, H., Deng, R., and Wang, Y. (2022). The anti-angiogenesis mechanism of Geniposide on rheumatoid arthritis is related to the regulation of PTEN. *Inflammopharmacology* 30 (3), 1047–1062. doi:10.1007/s10787-022-00975-3

Buhrmann, C., Mobasheri, A., Matis, U., and Shakibaei, M. (2010). Curcumin mediated suppression of nuclear factor-κB promotes chondrogenic differentiation of mesenchymal stem cells in a high-density co-culture microenvironment. *Arthritis Res. Ther.* 12 (4), R127. doi:10.1186/ar3065

Bustamante, M. F., Garcia-Carbonell, R., Whisenant, K. D., and Guma, M. (2017). Fibroblast-like synoviocyte metabolism in the pathogenesis of rheumatoid arthritis. *Arthritis Res. Ther.* 19 (1), 110. doi:10.1186/s13075-017-1303-3

Cai, L., Mu, Y. R., Liu, M. M., Zhou, M. Y., Meng, B., Liu, F. Y., et al. (2021). Penta-acetyl Geniposide suppresses migration, invasion, and inflammation of TNF-α-stimulated rheumatoid arthritis fibroblast-like synoviocytes involving wnt/β-catenin signaling pathway. *Inflammation* 44 (6), 2232–2245. doi:10.1007/s10753-021-01495-y

Cai, L., Zhou, M. Y., Hu, S., Liu, F. Y., Wang, M. Q., Wang, X. H., et al. (2022). Umbelliferone inhibits migration, invasion and inflammation of rheumatoid arthritis fibroblast-like synoviocytes and relieves adjuvant-induced arthritis in rats by blockade of wnt/β-catenin signaling pathway. *Am. J. Chin. Med.* 50 (7), 1945–1962. doi:10.1142/S0192415X22500835

Cai, L., Zong, P., Zhou, M. Y., Liu, F. Y., Meng, B., Liu, M. M., et al. (2022). 7-Hydroxycoumarin mitigates the severity of collagen-induced arthritis in rats by inhibiting proliferation and inducing apoptosis of fibroblast-like synoviocytes via suppression of Wnt/β-catenin signaling pathway. *Phytomedicine* 94, 153841. doi:10.1016/j.phymed.2021.153841

Cao, C., Zeng, L., and Rong, X. (2022). Therapeutic mechanism of emodin for treatment of rheumatoid arthritis: A network pharmacology-based analysis. *Nan Fang. Yi Ke Da Xue Xue Bao* 42 (6), 913–921. doi:10.12122/j.issn.1673-4254.2022.06.16

Cao, D., Fan, Q., Li, Z., Chen, M., Jiang, Y., Lin, R., et al. (2022). Transcriptomic profiling revealed the role of apigenin-4'-O-α-L-rhamnoside in inhibiting the activation of rheumatoid arthritis fibroblast-like synoviocytes via MAPK signaling pathway. *Phytomedicine* 102, 154201. doi:10.1016/j.phymed.2022.154201

Carter, A. B., Knudtson, K. L., Monick, M. M., and Hunninghake, G. W. (1999). The p38 mitogen-activated protein kinase is required for NF-kappaB-dependent gene expression. The role of TATA-binding protein (TBP). *J. Biol. Chem.* 274 (43), 30858–30863. doi:10.1074/jbc.274.43.30858

Chen, D. P., Wong, C. K., Leung, P. C., Fung, K. P., Lau, C. B., Lau, C. P., et al. (2011). Anti-inflammatory activities of Chinese herbal medicine sinomenine and Liang Miao San on tumor necrosis factor-α-activated human fibroblast-like synoviocytes in rheumatoid arthritis. *J. Ethnopharmacol.* 137 (1), 457–468. doi:10.1016/j.jep.2011.05.048

Chen, H., Tao, L., Liang, J., Pan, C., and Wei, H. (2023). Ubiquitin D promotes the progression of rheumatoid arthritis via activation of the p38 MAPK pathway. *Mol. Med. Rep.* 27 (2), 53. doi:10.3892/mmr.2023.12940

Chen, J., Lin, X., He, J., Liu, D., He, L., Zhang, M., et al. (2022). Artemisitene suppresses rheumatoid arthritis progression via modulating METTL3-mediated N6-methyladenosine modification of ICAM2 mRNA in fibroblast-like synoviocytes. *Clin. Transl. Med.* 12 (12), e1148. doi:10.1002/ctm2.1148

Chen, K., Lin, Z. W., He, S. M., Wang, C. Q., Yang, J. C., Lu, Y., et al. (2019). Metformin inhibits the proliferation of rheumatoid arthritis fibroblast-like synoviocytes through IGF-IR/PI3K/AKT/m-TOR pathway. *Biomed. Pharmacother.* 115, 108875. doi:10.1016/j.biopha.2019.108875

Chen, X., Lin, H., Chen, J., Wu, L., Zhu, J., Ye, Y., et al. (2021). Paclitaxel inhibits synoviocyte migration and inflammatory mediator production in rheumatoid arthritis. *Front. Pharmacol.* 12, 714566. doi:10.3389/fphar.2021.714566

Chen, Y., Wang, Y., Liu, M., Zhou, B., and Yang, G. (2020). Diosmetin exhibits anti-proliferative and anti-inflammatory effects on TNF-α-stimulated human rheumatoid arthritis fibroblast-like synoviocytes through regulating the Akt and NF-κB signaling pathways. *Phytother. Res.* 34 (6), 1310–1319. doi:10.1002/ptr.6596

Cheng, Y. C., Zhang, X., Lin, S. C., Li, S., Chang, Y. K., Chen, H. H., et al. (2022). Echinocystic acid ameliorates arthritis in SKG mice by suppressing Th17 cell differentiation and human rheumatoid arthritis fibroblast-like synoviocytes inflammation. *J. Agric. Food Chem.* 70 (51), 16176–16187. doi:10.1021/acs.jafc.2c05802

Choe, J. Y., Lee, S. J., Park, S. H., and Kim, S. K. (2012). Tacrolimus (FK506) inhibits interleukin-1β-induced angiopoietin-1, Tie-2 receptor, and vascular endothelial growth factor through down-regulation of JNK and p38 pathway in human rheumatoid fibroblast-like synoviocytes. *Jt. Bone Spine* 79 (2), 137–143. doi:10.1016/j.jbspin.2011.03.018

Choi, Y., Lee, E. G., Jeong, J. H., and Yoo, W. H. (2021). 4-Phenylbutyric acid, a potent endoplasmic reticulum stress inhibitor, attenuates the severity of collagen-induced arthritis in mice via inhibition of proliferation and inflammatory responses of synovial fibroblasts. *Kaohsiung J. Med. Sci.* 37 (7), 604–615. doi:10.1002/kjm2.12376

Cici, D., Corrado, A., Rotondo, C., and Cantatore, F. P. (2019). Wnt signaling and biological therapy in rheumatoid arthritis and spondyloarthritis. *Int. J. Mol. Sci.* 20 (22), 5552. doi:10.3390/ijms20225552

Dai, Y., Sheng, J., He, S., Wu, Q., Wang, Y., and Su, L. (2022). Dehydroevodiamine suppresses inflammatory responses in adjuvant-induced arthritis rats and human fibroblast-like synoviocytes. *Bioengineered* 13 (1), 268–279. doi:10.1080/21655979.2021.1999554

Davis, J. M., 3rd, and Matteson, E. L.American College of Rheumatology; European League Against Rheumatism (2012). My treatment approach to rheumatoid arthritis. *Mayo Clin. Proc.* 87 (7), 659–673. doi:10.1016/j.mayocp.2012.03.011

De, A. (2011). Wnt/Ca2+ signaling pathway: A brief overview. *Acta Biochim. Biophys. Sin. (Shanghai)* 43 (10), 745–756. doi:10.1093/abbs/gmr079

Deal, C. (2012). Bone loss in rheumatoid arthritis: Systemic, periarticular, and focal. *Curr. Rheumatol. Rep.* 14 (3), 231–237. doi:10.1007/s11926-012-0253-7

Deng, H., Zheng, M., Hu, Z., Zeng, X., Kuang, N., and Fu, Y. (2020). Effects of daphnetin on the autophagy signaling pathway of fibroblast-like synoviocytes in rats with collagen-induced arthritis (CIA) induced by TNF-α. *Cytokine* 127, 154952. doi:10.1016/j.cyto.2019.154952

Di Benedetto, P., Ruscitti, P., Berardicurti, O., Panzera, N., Grazia, N., Di Vito Nolfi, M., et al. (2021). Blocking Jak/STAT signalling using tofacitinib inhibits angiogenesis in experimental arthritis. *Arthritis Res. Ther.* 23 (1), 213. doi:10.1186/s13075-021-02587-8

Diller, M., Hasseli, R., Hülser, M. L., Aykara, I., Frommer, K., Rehart, S., et al. (2019). Targeting activated synovial fibroblasts in rheumatoid arthritis by peficitinib. *Front. Immunol.* 10, 541. doi:10.3389/fimmu.2019.00541

Ding, Q., Hu, W., Wang, R., Yang, Q., Zhu, M., Li, M., et al. (2023). Signaling pathways in rheumatoid arthritis: Implications for targeted therapy. *Signal Transduct. Target Ther.* 8 (1), 68. doi:10.1038/s41392-023-01331-9

Doss, H. M., Ganesan, R., and Rasool, M. (2016). Trikatu, an herbal compound ameliorates rheumatoid arthritis by the suppression of inflammatory immune responses in rats with adjuvant-induced arthritis and on cultured fibroblast like synoviocytes via the inhibition of the NFκB signaling pathway. *Chem. Biol. Interact.* 258, 175–186. doi:10.1016/j.cbi.2016.09.003

Du, H., Wang, Y., Zeng, Y., Huang, X., Liu, D., Ye, L., et al. (2020). Tanshinone IIA suppresses proliferation and inflammatory cytokine production of synovial fibroblasts from rheumatoid arthritis patients induced by TNF-α and attenuates the inflammatory response in AIA mice. *Front. Pharmacol.* 11, 568. doi:10.3389/fphar.2020.00568

Du, H., Zhang, X., Zeng, Y., Huang, X., Chen, H., Wang, S., et al. (2019). A novel phytochemical, DIM, inhibits proliferation, migration, invasion and TNF-α induced inflammatory cytokine production of synovial fibroblasts from rheumatoid arthritis patients by targeting MAPK and AKT/mTOR signal pathway. *Front. Immunol.* 10, 1620. doi:10.3389/fimmu.2019.01620

Emori, T., Kasahara, M., Sugahara, S., Hashimoto, M., Ito, H., Narumiya, S., et al. (2020). Role of JAK-STAT signaling in the pathogenic behavior of fibroblast-like synoviocytes in rheumatoid arthritis: Effect of the novel JAK inhibitor peficitinib. *Eur. J. Pharmacol.* 882, 173238. doi:10.1016/j.ejphar.2020.173238

Fang, Z., He, D., Yu, B., Liu, F., Zuo, J., Li, Y., et al. (2017). High-throughput study of the effects of celastrol on activated fibroblast-like synoviocytes from patients with rheumatoid arthritis. *Genes (Basel)* 8 (9), 221. doi:10.3390/genes8090221

Feng, Y., Mei, L., Wang, M., Huang, Q., and Huang, R. (2021). Anti-inflammatory and pro-apoptotic effects of 18beta-glycyrrhetinic acid *in vitro* and *in vivo* models of rheumatoid arthritis. *Front. Pharmacol.* 12, 681525. doi:10.3389/fphar.2021.681525

Firestein, G. S. (2003). Evolving concepts of rheumatoid arthritis. *Nature* 423 (6937), 356–361. doi:10.1038/nature01661

Gan, K., Xu, L., Feng, X., Zhang, Q., Wang, F., Zhang, M., et al. (2015). Celastrol attenuates bone erosion in collagen-Induced arthritis mice and inhibits osteoclast differentiation and function in RANKL-induced RAW264.7. *Int. Immunopharmacol.* 24 (2), 239–246. doi:10.1016/j.intimp.2014.12.012

Gao, X., Kang, X., Lu, H., Xue, E., Chen, R., Pan, J., et al. (2022). Piceatannol suppresses inflammation and promotes apoptosis in rheumatoid arthritis-fibroblast-like synoviocytes by inhibiting the NF-κB and MAPK signaling pathways. *Mol. Med. Rep.* 25 (5), 180. doi:10.3892/mmr.2022.12696

Gharib, M., Elbaz, W., Darweesh, E., Sabri, N. A., and Shawki, M. A. (2021). Efficacy and safety of Metformin use in rheumatoid arthritis: A randomized controlled study. *Front. Pharmacol.* 12, 726490. doi:10.3389/fphar.2021.726490

Guma, M., Hammaker, D., Topolewski, K., Corr, M., Boyle, D. L., Karin, M., et al. (2012). Antiinflammatory functions of p38 in mouse models of rheumatoid arthritis: Advantages of targeting upstream kinases MKK-3 or MKK-6. *Arthritis Rheum.* 64 (9), 2887–2895. doi:10.1002/art.34489

Guo, Q., Zhang, S., Huang, J., and Liu, K. (2020). Alogliptin inhibits IL-1β-induced inflammatory response in fibroblast-like synoviocytes. *Int. Immunopharmacol.* 83, 106372. doi:10.1016/j.intimp.2020.106372

Gutierrez-Urena, S. R., Amaya-Cabrera, E. L., Uribe-Martinez, J. F., Ventura-Valenzuela, M. E., Rosal-Arteaga, C., Martinez-Bonilla, G. E., et al. (2020). Peficitinib hydrobromide to treat rheumatoid arthritis. *Drugs Today (Barc)* 56 (8), 505–514. doi:10.1358/dot.2020.56.8.3123469

Hammaker, D., Topolewski, K., Edgar, M., Yoshizawa, T., Fukushima, A., Boyle, D. L., et al. (2012). Decreased collagen-induced arthritis severity and adaptive immunity in MKK-6-deficient mice. *Arthritis Rheum.* 64 (3), 678–687. doi:10.1002/art.33359

Harigai, M., Hara, M., Kawamoto, M., Kawaguchi, Y., Sugiura, T., Tanaka, M., et al. (2004). Amplification of the synovial inflammatory response through activation of mitogen-activated protein kinases and nuclear factor kappaB using ligation of CD40 on CD14+ synovial cells from patients with rheumatoid arthritis. *Arthritis Rheum.* 50 (7), 2167–2177. doi:10.1002/art.20340

Hu, X., Tang, J., Zeng, G., Hu, X., Bao, P., Wu, J., et al. (2019). RGS1 silencing inhibits the inflammatory response and angiogenesis in rheumatoid arthritis rats through the

inactivation of Toll-like receptor signaling pathway. *J. Cell Physiol.* 234 (11), 20432–20442. doi:10.1002/jcp.28645

Huang, M., Wu, K., Zeng, S., Liu, W., Cui, T., Chen, Z., et al. (2021). Punicalagin inhibited inflammation and migration of fibroblast-like synoviocytes through NF-κB pathway in the experimental study of rheumatoid arthritis. *J. Inflamm. Res.* 14, 1901–1913. doi:10.2147/JIR.S302929

Huang, Q., Xiao, X., Yu, J., Yang, Y., Yu, J., Liu, Y., et al. (2022). Tectoridin exhibits anti-rheumatoid arthritis activity through the inhibition of the inflammatory response and the MAPK pathway *in vivo* and *in vitro*. *Arch. Biochem. Biophys.* 727, 109328. doi:10.1016/j.abb.2022.109328

Jay, G. D., Britt, D. E., and Cha, C. J. (2000). Lubricin is a product of megakaryocyte stimulating factor gene expression by human synovial fibroblasts. *J. Rheumatol.* 27 (3), 594–600.

Ji, W., and Xu, W. (2022). Orientin inhibits the progression of fibroblast-like synovial cells in rheumatoid arthritis by regulating MAPK-signaling pathway. *Allergol. Immunopathol. Madr.* 50 (6), 154–162. doi:10.15586/aei.v50i6.742

Ji, Y. R., Chen, Y., Chen, Y. N., Qiu, G. L., Wen, J. G., Zheng, Y., et al. (2020). Dexmedetomidine inhibits the invasion, migration, and inflammation of rheumatoid arthritis fibroblast-like synoviocytes by reducing the expression of NLRC5. *Int. Immunopharmacol.* 82, 106374. doi:10.1016/j.intimp.2020.106374

Jia, N., Ma, H., Zhang, T., Wang, L., Cui, J., Zha, Y., et al. (2022). Gentiopicroside attenuates collagen-induced arthritis in mice via modulating the CD147/p38/NF-κB pathway. *Int. Immunopharmacol.* 108, 108854. doi:10.1016/j.intimp.2022.108854

Jo, S., Samarpita, S., Lee, J. S., Lee, Y. J., Son, J. E., Jeong, M., et al. (2022). 8-Shogaol inhibits rheumatoid arthritis through targeting TAK1. *Pharmacol. Res.* 178, 106176. doi:10.1016/j.phrs.2022.106176

Kadkhoda, Z., Amirzargar, A., Esmaili, Z., Vojdanian, M., and Akbari, S. (2016). Effect of TNF-α blockade in gingival crevicular fluid on periodontal condition of patients with rheumatoid arthritis. *Iran. J. Immunol.* 13 (3), 197–203.

Kaminska, B. (2005). MAPK signalling pathways as molecular targets for anti-inflammatory therapy--from molecular mechanisms to therapeutic benefits. *Biochim. Biophys. Acta* 1754 (1-2), 253–262. doi:10.1016/j.bbapap.2005.08.017

Kaneko, Y., Kawahito, Y., Kojima, M., Nakayama, T., Hirata, S., Kishimoto, M., et al. (2021). Efficacy and safety of tacrolimus in patients with rheumatoid arthritis - a systematic review and meta-analysis. *Mod. Rheumatol.* 31 (1), 61–69. doi:10.1080/14397595.2020.1719607

Kim, J., Kim, J., Kim, D. W., Ha, Y., Ihm, M. H., Kim, H., et al. (2010). Wnt5a induces endothelial inflammation via beta-catenin-independent signaling. *J. Immunol.* 185 (2), 1274–1282. doi:10.4049/jimmunol.1000181

Kim, M., Sur, B., Villa, T., Nah, S. Y., and Oh, S. (2021). Inhibitory activity of gintonin on inflammation in human IL-1β-stimulated fibroblast-like synoviocytes and collagen-induced arthritis in mice. *J. Ginseng Res.* 45 (4), 510–518. doi:10.1016/j.jgr.2020.12.001

Kim, M., Sur, B., Villa, T., Yun, J., Nah, S. Y., and Oh, S. (2021). Gintonin regulates inflammation in human IL-1β-stimulated fibroblast-like synoviocytes and carrageenan/kaolin-induced arthritis in rats through LPAR2. *J. Ginseng Res.* 45 (5), 575–582. doi:10.1016/j.jgr.2021.02.001

Kim, S. K., Park, K. Y., Yoon, W. C., Park, S. H., Park, K. K., Yoo, D. H., et al. (2011). Melittin enhances apoptosis through suppression of IL-6/sIL-6R complex-induced NF-κB and STAT3 activation and Bcl-2 expression for human fibroblast-like synoviocytes in rheumatoid arthritis. *Jt. Bone Spine* 78 (5), 471–477. doi:10.1016/j.jbspin.2011.01.004

Kitanaga, Y., Imamura, E., Nakahara, Y., Fukahori, H., Fujii, Y., Kubo, S., et al. (2020). *In vitro* pharmacological effects of peficitinib on lymphocyte activation: A potential treatment for systemic sclerosis with JAK inhibitors. *Rheumatol. Oxf.* 59 (8), 1957–1968. doi:10.1093/rheumatology/kez526

Kubo, S., Nakayamada, S., and Tanaka, Y. (2023). JAK inhibitors for rheumatoid arthritis. *Expert Opin. Investig. Drugs* 32 (4), 333–344. doi:10.1080/13543784.2023.2199919

Kwon, Y. J., Yoon, C. H., Lee, S. W., Park, Y. B., Lee, S. K., and Park, M. C. (2014). Inhibition of glycogen synthase kinase-3β suppresses inflammatory responses in rheumatoid arthritis fibroblast-like synoviocytes and collagen-induced arthritis. *Jt. Bone Spine* 81 (3), 240–246. doi:10.1016/j.jbspin.2013.09.006

Lampropoulos, C. E., Orfanos, P., Bournia, V. K., Karatsourakis, T., Mavragani, C., Pikazis, D., et al. (2015). Adverse events and infections in patients with rheumatoid arthritis treated with conventional drugs or biologic agents: A real world study. *Clin. Exp. Rheumatol.* 33 (2), 216–224.

Leah, E. (2011). Crosstalk in RA synovia-TLR3-BAFF axis sustains B-cell activation. *Nat. Rev. Rheumatol.* 7 (10), 559. doi:10.1038/nrrheum.2011.122

Li, F., Dai, M., Wu, H., Deng, R., Fu, J., Zhang, Z., et al. (2018). Immunosuppressive effect of Geniposide on mitogen-activated protein kinase signalling pathway and their cross-talk in fibroblast-like synoviocytes of adjuvant arthritis rats. *Molecules* 23 (1), 91. doi:10.3390/molecules23010091

Li, J., Pang, J., Liu, Z., Ge, X., Zhen, Y., Jiang, C. C., et al. (2021). Shikonin induces programmed death of fibroblast synovial cells in rheumatoid arthritis by inhibiting energy pathways. *Sci. Rep.* 11 (1), 18263. doi:10.1038/s41598-021-97713-6

Li, N., Li, X., Deng, L., Yang, H., Gong, Z., Wang, Q., et al. (2023). 6-Shogaol inhibits the proliferation, apoptosis, and migration of rheumatoid arthritis fibroblast-like synoviocytes via the PI3K/AKT/NF-κB pathway. *Phytomedicine* 109, 154562. doi:10.1016/j.phymed.2022.154562

Li, X., He, L., Hu, Y., Duan, H., Li, X., Tan, S., et al. (2013). Sinomenine suppresses osteoclast formation and *Mycobacterium tuberculosis* H37Ra-induced bone loss by modulating RANKL signaling pathways. *PLoS One* 8 (9), e74274. doi:10.1371/journal.pone.0074274

Li, X., and Wang, Y. (2020). Cinnamaldehyde attenuates the progression of rheumatoid arthritis through down-regulation of PI3K/AKT signaling pathway. *Inflammation* 43 (5), 1729–1741. doi:10.1007/s10753-020-01246-5

Li, Z., Chen, M., Wang, Z., Fan, Q., Lin, Z., Tao, X., et al. (2023). Berberine inhibits RA-FLS cell proliferation and adhesion by regulating RAS/MAPK/FOXO/HIF-1 signal pathway in the treatment of rheumatoid arthritis. *Bone Jt. Res.* 12 (2), 91–102. doi:10.1302/2046-3758.122.BJR-2022-0269.R1

Lian-Hua, H. E., Qian-Qian, W., Cong-Cong, S., Na, L., and Chun-Fang, L. (2020). Effect of shikonin on function of rheumatoid arthritis fibroblast like synoviocytes. *Zhongguo Zhong Yao Za Zhi* 45 (19), 4712–4718. doi:10.19540/j.cnki.cjcmm.20200506.401

Liang, J., Cai, Y., Zhang, J., Jing, Z., Lv, L., Zhang, G., et al. (2023). Metformin treatment reduces the incidence of rheumatoid arthritis: A two-sample mendelian randomized study. *J. Clin. Med.* 12 (7), 2461. doi:10.3390/jcm12072461

Lin, W., Chen, G., Mao, Y., Ma, X., Zhou, J., Yu, X., et al. (2022). Imperatorin inhibits proliferation, migration, and inflammation *via* blocking the NF-κB and MAPK pathways in rheumatoid fibroblast-like synoviocytes. *ACS Omega* 7 (34), 29868–29876. doi:10.1021/acsomega.2c02766

Lin, X., Chen, J., Tao, C., Luo, L., He, J., and Wang, Q. (2023). Osthole regulates N6-methyladenosine-modified TGM2 to inhibit the progression of rheumatoid arthritis and associated interstitial lung disease. *MedComm* 4(2), e219. doi:10.1002/mco2.219

Lin, Y., He, F., Wu, L., Xu, Y., and Du, Q. (2022). Matrine exerts pharmacological effects through multiple signaling pathways: A comprehensive review. *Drug Des. Devel Ther.* 16, 533–569. doi:10.2147/DDDT.S349678

Liu, J., Zhao, N., Su, S. H., Gao, Y., and Qi, B. (2023). Anti-arthritic effect of edaravone against complete freund adjuvant induced arthritis via osteoclast differentiation and HIF-1α-VEGF-ANG-1 Axis. *Drug Des. Devel Ther.* 17, 519–534. doi:10.2147/DDDT.S391606

Liu, R., Song, Y., Li, C., Zhang, Z., Xue, Z., Huang, Q., et al. (2022). The naturally occurring flavonoid nobiletin reverses methotrexate resistance via inhibition of P-glycoprotein synthesis. *J. Biol. Chem.* 298 (4), 101756. doi:10.1016/j.jbc.2022.101756

Liu, T., Zhang, L., Joo, D., and Sun, S. C. (2017). NF-κB signaling in inflammation. *Signal Transduct. Target Ther.* 2, 17023. doi:10.1038/sigtrans.2017.23

Liu, X. G., Zhang, Y., Ju, W. F., Li, C. Y., and Mu, Y. C. (2019). MiR-21 relieves rheumatoid arthritis in rats via targeting Wnt signaling pathway. *Eur. Rev. Med. Pharmacol. Sci.* 23 (3), 96–103. doi:10.26355/eurrev_201908_18635

Liu, Y., Peng, J., Xiong, X., Cheng, L., and Cheng, X. (2022). Tofacitinib enhances IGF1 via inhibiting STAT6 transcriptionally activated-miR-425-5p to ameliorate inflammation in RA-FLS. *Mol. Cell Biochem.* 477 (10), 2335–2344. doi:10.1007/s11010-022-04444-x

Luczkiewicz, P., Kokotkiewicz, A., Dampc, A., and Luczkiewicz, M. (2014). Mangiferin: A promising therapeutic agent for rheumatoid arthritis treatment. *Med. Hypotheses* 83 (5), 570–574. doi:10.1016/j.mehy.2014.08.021

Lv, M., Liang, Q., Luo, Z., Han, B., Ni, T., Wang, Y., et al. (2022). UPLC-LTQ-Orbitrap-Based cell metabolomics and network pharmacology analysis to reveal the potential antiarthritic effects of pristimerin: *In vitro*, in silico and *in vivo* study. *Metabolites* 12 (9), 839. doi:10.3390/metabo12090839

Lv, Q., Zhu, X. Y., Xia, Y. F., Dai, Y., and Wei, Z. F. (2015). Tetrandrine inhibits migration and invasion of rheumatoid arthritis fibroblast-like synoviocytes through down-regulating the expressions of Rac1, Cdc42, and RhoA GTPases and activation of the PI3K/Akt and JNK signaling pathways. *Chin. J. Nat. Med.* 13 (11), 831–841. doi:10.1016/S1875-5364(15)30087-X

Maeda, K., Takahashi, N., and Kobayashi, Y. (2013). Roles of Wnt signals in bone resorption during physiological and pathological states. *J. Mol. Med. Berl.* 91 (1), 15–23. doi:10.1007/s00109-012-0974-0

Mahmoud, D. E., Kaabachi, W., Sassi, N., Tarhouni, L., Rekik, S., Jemmali, S., et al. (2022). The synovial fluid fibroblast-like synoviocyte: A long-neglected piece in the puzzle of rheumatoid arthritis pathogenesis. *Front. Immunol.* 13, 942417. doi:10.3389/fimmu.2022.942417

Malemud, C. J. (2013). Intracellular signaling pathways in rheumatoid arthritis. *J. Clin. Cell Immunol.* 4, 160. doi:10.4172/2155-9899.1000160

Mardani, M., Mohammadshahi, J., Abolghasemi, S., and Teimourpour, R. (2023). Drug-induced liver injury due to tofacitinib: A case report. *J. Med. Case Rep.* 17 (1), 97. doi:10.1186/s13256-023-03821-4

Mavers, M., Ruderman, E. M., and Perlman, H. (2009). Intracellular signal pathways: Potential for therapies. *Curr. Rheumatol. Rep.* 11 (5), 378–385. doi:10.1007/s11926-009-0054-9

Meng, M., Yue, Z., Chang, L., Liu, Y., Hu, J., Song, Z., et al. (2021). Anti-rheumatoid arthritic effects of Paris saponin VII in human rheumatoid arthritis fibroblast-like synoviocytes and adjuvant-induced arthritis in rats. *Front. Pharmacol.* 12, 683698. doi:10.3389/fphar.2021.683698

Meng, Y., Ji, J., Xiao, X., Li, M., Niu, S., He, Y., et al. (2021). Ononin induces cell apoptosis and reduces inflammation in rheumatoid arthritis fibroblast-like synoviocytes by alleviating MAPK and NF-κB signaling pathways. *Acta Biochim. Pol.* 68 (2), 239–245. doi:10.18388/abp.2020_5528

Miao, C. G., Yang, Y. Y., He, X., Li, X. F., Huang, C., Huang, Y., et al. (2013). Wnt signaling pathway in rheumatoid arthritis, with special emphasis on the different roles in synovial inflammation and bone remodeling. *Cell Signal* 25 (10), 2069–2078. doi:10.1016/j.cellsig.2013.04.002

Min, H. K., Kim, S. H., Won, J. Y., Kim, K. W., Lee, J. Y., Lee, S. H., et al. (2023). Dasatinib, a selective tyrosine kinase inhibitor, prevents joint destruction in rheumatoid arthritis animal model. *Int. J. Rheum. Dis.* 26 (4), 718–726. doi:10.1111/1756-185X.14627

Mohammadian Haftcheshmeh, S., Khosrojerdi, A., Aliabadi, A., Lotfi, S., Mohammadi, A., and Momtazi-Borojeni, A. A. (2021). Immunomodulatory effects of curcumin in rheumatoid arthritis: Evidence from molecular mechanisms to clinical outcomes. *Rev. Physiol. Biochem. Pharmacol.* 179, 1–29. doi:10.1007/112_2020_54

Mori, M., Hashimoto, M., Matsuo, T., Fujii, T., Furu, M., Ito, H., et al. (2017). Cell-contact-dependent activation of CD4(+) T cells by adhesion molecules on synovial fibroblasts. *Mod. Rheumatol.* 27 (3), 448–456. doi:10.1080/14397595.2016.1220353

Müller-Ladner, U., Ospelt, C., Gay, S., Distler, O., and Pap, T. (2007). Cells of the synovium in rheumatoid arthritis. Synovial fibroblasts. *Arthritis Res. Ther.* 9 (6), 223. doi:10.1186/ar2337

Neumann, E., Lefèvre, S., Zimmermann, B., Gay, S., and Müller-Ladner, U. (2010). Rheumatoid arthritis progression mediated by activated synovial fibroblasts. *Trends Mol. Med.* 16 (10), 458–468. doi:10.1016/j.molmed.2010.07.004

Niu, X., Song, H., Xiao, X., Yang, Y., Huang, Q., Yu, J., et al. (2022). Tectoridin ameliorates proliferation and inflammation in TNF-α-induced HFLS-RA cells via suppressing the TLR4/NLRP3/NF-κB signaling pathway. *Tissue Cell* 77, 101826. doi:10.1016/j.tice.2022.101826

Nygaard, G., and Firestein, G. S. (2020). Restoring synovial homeostasis in rheumatoid arthritis by targeting fibroblast-like synoviocytes. *Nat. Rev. Rheumatol.* 16 (6), 316–333. doi:10.1038/s41584-020-0413-5

O'Shea, J. J., Laurence, A., and McInnes, I. B. (2013). Back to the future: Oral targeted therapy for RA and other autoimmune diseases. *Nat. Rev. Rheumatol.* 9 (3), 173–182. doi:10.1038/nrrheum.2013.7

Palmroth, M., Kuuliala, K., Peltomaa, R., Virtanen, A., Kuuliala, A., Kurttila, A., et al. (2021). Tofacitinib suppresses several JAK-STAT pathways in rheumatoid arthritis *in vivo* and baseline signaling profile associates with treatment response. *Front. Immunol.* 12, 738481. doi:10.3389/fimmu.2021.738481

Pan, D., Li, N., Liu, Y., Xu, Q., Liu, Q., You, Y., et al. (2018). Kaempferol inhibits the migration and invasion of rheumatoid arthritis fibroblast-like synoviocytes by blocking activation of the MAPK pathway. *Int. Immunopharmacol.* 55, 174–182. doi:10.1016/j.intimp.2017.12.011

Pan, F., Zhu, L., Lv, H., and Pei, C. (2016). Quercetin promotes the apoptosis of fibroblast-like synoviocytes in rheumatoid arthritis by upregulating lncRNA MALAT1. *Int. J. Mol. Med.* 38 (5), 1507–1514. doi:10.3892/ijmm.2016.2755

Panchal, V., Vyas, B. H., Sivasubramanian, B. P., Panchal, K., and Patel, H. (2023). A meta-analysis evaluating the effectiveness and safety of upadacitinib in treating rheumatoid arthritis in patients with inadequate response to disease-modifying anti-rheumatic drugs. *Cureus* 15 (1), e34384. doi:10.7759/cureus.34384

Panipinto, P. M., Singh, A. K., Shaikh, F. S., Siegel, R. J., Chourasia, M., and Ahmed, S. (2021). Takinib inhibits inflammation in human rheumatoid arthritis synovial fibroblasts by targeting the janus kinase-signal transducer and activator of transcription 3 (JAK/STAT3) pathway. *Int. J. Mol. Sci.* 22 (22), 12580. doi:10.3390/ijms222212580

Park, J. K., Jang, Y. J., Oh, B. R., Shin, J., Bae, D., Ha, N., et al. (2020). Therapeutic potential of CKD-506, a novel selective histone deacetylase 6 inhibitor, in a murine model of rheumatoid arthritis. *Arthritis Res. Ther.* 22 (1), 176. doi:10.1186/s13075-020-02258-0

Qin, Y., Cai, M. L., Jin, H. Z., Huang, W., Zhu, C., Bozec, A., et al. (2022). Age-associated B cells contribute to the pathogenesis of rheumatoid arthritis by inducing activation of fibroblast-like synoviocytes via TNF-α-mediated ERK1/2 and JAK-STAT1 pathways. *Ann. Rheum. Dis.* 81 (11), 1504–1514. doi:10.1136/ard-2022-222605

Rabelo Fde, S., da Mota, L. M., Lima, R. A., Lima, F. A., Barra, G. B., de Carvalho, J. F., et al. (2010). The Wnt signaling pathway and rheumatoid arthritis. *Autoimmun. Rev.* 9 (4), 207–210. doi:10.1016/j.autrev.2009.08.003

Ruscitti, P., Liakouli, V., Panzera, N., Angelucci, A., Berardicurti, O., Di Nino, E., et al. (2022). Tofacitinib may inhibit myofibroblast differentiation from rheumatoid-fibroblast-like synoviocytes induced by TGF-beta and IL-6. *Pharm. (Basel)* 15 (5), 622. doi:10.3390/ph15050622

Samarpita, S., Ganesan, R., and Rasool, M. (2020). Cyanidin prevents the hyperproliferative potential of fibroblast-like synoviocytes and disease progression via targeting IL-17A cytokine signalling in rheumatoid arthritis. *Toxicol. Appl. Pharmacol.* 391, 114917. doi:10.1016/j.taap.2020.114917

Samarpita, S., Kim, J. Y., Rasool, M. K., and Kim, K. S. (2020). Investigation of toll-like receptor (TLR) 4 inhibitor TAK-242 as a new potential anti-rheumatoid arthritis drug. *Arthritis Res. Ther.* 22 (1), 16. doi:10.1186/s13075-020-2097-2

Samarpita, S., and Rasool, M. (2021). Cyanidin attenuates IL-17A cytokine signaling mediated monocyte migration and differentiation into mature osteoclasts in rheumatoid arthritis. *Cytokine* 142, 155502. doi:10.1016/j.cyto.2021.155502

Saravanan, S., Islam, V. I., Babu, N. P., Pandikumar, P., Thirugnanasambantham, K., Chellappandian, M., et al. (2014). Swertiamarin attenuates inflammation mediators via modulating NF-κB/I κB and JAK2/STAT3 transcription factors in adjuvant induced arthritis. *Eur. J. Pharm. Sci.* 56, 70–86. doi:10.1016/j.ejps.2014.02.005

Shang, W., Zhao, L. J., Dong, X. L., Zhao, Z. M., Li, J., Zhang, B. B., et al. (2016). Curcumin inhibits osteoclastogenic potential in PBMCs from rheumatoid arthritis patients via the suppression of MAPK/RANK/c-Fos/NFATc1 signaling pathways. *Mol. Med. Rep.* 14 (4), 3620–3626. doi:10.3892/mmr.2016.5674

Shao, N., Feng, Z., and Li, N. (2022). Isoginkgetin inhibits inflammatory response in the fibroblast-like synoviocytes of rheumatoid arthritis by suppressing matrix metallopeptidase 9 expression. *Chem. Biol. Drug Des.* 99 (6), 923–929. doi:10.1111/cbdd.14049

Shen, P., Jiao, Y., Miao, L., Chen, J. H., and Momtazi-Borojeni, A. A. (2020). Immunomodulatory effects of berberine on the inflamed joint reveal new therapeutic targets for rheumatoid arthritis management. *J. Cell Mol. Med.* 24 (21), 12234–12245. doi:10.1111/jcmm.15803

Shen, Y., Fan, X., Qu, Y., Tang, M., Huang, Y., Peng, Y., et al. (2022). Magnoflorine attenuates inflammatory responses in RA by regulating the PI3K/Akt/NF-κB and Keap1-Nrf2/HO-1 signalling pathways *in vivo* and *in vitro*. *Phytomedicine* 104, 154339. doi:10.1016/j.phymed.2022.154339

Shen, Y., Teng, L., Qu, Y., Liu, J., Zhu, X., Chen, S., et al. (2022). Anti-proliferation and anti-inflammation effects of corilagin in rheumatoid arthritis by downregulating NF-κB and MAPK signaling pathways. *J. Ethnopharmacol.* 284, 114791. doi:10.1016/j.jep.2021.114791

Sheng, S., Wang, X., Liu, X., Hu, X., Shao, Y., Wang, G., et al. (2022). The role of resveratrol on rheumatoid arthritis: From bench to bedside. *Front. Pharmacol.* 13, 829677. doi:10.3389/fphar.2022.829677

Shimizu, T., Kawashiri, S. Y., Morimoto, S., Kawazoe, Y., Kuroda, S., Kawasaki, R., et al. (2023). Efficacy and safety of selective JAK 1 inhibitor filgotinib in active rheumatoid arthritis patients with inadequate response to methotrexate: Comparative study with filgotinib and tocilizumab examined by clinical index as well as musculoskeletal ultrasound assessment (TRANSFORM study): Study protocol for a randomized, open-label, parallel-group, multicenter, and non-inferiority clinical trial. *Trials* 24 (1), 161. doi:10.1186/s13063-023-07176-5

Shu, C., Chen, J., Lv, M., Xi, Y., Zheng, J., and Xu, X. (2022). Plumbagin relieves rheumatoid arthritis through nuclear factor kappa-B (NF-κB) pathway. *Bioengineered* 13 (5), 13632–13642. doi:10.1080/21655979.2022.2081756

Smolen, J. S., Aletaha, D., Barton, A., Burmester, G. R., Emery, P., Firestein, G. S., et al. (2018). Rheumatoid arthritis. *Nat. Rev. Dis. Prim.* 4, 18001. doi:10.1038/nrdp.2018.1

Song, X., Zhang, Y., Dai, E., Wang, L., and Du, H. (2020). Prediction of triptolide targets in rheumatoid arthritis using network pharmacology and molecular docking. *Int. Immunopharmacol.* 80, 106179. doi:10.1016/j.intimp.2019.106179

Srivastava, S., Samarpita, S., Ganesan, R., and Rasool, M. (2022). CYT387 inhibits the hyperproliferative potential of fibroblast-like synoviocytes via modulation of IL-6/JAK1/STAT3 signaling in rheumatoid arthritis. *Immunol. Invest.* 51 (6), 1582–1597. doi:10.1080/08820139.2021.1994589

Sugiura, T., Kamino, H., Nariai, Y., Murakawa, Y., Kondo, M., Kawakami, M., et al. (2020). Screening of a panel of low molecular weight compounds that inhibit synovial fibroblast invasion in rheumatoid arthritis. *J. Immunol.* 205 (12), 3277–3290. doi:10.4049/jimmunol.1901429

Sujitha, S., Dinesh, P., and Rasool, M. (2020). Berberine encapsulated PEG-coated liposomes attenuate Wnt1/β-catenin signaling in rheumatoid arthritis via miR-23a activation. *Eur. J. Pharm. Biopharm.* 149, 170–191. doi:10.1016/j.ejpb.2020.02.007

Sun, Y., and Li, L. (2018). Cyanidin-3-glucoside inhibits inflammatory activities in human fibroblast-like synoviocytes and in mice with collagen-induced arthritis. *Clin. Exp. Pharmacol. Physiol.* 45 (10), 1038–1045. doi:10.1111/1440-1681.12970

Sur, B., Kim, M., Villa, T., and Oh, S. (2020). Benzylideneacetophenone derivative alleviates arthritic symptoms via modulation of the MAPK signaling pathway. *Molecules* 25 (15), 3319. doi:10.3390/molecules25153319

Taldaev, A., Rudnev, V. R., Nikolsky, K. S., Kulikova, L. I., and Kaysheva, A. L. (2021). Molecular modeling insights into upadacitinib selectivity upon binding to JAK protein family. *Pharm. (Basel)* 15 (1), 30. doi:10.3390/ph15010030

Tang, M., Zhu, W. J., Yang, Z. C., and He, C. S. (2019). Brucine inhibits TNF-α-induced HFLS-RA cell proliferation by activating the JNK signaling pathway. *Exp. Ther. Med.* 18 (1), 735–740. doi:10.3892/etm.2019.7582

Tang, Y., Liu, Q., Feng, Y., Zhang, Y., Xu, Z., Wen, C., et al. (2020). Tripterygium ingredients for pathogenicity cells in rheumatoid arthritis. *Front. Pharmacol.* 11, 583171. doi:10.3389/fphar.2020.583171

Taylor, P. C., Bieber, T., Alten, R., Witte, T., Galloway, J., Deberdt, W., et al. (2023). Baricitinib safety for events of special interest in populations at risk: Analysis from randomised trial data across rheumatologic and dermatologic indications. *Adv. Ther.* 40 (4), 1867–1883. doi:10.1007/s12325-023-02445-w

Terabe, K., Takahashi, N., Asai, S., Hirano, Y., Kanayama, Y., Yabe, Y., et al. (2023). Effectiveness of tacrolimus concomitant with biological disease-modifying antirheumatic drugs in patients with rheumatoid arthritis. *Mod. Rheumatol.* 33 (2), 292–301. doi:10.1093/mr/roac025

Tran, C. N., Davis, M. J., Tesmer, L. A., Endres, J. L., Motyl, C. D., Smuda, C., et al. (2007). Presentation of arthritogenic peptide to antigen-specific T cells by fibroblast-like synoviocytes. *Arthritis Rheum.* 56 (5), 1497–1506. doi:10.1002/art.22573

Tran, C. N., Thacker, S. G., Louie, D. M., Oliver, J., White, P. T., Endres, J. L., et al. (2008). Interactions of T cells with fibroblast-like synoviocytes: Role of the B7 family costimulatory ligand B7-H3. *J. Immunol.* 180 (5), 2989–2998. doi:10.4049/jimmunol.180.5.2989

Tu, J., Huang, W., Zhang, W., Mei, J., and Zhu, C. (2022). Two main cellular components in rheumatoid arthritis: Communication between T cells and fibroblast-like synoviocytes in the joint synovium. *Front. Immunol.* 13, 922111. doi:10.3389/fimmu.2022.922111

Tucci, G., Garufi, C., Pacella, I., Zagaglioni, M., Pinzon Grimaldos, A., Ceccarelli, F., et al. (2022). Baricitinib therapy response in rheumatoid arthritis patients associates to STAT1 phosphorylation in monocytes. *Front. Immunol.* 13, 932240. doi:10.3389/fimmu.2022.932240

Villa, T., Kim, M., and Oh, S. (2020). Fangchinoline has an anti-arthritic effect in two animal models and in IL-1β-stimulated human FLS cells. *Biomol. Ther. Seoul.* 28 (5), 414–422. doi:10.4062/biomolther.2020.113

Vomero, M., Caliste, M., Barbati, C., Speziali, M., Celia, A. I., Ucci, F., et al. (2022). Tofacitinib decreases autophagy of fibroblast-like synoviocytes from rheumatoid arthritis patients. *Front. Pharmacol.* 13, 852802. doi:10.3389/fphar.2022.852802

Walsh, N. C., Reinwald, S., Manning, C. A., Condon, K. W., Iwata, K., Burr, D. B., et al. (2009). Osteoblast function is compromised at sites of focal bone erosion in inflammatory arthritis. *J. Bone Min. Res.* 24 (9), 1572–1585. doi:10.1359/jbmr.090320

Wang, C. H., Yao, H., Chen, L. N., Jia, J. F., Wang, L., Dai, J. Y., et al. (2012). CD147 induces angiogenesis through a vascular endothelial growth factor and hypoxia-inducible transcription factor 1α-mediated pathway in rheumatoid arthritis. *Arthritis Rheum.* 64 (6), 1818–1827. doi:10.1002/art.34341

Wang, D. D., Jiang, M. Y., Wang, W., Zhou, W. J., Zhang, Y. W., Yang, M., et al. (2020). Paeoniflorin-6'-O-benzene sulfonate down-regulates CXCR4-Gβγ-PI3K/AKT mediated migration in fibroblast-like synoviocytes of rheumatoid arthritis by inhibiting GRK2 translocation. *Biochem. Biophys. Res. Commun.* 526 (3), 805–812. doi:10.1016/j.bbrc.2020.03.164

Wang, G., Xie, X., Yuan, L., Qiu, J., Duan, W., Xu, B., et al. (2020). Resveratrol ameliorates rheumatoid arthritis via activation of SIRT1-Nrf2 signaling pathway. *Biofactors* 46 (3), 441–453. doi:10.1002/biof.1599

Wang, H., Mei, D., Liang, F. Q., Xue, Z. Y., Wang, P., Liu, R. J., et al. (2023). BAFF promotes FLS activation through BAFFR-mediated non-canonical NF-κB pathway and the effects of CP-25. *Inflammation* 46, 861–875. doi:10.1007/s10753-022-01774-2

Wang, H., Tu, S., Yang, S., Shen, P., Huang, Y., Ba, X., et al. (2019). Berberine modulates LPA function to inhibit the proliferation and inflammation of FLS-RA via p38/ERK MAPK pathway mediated by LPA(1). *Evid. Based Complement. Altern. Med.* 2019, 2580207. doi:10.1155/2019/2580207

Wang, J., Lian, J., Kong, X., and Lin, N. (2010). Effects of triptolide on cell proliferation and regulation of Ras-MAPKs pathway in synoviocytes induced by tumor necrosis factor. *Zhongguo Zhong Yao Za Zhi* 35 (7), 888–891. doi:10.4268/cjcmm20100719

Wang, M., Dai, T., Li, S., and Wang, W. (2022). Eugenol suppresses the proliferation and invasion of TNF-α-induced fibroblast-like synoviocytes via regulating NF-κB and COX-2. *Biochem. Biophys. Res. Commun.* 612, 63–69. doi:10.1016/j.bbrc.2022.04.074

Wang, R., Liu, J., Wang, Z., Wu, X., Guo, H., Jiao, X., et al. (2021). Mangiferin exert protective effects on joints of adjuvant-induced arthritis rats by regulating the MAPKs/NF-κB pathway of fibroblast-like synoviocytes. *Int. Immunopharmacol.* 101, 108352. doi:10.1016/j.intimp.2021.108352

Wang, S., Du, Q., Sun, J., Geng, S., and Zhang, Y. (2022). Investigation of the mechanism of Isobavachalcone in treating rheumatoid arthritis through a combination strategy of network pharmacology and experimental verification. *J. Ethnopharmacol.* 294, 115342. doi:10.1016/j.jep.2022.115342

Wang, X. H., Dai, C., Wang, J., Liu, R., Li, L., and Yin, Z. S. (2021). Therapeutic effect of neohesperidin on TNF-α-stimulated human rheumatoid arthritis fibroblast-like synoviocytes. *Chin. J. Nat. Med.* 19 (10), 741–749. doi:10.1016/S1875-5364(21)60107-3

Wang, Z. Z., Huang, T. Y., Gong, Y. F., Zhang, X. M., Feng, W., and Huang, X. Y. (2020). Effects of sorafenib on fibroblast-like synoviocyte apoptosis in rats with adjuvant arthritis. *Int. Immunopharmacol.* 83, 106418. doi:10.1016/j.intimp.2020.106418

Wendel, H. G., De Stanchina, E., Fridman, J. S., Malina, A., Ray, S., Kogan, S., et al. (2004). Survival signalling by Akt and eIF4E in oncogenesis and cancer therapy. *Nature* 428 (6980), 332–337. doi:10.1038/nature02369

Wendling, D., Prati, C., Toussirot, E., and Herbein, G. (2010). Targeting intracellular signaling pathways to treat rheumatoid arthritis: Pandora's box? *Jt. Bone Spine* 77 (2), 96–98. doi:10.1016/j.jbspin.2010.01.004

Westhovens, R. (2023). Filgotinib in rheumatoid arthritis. *Expert Rev. Clin. Immunol.* 19 (2), 135–144. doi:10.1080/1744666X.2023.2149495

Weston, S., Macdonald, J. L., Williams, L. M., Roussou, E., Kang, N. V., Kiriakidis, S., et al. (2022). The JAK inhibitor baricitinib inhibits oncostatin M induction of proinflammatory mediators in *ex-vivo* synovial derived cells. *Clin. Exp. Rheumatol.* 40 (9), 1620–1628. doi:10.55563/clinexprheumatol/cfsajk

Wu, Z. M., Xiang, Y. R., Zhu, X. B., Shi, X. D., Chen, S., Wan, X., et al. (2022). Icariin represses the inflammatory responses and survival of rheumatoid arthritis fibroblast-like synoviocytes by regulating the TRIB1/TLR2/NF-kB pathway. *Int. Immunopharmacol.* 110, 108991. doi:10.1016/j.intimp.2022.108991

Xie, C., Jiang, J., Liu, J., Yuan, G., and Zhao, Z. (2019). Triptolide suppresses human synoviocyte MH7A cells mobility and maintains redox balance by inhibiting autophagy. *Biomed. Pharmacother.* 115, 108911. doi:10.1016/j.biopha.2019.108911

Xu, R., Liu, Z., Hou, J., Huang, T., and Yang, M. (2018). Osthole improves collagen-induced arthritis in a rat model through inhibiting inflammation and cellular stress. *Cell Mol. Biol. Lett.* 23, 19. doi:10.1186/s11658-018-0086-0

Xu, Z., Shang, W., Zhao, Z., Zhang, B., Liu, C., and Cai, H. (2022). Curcumin alleviates rheumatoid arthritis progression through the phosphatidylinositol 3-kinase/protein kinase B pathway: An *in vitro* and *in vivo* study. *Bioengineered* 13 (5), 12899–12911. doi:10.1080/21655979.2022.2078942

Yalcin Kehribar, D., Ozgen, M., Yolbas, S., Yildirim, A., Onalan Etem, E., Ciftci, O., et al. (2021). The inhibition of Src kinase suppresses the production of matrix metalloproteinases in from synovial fibroblasts and inhibits MAPK and STATs pathways. *Turk J. Med. Sci.* 51 (4), 2142–2149. doi:10.3906/sag-2008-274

Yamada, H. (2023). The search for the pathogenic T cells in the joint of rheumatoid arthritis: Which T-cell subset drives autoimmune inflammation? *Int. J. Mol. Sci.* 24 (8), 6930. doi:10.3390/ijms24086930

Yang, F., Shen, J., and Cai, H. (2022). Paeoniflorin inhibits Wnt1/beta-catenin pathway and promotes apoptosis of fibroblast-like synoviocytes in patients with rheumatoid arthritis by upregulating lncRNA MALAT1. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi* 38 (8), 692–698.

Yang, J., Liu, J., Li, J., Jing, M., Zhang, L., Sun, M., et al. (2022). Celastrol inhibits rheumatoid arthritis by inducing autophagy via inhibition of the PI3K/AKT/mTOR signaling pathway. *Int. Immunopharmacol.* 112, 109241. doi:10.1016/j.intimp.2022.109241

Yang, Y., Dong, Q., and Li, R. (2017). Matrine induces the apoptosis of fibroblast-like synoviocytes derived from rats with collagen-induced arthritis by suppressing the activation of the JAK/STAT signaling pathway. *Int. J. Mol. Med.* 39 (2), 307–316. doi:10.3892/ijmm.2016.2843

Yang, Y. P., Jian, Y. Q., Liu, Y. B., Ismail, M., Xie, Q. L., Yu, H. H., et al. (2021). Triterpenoids from kadsura coccinea with their anti-inflammatory and inhibited proliferation of rheumatoid arthritis-fibroblastoid synovial cells activities. *Front. Chem.* 9, 808870. doi:10.3389/fchem.2021.808870

Yang, Y. P., Jian, Y. Q., Liu, Y. B., Xie, Q. L., Yu, H. H., Wang, B., et al. (2022). Heilaohuacid G, a new triterpenoid from Kadsura coccinea inhibits proliferation, induces apoptosis, and ameliorates inflammation in RA-FLS and RAW 264.7 cells via suppressing NF-?B pathway. *Phytother. Res.* 36 (10), 3900–3910. doi:10.1002/ptr.7527

Yang, Y., Ye, Y., Qiu, Q., Xiao, Y., Huang, M., Shi, M., et al. (2016). Triptolide inhibits the migration and invasion of rheumatoid fibroblast-like synoviocytes by blocking the activation of the JNK MAPK pathway. *Int. Immunopharmacol.* 41, 8–16. doi:10.1016/j.intimp.2016.10.005

Yao, R. B., Zhao, Z. M., Zhao, L. J., and Cai, H. (2017). Sinomenine inhibits the inflammatory responses of human fibroblast-like synoviocytes *via* the TLR4/MyD88/NF-κB signaling pathway in rheumatoid arthritis. *Pharmazie* 72 (6), 355–360. doi:10.1691/ph.2017.6946

Yi, L., Ke, J., Liu, J., Lai, H., Lv, Y., Peng, C., et al. (2021). Sinomenine increases adenosine A_{2A} receptor and inhibits NF-κB to inhibit arthritis in adjuvant-induced-arthritis rats and fibroblast-like synoviocytes through α7nAChR. *J. Leukoc. Biol.* 110 (6), 1113–1120. doi:10.1002/JLB.3MA0121-024RRRR

Yin, G., Wang, Y., Cen, X. M., Yang, M., Liang, Y., and Xie, Q. B. (2015). Lipid peroxidation-mediated inflammation promotes cell apoptosis through activation of NF-κB pathway in rheumatoid arthritis synovial cells. *Mediat. Inflamm.* 2015, 460310. doi:10.1155/2015/460310

Yoon, H. Y., Lee, E. G., Lee, H., Cho, I. J., Choi, Y. J., Sung, M. S., et al. (2013). Kaempferol inhibits IL-1β-induced proliferation of rheumatoid arthritis synovial fibroblasts and the production of COX-2, PGE2 and MMPs. *Int. J. Mol. Med.* 32 (4), 971–977. doi:10.3892/ijmm.2013.1468

Yu, H. H., Li, M., Li, Y. B., Lei, B. B., Yuan, X., Xing, X. K., et al. (2020). Benzoylaconitine inhibits production of IL-6 and IL-8 via MAPK, Akt, NF-κB signaling in IL-1β-induced human synovial cells. *Biol. Pharm. Bull.* 43 (2), 334–339. doi:10.1248/bpb.b19-00719

Yu, X., Zhou, J., Zhao, F., Liu, X., Mao, Y., Diao, L., et al. (2021). Tomatidine suppresses the destructive behaviors of fibroblast-like synoviocytes and ameliorates type

II collagen-induced arthritis in rats. *Front. Pharmacol.* 12, 670707. doi:10.3389/fphar.2021.670707

Yu, Y., Koehn, C. D., Yue, Y., Li, S., Thiele, G. M., Hearth-Holmes, M. P., et al. (2015). Celastrol inhibits inflammatory stimuli-induced neutrophil extracellular trap formation. *Curr. Mol. Med.* 15 (4), 401–410. doi:10.2174/1566524015666150505160743

Zeng, S., Wang, K., Huang, M., Qiu, Q., Xiao, Y., Shi, M., et al. (2017). Halofuginone inhibits TNF-α-induced the migration and proliferation of fibroblast-like synoviocytes from rheumatoid arthritis patients. *Int. Immunopharmacol.* 43, 187–194. doi:10.1016/j.intimp.2016.12.016

Zhai, K. F., Duan, H., Cui, C. Y., Cao, Y. Y., Si, J. L., Yang, H. J., et al. (2019). Liquiritin from Glycyrrhiza uralensis attenuating rheumatoid arthritis via reducing inflammation, suppressing angiogenesis, and inhibiting MAPK signaling pathway. *J. Agric. Food Chem.* 67 (10), 2856–2864. doi:10.1021/acs.jafc.9b00185

Zhang, D., Ning, T., and Wang, H. (2022). Vitexin alleviates inflammation and enhances apoptosis through the regulation of the JAK/STAT/SOCS signaling pathway in the arthritis rat model. *J. Biochem. Mol. Toxicol.* 36 (12), e23201. doi:10.1002/jbt.23201

Zhang, G., Liu, B., Zeng, Z., Chen, Q., Feng, Y., and Ning, X. (2021). Oxymatrine hydrazone (OMTH) synthesis and its protective effect for rheumatoid arthritis through downregulation of MEK/NF-κB pathway. *Environ. Toxicol.* 36 (12), 2448–2453. doi:10.1002/tox.23357

Zhang, L., Lin, Y., Xu, X., Liu, H., Wang, X., and Pan, J. (2023). Telotristat Etiprate alleviates rheumatoid arthritis by targeting LGALS3 and affecting MAPK signaling. *Intractable Rare Dis. Res.* 12 (1), 45–57. doi:10.5582/irdr.2022.01121

Zhang, Q., Liu, J., Zhang, M., Wei, S., Li, R., Gao, Y., et al. (2019). Apoptosis induction of fibroblast-like synoviocytes is an important molecular-mechanism for herbal medicine along with its active components in treating rheumatoid arthritis. *Biomolecules* 9 (12), 795. doi:10.3390/biom9120795

Zhang, X., Guan, X., Piao, Y., Che, X., Si, M., and Jin, J. (2022). Baicalein induces apoptosis of rheumatoid arthritis synovial fibroblasts through inactivation of the PI3K/Akt/mTOR pathway. *Evid. Based Complement. Altern. Med.* 2022, 3643265. doi:10.1155/2022/3643265

Zhang, X., Ye, G., Wu, Z., Zou, K., He, X., Xu, X., et al. (2020). The therapeutic effects of edaravone on collagen-induced arthritis in rats. *J. Cell Biochem.* 121 (2), 1463–1474. doi:10.1002/jcb.29382

Zhang, Y., Tang, L. D., Wang, J. Y., Wang, H., Chen, X. Y., Zhang, L., et al. (2022). Anti-inflammatory effects of aucubin in cellular and animal models of rheumatoid arthritis. *Chin. J. Nat. Med.* 20 (6), 458–472. doi:10.1016/S1875-5364(22)60182-1

Zhong, B., Guo, S., Yang, Z., Han, L., Du, J., Chen, J., et al. (2021). Roflumilast reduced the IL-18-induced inflammatory response in fibroblast-like synoviocytes (FLS). *ACS Omega* 6 (3), 2149–2155. doi:10.1021/acsomega.0c05281

Zhong, Z., Qian, Z., Zhang, X., Chen, F., Ni, S., Kang, Z., et al. (2019). Tetrandrine prevents bone loss in ovariectomized mice by inhibiting RANKL-induced osteoclastogenesis. *Front. Pharmacol.* 10, 1530. doi:10.3389/fphar.2019.01530

Zhou, H., Liu, J. X., Luo, J. F., Cheng, C. S., Leung, E. L., Li, Y., et al. (2017). Suppressing mPGES-1 expression by sinomenine ameliorates inflammation and arthritis. *Biochem. Pharmacol.* 142, 133–144. doi:10.1016/j.bcp.2017.07.010

Zhou, J., Mao, Y., Shi, X., Zhang, Y., Yu, X., Liu, X., et al. (2022). Peimine suppresses collagen-induced arthritis, activated fibroblast-like synoviocytes and TNFα-induced MAPK pathways. *Int. Immunopharmacol.* 111, 109181. doi:10.1016/j.intimp.2022.109181

Zhou, Y. R., Zhao, Y., Bao, B. H., and Li, J. X. (2015). SND-117, a sinomenine bivalent alleviates type II collagen-induced arthritis in mice. *Int. Immunopharmacol.* 26 (2), 423–431. doi:10.1016/j.intimp.2015.04.006

Zuo, J., Xia, Y., Li, X., Ou-Yang, Z., and Chen, J. W. (2015). Selective modulation of MAPKs contribute to the anti-proliferative and anti-inflammatory activities of 1,7-dihydroxy-3,4-dimethoxyxanthone in rheumatoid arthritis-derived fibroblast-like synoviocyte MH7A cells. *J. Ethnopharmacol.* 168, 248–254. doi:10.1016/j.jep.2015.03.069

Glossary

RA	Rheumatoid arthritis
NFATc1	c-Fos and nuclear factor of activated T cells c1
FLS	Fibroblast-like synoviocyte
ATF2	Activating transcription factor-2
NSAIDs	Non-steroidal anti-inflammatory drugs
PGE (2)	Prostaglandin E (2)
DMARDs	Disease-modifying anti-rheumatic drugs
ROS	Reactive oxygen species
MTX	Methotrexate
HIF1	Hypoxia-inducible factor 1
bDMARDs	Biologic disease-modifying anti-rheumatic drugs
CIA	Collagen-induced arthritis
ECM	Extracellular matrix
IκB	Inhibitor of κB
MAPK	Mitogen-activated protein kinase
Bcl-2	B-cell lymphoma-2
NF-κB	Nuclear factor kappa-B
Bax	Bcl-2-associated X
JAK	Janus kinase
AA	Adjuvant-induced arthritic
STAT	Signal transducers and activators of transcription
OPG	Osteoprotegerin
TNF	Tumor necrosis factor
MEKK	Mitogen-activated protein kinase kinase
IL	Interleukin
IKK	IκB kinase
NOS	Nitric oxide synthase
TGM2	Transglutaminase 2
COX-2	Cyclooxygenase-2
NLS	Nuclear-localization sequence
RANK	Receptor activator of NF-κB
HDAC	Histone deacetylases
RANKL	Receptor activator of NF-κB ligand
GRK2	G protein-coupled receptor kinase 2
VEGF	Vascular endothelial growth factor
M-CSF	Macrophage colony stimulating factor
MMPs	Matrix metalloproteinases
MDA	Malondialdehyde
PDGF	Platelet-derived growth factor
TRIB1	Tribbles pseudokinase 1
TGF-β	Transforming growth factor-β
NFATc1	Nuclear factor of activated T cells
FLIP	Anti-apoptotic molecule FLICE inhibitory protein
NLRP3	NOD-like receptor protein 3
TLRs	Toll-like receptors
HO-1	Heme oxygenase
DKK-1	Dickkopf-1
SIRT1	Silent information regulator 1
CXCL-8	CXC motif chemokine 8
MCP-1	Monocyte chemotactic protein-1
CCL2	CC motif chemokine ligand 2
OPN	Osteopontin
MHCII	Major histocompatibility complex class II
ATP	Adenosine triphosphate
FcγRs	Fc-gamma receptors
α7nAChR	α7-nicotinic acetylcholine receptor
APRIL	A proliferation-inducing ligand
mPGES-1	Microsomal prostaglandin E synthase 1
ABCs	Age-associated B cells
AP-1	Activated protein-1
VCAM-1	Vascular cell adhesion molecule-1
FDA	Food and Drug Administration
ICAM-1	Intercellular cell adhesion molecule-1
EMA	European Medicines Agency
LFA-1	Lymphocyte function-associated antigen
csDMARDs	Conventional synthetic disease-modifying anti-rheumatic drugs
JNK	c-Jun N-terminal kinase
TNFSF11	TNF Superfamily Member 11
ERK	Extracellular regulated protein kinase
AESI	Adverse events of special interest
Bcl-2	B-cell lymphoma 2
OSM	Oncostatin M
Ang1	Angiopoietin-1
α-SMA	Smooth muscle alpha-actin
Tie-2	Tyrosine-protein kinase receptor
SOCS	Suppressor of cytokine signaling
GSK-3	Glycogen synthase kinase 3
TAK	TGF β-activated kinase
MKK	Mitogen-activated protein kinase kinase
mTORC1	mTOR complex 1
mTOR	Mammalian target of rapamycin

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Ursolic acid reduces oxidative stress injury to ameliorate experimental autoimmune myocarditis by activating Nrf2/HO-1 signaling pathway

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Background: Oxidative stress is crucial in experimental autoimmune myocarditis (EAM)-induced inflammatory myocardial injury. Ursolic acid (UA) is an antioxidant-enriched traditional Chinese medicine formula. The present study aimed to investigate whether UA could alleviate inflammatory cardiac injury and determine the underlying mechanisms.

Methods: Six-week-old male BALB/c mice were randomly assigned to one of the three groups: Sham, EAM group, or UA intervention group (UA group) by gavage for 2 weeks. An EAM model was developed by subcutaneous injection of α-myosin heavy chain derived polypeptide (α-MyHC peptide) into lymph nodes on days 0 and 7. Echocardiography was used to assess cardiac function on day 21. The inflammation level in the myocardial tissue of each group was compared using hematoxylin and eosin staining (HE) of heart sections and Interleukin-6 (IL-6) immunohistochemical staining. Masson staining revealed the degree of cardiac fibrosis. Furthermore, Dihydroethidium staining, Western blot, immunohistochemistry, and enzyme-linked immunosorbent assay (ELISA) were used to determine the mechanism of cardioprotective effects of UA on EAM-induced cardiac injury, and the level of IL-6, Nrf2, and HO-1.

Results: In EAM mice, UA intervention significantly reduced the degree of inflammatory infiltration and myocardial fibrosis while improving cardiac function. Mechanistically, UA reduced myocardial injury by inhibiting oxidative stress (as demonstrated by a decrease of superoxide and normalization of pro- and antioxidant enzyme levels). Interestingly, UA intervention upregulated the expression of antioxidant factors such as Nrf2 and HO-1. *In vitro* experiments, specific Nrf2 inhibitors reversed the antioxidant and antiapoptotic effects of

Abbreviations: UA, ursolic acid; EAM, experimental autoimmune myocarditis; Nrf2, Nuclear factor erythroid-related factor 2; HO-1, Heme oxygenase-1; NQO1, NAD(P)H quinone dehydrogenase 1; GSH-Px, Glutathione peroxidase; Elisa, enzyme-linked immunosorbent assay; DHE, Dihydroethidium; MDA, Malondialdehyde; ROS, Reactive oxygen species; AREs, antioxidant response elements; EF, ejection fraction; FS, fractional shortening; LV, left ventricle; cTnI, cardiac troponin I; BNP, brain natriuretic peptide.

ursolic acid, which further suggested that the amelioration of EAM by UA was in a Nrf2/HO-1 pathway-dependent manner.

Conclusion: These findings indicate that UA is a cardioprotective traditional Chinese medicine formula that reduces EAM-induced cardiac injury by up-regulating Nrf2/HO-1 expression and suppressing oxidative stress, making it a promising therapeutic strategy for the treatment of EAM.

KEYWORDS

experimental autoimmune myocarditis, ursolic acid, oxidative stress, Nrf2, HO-1

Introduction

Myocarditis is an inflammation of the myocardium caused by viruses, bacteria, or parasites, as well as non-infectious causes such as autoimmunity, hypersensitivity, and cardiotoxicity (Frustaci et al., 2009; Caforio et al., 2015). It can result in sudden cardiac death or dilated cardiomyopathy (Felker et al., 2000). Currently, most myocarditis treatments are based on supportive and immunosuppressive therapy (Jensen and Marchant, 2016). However, effective therapies against myocarditis are lacking. The prevailing view indicates that oxidative stress exacerbates the autoimmune process in myocarditis (Tada and Suzuki, 2016). Moreover, long-term oxidative stress and antioxidant system suppression may result in cardiac remodeling in inflammatory cardiomyopathy (Tada and Suzuki, 2016). Oxidative stress is considered a potential therapeutic target to treat myocarditis. One of the hallmark changes in the myocarditis heart is increased oxidative stress, which leads to cardiac apoptosis. Nuclear factor erythroid-related factor 2 (Nrf2) is a transcription factor that activates under conditions of high oxidative stress and regulates many antioxidant and detoxification genes (Dinkova-Kostova et al., 2018). An imbalance between active oxidants and antioxidants causes oxidative stress. Under oxidative conditions, Nrf2 is released upon dissociation from kelch like ECH associated protein 1(Keap1) and translocated to the nucleus (Kobayashi et al., 2006). In the nucleus, it binds to antioxidant response elements (AREs), the promoter regions of its target genes. All of these genes, including Heme oxygenase-1 (HO-1), NAD(P)H quinone dehydrogenase 1 (NQO1), and Glutathione peroxidase (GSH-Px), have AREs in their promoter regions.

Traditional Chinese medicine has long been uniquely effective in treating inflammatory diseases. Ursolic acid (UA) is a pentacyclic triterpenoid compound found in many natural plants, such as apple peels, herbal medicines, and other edible plants (Cui et al., 2006; Seeram, 2008; Tannock, 2011). UA has numerous biological properties, including anti-tumor, anti-oxidation, anti-inflammation, anti-fibrosis, and anti-atherosclerosis, and can be used to treat various diseases (Wang et al., 2011; Silva et al., 2016). Some of these effects of UA are determined by its potent antioxidant properties (López-Hortas et al., 2018). Moreover, mounting evidence suggests the versatile roles of UA in the cardiovascular system (Saravanan, Pugalendi, 2006). Previous studies have confirmed that UA is a cardioprotective compound due to its antioxidant properties (Liobikas et al., 2011). However, whether UA can improve experimental autoimmune myocarditis (EAM) is unknown.

Several works have demonstrated the modulation of Nrf2 by UA as one of the main mechanisms behind their beneficial effects in pathological models like cigarette smoke-induced emphysema (Lin et al., 2017) and renal tubular epithelial cell damage induced by calcium oxalate monohydrate. Nevertheless, the regulation of this antioxidant pathway in cardiac pathology remains to be further investigated (Jia et al., 2021). The Nrf2 pathway has been shown to play a critical role in EAM as it reduces DNA damage induced by oxidative stress in EAM mice (Jaén et al., 2020). However, it is unknown whether UA can alleviate inflammation and oxidative injury in EAM through Nrf2/HO-1 signaling pathway.

In the present study, an EAM murine model was developed to investigate the regulatory role of UA in anti-inflammatory and cardiac antioxidant pathways. The present study investigated whether UA can reduce EAM-induced oxidative stress injury and whether this effect is mediated through Nrf2 and HO-1 signaling pathways. The present study provides new insights for developing therapeutic targets to improve the prognosis and treatment of myocarditis patients.

Materials and methods

Experimental animals and EAM model

Male BALB/c mice aged 6–7 weeks (weighing 18–20 g) were obtained from Beijing Vital River Laboratory Animal Technology Co., LTD. (China) and housed at 25°C ± 2°C with free access to water and food on a 12 h light/dark cycle. The Animal Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology (China) approved all experimental procedures that complied with the National Institutes of Health Guidelines on the Use of Laboratory Animals (Approval code: S3136; The validity of approval is from 01/01/2022-12/31/2024).

All mice were anesthetized using 1.5% isoflurane before the procedure to minimize animal stress. Mice were immunized with a cardiac-specific peptide (α-MyHC peptide: Ac-RSLKLMATLFSTYASADR-OH) purchased from GL Biochem Ltd (China). The peptide was dissolved in saline and emulsified in a 1:1 ratio with Complete Freund's Adjuvant (Sigma, United States) (n = 30). On day 0, 200 μg peptide in 0.2 mL of the emulsion was injected subcutaneously into one side of the axillary and inguinal lymph node region. On day 7, the same dose was injected subcutaneously into the opposite side. Sham group (n = 12) was injected with a physiological saline solution instead of myosin. Mice immunized with cardiac peptide were randomly divided into UA treatment (n = 16) and PBS (n = 14)

groups. From day 7–21, mice were given intragastric gavage of UA ((U6753, Sigma, United States), 100 mg/kg) (UA intervention group) or PBS containing 7.5% DMSO (D2650, Sigma, United States) (sham group and peptide–immunized mice without UA). Therefore, three experimental groups were formed: 1) a sham group, 2) an EAM group, and 3) an EAM + UA group. On day 21, all mice underwent echocardiography. The blood was then obtained through cardiac puncture. Body weight (BW) and tibial length (TL) were measured. Finally, hearts were removed from the chest and subjected to subsequent experiments.

Transthoracic echocardiography

On day 21, the cardiac function was assessed using an echocardiographic imaging system (Vevo 1,100, Canada) with a transducer MS400 (30 MHz). The mice were anesthetized with 1.5% isoflurane, and two-dimensional echocardiographic views of the mid-ventricular short axis at the papillary muscle tips below the mitral valve were obtained. The Vevo 1,100 software calculated the left ventricular ejection fraction (EF) and fractional shortening (FS).

Measurement of cytokines by enzyme-linked immunosorbent assay (ELISA)

Tissue (10 mg) was homogenized in PBS on ice for ELISA. The samples were centrifuged for 10 min at 5,000 × g to remove any insoluble material. Plasma samples were collected by cardiac puncture. Interleukin-6 (IL-6), cardiac troponin I (cTnI), and brain natriuretic peptide (BNP) levels were measured using ELISA kits (mlbio, China) according to the manufacturer's instructions.

Malondialdehyde (MDA) assay

MDA was measured using the MDA assay kit (Beyotime, China) as directed by the manufacturer. Plasma (100 μL) or homogenized cells (1×10^6) samples were added to 200 μL MDA Lysis Buffer. The samples were incubated at 100°C for 15 min before being cooled in an ice bath to room temperature. The samples were centrifuged at 1,000 *g* for 10 min at room temperature to remove any insoluble material. Then, 200 μL of supernatant was pipetted into a 96-well plate, and the absorbance was measured at 532 nm (A_{532}).

Cell culture and *in vitro* experimental protocols

H9c2 cells were obtained from the cell bank of the Chinese Academy of Sciences (China). The cells were cultured in dulbecco's modified eagle medium (DMEM) (high glucose) medium (Gibco, United States) supplemented with 10% fetal bovine serum (FBS) (Gibco, United States)and 1% of penicillin-streptomycin (Gibco, United States) in a humidified atmosphere of 5% CO_2 at 37°C. Cells were maintained in a proliferative state (i.e., undifferentiated state). To induce inflammation, cells were rendered quiescent by serum starvation for 12 h prior to stimulation with rIL-6 (25 ng/mL) for 1 h (Shi et al., 2020). In order to study the antioxidant effect of ursolic acid, 10 μM, 20 μM, 30 μM, 40 μM, and 50 μM of UA was added to the cells culture 4 h after the addition of rIL-6. For Nrf2 inhibitor experiments, cultures were co-incubated by 30 μM UA and 5 μM ML385 for 4 h (HY-100523, MCE, United States) (Chen et al., 2021). The cells were split into four groups: 1) Sham group; 2) IL-6 group; 3) UA group (IL-6 + UA); 4) ML385 group (IL-6 + UA + ML385).

TABLE 1 RT q-PCR primer sequences.

	Primer sequences (5′-3′)
GAPDH	Forward: 5′-ACTCCACTCACGGCAAATTCA-3′
	Reverse: 5′-GGCCTCACCCCATTTGATG-3′
IL-6	Forward: 5′-ACAACCACGGCCTTCCCTACT-3′
	Reverse: 5′-CTCATTTCCACGATTTCCCAGA-3′
Col1a1	Forward: 5′-AATGGCACGGCTGTGTGCGA-3′
	Reverse: 5′-AGCACTCGCCCTCCCGTCTT-3′
Col3a1	Forward: 5′-CCTGGCTCAAATGGCTCAC-3′
	Reverse: 5′- GACCTCGTGTTCCGGGTAT-3′

Cell viability assay

Cell viability was determined using the Cell Counting Kit-8 (CCK-8) (Biosharp, China) assay following the manufacturer's instructions. The cells were seeded at a density of 5,000 cells/well in the 96-well plates. After 24 h, cells were treated with different concentrations of UA. The CCK-8 solution was added to each well, and the cells were incubated at 37°C for another 2 h. The absorbance was measured at 450 nm (A_{450}). Each sample was replicated thrice. The cells that stained positive for CCK-8 solution was thought to be viable. The results are presented as a percentage compared with sham group.

Histopathology and immunohistochemistry

Myocardial tissues from left ventricle (LV) of mice at the papillary muscle level were harvested, fixed in fresh 4% paraformaldehyde, embedded in paraffin, and serially sectioned (5–6 μm). Dewaxed and hydrated heart sections were stained with hematoxylin and eosin staining (HE) for general morphology or with Masson trichrome stain kit for fibrosis detection. The blue stained area (collagen deposition) was quantified and normalized to the total section area using ImageJ (1.8.0, National Institutes of Health) software to determine cardiac fibrosis.

Immunofluorescence staining

The paraffin sections were baked for 1 h in a 65°C oven. The sections were dewaxed and antigen repaired before incubating in 3% H_2O_2 for 10 min to remove endogenous enzymes. After 1 h of

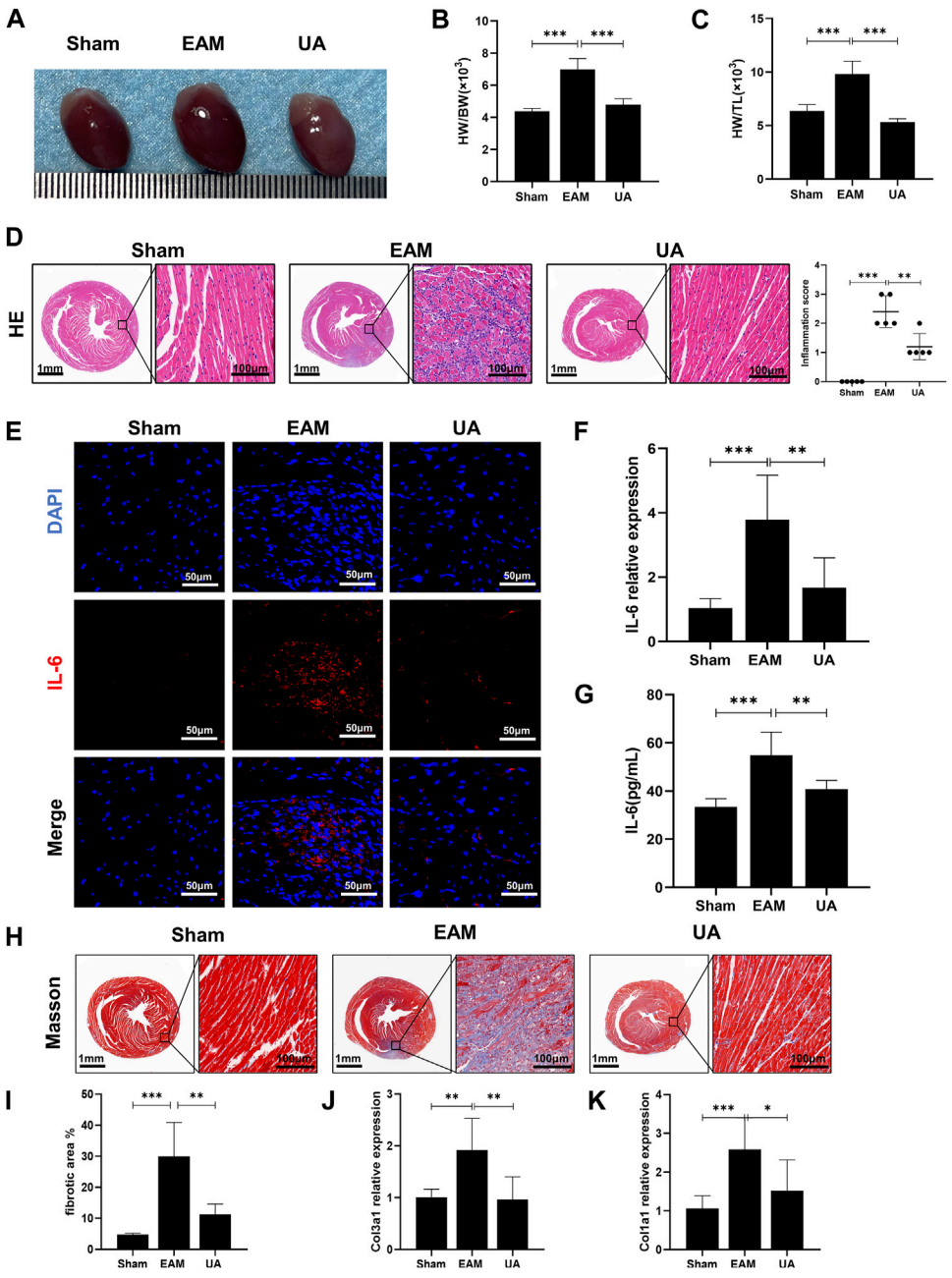

FIGURE 1
UA mitigated EAM-induced cardiac inflammation and fibrosis. **(A)** Heart images representing each experimental group. **(B)** Heart weight to body weight ratio (HW/BW). **(C)** Heart weight to tibial length ratio (HW/TL) from all experimental groups. **(D)** Representative histological images of immune cell infiltration in cardiac tissue from the indicated groups and the corresponding quantification (n = 5). Scale bars: left 1mm, inset 100 μm. **(E)** Representative immunofluorescence images of cardiac tissue stained red with IL-6 and blue with DAPI. Scale bars: 50 μm. **(F)** RT-qPCR detected the mRNA levels of IL-6 in cardiac tissue. **(G)** ELISA detected IL-6 levels in cardiac tissue from all experimental groups at day 21. **(H, I)** Representative Masson staining images indicating collagen fiber deposition in blue and the corresponding quantification (n = 8). Scale bars: left 1 mm, inset 100 μm. **(J, K)** Col1a1 and Col3a1 mRNA expression levels in cardiac tissue from the indicated groups at day 21 (n = 9). (Data were shown as the mean ± SD, ordinary one-way analysis of variance, Tukey's multiple comparisons test and Games-Howell's multiple comparisons test; ns = not significant, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$).

blocking with goat serum at room temperature, heart slides were incubated overnight at 4°C with the following primary antibodies: α-Smooth Muscle Actin (α-SMA) (1:100) (#7817, Abcam, United Kingdom), IL-6 (1:50) (#290735, Abcam, United Kingdom). Samples were incubated with secondary antibodies combined with Alexa Fluor 488 and 555 (Beyotime, China) for 1 h at 1:500, followed by 4′,6-diamidino-2-phenylindole (DAPI) (Beyotime, China) for 15 min at room temperature. Finally, the sections were washed with PBS 1x and covered with coverslips.

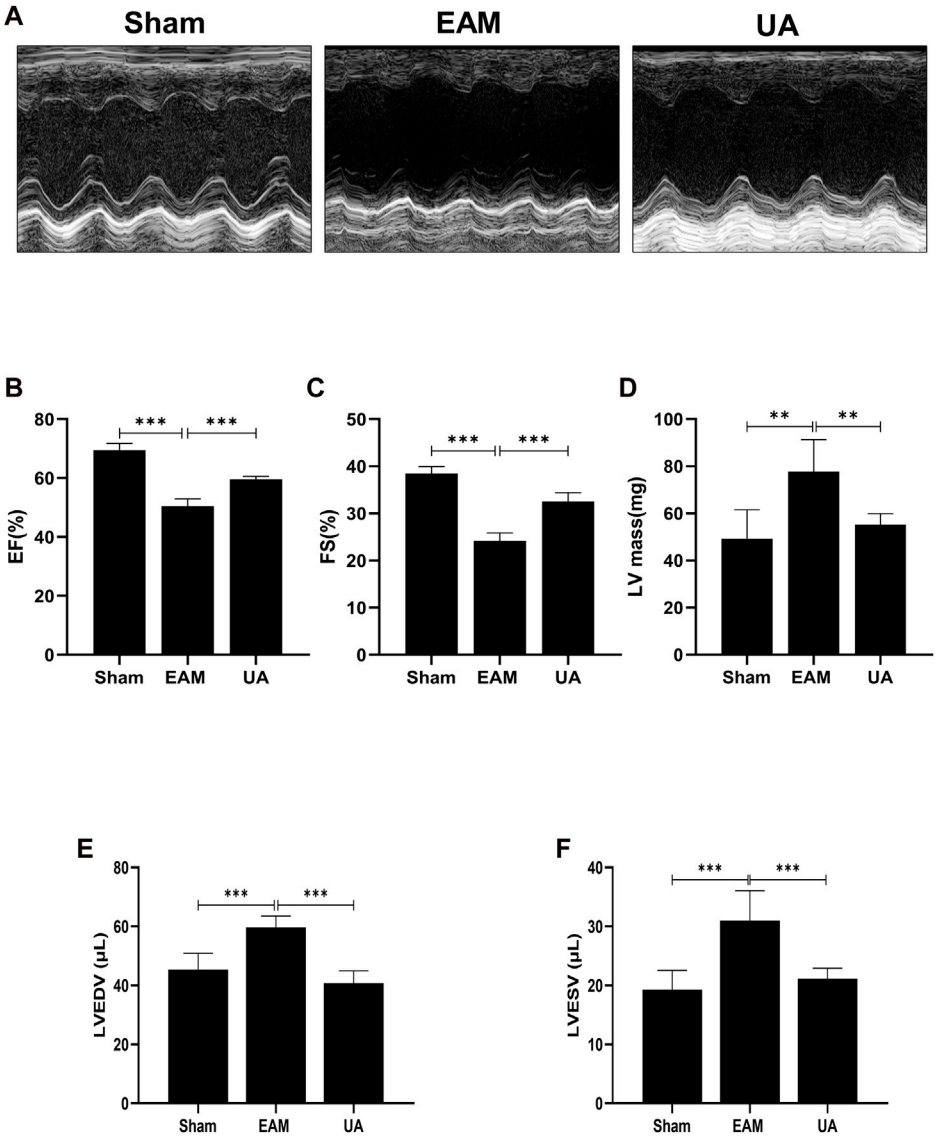

FIGURE 2
UA improved EAM-induced left ventricular dysfunction. **(A)** Representative echocardiography M-mode images from the indicated groups on day 21. **(B, C)** Statistical analysis of ejection fractions (EF) and fractional shorting (FS) in indicated groups. **(D, E, F)** Left ventricular function-related ultrasonic parameters in each group (n = 6). (Data were shown as the mean ± SD, ordinary one-way analysis of variance, Tukey's multiple comparisons test; ns = not significant, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$).

TUNEL assay

Myocardial apoptosis was determined by terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining by TUNEL Apoptosis Assay Kit with fluorescein (Beyotime, China) following the manufacturer's instructions. Green fluorescein staining was used to identify apoptotic nuclei, while DAPI stained total nuclei. The apoptotic index was expressed as the percentage of apoptotic nuclei to total nuclei.

Western blot analysis

Cardiac tissues or cells were homogenized in ripa lysis buffer (include protease and phosphatase Inhibitor) with a sonicator on ice, and the total protein concentrations were measured using the BCA Protein Assay Kit (Beyotime, China). As previously reported, cytosolic and nucleic proteins were separated for analysis of Nrf2 release. Depending on the molecular weight of target proteins, equal amounts of protein lysates (20–40 μg/lane) were subjected to 10%–12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotted to PVDF membranes (MerckMillipore, Germany). After blocking with 5% skim milk for 1 h at room temperature, the membranes were incubated at 4°C overnight with respective primary antibodies against Nrf2 (1:1,000) (16,396, proteintech, China), HO-1 (1:1,000, ab52947), LaminB1 (1:1,000, ab133741), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:2000, CST#5174), β-actin (1:

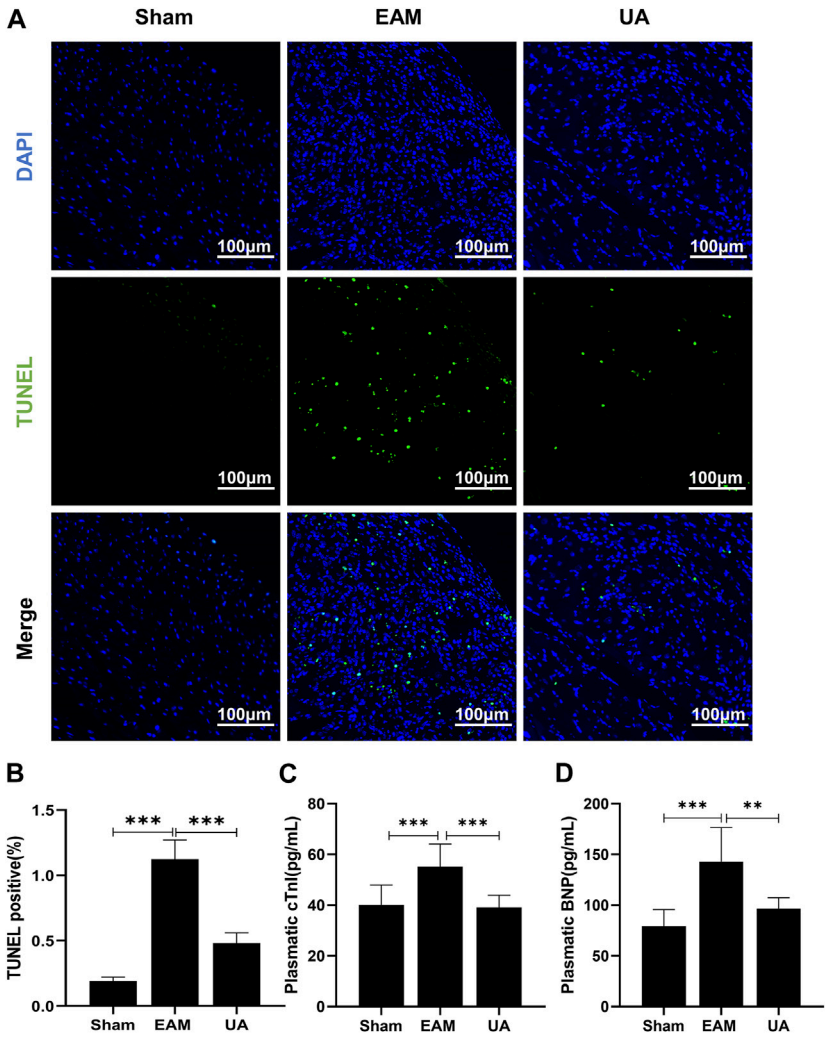

FIGURE 3
UA reduced EAM-induced cardiomyocyte apoptosis. **(A)** TUNLE staining images of cardiac tissue stained with TUNEL-positive nuclei in green and DAPI in blue. Scale bars: 100 μm. **(B)** Quantitative analysis of apoptotic index among the indicated groups (n = 5). **(C, D)** cTnI and BNP levels detected by ELISA in cardiac tissue from the indicated groups (n = 9). (Data were shown as the mean ± SD, ordinary one-way analysis of variance, Tukey's multiple comparisons test and Games-Howell's multiple comparisons test; ns = not significant, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$).

TABLE 2 Cardiac function parameters obtained from echocardiography.

	Sham	EAM	UA
Heart rate (bpm)	401 ± 86	410 ± 21	382 ± 26
Ejection fraction%	69.5 ± 2	50 ± 2.5***	59.5 ± 1###
Fractional shortening%	38 ± 1.5	24 ± 2***	32 ± 1.5###
LVEDV (μL)	46 ± 6	60 ± 4***	41 ± 4###
LVESV (μL)	19 ± 3	31 ± 5***	21 ± 2###
LV mass (mg)	49 ± 12	78 ± 14**	55 ± 4.5##

Data are presented as mean ± SD, ordinary one-way analysis of variance, Tukey's multiple comparisons test.
*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$ vs. Control.
#$p < 0.05$.
##$p < 0.01$.
###$p < 0.001$ vs EAM. LVEDV, Left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume.

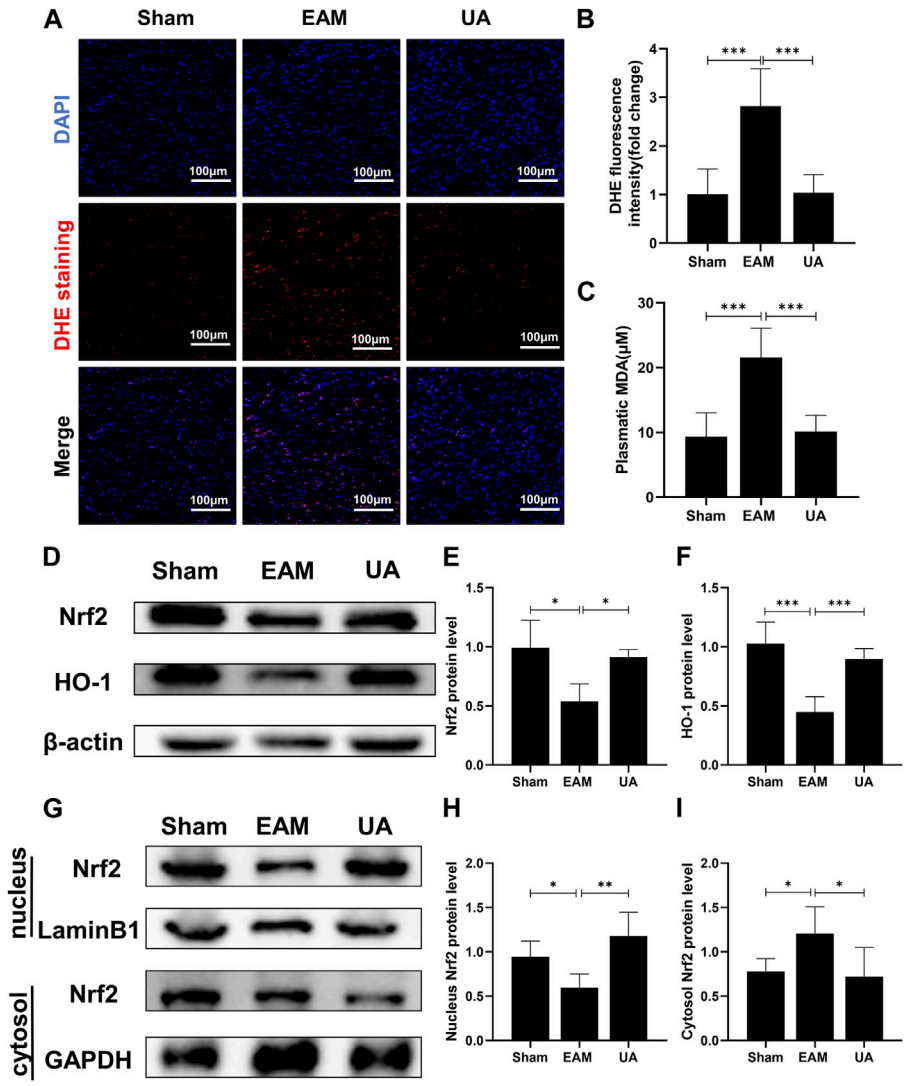

FIGURE 4
UA reduced the EAM-induced cardiac oxidative stress by regulating Nrf2 levels. **(A)** Heart images of DHE staining in the indicated groups. Scale bars: 100 μm. **(B)** The fluorescence intensity of DHE staining was measured and expressed as a fold change (n = 3). **(C)** An MDA assay measured serum MDA levels in each group. **(D,E, F)** The total cellular Nrf2 and HO-1 protein expression levels in each group were measured with Western blot and the representative quantification (n = 6). **(G,H, I)** Protein expressions of Nrf2 in cytoplasmic and nuclear cell lysates in mouse hearts of the different groups. (Data were shown as the mean ± SD, ordinary one-way analysis of variance, Tukey's multiple comparisons test and Games-Howell's multiple comparisons test; ns = not significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

1,000, ab8226) and washed. The protein bands were visualized using enhanced chemiluminescence (Pierce, United States) after incubation with the corresponding secondary HRP-conjugated antibodies. Densitometry values were determined using ImageJ software (1.8.0, National Institutes of Health).

Real-time quantitative polymerase chain reaction (RT q-PCR)

TRIzol Reagent (Invitrogen, United States) was used to isolate total RNA from flash-frozen myocardial tissues or H9c2 cells. cDNA was synthesized using a reverse transcription reagent kit (Takara Biotechnology, Japan) and then amplified in the Bio-rad CXF CONNECT Detector system using the SYBR® Premix Ex TaqTM Perfect Real Time Kit (Takara Biotechnology, Japan). Relative expression was computed using the CT method. Table 1 lists the SYBR Green real-time PCR primers for mouse gene expression.

Molecular docking

The crystal structure of Nrf2 protein (PDB ID: 7O7B) has been obtained from the RCSB protein data bank. For protein preparation, the natural ligand was extracted and the resulting crystal structure was then freed of the water molecules and added the polar hydrogen atoms. The 3D structure of ursolic acid has

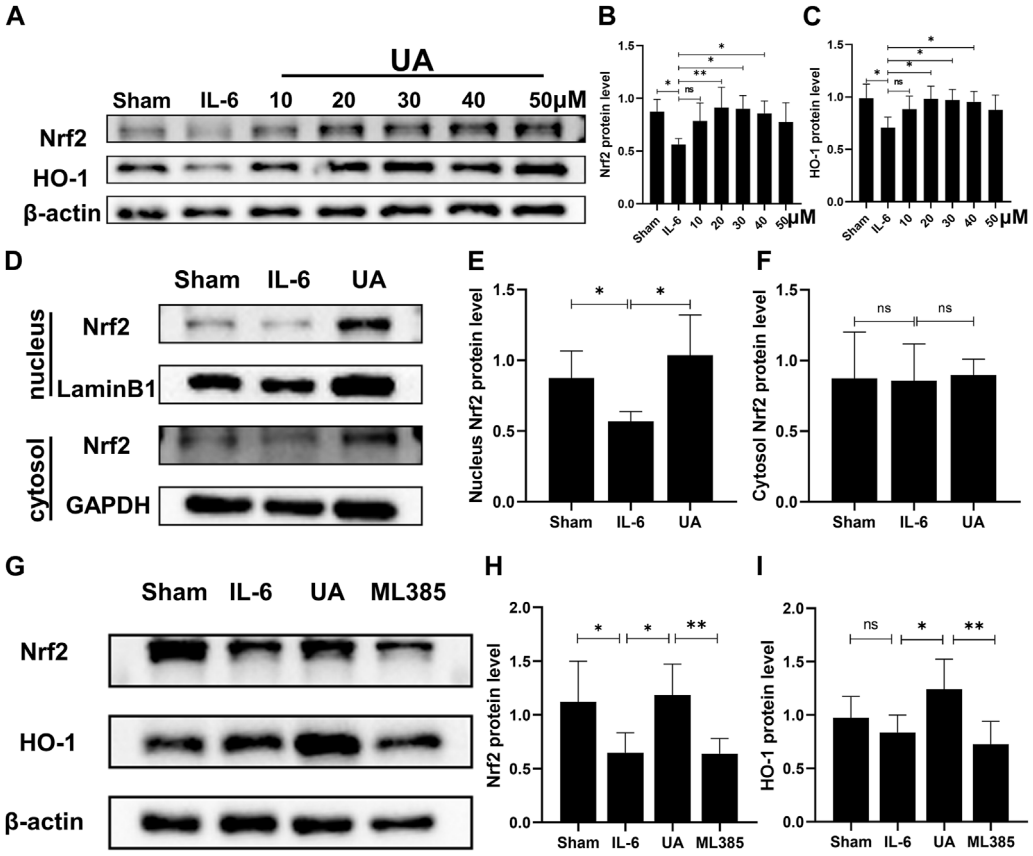

FIGURE 5
UA upregulated Nrf2/HO-1 signaling pathway *in vitro*. **(A, B, C)** Representative Western blot images and quantification of Nrf2 and HO-1 protein levels of H9c2 cells treated with UA at various concentrations (n = 6). **(D, E, F)** Protein expression of Nrf2 in cytoplasmic and nuclear cell lysate from different groups of H9c2 cells (UA = 30 μM). **(G, H, I)** The Nrf2 and HO-1 protein expression in H9c2 cells were measured with Western blot and the representative quantification (n = 6). (Data were shown as the mean ± SD, ordinary one-way analysis of variance, Tukey's multiple comparisons test and Games-Howell's multiple comparisons test; ns = not significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

been obtained from pubchme database. This structure was optimized and polar hydrogen atoms are added. Subsequently, the docking procedure was conducted by using Autodock software and its conformation was optimized by using a genetic algorithm based on the principle of minimizing docking energy.

Statistical analysis

The data were analyzed with GraphPad Prism software (version 8.0 for Windows, San Diego, CA, United States). All data are normally distributed. The multiple-group comparisons were performed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test for homogeneity of variance and Games-Howell's multiple comparisons test for heterogeneity of varianc. All values were presented as mean ± SD. The differences were considered statistically significant with a *p*-value <0.05.

Results

Ursolic acid ameliorated myocardial damage in EAM mice

The ratios of heart weight/body weight (HW/BW) and heart weight/tibia length (HW/TL) were elevated and heart was enlarged in EAM mice compared to sham group, which was alleviated by UA treatment (Figures 1A–C). H&E-stained EAM heart sections revealed a high inflammation score, which was reduced by UA treatment (Figure 1D). Immunofluorescence staining was used to characterize the immune cells in the infiltrated area. Compared with sham group, IL-6 was significantly increased in EAM group, while other inflammatory factors IL-1β and TNF-α had no significant difference (Figure 1E; Supplementary Figure S1A,B). In addition, UA could partially normalize IL-6 mRNA expression and the secretion level of IL-6 in EAM mouse heart tissues (Figures 1F,G).

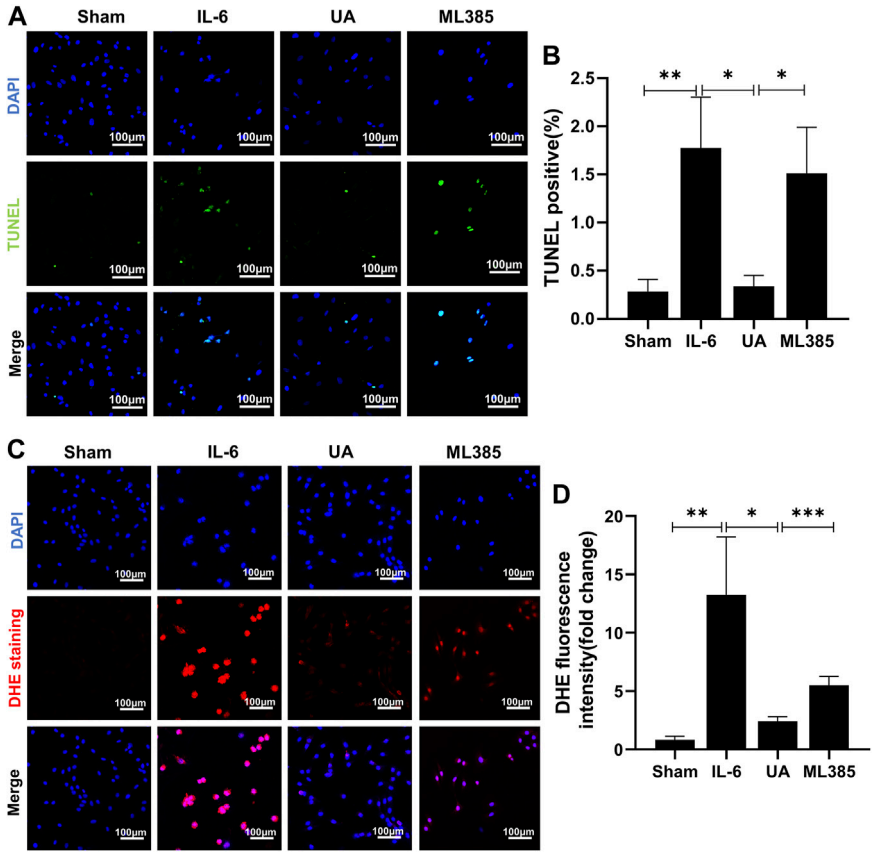

FIGURE 6
ML385 counteracted the anti-apoptosis and antioxidant effects of Ursolic Acid. **(A)** TUNEL staining images of H9c2 cells stained with TUNEL-positive nuclei in green and DAPI in blue. Scale bars: 100 μm. **(B)** Quantitative analysis of apoptotic index among the indicated groups (n = 5). **(C)** Representative images of DHE staining in H9c2 cells. Scale bars: 100 μm. **(D)** Fluorescence intensity of DHE staining was measured, and the results were expressed as a fold change (n = 6). (Data were shown as the mean ± SD, ordinary one-way analysis of variance, Tukey's multiple comparisons test and Games-Howell's multiple comparisons test; ns = not significant, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$).

In EAM model, heart sections stained with Masson's trichrome to detect collagen fiber deposition revealed some peripheral fibrotic areas in EAM mice that were reduced by UA administration (Figures 1H,I). Immunofluorescence staining with α-SMA, a fibroblast activation marker, was also increased in EAM animals, whereas it was deceased in UA-treated mice (Supplementary Figure S1C). The fibrosis analysis was supplemented by estimating mRNA levels of mediators involved in the development of cardiac fibrosis. In the present study, EAM mice had higher levels of collagen 1 (Col1a1) and 3 (Col3a1) cardiac mRNA that significantly reduced in UA-treated EAM mice (Figure 1J,K). Ursolic acid intervention alleviated myocardial inflammation and fibrosis in mice with EAM.

Ursolic acid treatment mitigated EAM-Induced systolic dysfunction

On day 21, echocardiography was performed to assess cardiac function in each group (Figure 2; Table 2). Figure 2A depicts the representative M-mode echocardiograms for each group. Cardiac systolic dysfunction, as indicated by left ventricular EF and FS, decreased significantly in the EAM group but not in the UA-treated group (Figures 2B,C). When compared to sham group, the EAM group had substantially higher levels of left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV), and left ventricular mass (LV Mass), which was mitigated in the UA-treated group (Figures 2D–F). Other factors, such as heart rate (HR), did not differ between the groups (Table 2). Ursolic acid reduced left ventricular dysfunction induced by EAM.

Ursolic acid treatment reduced cardiac apoptosis

Cardiac dysfunction has been associated with increased cardiomyocytes death, which eventually debilitates the contractile capacity of the heart. Findings indicated that the proportion of TUNEL-positive nuclei increased significantly in EAM group (Figures 3A,B). In addition, increased plasmatic levels of the cardiac damage marker cTnI (Figure 3C) and BNP (Figure 3D) were found in EAM mice. However, both parameters were significantly lower in UA-treated EAM mice.

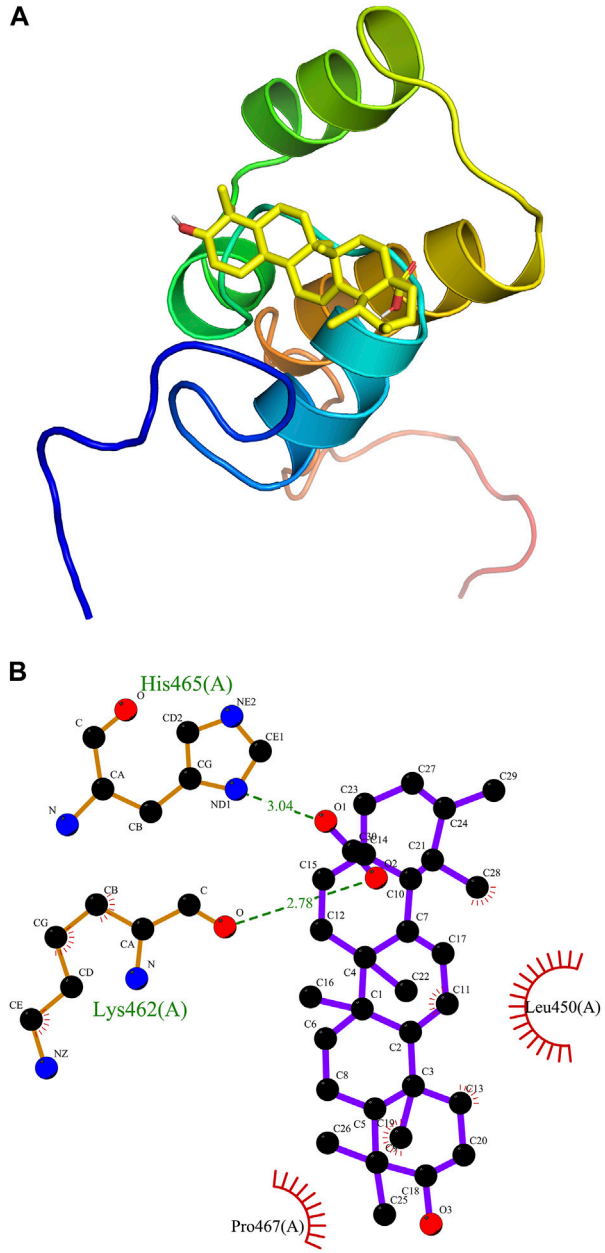

FIGURE 7
Molecular docking. Predicted docking pose of ursolic acid in complex with Nrf2 (PDB ID: 7O7B). **(A)** Overall structure of Nrf2/Ursolic acid complex. **(B)** LigPlot 2D structure analysis of protein-ligand interaction showing interactions between Nrf2 and ursolic acid. Green dashed lines indicate hydrogen bonds. In addition, ursoilc acid also interacts with Pro467 and Leu 450 via hydrophobic interactions, which are shown as arcs (red).

Effects of ursolic acid in the cardiac oxidative stress development: Nrf2 signaling pathway

Hearts from EAM animals were also exposed to elevated levels of oxidative stress that overwhelms the capacity of antioxidant systems to cope with this severe inflammatory scenario. Therefore, cytosolic reactive oxygen species (ROS) sensor DHE was used to characterize intracellular ROS, and MDA content was measured to assess lipid peroxidation end products. The findings revealed that EAM mice had higher ROS levels and lipid peroxidation levels than sham group, while UA-treated mice had lower levels (Figure 4A–C).

Nrf2 is an important transcriptional factor and the Nrf2/HO-1 axis is an important antioxidative stress pathway in the body. The present study identified that EAM reduced Nrf2/HO-1 protein expression of mice while UA could restore it (Figure 4D–F). Furthermore, UA treatment activated total cellular and nuclear Nrf2 in mouse heart but not cytoplasmic Nrf2 (Figure 4G–I). These findings demonstrate that UA's cardio-protective effect is partially due to the induction of the antioxidant response via the Nrf2 pathway activation.

Ursolic acid upregulated Nrf2/HO-1 signaling pathway *in Vitro*

To further confirm the molecular mechanism of UA in the inflammatory injury of cardiomyocytes, H9c2 cell was used *in vitro* experiments. To investigate the role of Nrf2 in the UA-mediated beneficial effects, we performed a set of experiments in which we exposed IL-6-stimulated cells to UA (10 μM, 20 μM, 30 μM, 40 μM, and 50 μM) for 4 h and quantified Nrf2 total protein levels in H9c2 cell (Figure 5A–C). To investigate the toxicity of UA to cells, we examined cell survival at various concentration points after 24 h of UA intervention (Supplementary Figure S1D). Findings revealed that cell survival was significantly reduced after 24 h of treatment with 50 μM UA. However, the cell survival rate did not change significantly when the concentration of UA less than 50 μM. In conclusion, 30 μM UA was selected for further experiments. The inflammatory cells stimulated by IL-6 were separated nucleoprotein from cytoplasmic protein by nuclear protein extraction kit. In IL-6-stimulated cells, the expression level of Nrf2 in nucleoprotein increased significantly after UA treatment. Meanwhile, there were no significant differences in the expression of Nrf2 in the cytoplasm between the groups (Figure 5D–F).

To further testify that the effects of UA were Nrf2/HO-1 pathway-dependent, specifc Nrf2 inhibitor ML385 was utilized *in vitro*. Protein expression of both Nrf2 and HO-1 was reduced after co-incubation of ML385 with UA in cardiomyocytes (Figure 5G–I; Supplementary Figure S1E–G).

ML385 counteracted the anti-apoptosis and antioxidant effects of ursolic acid

Inflammation increased the percentage of apoptotic cells and intracellular ROS. UA attenuated inflammation-induced apoptosis and ROS accumulation, which were nearly abrogated by administration of ML385 (Figure 6). It suggested that the anti-apoptotic and antioxidant effects of UA were mainly achieved by activating Nrf2.

Docking analysis

For a better understanding of the correlation between ursolic acid and Nrf2 domain, AutoDock Vina (Molecular Graphics Lab at the Scripps Research Institute) was employed for the receptor and

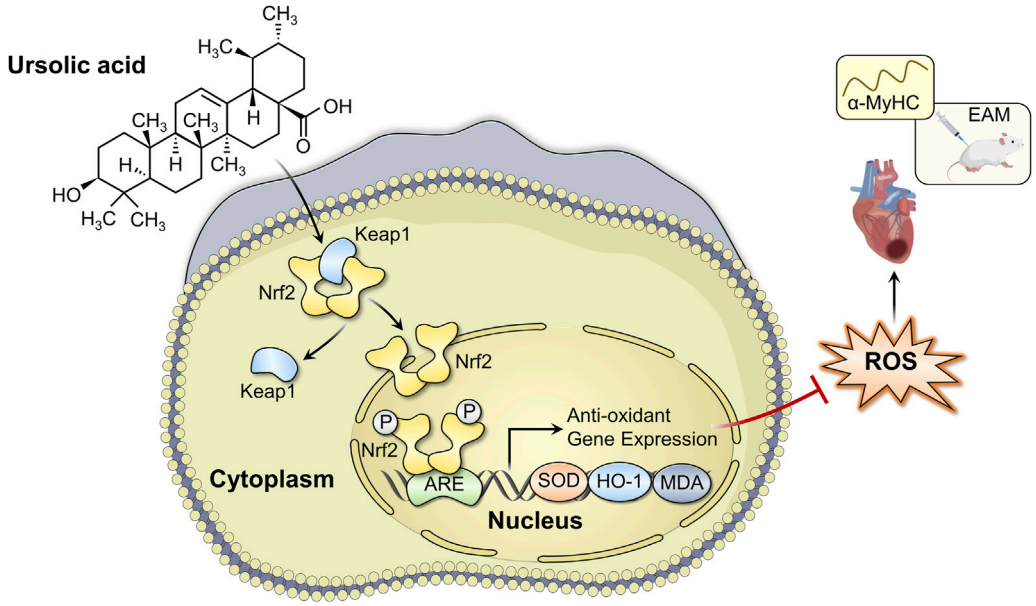

FIGURE 8
Schematic diagram of the mechanism of ursolic acid in the treatment of experimental autoimmune myocarditis.

ligand docking analysis. An interacting complex of Nrf2 and ursolic acid was obtained after docking the molecular proteins with different conformations and poses. The 3D molecular simulation illustrate the binding modes of ursoilc acid with Nrf2 via the hydrogen bond and other interactions (Figure 7A). The binding energy between Nrf2 and ursolic is −6.9 kCal/mol. In Figure 7B, Nrf2 primarily relied on hydrogen bonds to interact with ursolic acid, ursolic acid forms a hydrogen bond (2.78 Å) with Lys462 and a hydrogen bond (3.04 Å). In addition, ursoilc acid also interacts with Pro467 and Leu 450 via hydrophobic interactions, which are shown as arcs (red) in Figure 7B.

Discussion

In the present study, we found that UA could reduce EAM-induced myocardial injury through the oxidative stress pathway. UA ameliorated EAM-induced myocardial injury by scavenging ROS and activating the Nrf2/HO-1 pathway.

Despite the host-activated immune response, many studies have confirmed the presence of multiple anti-myocardial antibodies in patients with myocarditis and DCM, the most common of which are anti-cardiac myosin antibodies (Caforio et al., 2008). In the present study, the EAM model in mice is induced by immunizing mice with myocardial self-antigens, which simulates the autoimmunological process of acute myocarditis (Pando et al., 2010; Zempo et al., 2015). An autoimmune response is a common pathological process of myocarditis caused by various pathogenic factors. The EAM model has been used in many studies to investigate the pathogenesis of clinical myocarditis and post-myocarditis cardiac dysfunction (Tada and Suzuki, 2016). Therefore, we select the EAM model to further investigate the role and potential mechanisms of UA in protection against myocarditis.

In an abnormal immune response in the myocardium, antigen presence stimulates the differentiation and proliferation of many T cells in the peripheral immune organs, which migrates from the blood circulation to the heart. Lymphocytes infiltrate the myocardium and release inflammatory cytokines such as IL-6, IL-17, and IL-23, causing cardiomyocyte hypertrophy or death, resulting in myocardial hypertrophy or fibrosis and cardiac systolic or diastolic dysfunction (Vdovenko and Eriksson, 2018; Chang et al., 2019). IL-6 plays an important role in the inflammatory storm of myocarditis. Yamashita et al. demonstrated that blocking IL-6 receptors prior to immunization inhibited the development of autoimmune myocarditis in mice. In a study of autoimmune myocarditis in IL-6-deficient mice, it was found that the prevalence and severity of myocarditis were markedly reduced in the absence of IL-6. These may provide evidences for our selection of IL-6 as the primary inflammatory agent. Therefore, in the present study, we provide experimental evidence that IL-6 stimulation of H9c2 cells causes ROS production and cell apoptosis (Figure 6).

In recent years, Nrf2 has been shown to have a protective role in major cardiovascular diseases (CVDs) in murine models. It can improve cardiac function and reduce efferocytosis and the release of pro-inflammatory cytokines, thereby improving the pathological phenotype (Gu et al., 2017; Shen et al., 2019; Chen et al., 2021). Nrf2 is the master regulator of antioxidant responses. The activation of the Nrf2-dependent antioxidant system is important in cellular defense against oxidative stress damage, and the deficiency of the Nrf2 system may pose a significant risk to the cardiovascular system (da Costa et al., 2019). Curcumin, a natural Nrf2 activator, inhibits oxidative stress and inflammation caused by free fatty acid treatment of cardiomyocytes, implying that Nrf2 is closely associated with the risk of obesity-related metabolic cardiovascular disease (Zeng et al., 2015). In experimental diabetic cardiomyopathy, reduced Nrf2 activity was

observed, as was antioxidant enzyme activity downstream and increased oxidative stress (Wang et al., 2017). In addition, numerous studies have demonstrated that Nrf2 protects against atherosclerosis by reducing vascular smooth muscle cell (VSMC) migration, proliferation, calcification, and vascular remodeling (Duckers et al., 2001; Ashino et al., 2013; Ashino et al., 2016), although it has not been thoroughly studied that Nrf2-ARE may play a regulatory role in myocarditis (Tada and Suzuki, 2016). Our findings revealed that EAM downregulates the Nrf2 protein expression and reduces the nuclear entry of Nrf2. Moreover, EAM downregulates Nrf2 transcription regulation of phase II antioxidant enzyme HO-1, increases the oxidative stress-induced lipid peroxidation index Malondialdehyde (MDA) and cell apoptosis in heart. Under oxidative conditions, Nrf2 translocates from the cytoplasm to the nucleus, which binds to AREs to regulate downstream genes, such as HO-1, in the present study. These findings are consistent with a previous study that describe the role of Nrf2 upregulation in protecting the EAM-induced cardiomyocyte from oxidant stress damage (Jaén et al., 2020). We determined that UA can reduce damage associated with oxidative stress in the EAM model by activating the Nrf2 pathway and its antioxidant targets. To further validate this view, in the present study, we identified Nrf2 as the primary target of UA through the inhibition of Nrf2 by ML385, which nearly reversed the beneficial effects of UA on apoptosis and oxidative stress damage.

Currently, the prevailing view is that oxidative stress exacerbates the dysfunction and structural remodeling of myocarditis. There are also many studies for the effects of antioxidants. Meanwhile, more and more molecular targets and new drugs have been discovered. However, due to the difficulty of manufacturing the drugs and safety, clinical translation cannot be achieved. In contrast, ursolic acid has following advantages, which make it promising as an antioxidant to assist in EAM treatment: 1) UA is a natural product ingredient that can be extracted from natural plants (e.g., apple peels) in plentiful quantities and at a relatively cheap price; 2) UA probably provides a better safety profile in clinical use, even people with a high risk of myocarditis may benefit from taking UA as a health supplement. Furthermore, the findings of the present study on the regulatory role of UA in EAM broaden our understanding of the multiple functions of UA in the heart. These findings could serve as a model for drug development in autoimmune myocarditis. The results, along with previous studies on the role of UA in myocardial infarction and atherosclerosis, suggest that UA may be useful for various clinical indications in CVDs.

However, there are several limitations to present study. To further validate the role of Nrf2/HO-1 signaling pathway in H9c2, we selected Nrf2 inhibitor, ML385, rather than Nrf2 siRNA, which is relatively less persuasion. Furthermore, the antioxidant mechanisms of ursolic acid are complex and still need further study.

Conclusion

In conclusion, we have demonstrated that UA administration could alleviate EAM-induced myocardial injury, as evidenced by improvements in cardiac functions, cardiac inflammation, and structural remodeling, partially by attenuating oxidative stress in a Nrf2/HO-1 pathway-dependent manner (Figure 8). The present study indicates that UA may be a therapeutic candidate drug for the treatment of myocarditis.

Data availability statement

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

Ethics statement

The animal study was reviewed and approved by Institutional Animal Care and Use Committee, Huazhong University of Science and Technology.

Author contributions

YF, TL and JW designed this research; YF, SH, YB and JQ performed the experiments; YF, YZ, YT and JS analyzed and interpreted the results; YF drafted manuscript; TL, QJ and WD revised the manuscript; YC, JW and MX determined the final version of manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1189372/full#supplementary-material

References

Ashino, T., Yamamoto, M., and Numazawa, S. (2016). Nrf2/Keap1 system regulates vascular smooth muscle cell apoptosis for vascular homeostasis: Role in neointimal formation after vascular injury. *Sci. Rep.* 6, 26291. doi:10.1038/srep26291

Ashino, T., Yamamoto, M., Yoshida, T., and Numazawa, S. (2013). Redox-sensitive transcription factor Nrf2 regulates vascular smooth muscle cell migration and neointimal hyperplasia. *Arterioscler. Thromb. Vasc. Biol.* 33 (4), 760–768. doi:10.1161/atvbaha.112.300614

Caforio, A. L., Marcolongo, R., Basso, C., and Iliceto, S. (2015). Clinical presentation and diagnosis of myocarditis. *Heart (British Card. Soc.* 101 (16), 1332–1344. doi:10.1136/heartjnl-2014-306363

Caforio, A. L., Tona, F., Bottaro, S., Vinci, A., Dequal, G., Daliento, L., et al. (2008). Clinical implications of anti-heart autoantibodies in myocarditis and dilated cardiomyopathy. *Autoimmunity* 41 (1), 35–45. doi:10.1080/08916930701619235

Chang, H., Zhao, F., Xie, X., Liao, Y., Song, Y., Liu, C., et al. (2019). PPARα suppresses Th17 cell differentiation through IL-6/STAT3/RORγt pathway in experimental autoimmune myocarditis. *Exp. Cell. Res.* 375 (1), 22–30. doi:10.1016/j.yexcr.2018.12.005

Chen, X., Wan, W., Guo, Y., Ye, T., Fo, Y., Sun, Y., et al. (2021). Pinocembrin ameliorates post-infarct heart failure through activation of Nrf2/HO-1 signaling pathway. *Mol. Med. Camb. Mass)* 27 (1), 100. doi:10.1186/s10020-021-00363-7

Cui, T., Li, J. Z., Kayahara, H., Ma, L., Wu, L. X., and Nakamura, K. (2006). Quantification of the polyphenols and triterpene acids in Chinese hawthorn fruit by high-performance liquid chromatography. *J. Agric. food Chem.* 54 (13), 4574–4581. doi:10.1021/jf060310m

da Costa, R. M., Rodrigues, D., Pereira, C. A., Silva, J. F., Alves, J. V., Lobato, N. S., et al. (2019). Nrf2 as a potential mediator of cardiovascular risk in metabolic diseases. *Front. Pharmacol.* 10, 382. doi:10.3389/fphar.2019.00382

Dinkova-Kostova, A. T., Kostov, R. V., and Kazantsev, A. G. (2018). The role of Nrf2 signaling in counteracting neurodegenerative diseases. *FEBS J.* 285 (19), 3576–3590. doi:10.1111/febs.14379

Duckers, H. J., Boehm, M., True, A. L., Yet, S. F., San, H., Park, J. L., et al. (2001). Heme oxygenase-1 protects against vascular constriction and proliferation. *Nat. Med.* 7 (6), 693–698. doi:10.1038/89068

Felker, G. M., Thompson, R. E., Hare, J. M., Hruban, R. H., Clemetson, D. E., Howard, D. L., et al. (2000). Underlying causes and long-term survival in patients with initially unexplained cardiomyopathy. *N. Engl. J. Med.* 342 (15), 1077–1084. doi:10.1056/nejm200004133421502

Frustaci, A., Russo, M. A., and Chimenti, C. (2009). Randomized study on the efficacy of immunosuppressive therapy in patients with virus-negative inflammatory cardiomyopathy: The TIMIC study. *Eur. Heart J.* 30 (16), 1995–2002. doi:10.1093/eurheartj/ehp249

Gu, J., Cheng, Y., Wu, H., Kong, L., Wang, S., Xu, Z., et al. (2017). Metallothionein s downstream of Nrf2 and partially mediates sulforaphane prevention of diabetic cardiomyopathy. *Diabetes* 66 (2), 529–542. doi:10.2337/db15-1274

Jaén, R. I., Fernández-Velasco, M., Terrón, V., Sánchez-García, S., Zaragoza, C., Canales-Bueno, N., et al. (2020). BML-111 treatment prevents cardiac apoptosis and oxidative stress in a model of autoimmune myocarditis. *FASEB J. official Publ. Fed. Am. Soc. Exp. Biol.* 34 (8), 10531–10546. doi:10.1096/fj.202000611R

Jensen, L. D., and Marchant, D. J. (2016). Emerging pharmacologic targets and treatments for myocarditis. *Pharmacol. Ther.* 161, 40–51. doi:10.1016/j.pharmthera.2016.03.006

Jia, Z., Li, W., Bian, P., Yang, L., Liu, H., Pan, D., et al. (2021). Ursolic acid treats renal tubular epithelial cell damage induced by calcium oxalate monohydrate via inhibiting oxidative stress and inflammation. *Bioengineered* 12 (1), 5450–5461. doi:10.1080/21655979.2021.1955176

Kobayashi, A., Kang, M. I., Watai, Y., Tong, K. I., Shibata, T., Uchida, K., et al. (2006). Oxidative and electrophilic stresses activate Nrf2 through inhibition of ubiquitination activity of Keap1. *Mol. Cell. Biol.* 26 (1), 221–229. doi:10.1128/mcb.26.1.221-229.2006

Lin, L., Yin, Y., Hou, G., Han, D., Kang, J., and Wang, Q. (2017). Ursolic acid attenuates cigarette smoke-induced emphysema in rats by regulating PERK and Nrf2 pathways. *Pulm. Pharmacol. Ther.* 44, 111–121. doi:10.1016/j.pupt.2017.03.014

Liobikas, J., Majiene, D., Trumbeckaite, S., Kursvietiene, L., Masteikova, R., Kopustinskiene, D. M., et al. (2011). Uncoupling and antioxidant effects of ursolic acid in isolated rat heart mitochondria. *J. Nat. Prod.* 74 (7), 1640–1644. doi:10.1021/np200060p

López-Hortas, L., Pérez-Larrán, P., González-Muñoz, M. J., Falqué, E., and Domínguez, H. (2018). Recent developments on the extraction and application of ursolic acid. A review. *Food Res. Int. Ott. Ont)* 103, 130–149. doi:10.1016/j.foodres.2017.10.028

Pando, R., Barshack, I., Raz, A., Luboshits, G., Haklai, R., Maysel-Auslender, S., et al. (2010). The Ras antagonist farnesylthiosalicylic acid ameliorates experimental myocarditis in the rat. *Cardiovasc. pathology official J. Soc. Cardiovasc. Pathology* 19 (2), 94–101. doi:10.1016/j.carpath.2008.10.009

Saravanan, R., and Pugalendi, V. (2006). Impact of ursolic acid on chronic ethanol-induced oxidative stress in the rat heart. *Pharmacol. Rep. PR.* 58 (1), 41–47.

Seeram, N. P. (2008). Berry fruits for cancer prevention: Current status and future prospects. *J. Agric. food Chem.* 56 (3), 630–635. doi:10.1021/jf072504n

Shen, Y., Liu, X., Shi, J., and Wu, X. (2019). Involvement of Nrf2 in myocardial ischemia and reperfusion injury. *Int. J. Biol. Macromol.* 125, 496–502. doi:10.1016/j.ijbiomac.2018.11.190

Shi, W., Ma, H., Liu, T., Yan, D., Luo, P., Zhai, M., et al. (2020). Inhibition of Interleukin-6/glycoprotein 130 signalling by Bazedoxifene ameliorates cardiac remodelling in pressure overload mice. *J. Cell. Mol. Med.* 24 (8), 4748–4761. doi:10.1111/jcmm.15147

Silva, F. S., Oliveira, P. J., and Duarte, M. F. (2016). Oleanolic, ursolic, and betulinic acids as food supplements or pharmaceutical agents for type 2 diabetes: Promise or illusion? *J. Agric. food Chem.* 64 (15), 2991–3008. doi:10.1021/acs.jafc.5b06021

Tada, Y., and Suzuki, J. (2016). Oxidative stress and myocarditis. *Curr. Pharm. Des.* 22 (4), 450–471. doi:10.2174/1381612822666151222160559

Tannock, L. R. (2011). Ursolic acid effect on atherosclerosis: Apples and apples, or apples and oranges? *Atherosclerosis* 219 (2), 397–398. doi:10.1016/j.atherosclerosis.2011.09.029

Vdovenko, D., and Eriksson, U. (2018). Regulatory role of CD4(+) T cells in myocarditis. *J. Immunol. Res.* 2018, 4396351. doi:10.1155/2018/4396351

Wang, S., Wang, B., Wang, Y., Tong, Q., Liu, Q., Sun, J., et al. (2017). Zinc prevents the development of diabetic cardiomyopathy in db/db mice. *Int. J. Mol. Sci.* 18 (3), 580. doi:10.3390/ijms18030580

Wang, X., Ikejima, K., Kon, K., Arai, K., Aoyama, T., Okumura, K., et al. (2011). Ursolic acid ameliorates hepatic fibrosis in the rat by specific induction of apoptosis in hepatic stellate cells. *J. Hepatol.* 55 (2), 379–387. doi:10.1016/j.jhep.2010.10.040

Zempo, H., Sugita, Y., Ogawa, M., Watanabe, R., Suzuki, J., and Isobe, M. (2015). A P2X7 receptor antagonist attenuates experimental autoimmune myocarditis via suppressed myocardial CD4+ T and macrophage infiltration and NADPH oxidase 2/4 expression in mice. *Heart vessels* 30 (4), 527–533. doi:10.1007/s00380-014-0527-2

Zeng, C., Zhong, P., Zhao, Y., Kanchana, K., Zhang, Y., Khan, Z. A., et al. (2015). Curcumin protects hearts from FFA-induced injury by activating Nrf2 and inactivating NF-κB both *in vitro* and *in vivo*. *J. Mol. Cell. Cardiol.* 79, 1–12. doi:10.1016/j.yjmcc.2014.10.002

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Commentary: Identification of cuproptosis hub genes contributing to the immune microenvironment in ulcerative colitis using bioinformatic analysis and experimental verification

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KEYWORDS

inflammatory bowel disease, ulcerative colitis, cuproptosis, bioinformatics, FDX1

A Commentary on

Identification of cuproptosis hub genes contributing to the immune microenvironment in ulcerative colitis using bioinformatic analysis and experimental verification

by Yang C, Wang W, Li S, Qiao Z, Ma X, Yang M, Zhang J, Cao L, Yao S, Yang Z and Wang W (2023) *Front. Immunol.* 14:1113385. doi: 10.3389/fimmu.2023.1113385

Cuproptosis is an entirely novel copper-dependent regulated cell death that differs morphologically, biochemically, and genetically from apoptosis, autophagy and ferroptosis (1). Yang et al. (2) and Chen et al. (3) conducted studies that suggested a potential therapeutic role of cuproptosis in inflammatory bowel disease (IBD). They reached this conclusion by employing bioinformatics analysis and experimental validation using dextran sodium sulfate (DSS)-induced mouse models. Yang et al. confirmed the therapeutic effects of three cuproptosis-related genes (*DLAT*, *DLD*, and *PDHA1*) on immune infiltration in patients with ulcerative colitis (UC). Similarly, Chen et al. found that Crohn's disease (CD), UC, celiac disease (CEL), and IBD-induced cancer (IBD-CA) had common cuproptosis-related differentially expressed genes, including *DLAT*, *LIAS*, *DBT*, and *PDHA1*. Both these studies had positive implications for mechanism exploration; however, further analyses of their results are warranted.

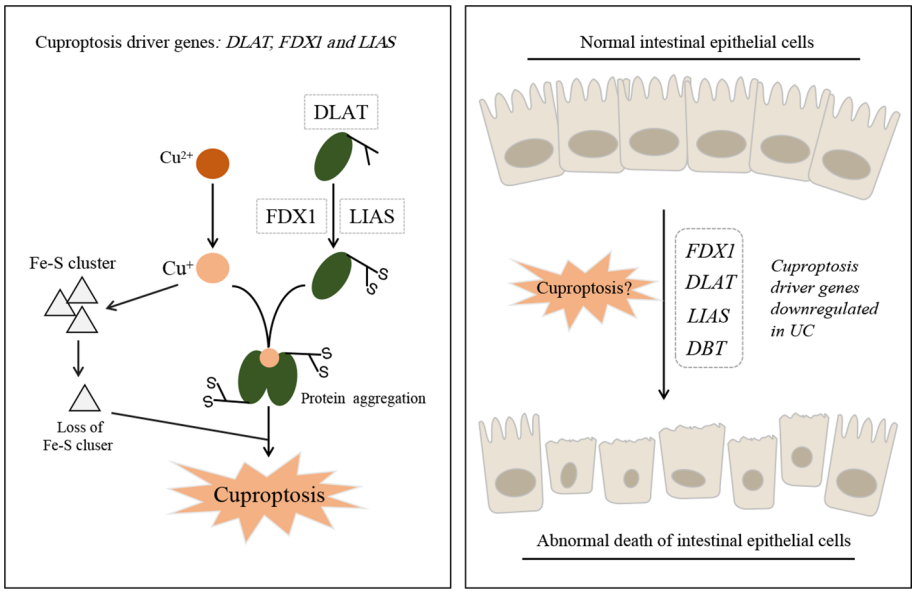

FIGURE 1
Schematic model of cuproptosis and ulcerative colitis. Cu^{+} binds to lipoylated mitochondrial enzymes DLAT and induced the aggregation of these proteins. Mitochondrial ferredoxin (FDX1) and lipoyl synthase (LIAS) are key driver genes of cuproptosis (Left). Abnormal death of intestinal epithelial cells can lead to the occurrence of ulcerative colitis, but the cuproptosis driver genes were identifiied as down-regulated in UC patients (Right).

Tsvetkov P et al. first discovered cuproptosis in 2022 (1), and since then, 12 cuproptosis-related genes, namely *FDX1, LIAS, DLAT, DLD, PDHA1, PDHB, MTF1, GLS, CDKN2A, LIPT1, ATP7B*, and *SLC31A1*, have been validated. This gene set is currently used in many disease studies using bioinformatics analysis. Especially, *DLAT, DLA, PDHA1, LIAS*, and *DBT* are known to promote cuproptosis (Figure 1); hence, the downregulation of these genes prevents cuproptosis. Of note, the promotion of tumor cell death and inhibition of non-tumor cell death are opposing treatment strategies. For example, ferroptosis inhibition alleviates experimental colitis (4) while ferroptosis promotion in tumor cells is a novel therapeutic approach for cancer (5). However, in the above-mentioned studies, the expression of these genes in the intestinal mucosa samples of IBD was found to be downregulated compared to individuals without IBD (Figure 1). According to Tsvetkov P et al. (1), *FDX1* is a key cuproptosis regulator that reduces Cu^{2+} to the more toxic Cu^{+}, thus promoting abnormal oligomerization of thioacylated proteins in the tricarboxylic acid cycle. However, Yang et al. (2) found *FDX1* expression to be downregulated in patients with IBD compared to normal individuals. This seems contradictory if we assume that cuproptosis occurs in the intestinal mucosa of patients with IBD. The contradictory results suggest two things: (I) the downregulation of these genes may not be related to cuproptosis, and may only be as a result of the disease itself; (II) The regulatory mechanism of cuproptosis in normal intestinal mucosal cells could be opposite to that in tumor cells. These hypotheses require further validation.

UC is characterized by recurring inflammatory episodes limited to the colon's mucosal layer (6). Promoting mucosal healing, even at the cell level, has been a potential strategy for IBD, including UC, treatment (7). Therefore, inhibiting abnormal intestinal epithelial cell death to maintain intestinal mucosal homeostasis is a potential intervention strategy. Both studies validated their results using DSS-induced colitis mouse models. Yang et al. conducted a quantitative analysis of four cuproptosis genes using western blot, which further validated the results from their bioinformatics analysis; however, whether the samples used in the experiment were whole colon tissue or intestinal mucosal cells has not been mentioned. The use of intestinal mucosal cells alone for validation will provide more accurate results, as the bioinformatics analysis samples were also from the intestinal mucosa of patients with IBD.

More studies are still needed to verify whether cuproptosis occurs in the intestinal mucosal cells of patients with IBD. Additionally, the detection of cell copper is also necessary.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

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References

1. Tsvetkov P, Coy S, Petrova B, Dreishpoon M, Verma A, Abdusamad M, et al. Copper induces cell death by targeting lipoylated TCA cycle proteins. *Science* (2022) 375(6586):1254–61. doi: 10.1126/science.abf0529

2. Yang C, Wang W, Li S, Qiao Z, Ma X, Yang M, et al. Identification of cuproptosis hub genes contributing to the immune microenvironment in ulcerative colitis using bioinformatic analysis and experimental verification. *Front Immunol* (2023) 14:1113385. doi: 10.3389/fimmu.2023.1113385

3. Chen Y, Li X, Sun R, Ji J, Yang F, Tian W, et al. A broad cuproptosis landscape in inflammatory bowel disease. *Front Immunol* (2022) 13:1031539. doi: 10.3389/fimmu.2022.1031539

4. Xu M, Tao J, Yang Y, Tan S, Liu H, Jiang J, et al. Ferroptosis involves in intestinal epithelial cell death in ulcerative colitis. *Cell Death Dis* (2020) 11(2):86. doi: 10.1038/s41419-020-2299-1

5. Lei G, Zhuang L, Gan B. Targeting ferroptosis as a vulnerability in cancer. *Nat Rev Cancer* (2022) 22(7):381–96. doi: 10.1038/s41568-022-00459-0

6. Ordás I, Eckmann L, Talamini M, Baumgart DC, Sandborn WJ. Ulcerative colitis. *Lancet* (2012) 380(9853):1606–19. doi: 10.1016/S0140-6736(12)60150-0

7. Le Berre C, Ricciuto A, Peyrin-Biroulet L, Turner D. Evolving short- and long-term goals of management of inflammatory bowel diseases: getting it right, making it last. *Gastroenterology* (2022) 162(5):1424–38. doi: 10.1053/j.gastro.2021.09.076

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Baricitinib treatment for refractory skin changes in POEMS syndrome: a case report

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Polyneuropathy, organomegaly, endocrinopathy, monoclonal plasma cell disorder, and skin changes (POEMS) syndrome is a multisystem disorder that has limited treatment options. Here, we described a case of a 55-year-old female subject who was treated for multiple drugs, but the skin symptoms continued to progress; the patient responded well to baricitinib. This suggests that JAK/STAT signaling pathways play an essential role in the pathological process of POEMS syndrome.

KEYWORDS

POEMS syndrome, Janus kinase inhibitor (JAKI), JAK/STAT signaling, skin changes, case report

1 Introduction

Polyneuropathy, organomegaly, endocrinopathy, monoclonal plasma cell disorder, and skin changes (POEMS) syndrome is a multisystem disorder that has limited treatment options (Brown and Ginsberg, 2019). Majority of patients with POEMS syndrome present with hyperpigmentation, hemangiomas, and hypertrichosis. However, most patients do not benefit from the current approaches available for the treatment of these cutaneous symptoms. Herein, we report the case of a patient with POEMS syndrome who presented with six types of skin changes and responded well to baricitinib, with all skin lesions resolving over the treatment period.

2 Case report

The patient is a 55-year-old female subject, who, in 2014, was initially diagnosed with systemic sclerosis due to pulmonary fibrosis and skin changes, including hypertrichosis of the limbs and jaws, thickening and swelling of the skin, hyperpigmentation, hemangiomas, Raynaud's phenomenon, and swelling of the back and lower limbs. Skin biopsy of the right leg revealed hyperkeratosis of the epidermis, mild hyperplasia of the dermis, and sparse collagen fibers in the middle without dermal vascularization. In 2016, the patient complained of bilateral lower limb pain, numbness, and paresthesia. An electrophysiological examination suggested multiple peripheral neuropathies. Ultrasonography revealed bilateral lymphadenopathy in the neck and supraclavicular, axillary, inguinal, mesenteric, and retroperitoneal regions. Laboratory tests revealed diabetes mellitus and mild renal dysfunction, as well as serum vascular endothelial growth factor (VEGF) levels of 7,924 pg/mL (normal range: 0–600 pg/mL). Furthermore, serum protein electrophoresis revealed a monoclonal band of IgA lambda +. Consequently, the patient was diagnosed with

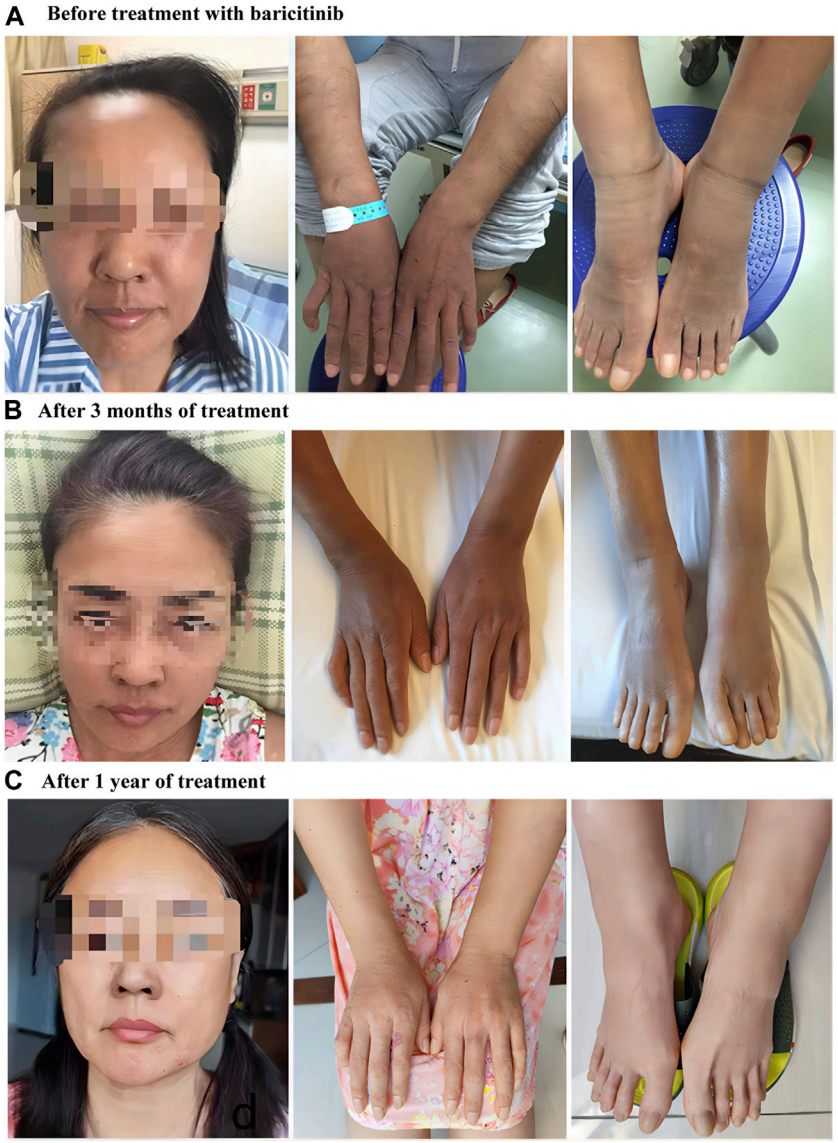

FIGURE 1
Prior to the treatment with baricitinib, hyperpigmentation, skin thickening on the face, upper and lower limbs with hypertrichosis and hemangiomas in the extensor lateral forearms, and swollen lower limbs were observed **(A)**. The skin changes have partially dissipated after 3 months of treatment **(B)** and have been almost fully reversed after 1 year **(C)**.

POEMS syndrome due to multiple peripheral neuropathy symptoms, skin changes, and a high level of VEGF, based on the current diagnostic criteria for POEMS syndrome, i.e., the Dispenzieri diagnostic criteria (Miest et al., 2013).

The patient refused bortezomib; therefore, an alternative treatment regimen was adopted. Despite treatment with multiple drugs, including thalidomide, cyclophosphamide, glucocorticoids, and Kunxian capsules, supplemented with the usual dose, the skin symptoms continued to progress, while other non-skin symptoms improved. Hence, the treatment plan was modified, and oral baricitinib (2 mg/day) was administered. The cutaneous symptoms improved after 3 months of baricitinib treatment, and full recovery was noted after 1 year (Figure 1). The patient showed sustained improvement in the skin symptoms over 18 months of follow-up, and serum VEGF levels decreased considerably to 50.62 pg/mL.

3 Discussion

The Janus kinase (JAK)/STAT pathway is involved in various skin disorders, including alopecia areata, atopic dermatitis, lupus erythematosus, dermatomyositis, psoriasis, and vitiligo (Solimani et al., 2019). Vitiligo is an autoimmune skin disease in which melanocytes are reduced in association with the activation of the interferon-γ (IFN-γ) and CXCL-10 signaling pathways (Frisoli et al., 2020). The JAK inhibitor baricitinib, which has been shown to block IFN-γ signaling and contribute to re-pigmentation, has been approved for treatment of patients with vitiligo (Qi et al., 2021; Yan et al., 2022). Interestingly, in the present case, hyperpigmentation was treated with baricitinib, which is in contrast to the skin symptoms of vitiligo.

To the best of our knowledge, this is the first report on the successful use of baricitinib to treat skin symptoms associated

with POEMS syndrome. The pathogenic mechanisms underlying the skin symptoms associated with POEMS syndrome are poorly understood. However, VEGF has been identified as a key cytokine involved in the pathogenesis of this disorder and is known to reflect the disease activity (Dispenzieri, 2017). Du et al. reported that VEGF expression is associated with JAK/STAT signaling in psoriasis (Du et al., 2020). The role of JAK/STAT in rheumatoid arthritis and new vessel formation has also been reported. Paola et al. explored the anti-angiogenic role of tofacitinib, another JAK inhibitor, in experimental arthritis, which was attributed to inhibition of the pro-angiogenic effects of VEGF (Di Benedetto et al., 2021). Further evidence has confirmed that among the tyrosine kinase cell receptor (VEGFR) 2-mediated signaling pathways, the JAK/STAT signaling pathway, especially the STAT3 signaling pathway, is a critical target and biomarker of angiogenesis (Zhang et al., 2011). Given these findings and our observation with the present case, we propose that angiogenesis is the primary process involved in the pathogenesis of cutaneous changes in patients with POEMS syndrome.

4 Conclusion

Baricitinib is a promising treatment for skin symptoms in patients with POEMS, which can be attributed to the targeting of JAK/STAT signaling to inhibit angiogenesis. However, further studies are required to better understand the pathogenic mechanisms underlying POEMS syndrome, specifically the role of JAK/STAT3 signaling and angiogenesis.

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 authors.

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article. Written informed consent was obtained from the participant/patient(s) for the publication of this case report.

Author contributions

JX, ZL, and YJ prepared the study documents. DT, XQ, and EJ were responsible for patient contact and data collection. JX and JZ drafted the manuscript. All authors contributed to the article and approved the submitted version.

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References

Brown, R., and Ginsberg, L. (2019). POEMS syndrome: clinical update. *J. Neurol.* 266, 268–277. (1432-1459 (Electronic)). doi:10.1007/s00415-018-9110-6

Di Benedetto, P., Ruscitti, P., Berardicurti, O. A-O., Panzera, N., Grazia, N., Di Vito Nolfi, M., et al. (2021). Blocking Jak/STAT signalling using tofacitinib inhibits angiogenesis in experimental arthritis. *Arthritis Res. Ther.* 23, 213. (1478-6362 (Electronic)). doi:10.1186/s13075-021-02587-8

Dispenzieri, A. (2017). POEMS syndrome: 2017 update on diagnosis, risk stratification, and management. *Am. J. Hematol.* 92, 814–829. (1096-8652 (Electronic)). doi:10.1002/ajh.24802

Du, Y., Jiang, S., Cheng, L., and Liu, J. (2020). JAK/STAT and VEGF/PAK1 signaling as emerging targets for topical treatment of psoriasis: a pilot study. *Int. J. Clin. Exp. Pathol.* 13, 3111–3119. (1936-2625 (Electronic)).

Frisoli, M. L., Essien, K., and Harris, J. E. (2020). Vitiligo: mechanisms of pathogenesis and treatment. *Annu. Rev. Immunol.* 38, 621–648. (1545-3278 (Electronic)). doi:10.1146/annurev-immunol-100919-023531

Miest, R. Y., Comfere, N., Dispenzieri, A., Fau-Lohse, C. M., and el-Azhary, R. A. (2013). Cutaneous manifestations in patients with POEMS syndrome. *Int. J. dermatology* 52, 1349–1356. (1365-4632 (Electronic)). doi:10.1111/j.1365-4632.2012.05648.x

Qi, F., Liu, F., and Gao, L. (2021). Janus kinase inhibitors in the treatment of vitiligo: a review. *Front. Immunol.* 12, 790125. (1664-3224 (Electronic)). doi:10.3389/fimmu.2021.790125

Solimani, F., Meier, K., and Ghoreschi, K. (2019). Emerging topical and systemic JAK inhibitors in dermatology. *Front. Immunol.* 10, 2847. (1664-3224 (Electronic)). doi:10.3389/fimmu.2019.02847

Yan, T. M., Zhang, H., Wu, X. Y., and Zhang, Z. Y. (2022). Successful treatment of generalized granuloma annulare with baricitinib. *J. Eur. Acad. Dermatol Venereol.* 36, e500–e502. (1468-3083 (Electronic)). doi:10.1111/jdv.18031

Zhang, X., Song, Y., Wu, Y., Dong, Y., Lai, L., Zhang, J., et al. (2011). Indirubin inhibits tumor growth by antitumor angiogenesis via blocking VEGFR2-mediated JAK/STAT3 signaling in endothelial cell. *Int. J. Cancer* 129, 2502–2511. (1097-0215 (Electronic)). doi:10.1002/ijc.25909

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Tangeretin attenuates bleomycin-induced pulmonary fibrosis by inhibiting epithelial-mesenchymal transition via the PI3K/Akt pathway

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Background: Pulmonary fibrosis (PF) is a terminal pathological change in a variety of lung diseases characterized by excessive deposition of extracellular matrix, for which effective treatment is lacking. Tangeretin (Tan), a flavonoid derived from citrus, has been shown to have a wide range of pharmacological effects. This study aimed to investigate the role and potential mechanisms of Tan on pulmonary fibrosis.

Methods: A model of pulmonary fibrosis was established by administering bleomycin through tracheal drip, followed by administering Tan or pirfenidone through gavage. HE and Masson staining were employed to assess the extent of pulmonary fibrosis. Subsequently, Western blot, enzyme-linked immunosorbent assay (ELISA), RNA sequencing, and immunohistochemistry techniques were employed to uncover the protective mechanism of Tan in PF mice. Furthermore, A549 cells were stimulated with TGF-β1 to induce epithelial-mesenchymal transition (EMT) and demonstrate the effectiveness of Tan in mitigating PF.

Results: Tan significantly ameliorated bleomycin-induced pulmonary fibrosis, improved fibrotic pathological changes, and collagen deposition in the lungs, and reduced lung inflammation and oxidative stress. The KEGG pathway enrichment analysis revealed a higher number of enriched genes in the PI3K/Akt pathway. Additionally, Tan can inhibit the EMT process related to pulmonary fibrosis.

Conclusion: Taken together, the above research results indicate that Tan suppresses inflammation, oxidative stress, and EMT in BLM-induced pulmonary fibrosis via the PI3K/Akt pathway and is a potential agent for the treatment of pulmonary fibrosis.

KEYWORDS

pulmonary fibrosis, tangeretin, PI3K/Akt signaling pathway, epithelial-mesenchymal transition, bleomycin

1 Introduction

Pulmonary fibrosis (PF) is a chronic and lethal lung disease that is characterized by the excessive deposition of extracellular matrix (ECM), resulting in impaired gas exchange and lung function (Moss et al., 2022; C; Wang and Yang, 2022; J; Yang et al., 2033a). The most prevalent form of PF is idiopathic pulmonary fibrosis, which has a bleak prognosis and a median survival rate of 2.5–3.5 years following diagnosis (Strongman et al., 2018). Pulmonary fibrosis is associated with multiple risk factors, such as genetic predisposition, exposure to radiation and environmental toxins, adverse drug reactions, and age and gender (Mathai and Schwartz, 2019). Additionally, various respiratory viruses, including the common influenza virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and respiratory syncytial virus, have been identified as potential causative agents of pulmonary fibrosis (Wang et al., 2017; Chiou et al., 2023; W; Yang et al., 2022). Currently, pulmonary fibrosis is treated with pirfenidone (PFD) and nintedanib, which have limited efficacy in halting the progression of fibrosis and are associated with varying degrees of adverse effects, thereby hindering their widespread use (Trachalaki et al., 2021). Consequently, novel therapeutic approaches are warranted for the prevention and management of pulmonary fibrosis.

The pathogenesis of pulmonary fibrosis remains uncertain; however, current consensus suggests that it initiates with anomalous tissue restoration following injury (Sun et al., 2023; H; Li, Zhao, Tian, et al., 2020). Following lung injury, alveolar type II (AT2) cells participate in the repair process through self-renewal and differentiation into alveolar type I epithelial cells (AT1)(L. Shao et al., 2021; P; Wang, Yan, et al., 2022; Tan et al., 2021). Furthermore, AT2 cells may acquire a mesenchymal phenotype via epithelial-mesenchymal transition (EMT), stimulate fibroblast activation and differentiation, and generate a substantial quantity of extracellular matrix (ECM) molecules implicated in pulmonary fibrosis (Olajuyin et al., 2019; Zhou et al., 2022). Transforming growth factor-β1 (TGF-β1) is acknowledged as a pro-fibrotic factor in various organs (Meng et al., 2016). Its mechanism of action involves binding to the TGF-β type II receptor on the AT2 cell membrane and recruiting the TGF-β type I receptor to the plasma membrane, resulting in the formation of a heterotrimer that activates the downstream Smads signaling pathway and the expression of EMT-related transcription factors Snail, Slug, and Twist1(P. Wang, Yan, et al., 2022; Garg, 2013). Additionally, TGF-β1 can induce EMT through a non-Smad-dependent pathway, whereby it stimulates the expression of the PI3K subunit p110 and phosphorylation of Akt to promote EMT (Y.E. Zhang, 2017; Saito et al., 2017). The activation of Akt is facilitated through the regulation of its downstream proteins, such as GSK-3β, mTOR, HIF-1α, and NF-κB, which are implicated in the pathogenesis of pulmonary fibrosis (Qin et al., 2021; Peng et al., 2022; Xu et al., 2022).

Tangeretin (Tan) is a naturally occurring flavonoid mainly found in the peel peel of citrus plants. It exhibits various pharmacological properties, such as antioxidant and anti-inflammatory effects (Xin, et al., 2019; Sedik and Elgohary, 2023).Tan is absorbed by the gastrointestinal tract and primarily metabolized to 4′-demethyltangeretin by CYP1A1 and CYP1A2 enzymes (Surichan et al., 2018). Tan has the potential to mitigate acute lung injury induced by LPS through the inhibition of the Th17 response, TNF-α, and MPO activity via the Notch signaling pathway (M. Li, Zhao, Qi, et al., 2020). Previous studies have demonstrated that Tanis able to prevent podocyte injury and renal fibrosis by effectively blocking glucose-induced oxidative stress and hypoxia-induced EMT in podocytes (Kang et al., 2020). In addition, Tan effectively suppressed the activation of JAK2/STAT3, Wnt, and EGFR signaling pathways, as well as lung fibroblast activation (F. Yang J. et al., 2023; D; Shao et al., 2022). However, the potential mechanism of Tan in the treatment of pulmonary fibrosis remains incompletely understood. In this study, we employed network pharmacology to predict the potential biological pathways of Tan in the management of pulmonary fibrosis. Additionally, we conducted experimental investigations to investigate the function of Tan in pulmonary fibrosis.

2 Materials and methods

2.1 Chemicals and reagents

Tan was purchased from Chengdu Must Biotechnology Co., Ltd. (purity≥98%, Chengdu, China). Bleomycin sulfate (BLM) was obtained from Macklin (Shanghai, China). Pirfenidone was purchased from Solarbio (Beijing, China). The primary antibodies against E-cadherin, N-cadherin, MMP9, and collagen I were purchased from Immunoway (Plano, TX, United States). p-PI3K, PI3K, p-Akt1, and Akt1 were from Affinity Bioscience (Changzhou, China). TGF-β1, TGFBR2, α-SMA, β-actin, and all the secondary antibodies were from the Proteintech Group (Wuhan, China).

2.2 Animal model and treatment

Thirty-six 6–8 weeks old male C57BL/6 mice (weight 18–22 g) were purchased from Liaoning Changsheng Biotechnology Co., Ltd. and randomly divided into 6 groups: control, BLM, BLM + Tan (10 mg/kg, Tan 10), BLM + Tan (20 mg/kg, Tan 20), BLM + Tan (40 mg/kg, Tan 40), and BLM + pirfenidone (PFD). The pulmonary fibrosis mice model was constructed by intra-tracheal drip with 5 mg/kg of BLM at day 0. The control mice received an equal amount of saline intravenously. The following day after BLM treatment by gavage, once daily for 21 days: the positive drug group was given pirfenidone by gavage (200 mg/kg/d), Tan powder dissolved in 0.5% sodium carboxymethylcellulose (CMC-Na) solution by gavage (10 mg/kg/d, 20 mg/kg/d, 40 mg/kg/d), and the control and BLM groups were given equal volumes of 0.5% CMC-Na. Body weights were measured every 7 days. On the last day, the mice were euthanized and lung tissues were collected for subsequent experiments. All experiments were conducted in strict accordance with the Jilin University Guide for the Use and Welfare of Laboratory Animals and were approved by the Jilin University Animal Testing Ethics Committee (approval number: SY202212001).

TABLE 1 Primers sequences used for qPCR.

Gene Name	Forward primer (5′to 3′)	Reverse primer (5′to 3′)
GAPDH	GAATGGGCAGCCGTTAGGAA	AGGAGAAATCGGGCCAGCTA
IL-1β	TGCCACCTTTTGACAGTGATG	TGATGTGCTGCTGCGAGATT
IL-6	TGGTCTTCTGGAGTACCATAGC	TGTGACTCCAGCTTATCTCTTGG
TNF-α	GAGGCCAAGCCCTGGTATG	CGGGCCGATTGATCTCAGC
TGF-β1	ACAATTCCTGGCGTTACCTT	AGCCCTGTATTCCGTCTCC
collagen I	GTGTTCCCTACTCAGCCGTC	ACTCGAACGGGAATCCATCG
MMP9	AACCTCCAACCTCACGGAC	CAGCGTGGTGTTCGAATGG
E-cadherin	CAGGTCTCCTCATGGCTTTGC	CTTCCGAAAAGAAGGCTGTCC
N-cadherin	AGGCTTCTGGTGAAATTGCAT	GTCCACCTTGAAATCTGCTGG
α-SMA	AGCGGGCATCCACGAAAC	TTGATCTTCATGGTGCTGGGT

2.3 Cell culture and treatment

A549 cells were bought from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Cells were cultured at 37°C under 5% CO_2 in Dulbecco's Modified Eagle Media (DMEM; HyClone) with 10% fetal bovine serum (Biological Industries) and 100 U/mL penicillin and 100 μg/mL streptomycin. The cytotoxicity of Tan to A549 cells was determined by the CCK-8 assay. An *in vitro* pulmonary fibrosis model was established using 5 ng/mL TGF-β1 stimulated A549 cells as described previously (Weng et al., 2018; W; Liu et al., 2022). Briefly, A549 cells were seeded into 6-well plates and starved for 24 h following co-culture for 24 h at different Tan concentrations with or without TGF-β1 (Peprotech, New Jersey, United States).

2.4 Histopathological analysis of lung tissues

The left lung was fixed in 4% paraformaldehyde for 24 h, embedded in paraffin, and cut to 4 μm. The sections were then stained with hematoxylin-eosin (H&E) and Masson's trichrome stain. Fibrosis scoring and pulmonary fibrosis area assessment were performed as described previously (Hübner et al., 2008; C.-Y; Chen et al., 2010). Briefly, the area of blue collagen fibers in the whole Masson stained scanned section was quantified using ImageJ software in a 2 × field of view, with the degree of pulmonary fibrosis expressed as a percentage of collagen fiber area. The area fraction of fibrosis = collagen fiber area/lung tissue area × 100%.

2.5 Determination of hydroxyproline, oxidative stress, and inflammatory cytokines in lung tissues

The hydroxyproline, SOD, CAT, and MDA (Nanjing Jiancheng, Nanjing, China) contents in the mice lung tissues were detected according to the manufacturer's instructions. The levels of IL-1β, IL-6, and TNF-α (BioLegend, United States) in lung tissue homogenates were measured using ELISA kits according to the manufacturer's instructions.

2.6 Quantitative real-time polymerase chain reaction

Total RNA was extracted using TRIzol® Reagent (Magen) and then reverse transcribed into cDNA using a TransScript® Uni All-in-One First-Strand cDNA Synthesis SuperMix kit (TransGen Biotech, China). The gene expression was detected by qPCR with FastStart universal SYBR Green Master (Roche, United States) using QuantStudio 1 (Thermo Fisher, United States). GAPDH was used as an internal control. The target genes' relative mRNA expression level was quantified with the $2^{-\Delta\Delta CT}$ method. The primers used (Sangon Biotech, China) are shown in Table 1.

2.7 RNA sequencing analysis

Total RNA was extracted from the lung tissues using TRIzol® Reagent according the manufacturer's instructions (Magen). RNA samples were detected based on the A260/A280 absorbance ratio with a Nanodrop ND-2000 system (Thermo Scientific, United States), and the RIN of RNA was determined by an Agilent Bioanalyzer 4150 system (Agilent Technologies, CA, United States). Only qualified samples will be used for library construction. Paired-end libraries were prepared using a ABclonal mRNA-seq Lib Prep Kit (ABclonal, China) following the manufacturer's instructions. The mRNA was purified from 1 μg total RNA using oligo (dT) magnetic beads followed by fragmentation carried out using divalent cations at elevated temperatures in ABclonal First Strand Synthesis Reaction Buffer. Subsequently, first-strand cDNAs were synthesized with random hexamer primers and Reverse Transcriptase (RNase H) using mRNA fragments as templates, followed by second-strand cDNA synthesis using DNA polymerase I, RNAseH, buffer, and dNTPs. The synthesized double stranded cDNA fragments were then adapterligated for preparation of the paired-end library. Adaptor-ligated cDNA were used for PCR amplification. PCR products were purified (AMPure XP system) and library quality was assessed on an Agilent Bioanalyzer 4150 system. Finally, the

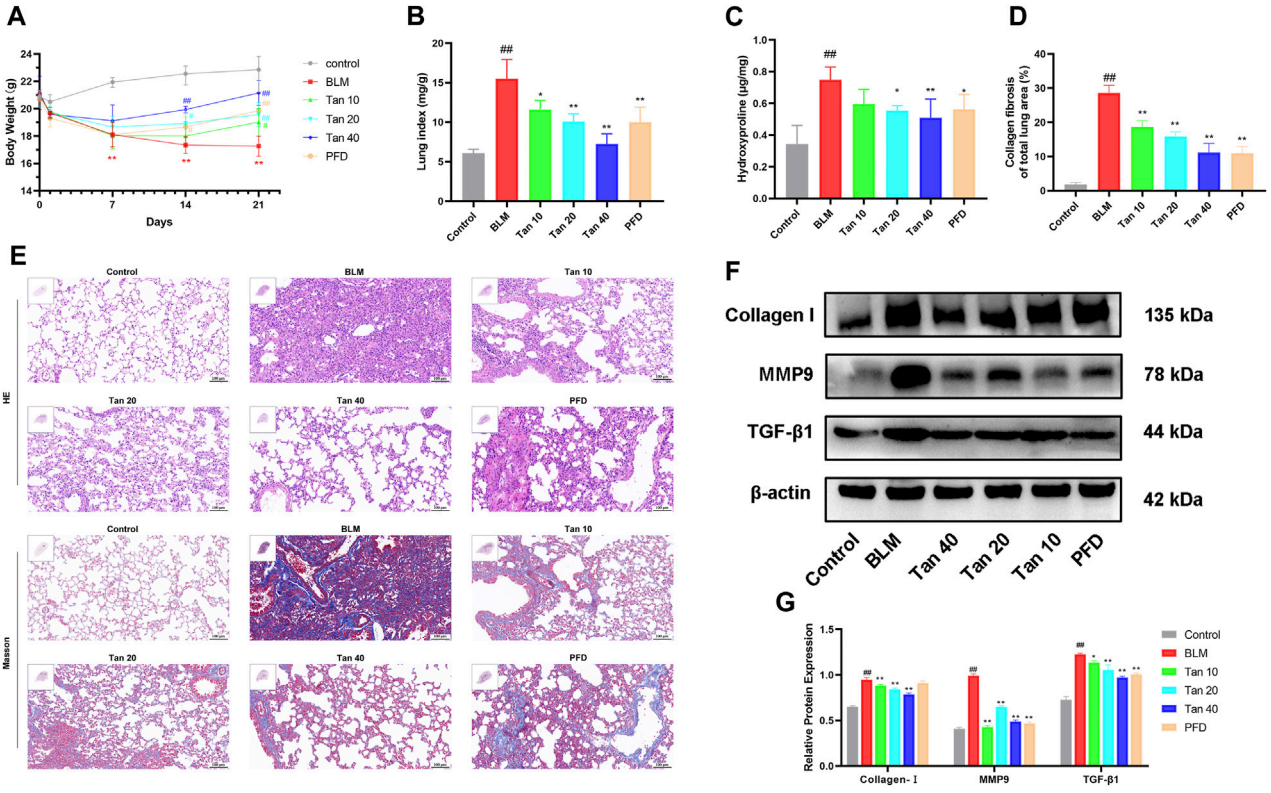

FIGURE 1
Tan attenuated BLM-induced pulmonary fibrosis in mice. **(A)** Body weight changes in various groups of mice at different time points (n = 6). **(B)** Lung index on day 21 (n = 6). **(C)** The hydroxyproline content in the mice lung tissues (n = 6). **(D)** Area statistics of blue collagen fibers in lung tissue as indicated in Materials and methods. **(E)** The images of H&E and Masson staining, scale bar, 100 μm. **(F)** Western blotting of collagen I, MMP9, and TGF-β1 proteins in mice lung. **(G)** Quantification of Western blot bands using ImageJ (n = 3). The data represent the mean ± SD. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, compared with the control group. $^{*}p < 0.05$, $^{**}p < 0.01$, compared with the model group.

library preparations were sequenced on an Illumina Novaseq 6000 and 150 bp paired-end reads were generated. The data generated from Illumina platform were used for bioinformatics analysis. All of the analyses were performed using an in-house pipeline from Shanghai Applied Protein Technology. FeatureCounts (http://subread.sourceforge.net/) was used to count the reads numbers mapped to each gene. And then FPKM of each gene was calculated based on the length of the gene and reads count mapped to this gene. Differential expression analysis was performed using the DESeq2 (http://bioconductor.org/packages/release/bioc/html/DESeq2.html), DEGs with | log2FC | > 1 and Padj <0.05 were considered to be significantly different expressed genes.

2.8 Immunohistochemistry

The lung tissue sections underwent de-paraffinization and hydration, followed by antigen retrieval in 3% H_2O_2 for 10 min. Subsequently, the sections were incubated with goat serum and then exposed to α-SMA antibody (1:1500) or E-cadherin antibody (1: 500) at 4°C overnight. The sections were then incubated with goat anti-rabbit IgG, followed by incubation with 0.05% diaminobenzidine and restained with hematoxylin.

2.9 Western blot analysis

Total proteins from lung tissue and cells were extracted using RIPA lysis buffer (Thermo Fisher, United States) supplemented with a phosphatase and protease inhibitor cocktail. Then, the samples were lysed on ice for 20 min and centrifuged at 12,000 rpm for 10 min. The total protein concentrations in the supernatant were measured by the BCA protein kit (Thermo Fisher, United States). The proteins were separated by SDS-PAGE and transferred onto PVDF membranes (Millipore, United States). After blocking the membranes with 5% skimmed milk for 2 h, the membranes were sealed with different primary antibodies against TGF-β1, TGFBR2, α-SMA, E-cadherin, N-cadherin, collagen I, p-PI3K, PI3K, p-Akt1, Akt1, and β-actin at 4°C overnight. The membranes were then incubated for 2 h at room temperature with horseradish peroxidase (HRP)-coupled goat anti-mouse or goat anti-rabbit IgG secondary antibody. The protein bands were visualized using the ECL luminescence detection kit, and the grayscale values of the protein bands were analyzed using ImageJ software.

2.10 Statistical analysis

All data were presented as mean ± standard deviation (SD). GraphPad Prism 8.0 software was used to perform One-way

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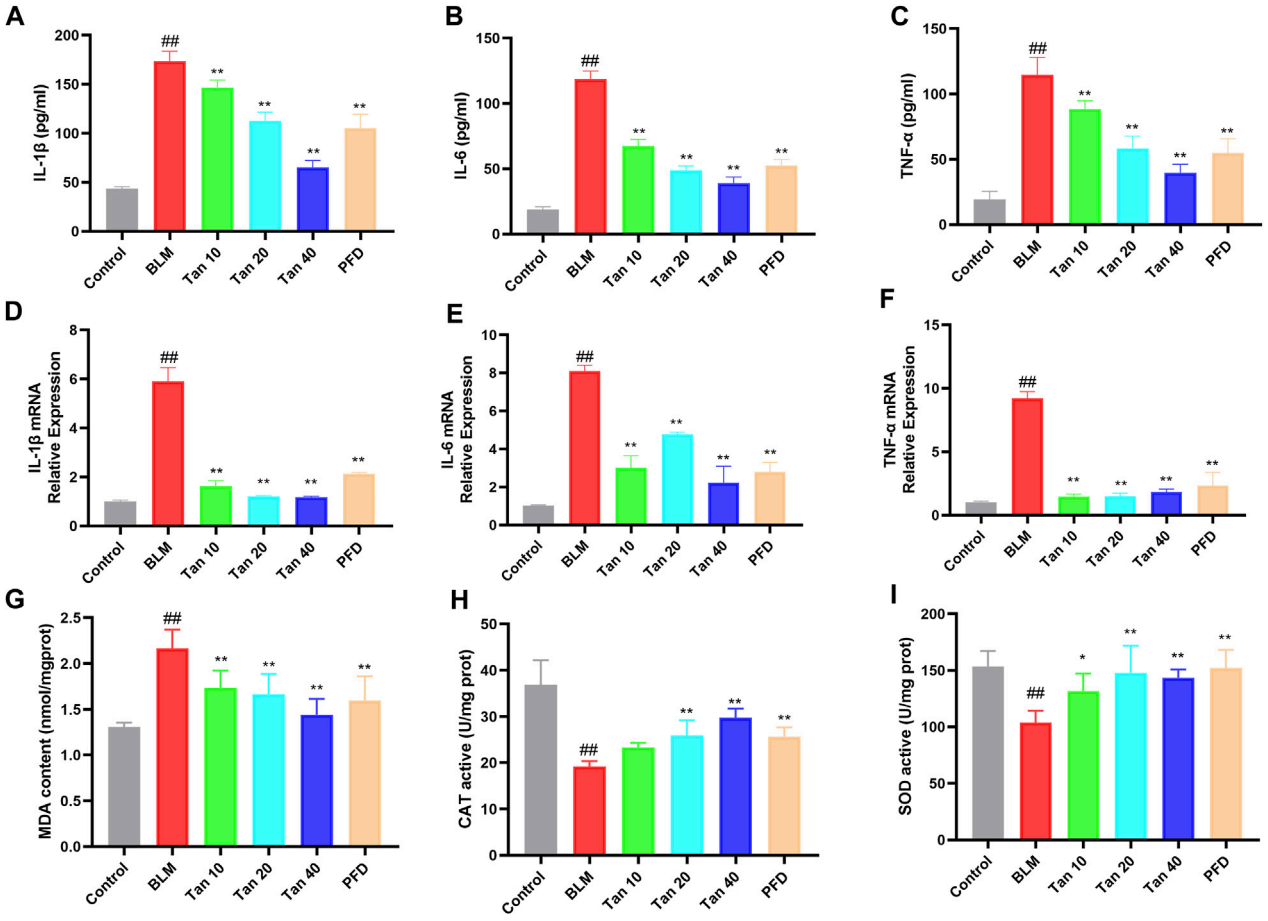

FIGURE 2
Tan reduced inflammation and oxidative stress in mice lung tissues. The secretion of IL-1β **(A)**, IL-6 **(B)**, and TNF-α **(C)** in lung tissue homogenates were measured by ELISA (n = 6). The mRNA levels of IL-1β **(D)**, IL-6 **(E)**, and TNF-α **(F)** in lung tissue were measured by qPCR (n = 3). The MDA content **(G)**, CAT **(H)**, and SOD **(I)** activity were determined in the mice lung tissues (n = 6). $^{\#\#}p < 0.01$, compared with the control group. $^{*}p < 0.05$, $^{**}p < 0.01$, compared with the model group.

ANOVA analysis and to graph. $p < 0.05$ was considered statistically significant.

3 Results

3.1 Tan attenuated bleomycin-induced pulmonary fibrosis in mice

To investigate the role of Tan in pulmonary fibrosis, a pulmonary fibrosis mouse model was established by tracheal drip injection of BLM. The results showed that the weight of BLM-treated mice decreased significantly, while Tan or PFD could increase the weight of the mice (Figure 1A). Meanwhile, Tan or PFD could effectively reduce the lung index and the hydroxyproline content of the lung tissue (Figures 1B,C). H&E and Masson staining results showed that, compared with the control group, the BLM group mice had severely damaged alveolar structures with massive inflammatory cell infiltration, alveolar wall thickening, and blue collagen deposition. These effects were significantly reduced by treatment with Tan or PFD (Figure 1E). Furthermore, Tan or PFD treatment reduced the Ashcroft score and the area of collagen fibers in the lung tissue of mice with pulmonary fibrosis (Figure 1D, Supplementary Figure S1A). Then, we measured the expression of collagen I, TGF-β1 and the core protein MMP9, markers of pulmonary fibrosis, by Western blotting. The data showed that BLM induced the expression of collagen I, TGF-β1, and MMP9, and Tan or PFD treatment significantly reduced this increase (Figures 1F,G). Consistent with the Western blotting results, qPCR results indicated that Tan intervention significantly reduced collagen I, TGF-β1, and MMP9 mRNA levels (Supplementary Figures S1B–D). Collectively, these results suggest that Tan could mitigate BLM-induced pulmonary fibrosis.

3.2 Tan reduced inflammation and oxidative stress in mice lung tissues

Studies have found that inflammation and oxidative stress also play an important role in the development of pulmonary fibrosis (Cameli et al., 2020; Q; Zhang et al., 2023). Hence, to evaluate the

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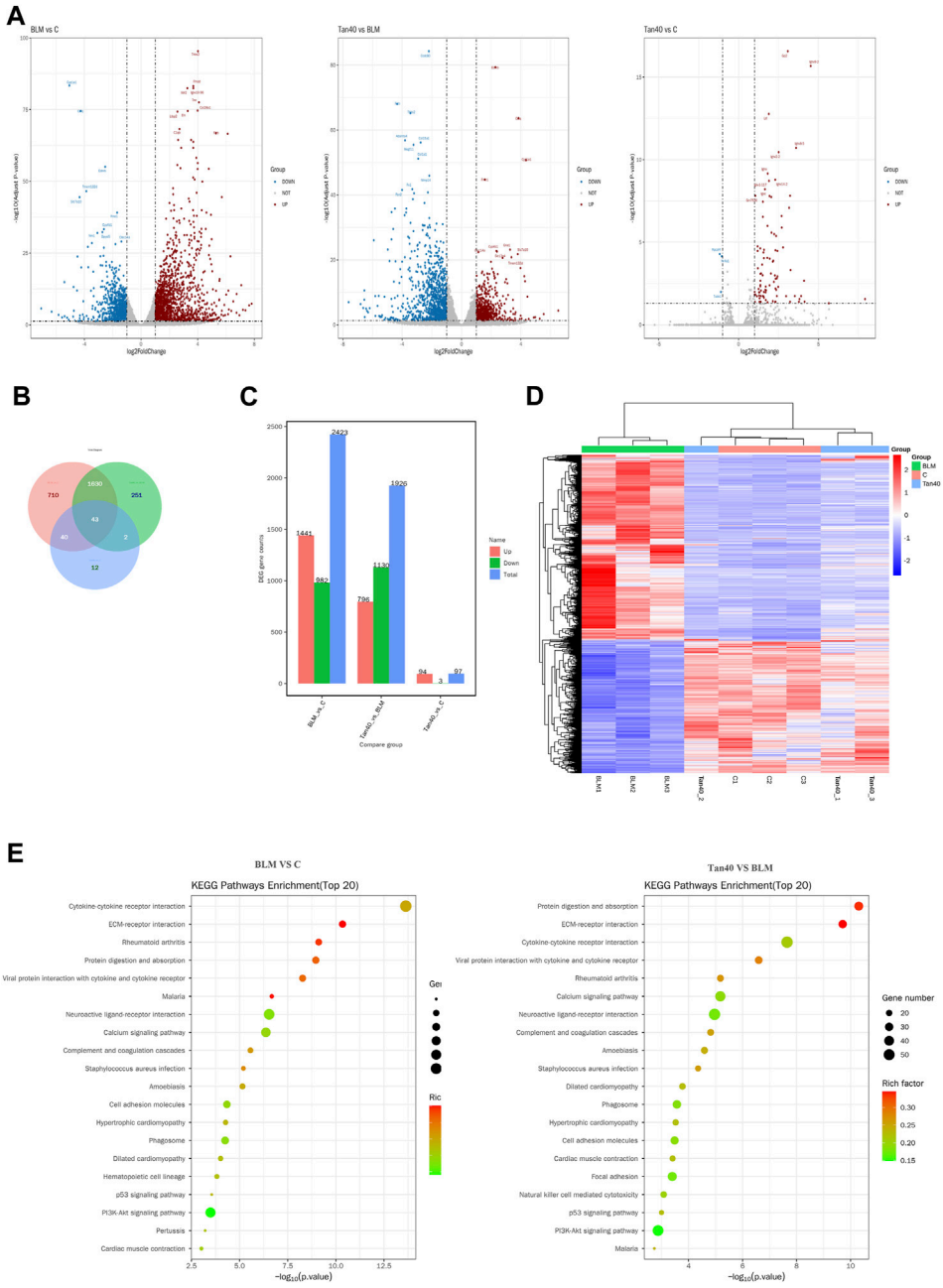

FIGURE 3
Transcriptomic effects of Tan on the lungs of mice with pulmonary fibrosis (n = 3). **(A)** Volcano plot of mRNA expression in each group of lung tissue. **(B)** Venn diagram of differentially expressed mRNAs between the three groups. **(C)** Number of DEGs (Differential Expressed Genes) in each group. **(D)** Heatmap showing the hierarchical clustering of mRNAs in the lung tissues of different groups. **(E)** The top 20 KEGG pathway enrichment analysis in control vs BLM, BLM vs Tan 40.

anti-inflammatory and antioxidant effects of Tan, we measured inflammation and oxidative stress-related indicators in mice lung tissues. The secretion and mRNA expression of IL-1β, IL-6, and TNF-α were significantly higher in the lung tissue of BLM-treated mice compared to the control group, and Tan decreased them in a dose-dependent manner (Figures 2A–F). In addition, the Tan intervention significantly reduced MDA levels and increased CAT and SOD activities (Figures 2G–I).

3.3 Transcriptomic analysis of Tan treating pulmonary fibrosis

We used DEseq2 for differential analysis, with the screening being $|\log_2 FC| > 1$ and Padj <0.05. Volcano plot showing changes in expression of different genes between the three groups (Figure 3A). Compared with the control group, a total of 2423 DEGs were identified in the BLM group, of which 1441 were upregulated

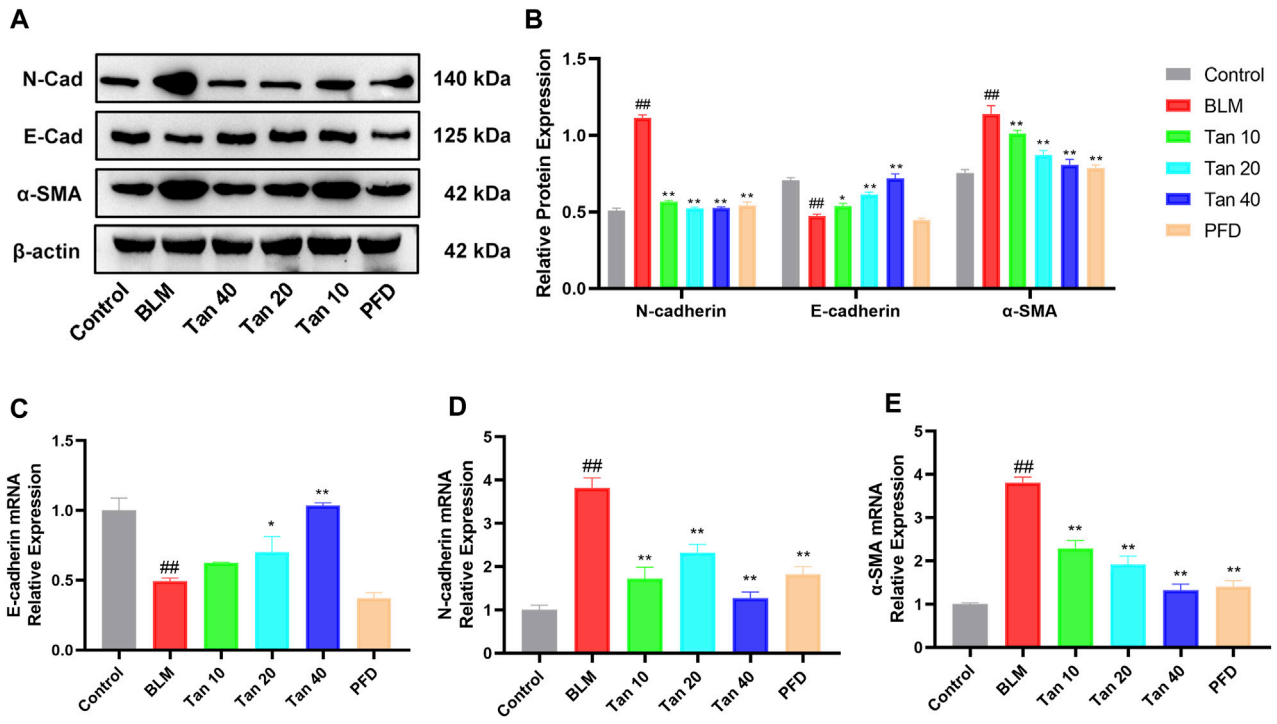

FIGURE 4
Tan reduced BLM-induced EMT in pulmonary fibrosis. **(A)** Western blotting of E-cadherin, N-cadherin, and α-SMA proteins in mice lung. **(B)** Grayscale analysis of target proteins using β-actin as an internal reference (n = 3). The mRNA levels of E-cadherin **(C)**, N-cadherin **(D)**, and α-SMA **(E)** in lung tissue were measured by qPCR (n = 3). $^{\#\#}p < 0.01$, compared with the control group. $^{*}p < 0.05$, $^{**}p < 0.01$, compared with the model group.

and 982 were downregulated. After treatment with Tan, a total of 1926 DEGs were identified. Among them, 796 genes were upregulated, while 1130 genes were downregulated (Figure 3B). The Venn diagram showed 43 DEGs between the groups, which may be the critical DEG for the Tan treatment of pulmonary fibrosis (Figure 3C). As shown in Figure 3D, heat map clustering analysis was performed on the DEGs. In addition, the KEGG pathway enrichment analysis showed that Tan treatment of pulmonary fibrosis mice mainly affected ECM-receptor interaction, PI3K/Akt signaling pathway, P53 signaling pathway and other related pathways. The top 20 KEGG pathways were showed in Figure 3E.

3.4 Tan inhibited BLM-Induced epithelial-mesenchymal transition process *in Vivo*

EMT is a critical pathological process in pulmonary fibrosis, manifested mainly in the transformation of epithelial cells into fibroblasts, leading to a downregulation of the expression of the epithelial marker E-cadherin and an upregulation of the mesenchymal phenotypic markers N-cadherin and α-SMA (Rout-Pitt et al., 2018; Miao et al., 2022). As shown in Figures 4A,B, the expression of E-cadherin was reduced and the expression of N-cadherin and α-SMA was increased in the lung tissue of BLM-treated mice compared to the control group. The qPCR results were consistent with the Western blotting results, which showed that Tan intervention significantly upregulated E-cadherin gene expression and downregulated N-cadherin and α-SMA gene expression (Figures 4C–E). Immunohistochemical staining revealed a significant increase in a-SMA expression and decrease in E-cadherin expression in the BLM group. However, intervention with Tan reversed these changes (Figures 5A, B).

3.5 Tan alleviated TGF-β1-induced epithelial-mesenchymal transition process in A549 cells

To further elucidate the mechanism of Tan in pulmonary fibrosis, we examined the effect of Tan on TGF-β1-induced EMT in A549 cells. The CCK-8 results showed no cytotoxic effect of Tan on A549 cells in the concentration range of 0–10 μM (Figure 6A). Tan effectively reduced the upregulation of TGFBR2 induced by 5 ng/mL TGF-β1 stimulation (Supplementary Figure S2). The results presented in Figures 6B–F indicate a significant increase in the protein expression of collagen I, N-cadherin, and α-SMA, along with a decrease in E-cadherin expression in A549 cells following TGF-β1 stimulation. In contrast, Tan administration decreased the expression of collagen I, N-cadherin, and α-SMA and increased the expression of E-cadherin. In conclusion, Tan can inhibit TGF-β1 induced EMT process to alleviate pulmonary fibrosis.

3.6 Tan inhibited PI3K/Akt signaling pathway *in Vitro* and *in Vivo*

Transcriptomic results showed that the PI3K/Akt signaling pathway was enriched with more DEGs. To investigate the potential

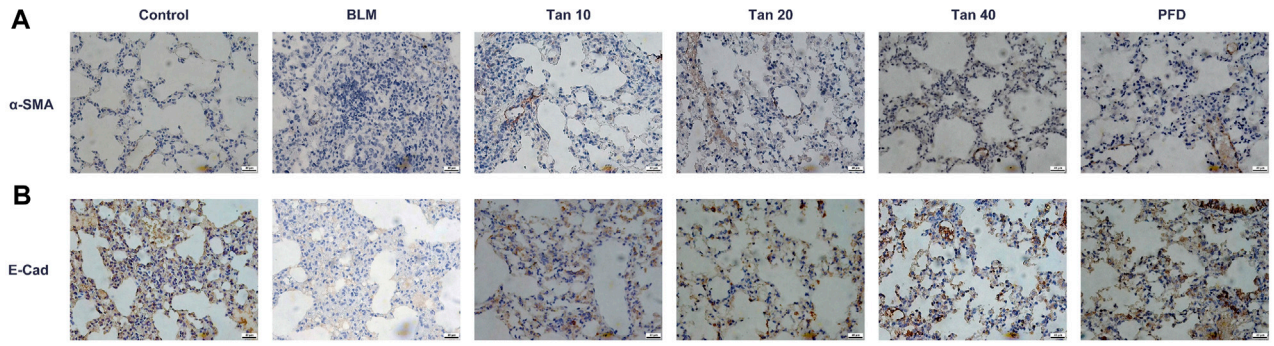

FIGURE 5
Immunohistochemical results of α-SMA **(A)** and E-cadherin **(B)** in lung tissue, scale bar, 40 μm.

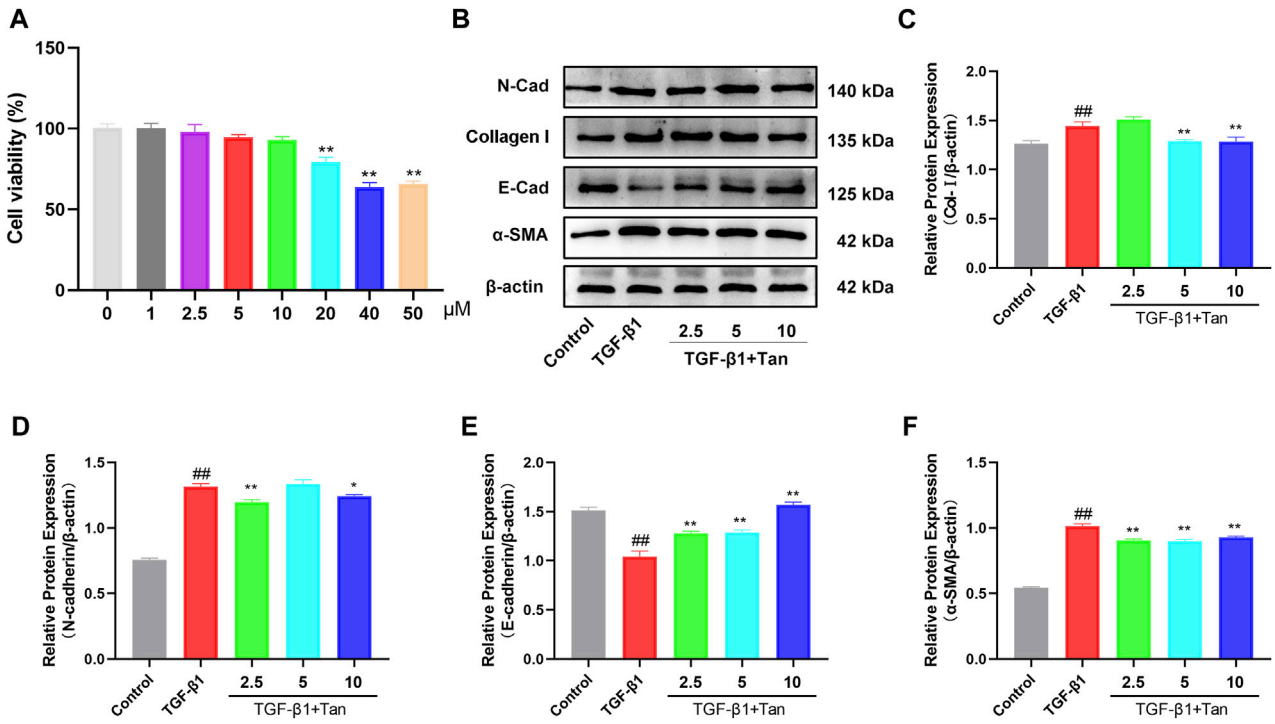

FIGURE 6
Tan alleviated the TGF-β1-induced EMT *in vitro*. **(A)** The effect of Tan on the viability of A549 cells. **(B)** Western blotting of collagen I, E-cadherin, N-cadherin, and α-SMA protein. **(C)** Quantification of the collagen I/β-actin ratio. **(D)** E-cadherin/β-actin ratio. **(E)** N-cadherin/β-actin ratio. **(F)** α-SMA/β-actin ratio. $^{\#\#}p < 0.01$, compared with the control group. $^{*}p < 0.05$, $^{**}p < 0.01$, compared with the model group.

relationship between the ameliorative effect of Tan on pulmonary fibrosis mice and the PI3K/Akt signaling pathway, we conducted Western blotting analysis to detect relevant proteins in this pathway. The results revealed a significant increase in the expression of p-PI3K and p-Akt1 in the lung tissues of pulmonary fibrosis mice. However, Tan treatment remarkably inhibited the expression of p-PI3K and p-Akt1 (Figures 7A–C). Furthermore, Tan also exhibited a suppressive effect on the expression of p-PI3K and p-Akt1 in TGF-β1-induced A549 cells (Figures 7D–F). These findings collectively suggest that Tan exerts its ameliorative effects on pulmonary fibrosis through the modulation of the PI3K/Akt signaling pathway.

4 Discussion

Pulmonary fibrosis represents the final stage of multiple acute and chronic lung ailments, culminating in respiratory failure and mortality. While pirfenidone and nintedanib have received FDA approval for pulmonary fibrosis treatment, they do not significantly reduce patient mortality (Trachalaki, Irfan, and Wells, 2021). Consequently, the quest for novel drugs to address pulmonary fibrosis is a pressing concern. Numerous studies have highlighted the antifibrotic advantages of the active constituents found in herbal medicines (Wang et al., 2021). Tan, an active constituent of the Chinese medicine Chen Pi, exhibits a diverse

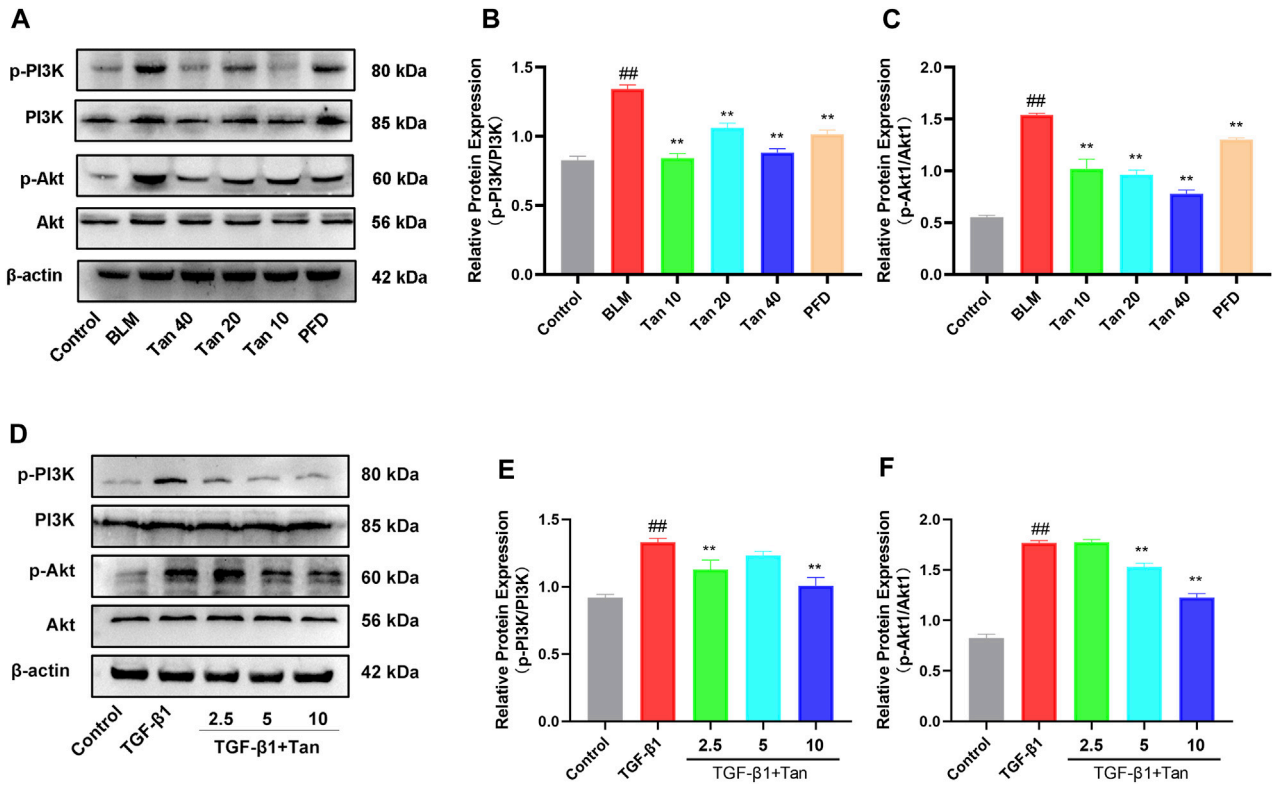

FIGURE 7
Tan inhibited the PI3K/Akt signaling pathway in pulmonary fibrosis. **(A)** Western blotting of p-PI3K, PI3K, p-Akt, and Akt proteins in mice lung. **(B)** Quantification of the p-PI3K/PI3K ratio. **(C)** p-Akt/Akt ratio. **(D)** Western blotting of p-PI3K, PI3K, p-Akt, and Akt proteins in TGF-β1-stimulated A549 cells. **(E)** p-PI3K/PI3K ratio. **(F)** p-Akt/Akt ratio. $^{\#\#}p < 0.01$, compared with the control group. $^{*}p < 0.05$, $^{**}p < 0.01$, compared with the model group.

range of pharmacological activities (Cheng et al., 2023). Research has demonstrated its potential as an antiviral agent by hindering the entry of the SARS-CoV-2 virus into cells (H. Wang et al., 2023). In this study, we elucidated the protective mechanism of Tan against pulmonary fibrosis. Transcriptome analysis of lung tissue samples from mice with pulmonary fibrosis identified several relevant DEGs. The findings suggest that Tan's amelioration of pulmonary fibrosis is associated with the PI3K/Akt signaling pathway. Further experimental validation showed that Tan inhibited TGF-β1-induced EMT in epithelial cells by suppressing PI3K/Akt signaling, which effectively attenuated pulmonary fibrosis.

BLM, an anti-tumor drug, has been utilized in animal experiments to induce pulmonary fibrosis due to its significant lung toxicity (Mouratis and Vassilis, 2011). After tracheal instillation of BLM, the lung epithelial cells undergo damage, resulting in a severe inflammatory response in the lungs. This response produces high levels of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, which in turn promote the recruitment of macrophages and lymphocytes, as well as the activation of fibroblasts (Xin et al., 2019; Lv et al., 2023). Our study found that the stimulation of BLM led to a significant increase in the secretion and expression of pro-inflammatory cytokines in lung tissue. However, the administration of Tan resulted in a significant reduction in the levels of these inflammatory factors. In addition, Tan was observed to alleviate the weight loss and elevated lung index caused by BLM in mice. Interestingly, the improvement observed with Tan treatment was more significant than that observed with pirfenidone treatment. The progression of pulmonary fibrosis is dependent on the proliferation and differentiation of fibroblasts. Myofibroblasts, which overexpress α-SMA, play a crucial role in this process by secreting Collagen I and Collagen III. This leads to an increase in extracellular matrix (ECM) deposition in the interstitial lung matrix, resulting in changes in matrix composition and increased lung tissue stiffness (X. Liu et al., 2018). The enzyme MMP9 is responsible for breaking down the extracellular matrix (ECM) and changing the balance between ECM and interstitial collagen, which can lead to the formation of fibrosis (G. Li et al., 2019a). The pathological histology analysis revealed severe damage to lung tissue, along with increased collagen deposition and higher hydroxyproline content in the mice with pulmonary fibrosis, which aligns with previous findings (Saito et al., 2017). However, Tan's intervention showed a significant improvement in lung histopathological damage and collagen deposition in the mice. Additionally, the study found that Tan reduced the expression of Collagen I, MMP9, and TGF-β1 in BLM-treated mice, which are all indicators of pulmonary fibrosis.

The role of oxidative stress in the development of pulmonary fibrosis is well established (Park et al., 2021). Studies have shown that exposure to BLM can lead to DNA damage, generation of reactive oxygen species, and a decrease in serum T-SOD, CAT, and GSH activities, while increasing MDA expression (Mouratis and Vassilis, 2011). Inhibition of oxidative stress has been shown to improve the pulmonary fibrosis process in mice. Our study found that compared to the control group, mice with pulmonary fibrosis treated with BLM had

significantly higher MDA levels and lower SOD and CAT activity in lung tissue. However, treatment with Tan significantly inhibited BLM-induced oxidative stress in the lung tissue of mice.

In addition to resident lung fibroblasts, EMT is believed to be the primary source of myofibroblasts in pulmonary fibrosis (P. Wang, Yan, et al., 2022). Studies have shown that about one-third of fibroblasts in pulmonary fibrosis originate from epithelial cells (Tanjore et al., 2009). These cells undergo structural and functional changes to become mesenchymal cells when exposed to oxidative stress or TGF-β1, leading to the development of EMT (Rout-Pitt et al., 2018). As a result, we examined the impact of Tan on EMT in our study. The study found that Tan increased the levels of protein and gene of E-cadherin and decreased the levels of protein and gene of N-cadherin and α-SMA. A549 cells were used to induce the phenotypic transformation caused by TGF-β1 and Western blotting was employed to analyze the effect of Tan on the epithelial-mesenchymal transition (EMT) phenotype The results indicated that consistent with previous studies, E-cadherin levels decreased and collagen I, N-cadherin, and α-SMA expression increased after TGF-β1 treatment, but improved after treatment with Tan(Q. Chen et al., 2023). Therefore, the research demonstrated that Tan can suppress EMT and inhibit pulmonary fibrosis.

The process of TGF-β1-induced EMT involves the activation of non-Smad-dependent signaling pathways such as PI3K/Akt, MAPK, RhoA, and NF-κB(Y.E. Zhang, 2017). PI3K/Akt signaling pathway has been identified as a crucial regulator of pulmonary fibrosis (J. Wang, et al., 2022). Studies have shown that inhibition of PI3K/Akt disrupts EMT and the use of Akt inhibitors can partially reverse EMT (Lin et al., 2014). TGF-β1 activates PI3K through the TGF-β receptor or EGF receptor. As a secondary messenger, PI3K activation causes the p110 subunit to bind with the p85 subunit, leading to the phosphorylation of the substrate PIP2. This conversion results in the formation of PIP3, which then binds to the PH structural domain of Akt, leading to its activation through phosphorylation (Hers et al., 2011; Saito et al., 2017). Activation of Akt can induce the expression of EMT-inducible transcription factors and phosphorylate Snail1 by inhibiting GSK-3β or activating NF-κB to promote EMT in squamous cell carcinoma cells (L. Zhang et al., 2013; Kaufhold and Bonavida, 2014). Moreover, sustained PI3K activation can aggravate BLM-induced pulmonary fibrosis by promoting the release of pro-inflammatory and pro-fibrotic factors (Kral et al., 2016). In conjunction with KEGG pathway enrichment analysis, we evaluated the expression of the PI3K/Akt pathway using Western blotting. The data show that Tan inhibits PI3K and Akt phosphorylation both *in vivo* and *in vitro*. Tan, a selective PI3K inhibitor, shows potential as a drug for treating upper respiratory tract infections (S. Chen et al., 2022). Furthermore, the inclusion of Tan in the diet can enhance the production of short-chain fatty acids. These fatty acids play a role in inhibiting the epithelial-mesenchymal transition (EMT) process by suppressing the PI3K/Akt/mTOR signaling cascade (B. Chen et al., 2021; D; Chen et al., 2020).Therefore, Tan may directly or indirectly act on PI3K, inhibiting the activation of the PI3K/Akt signaling pathway, and subsequently inhibiting the epithelial-mesenchymal transition (EMT) process in pulmonary fibrosis.

5 Conclusion

To conclude, this study provides evidence that Tan can improve pulmonary fibrosis both *in vivo* and *in vitro* by inhibiting EMT through the PI3K/Akt signaling pathway. The findings suggest that Tan could be a viable drug candidate for the treatment of pulmonary fibrosis.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: Sequence Read Archive (SRA)/ PRJNA987041.

Ethics statement

The animal study was approved by the Jilin University Animal Testing Ethics Committee. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

JL, QW, and PY designed and conceived the experiments., KS, YW, and YY conducted experiments., ML, JY, and GS performed the statistical analysis. JL wrote the manuscript. LP, BF, and PY revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

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SUPPLEMENTARY FIGURE S1

Tan improved lung tissue scores and reduced mRNA expression of pulmonary fibrosis markers in mice with pulmonary fibrosis. **(A)** Ashcroft score. The mRNA expression of collagen I **(B)**, MMP9 **(C)** and TGF-β1 **(D)** was detected by qPCR in the lung tissue in each group of mice. Data represent the mean ± SD. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with the control group. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the model group.

SUPPLEMENTARY FIGURE S2

Tan treatment resulted in a decrease in the expression of the TGF-β1 receptor, TGFBR2. **(A)** Representative western blotting images showing the expression of TGFBR2. **(B)** Quantification of TGFBR2/β-actin ratio. $^{\#\#}P < 0.01$, compared with the control group. $^{**}P < 0.01$, compared with the model group.

References

Cameli, P., Carleo, A., Bergantini, L., Landi, C., Prasse, A., and Bargagli, E. (2020). Oxidant/antioxidant disequilibrium in idiopathic pulmonary fibrosis pathogenesis. *Inflammation* 43 (1), 1–7. doi:10.1007/s10753-019-01059-1

Chen, B., Luo, J., Han, Y., Du, H., Liu, J., He, W., et al. (2021). Dietary tangeretin alleviated dextran sulfate sodium-induced colitis in mice via inhibiting inflammatory response, restoring intestinal barrier function, and modulating gut microbiota. *J. Agric. Food Chem.* 69 (27), 7663–7674. doi:10.1021/acs.jafc.1c03046

Chen, C-Y., Peng, W-H., Wu, L-C., and Hsu, S. L. (2010). Luteolin ameliorates experimental lung fibrosis both *in vivo* and *in vitro*: Implications for therapy of lung fibrosis. *J. Agric. Food Chem.* 58 (22), 11653–11661. doi:10.1021/jf1031668

Chen, D., Qiu, Y., Gao, Z., Wu, Y. X., Wan, B. B., Liu, G., et al. (2020). Sodium propionate attenuates the lipopolysaccharide-induced epithelial–mesenchymal transition via the PI3K/Akt/mTOR signaling pathway. *J. Agric. Food Chem.* 68 (24), 6554–6563. doi:10.1021/acs.jafc.0c01302

Chen, Q., Liao, X., Lin, L., Wu, L., and Tang, Q. (2023). FOXF1 attenuates TGF-β1-induced bronchial epithelial cell injury by inhibiting CDH11-mediated Wnt/β-catenin signaling. *Exp. Ther. Med.* 25 (3), 103. doi:10.3892/etm.2023.11802

Chen, S., Huang, W., Li, X., Gao, L., and Ye, Y. (2022). Identifying active compounds and mechanisms of citrus changshan-huyou Y. B. Chang against URTIs-associated inflammation by network pharmacology in combination with molecular docking. *Evid. Based Complement. Altern. Med.* 2022, 2156157. doi:10.1155/2022/2156157

Cheng, Y., Wu, C., Liu, Z., Song, P., Xu, B., and Chao, Z. (2023). Evaluation and optimization of quality based on the physicochemical characteristics and metabolites changes of qingpi during storage. *Foods* 12 (3), 463. doi:10.3390/foods12030463

Chiou, W. C., Huang, G. J., Chang, T. Y., Hsia, T. L., Yu, H. Y., Lo, J. M., et al. (2023). Ovatodiolide inhibits SARS-CoV-2 replication and ameliorates pulmonary fibrosis through suppression of the TGF-β/TβRs signaling pathway. *Biomed. Pharmacother.* 161, 114481. doi:10.1016/j.biopha.2023.114481

Garg, M. (2013). Epithelial-mesenchymal transition - activating transcription factors - multifunctional regulators in cancer. *World J. Stem Cells* 5 (4), 188–195. doi:10.4252/wjsc.v5.i4.188

Hers, I., Vincent, E. E., and Tavaré, J. M. (2011). Akt signalling in health and disease. *Cell Signal* 23 (10), 1515–1527. doi:10.1016/j.cellsig.2011.05.004

Hübner, R.-H., Gitter, W., Eddine El Mokhtari, N., Mathiak, M., Both, M., Bolte, H., et al. (2008). Standardized quantification of pulmonary fibrosis in histological samples. *BioTechniques* 44 (4), 507–511. doi:10.2144/000112729

Kang, M. K., Kim, S. I., Oh, S. Y., Na, W., and Kang, Y. H. (2020). Tangeretin ameliorates glucose-induced podocyte injury through blocking epithelial to mesenchymal transition caused by oxidative stress and hypoxia. *Int. J. Mol. Sci.* 21 (22), 8577. doi:10.3390/ijms21228577

Kaufhold, S., and Bonavida, B. (2014). Central role of Snail1 in the regulation of EMT and resistance in cancer: a target for therapeutic intervention. *J. Exp. Clin. Cancer Res.* 33 (1), 62. doi:10.1186/s13046-014-0062-0

Kral, J. B., Kuttke, M., Schrottmaier, W. C., Birnecker, B., Warszawska, J., Wernig, C., et al. (2016). Sustained PI3K Activation exacerbates BLM-induced Lung Fibrosis via activation of pro-inflammatory and pro-fibrotic pathways. *Sci. Rep.* 6, 23034. doi:10.1038/srep23034

Li, G., Jin, F., Du, J., He, Q., Yang, B., and Luo, P. (2019a). Macrophage-secreted TSLP and MMP9 promote bleomycin-induced pulmonary fibrosis. *Toxicol. Appl. Pharmacol.* 366, 10–16. doi:10.1016/j.taap.2019.01.011

Li, H., Zhao, C., Tian, Y., Lu, J., Zhang, G., Liang, S., et al. (2020). Src family kinases and pulmonary fibrosis: A review. *Biomed. Pharmacother.* 127, 110183. doi:10.1016/j.biopha.2020.110183

Li, M., Zhao, Y., Qi, D., He, J., and Wang, D. (2020). Tangeretin attenuates lipopolysaccharide-induced acute lung injury through Notch signaling pathway via suppressing Th17 cell response in mice. *Microb. Pathog.* 138, 103826. doi:10.1016/j.micpath.2019.103826

Li, X., Xie, P., Hou, Y., Chen, S., He, P., Xiao, Z., et al. (2019b). Tangeretin inhibits oxidative stress and inflammation via upregulating nrf-2 signaling pathway in collagen-induced arthritic rats. *Pharmacology* 104 (3-4), 187–195. doi:10.1159/000501163

Lin, G., Gai, R., Chen, Z., Wang, Y., Liao, S., Dong, R., et al. (2014). The dual PI3K/mTOR inhibitor NVP-BEZ235 prevents epithelial-mesenchymal transition induced by hypoxia and TGF-β1. *Eur. J. Pharmacol.* 729, 45–53. doi:10.1016/j.ejphar.2014.02.011

Liu, W, Han, X., Li, Q., Sun, L., and Wang, J. (2022). "Iguratimod ameliorates bleomycin-induced pulmonary fibrosis by inhibiting the EMT process and NLRP3 inflammasome activation". *Biomed. Pharmacother.* 153: 113460. doi:10.1016/j.biopha.2022.113460

Liu, X., Long, X., Liu, W., Zhao, Y., Hayashi, T., Yamato, M., et al. (2018). Type I collagen induces mesenchymal cell differentiation into myofibroblasts through YAP-induced TGF-β1 activation. *Biochimie* 150, 110–130. doi:10.1016/j.biochi.2018.05.005

Lv, K., Li, M., Sun, C., Miao, Y., Zhang, Y., Liu, Y., et al. (2023). Jingfang Granule alleviates bleomycin-induced acute lung injury via CD200-CD200R immunoregulatory pathway. *J. Ethnopharmacol.* 311, 116423. doi:10.1016/j.jep.2023.116423

Mathai, S. K., and Schwartz, D. A. (2019). Translational research in pulmonary fibrosis. *Transl. Res.* 209, 1–13. doi:10.1016/j.trsl.2019.02.001

Meng, X. M., Nikolic-Paterson, D. J., and Lan, H. Y. (2016). TGF-B: The master regulator of fibrosis. *Nat. Rev. Nephrol.* 12 (6), 325–338. doi:10.1038/nrneph.2016.48

Miao, Y., Li, X., Yang, Y., Zhang, J., Chen, L., Zhang, Q., et al. (2022). "Entrectinib ameliorates bleomycin-induced pulmonary fibrosis in mice by inhibiting TGF-β1 signaling pathway." *Int. Immunopharmacol.* 113: 109427. doi:10.1016/j.intimp.2022.109427

Moss, B. J., Ryter, S. W., and Rosas, I. O. (2022). Pathogenic mechanisms underlying idiopathic pulmonary fibrosis. *Annu. Rev. Pathol.* 17, 515–546. doi:10.1146/annurev-pathol-042320-030240

Mouratis, M. A., and Vassilis, A. (2011). Modeling pulmonary fibrosis with bleomycin. *Curr. Opin. Pulm. Med.* 17 (5), 355–361. doi:10.1097/MCP.0b013e328349ac2b

Olajuyin, A. M., Zhang, X., and Ji, H. L. (2019). Alveolar type 2 progenitor cells for lung injury repair. *Cell Death Discov.* 5, 63. doi:10.1038/s41420-019-0147-9

Park, S. J., Kim, T. H., Lee, K., Kang, M. A., Jang, H. J., Ryu, H. W., et al. (2021). Kurarinone attenuates BLM-induced pulmonary fibrosis via inhibiting TGF-β signaling pathways. *Int. J. Mol. Sci.* 22 (16), 8388. doi:10.3390/ijms22168388

Peng, Y., Wang, Y., Zhou, C., Mei, W., and Zeng, C. (2022). PI3K/Akt/mTOR pathway and its role in cancer therapeutics: Are we making headway? *Front. Oncol.* 12, 819128. doi:10.3389/fonc.2022.819128

Qin, W., Cao, L., and Massey, I. Y. (2021). Role of PI3K/Akt signaling pathway in cardiac fibrosis. *Mol. Cell. Biochem.* 476 (11), 4045–4059. doi:10.1007/s11010-021-04219-w

Rout-Pitt, N., Farrow, N., Parsons, D., and Donnelley, M. (2018). Epithelial mesenchymal transition (EMT): a universal process in lung diseases with implications for cystic fibrosis pathophysiology. *Respir. Res.* 19 (1), 136. doi:10.1186/s12931-018-0834-8

Saito, S., Zhuang, Y., Shan, B., Danchuk, S., Luo, F., Korfei, M., et al. (2017). Tubastatin ameliorates pulmonary fibrosis by targeting the TGFβ-PI3K-Akt pathway. *PLoS One* 12 (10), e0186615. doi:10.1371/journal.pone.0186615

Sedik, A. A., and Elgohary, R. (2023). Neuroprotective effect of tangeretin against chromium-induced acute brain injury in rats: targeting Nrf2 signaling pathway, inflammatory mediators, and apoptosis. *Inflammopharmacology* 31, 1465–1480. doi:10.1007/s10787-023-01167-3

Shao, D., Liu, X., Wu, J., Zhang, A., Bai, Y., Zhao, P., et al. (2022). Identification of the active compounds and functional mechanisms of Jinshui Huanxian formula in pulmonary fibrosis by integrating serum pharmacochemistry with network pharmacology. *Phytomedicine* 102, 154177. doi:10.1016/j.phymed.2022.154177

Shao, L., Zhang, Y., Shi, W., Ma, L., Xu, T., Chang, P., et al. (2021). Mesenchymal stromal cells can repair radiation-induced pulmonary fibrosis via a DKK-1-mediated Wnt/β-catenin pathway. *Cell Tissue Res.* 384 (1), 87–97. doi:10.1007/s00441-020-03325-3

Strongman, H., Kausar, I., and Maher, T. M. (2018). Incidence, prevalence, and survival of patients with idiopathic pulmonary fibrosis in the UK. *Adv. Ther.* 35 (5), 724–736. doi:10.1007/s12325-018-0693-1

Sun, T., Haihua, L., Yan, Z., Xiong, G., Liang, Y., Lu, F., et al. (2023). Inhibitory effects of 3-Cyclopropylmethoxy-4-(difluoromethoxy) benzoic acid on TGF-β1-induced epithelial–mesenchymal transformation of *in vitro* and bleomycin-induced pulmonary fibrosis *in vivo*. *Int. J. Mol. Sci.* 24, 6172. doi:10.3390/ijms24076172

Surichan, S., Arroo, R. R., Tsatsakis, A. M., and Androutsopoulos, V. P. (2018). Tangeretin inhibits the proliferation of human breast cancer cells via CYP1A1/CYP1B1 enzyme induction and CYP1A1/CYP1B1-mediated metabolism to the product 4' hydroxy tangeretin. *Toxicol Vitro* 50, 274–284. doi:10.1016/j.tiv.2018.04.001

Tanjore, H., Xu, X. C., Polosukhin, V. V., Degryse, A. L., Li, B., Han, W., et al. (2009). Contribution of epithelial-derived fibroblasts to bleomycin-induced lung fibrosis. *Am. J. Respir. Crit. care Med.* 180 (7), 657–665. doi:10.1164/rccm.200903-0322OC

Tan, W., Zhang, B., Liu, X., Zhang, C., Liu, J., and Miao, Q. (2021). Interleukin-33-Dependent accumulation of regulatory T cells mediates pulmonary epithelial regeneration during acute respiratory distress syndrome. *Front. Immunol.* 12, 653803. doi:10.3389/fimmu.2021.653803

Trachalaki, A., Irfan, M., and Wells, A. U. (2021). Pharmacological management of idiopathic pulmonary fibrosis: current and emerging options. *Expert Opin. Pharmacother.* 22 (2), 191–204. doi:10.1080/14656566.2020.1822326

Wang, C., and Yang, J. (2022). Mechanical forces: The missing link between idiopathic pulmonary fibrosis and lung cancer. *Eur. J. Cell Biol.* 101 (3), 151234. doi:10.1016/j.ejcb.2022.151234

Wang, H., Jia, Q., Feng, J., Miao, C., Ding, Y., Liu, S., et al. (2023). Establishment of angiotensin-converting enzyme 2 and cluster of differentiation 147 dual target cell membrane chromatography based on SNAP-tag technology for screening anti severe acute respiratory syndrome coronavirus 2 active components. *J. Chromatogr. A* 1693, 463903. doi:10.1016/j.chroma.2023.463903

Wang, J., Hu, K., Cai, X., Yang, B., He, Q., Wang, J., et al. (2022). Targeting PI3K/AKT signaling for treatment of idiopathic pulmonary fibrosis. *Acta Pharm. Sin. B* 12 (1), 18–32. doi:10.1016/j.apsb.2021.07.023

Wang, L., Cheng, W., and Zhang, Z. (2017). Respiratory syncytial virus infection accelerates lung fibrosis through the unfolded protein response in a bleomycin-induced pulmonary fibrosis animal model. *Mol. Med. Rep.* 16 (1), 310–316. doi:10.3892/mmr.2017.6558

Wang, L., Li, S., Yao, Y., Yin, W., and Ye, T. (2021). The role of natural products in the prevention and treatment of pulmonary fibrosis: a review. *Food & Funct.* 12 (3), 990–1007. doi:10.1039/D0FO03001E

Wang, P., Yan, Z., Zhou, P. K., and Gu, Y. (2022). The promising therapeutic approaches for radiation-induced pulmonary fibrosis: Targeting radiation-induced mesenchymal transition of alveolar type II epithelial cells. *Int. J. Mol. Sci.* 23 (23), 15014. doi:10.3390/ijms232315014

Weng, D., Chen, J., Li, H., Liu, F., Zhou, L. D., Liu, H. P., et al. (2018). 2-aminopurine suppresses the TGF-β1-induced epithelial–mesenchymal transition and attenuates bleomycin-induced pulmonary fibrosis. *Cell Death Discov.* 4 (1), 17. doi:10.1038/s41420-017-0016-3

Xin, X., Yao, D., Zhang, K., Han, S., Liu, D., Wang, H., et al. (2019). Protective effects of Rosavin on bleomycin-induced pulmonary fibrosis via suppressing fibrotic and inflammatory signaling pathways in mice. *Biomed. Pharmacother.* 115, 108870. doi:10.1016/j.biopha.2019.108870

Xu, Y., Wang, X., Han, D., Wang, J., Luo, Z., Jin, T., et al. (2022). Revealing the mechanism of Jiegeng decoction attenuates bleomycin-induced pulmonary fibrosis via PI3K/Akt signaling pathway based on lipidomics and transcriptomics. *Phytomedicine* 102, 154207. doi:10.1016/j.phymed.2022.154207

Yang, F., Hou, R., Liu, X., Tian, Y., Bai, Y., Li, J., et al. (2023b). Yangqing Chenfei formula attenuates silica-induced pulmonary fibrosis by suppressing activation of fibroblast via regulating PI3K/AKT, JAK/STAT, and Wnt signaling pathway. *Phytomedicine* 110, 154622. doi:10.1016/j.phymed.2022.154622

Yang, J., Liang, C., Liu, L., Wang, L., and Yu, G. (2023a). High-fat diet related lung fibrosis-epigenetic regulation matters. *Biomolecules* 13 (3), 558. doi:10.3390/biom13030558

Yang, W., Bai, X., Li, H., Li, H., Fan, W., Zhang, H., et al. (2022). Influenza A and B virus-triggered epithelial-mesenchymal transition is relevant to the binding ability of NA to latent TGF-β. *Front. Microbiol.* 13, 841462. doi:10.3389/fmicb.2022.841462

Zhang, Q., Luo, T., Yuan, D., Liu, J., Fu, Y., and Yuan, J. (2023). Qi-Long-Tian capsule alleviates pulmonary fibrosis development by modulating inflammatory response and gut microbiota. *Funct. Integr. Genomics* 23 (1), 64. doi:10.1007/s10142-023-00988-3

Zhang, L., Zhou, F., and ten Dijke, P. (2013). Signaling interplay between transforming growth factor-β receptor and PI3K/AKT pathways in cancer. *Trends Biochem. Sci.* 38 (12), 612–620. doi:10.1016/j.tibs.2013.10.001

Zhang, Y. E. (2017). Non-smad signaling pathways of the TGF-beta family. *Cold Spring Harb. Perspect. Biol.* 9 (2), a022129. doi:10.1101/cshperspect.a022129

Zhou, S., Zhu, J., Zhou, P. K., and Gu, Y. (2022). Alveolar type 2 epithelial cell senescence and radiation-induced pulmonary fibrosis. *Front. Cell Dev. Biol.* 10, 999600. doi:10.3389/fcell.2022.999600

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Progression of the PI3K/Akt signaling pathway in chronic obstructive pulmonary disease

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Chronic Obstructive Pulmonary Disease (COPD) is a chronic respiratory disease characterized by a slow progression and caused by the inhalation of harmful particulate matter. Cigarette smoke and air pollutants are the primary contributing factors. Currently, the pathogenesis of COPD remains incompletely understood. The PI3K/Akt signaling pathway has recently emerged as a critical regulator of inflammation and oxidative stress response in COPD, playing a pivotal role in the disease's progression and treatment. This paper reviews the association between the PI3K/Akt pathway and COPD, examines effective PI3K/Akt inhibitors and novel anti-COPD agents, aiming to identify new therapeutic targets for clinical intervention in this disease.

KEYWORDS

chronic obstructive pulmonary disease, PI3K/Akt signalling pathway, inhibitor, inflammation, oxidative stress

1 Introduction

COPD is a heterogeneous lung disease characterized by a variety of chronic respiratory symptoms, such as difficulty breathing, coughing, sputum production, and acute exacerbation. These symptoms arise from abnormal airways (bronchitis) and/or alveolar abnormalities (emphysema), resulting in persistent and frequently progressive airflow obstruction (Venkatesan, 2023). COPD ranks as the third leading cause of death globally, following ischemic heart disease and stroke (Obeidat et al., 2018). COPD arises from the complex interplay of multiple factors. Smoking stands as a significant risk factor for COPD, while environmental exposure and genetic variation can contribute to the development or exacerbation of the disease (Lareau et al., 2019). Presently, COPD is treated with a combination of medication and non-medication strategies, smoking cessation is the primary treatment, bronchodilators and glucocorticoids are the most commonly used drugs (Sandelowsky et al., 2021). Nevertheless, COPD commonly exhibits a progressive nature, and the conventional medications used to manage it entail notable side effects. These challenges emphasize the urgent need for exploring alternative treatment modalities for COPD.

The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB/Akt) signaling pathway is a critical cellular pathway that regulates multiple functions such as cell survival, growth, proliferation, metastasis, and metabolism (Engelman et al., 2006; Abeyrathna and Su, 2015). The abnormal activation of the PI3K/AKT pathway is associated with various human cancers (Noorolyai et al., 2019; Glaviano et al., 2023). Additionally, it is also involved in many chronic diseases such as diabetes (Savova et al.,

2023), cardiovascular diseases (Qin et al., 2021), neurological disorders (Wang Q. et al., 2022), autoimmune diseases (Cheng et al., 2022), inflammatory diseases (Xu et al., 2022), and liver diseases (Ye et al., 2023) et al. The PI3K/AKT pathway plays a crucial role in the onset and progression of numerous diseases. Recently, studies have demonstrated that inhibiting the PI3K/Akt signaling pathway reduces inflammation, apoptosis, and oxidative stress in cells, thereby playing a crucial role in COPD treatment (Sun et al., 2019).

This review comprehensively examines the structure and transduction of the PI3K/Akt signaling pathway. Additionally, it highlights the crucial role of PI3K/Akt signaling in COPD and presents a summary of potential drugs that target this pathway. The aim is to expand the therapeutic possibilities for COPD and offer innovative and effective targets for clinical intervention.

2 The PI3K/Akt signaling pathway

The PI3K/Akt signaling pathway plays a crucial role in various cellular regulatory processes such as cell growth, proliferation, migration, metabolism, and secretion. Furthermore, dysregulation of the PI3K/Akt signaling pathway is implicated in a diverse spectrum of human diseases, including cancer (Noorolyai et al., 2019), neurodegenerative disease (Rai et al., 2019), diabetes (Huang et al., 2018), and osteoarthritis (Sun et al., 2020).

2.1 The composition of PI3K

PI3Ks are a class of evolutionarily conserved intracellular lipid kinases called intracellular lipid kinases, classified into classes I, II, and III based on substrate specificity and sequence homology (Cantley, 2002). Type I PI3K can be divided into two subfamilies based on its coupling receptors. Type IA PI3K is a heterogeneous dimer composed of a p85-regulated subgroup and a p110 catalytic subgroup. It is activated by the growth factor receptor tyrosine kinase (RTK) (Katso et al., 2001). Type IB PI3K is a heterogeneous dimer composed of p101 regulatory subunits and p110-γ catalytic subunits. It is activated by G-protein-coupled receptors (GPCRs) (Voigt et al., 2006). Classes II and III PI3K are monomer types. Class II PI3K consists of a catalytic subunit similar to p110, while class III PI3K consists of a single member, Vps34 (Jean and Kiger, 2014). It is generally accepted that class I PI3K is the most widely studied, and class II and III PI3Ks have less knowledge about their specific functions. Here, we will focus on the role of Type I PI3K in COPD.

2.2 The composition of Akt

Akt, a serine/threonine kinase also known as PKB, belongs to the AGC protein kinase family. Three subtypes of Akt exist: Akt1 (PKBa), Akt2 (PKBb), and Akt3 (PKBc) (Risso et al., 2015). The three subtypes of Akt, closely related in mammals, possess three conserved domains: an amino terminal pleckstrin homology (PH) domain, a central kinase domain (excitation domain) highly similar to other AGC protein kinases like PKA and PKC (Peterson and Schreiber, 1999), and a carboxyl terminal regulatory domain that includes HM phosphorylation sites. The three Akt isomers exhibit high sequence similarity and structural resemblance, with Akt1 and Akt2 showing broader expression in mammals (He et al., 2021).

2.3 Mechanisms of PI3K/Akt pathway activation

Common mechanisms of PI3K activation involve the activation of receptor tyrosine kinase under physiological conditions. This leads to the phosphorylation of tyrosine residues and their subsequent binding to one or both SH2 domains of the PI3K splice subunit, resulting in allosteric activation of the PI3K catalytic subunit (Fruman et al., 2017). Additionally, activation of GPCR leads to allosteric activation of PI3K (Rathinaswamy et al., 2021). PI3K activation causes the transformation of PIP2 into PIP3 inside the plasma membrane, which binds specifically to the pleckstrin homlogy (PH) domain of two proteins, PDK-1 and Akt/PKB, to mediate PI3K signaling (Carnero et al., 2008).

The PI3K-dependent activation mechanism of Akt involves the interaction of Akt with PIP3, leading to its translocation to the medial membrane where its Thr308 and Ser473 sites become exposed. PDK1 phosphorylates Akt's Thr308 site, serving as the initial step in Akt activation. Subsequently, PDK2 phosphorylates the Ser473 sites, located at the hydrophobic carboxyl group's end, to achieve maximal Akt activation (Cole et al., 2019). Once fully activated, Akt phosphorylates downstream target proteins, thereby regulating various cellular functions such as angiogenesis, metabolism, growth, proliferation, protein synthesis, transcription, and apoptosis (Hemmings and Restuccia, 2015).

2.4 Regulation of the PI3K/Akt pathway

The PI3K/Akt signaling pathway is regulated by various factors, particularly a group of phosphatases that exert negative regulatory effects. These phosphatases include phosphatase and tensin homologues (PTEN), protein phosphatase 2 (PP2A), and protein phosphatase in the PH domain rich in repeated sequences of leucine (PHLPP1/2). PTEN plays a crucial role as an upstream component of the PI3K/Akt signaling pathway. It catalyzes the specific dephosphorylation of PIP3 to produce PIP2, thereby exerting a negative regulatory effect on Akt activation (Viennet et al., 2023). Additionally, Inositol polyphosphate 4-phosphatase type II (INPP4B) inhibits Akt signaling by dephosphorylating PIP2 into PIP (Gewinner et al., 2009). PP2A, a trimeric protein, combats Akt signaling by selectively inhibiting phosphorylation of Akt's Thr308 site (Zhang Y. et al., 2022) and dephosphorization of Akt's Ser473 site (Hwang et al., 2013). PHLPP1/2 primarily dephosphorylates Akt at Ser473 sites. Activation of the PI3K pathway leads to stable levels of PHLPP1 and a surge of PHLPP2, both of which attenuate Akt signaling (Chen et al., 2011). Therefore, in the absence of PTEN, PHLPP2 replaces its role in attenuating the output of the PI3K/Akt pathway (Figure 1) (Chen et al., 2014).

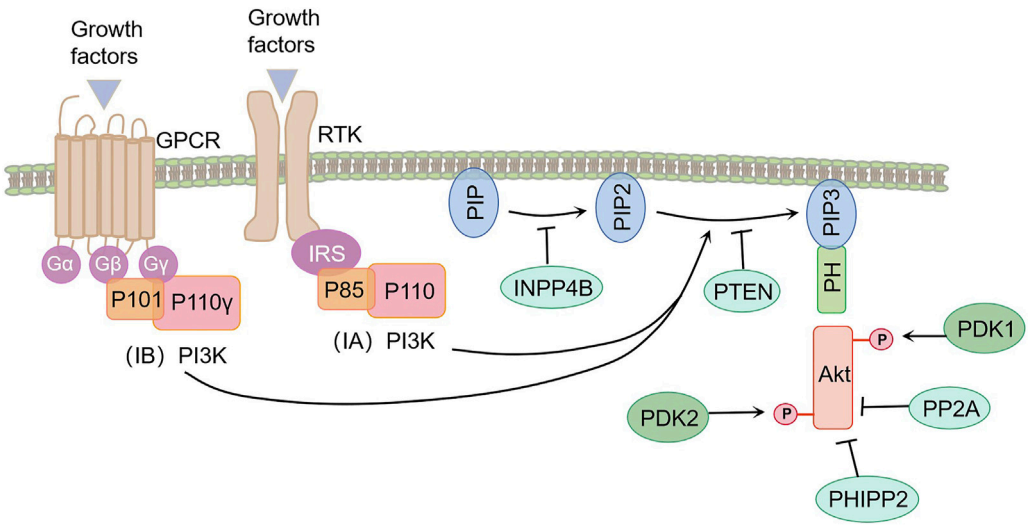

FIGURE 1
Transduction and regulatory pathways of PI3K/Akt pathway. Activation of growth factor receptor tyrosine kinase (RTK) and G-protein-coupled receptors (GPCRs) led to the activation of the PI3K catalytic subunit. Activated PI3K promotes the conversion of PIP2 to PIP3 in the medial membrane, a function that can be reversed by phosphatase and tensin homologues (PTEN). PIP3 activates Akt signaling by specifically binding to the pleckstrin homlogy (PH) domain of both PDK-1 and Akt proteins. Protein phosphatase 2 (PP2A) and the PH domain are rich in leucine repeats of the protein phosphatase (PHLPP1/2), negatively regulating the PI3K/Akt pathway.

3 The PI3K/Akt signaling pathway and its role in the pathogenesis of COPD

3.1 The interplay between inflammation and oxidative stress in the pathogenesis of COPD

Increasing evidence suggests that inflammation and oxidative stress are interconnected pathophysiological processes (Biswas, 2016). In COPD, activated inflammatory cells in the lungs release numerous inflammatory factors that trigger the production of oxygen free radicals, leading to oxidative stress (Guo et al., 2022). Moreover, oxidative stress can amplify lung inflammation by activating multiple signaling pathways within the cells (Barnes, 2022). This closely intertwined process frequently coexists across various chronic diseases, in addition to COPD (Neves et al., 2021), there are inflammatory bowel disease (Tian et al., 2017), alcoholic liver disease (Yang et al., 2022), diabetes (Grabež et al., 2022), neuroinflammatory disease (Xue et al., 2019) and other chronic inflammatory diseases.

3.2 The PI3K/Akt pathway and inflammation in COPD

COPD is a progressive inflammatory lung condition caused by the inhalation of cigarette smoke and other toxic external particulate matter, such as air pollution and biomass fuels. Chronic inflammation of the small airways, known as bronchiolitis, serves as the primary catalyst for COPD development (Brightling and Greening, 2019). Various cytokines secreted by alveolar macrophages, neutrophils, T lymphocytes, B lymphocytes, and structural cells like epithelial, endothelial, and fibroblasts contribute to this inflammatory response (Barnes, 2016). Recent research indicates that smoking acts as an initial trigger for activating innate immune system cells, with tobacco smoke stimulating the PI3K/Akt pathway and exacerbating the inflammatory response in monocytes. Furthermore, elevated PI3K signaling has been linked to sustained inflammation in individuals with COPD, just as shown in Figure 2 (Lee et al., 2018). Furthermore, inflammation underlies significant complications in COPD, including heart and lung diseases, respiratory failure, and cancer (Byrne et al., 2015).

Zhang et al. (2017) discovered that PI3K signaling was activated in alveolar macrophages of COPD mice, leading to a significant increase in pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, thereby enhancing inflammatory responses. Subsequent studies demonstrated that activation of the PI3K/Akt signaling pathway promoted polarization of macrophages in COPD from M1 to M2 phenotypes. The ratio of M1 to M2 macrophages following monocyte polarization has been linked to various inflammatory diseases (Zhang et al., 2023), and an elevated ratio of M2 macrophages has been implicated in lung inflammation (Lu et al., 2017). Neutrophil infiltration in the submucous membrane of the airway is the main driver of airway inflammation in COPD and is regulated by helper T cells 17 (Th17) and macrophages. Macrophages secrete inflammatory mediators that act as chemical inducers, increasing neutrophil infiltration in the airways and promoting lung injury and inflammatory responses in COPD patients (Holl et al., 2013). It is now evident that inflamed airways are exposed to hypoxia, triggering neutrophil degranulation and enhancing their potential for tissue damage. Hoenderdos et al. (2016) discovered that inhibiting the PI3K signaling pathway contributes to the suppression of neutrophil degranulation, suggesting that PI3K plays a crucial role in this process. Additionally, inhibiting Akt phosphorylation had no impact on degranulation regulation, implying that heightened

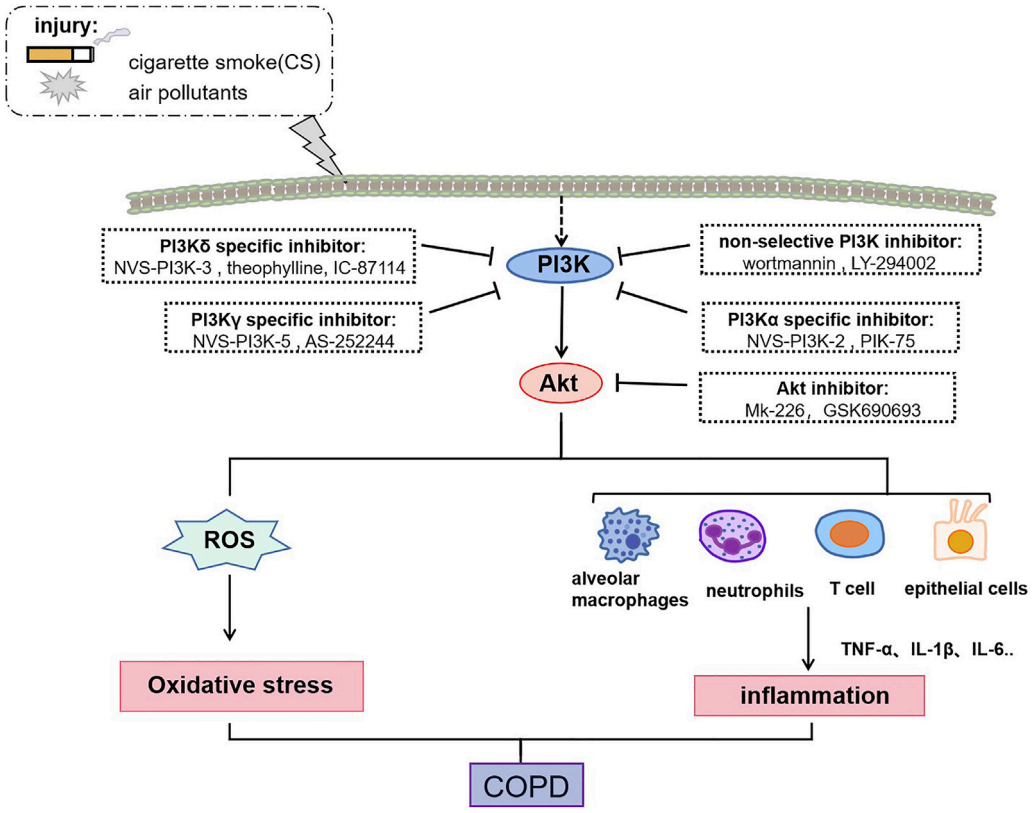

FIGURE 2
Role of PI3K /Akt in COPD regulation. The activation of PI3K/Akt is involved in COPD formation and can exacerbate COPD progression through inflammatory and oxidative stress. Specifically, activated PI3K/Akt promotes the release of ROS, as well as promotes the secretion of multiple pro-inflammatory cytokines by alveolar macrophages, neutrophils, T-lymphocytes, and epithelial cells to participate in COPD pathogenesis. Moreover, small molecule inhibitors targeting the PI3K/Akt signaling pathway can treat COPD through inhibiting oxidative stress and inflammation.

neutrophil reactivity is a result of early PI3K/Akt signaling. Yanagisawa et al. (2017) demonstrated that PI3K signaling is more active in bronchial epithelial cells of COPD patients. In contrast, the negative regulator of PI3K, PTEN, is frequently mutated or absent in the airway epithelial cells of smokers. Knockdown of PTEN leads to significant Akt phosphorylation and increased secretion of pro-inflammatory cytokines (e.g., IL-6, IL-B-induced CXCL8, etc.). Hence, activating PTEN could be an effective approach to impede the progression of COPD.

3.3 The role of the PI3K/Akt pathway in COPD oxidative stress

Oxidative stress arises from an imbalance between free radicals and antioxidants, playing a significant role in inflammatory diseases (Dandekar et al., 2015). Free radicals can originate from activated inflammatory cells, structural cells, cigarette smoke, indoor and outdoor air pollution, among other sources (Valavanidis et al., 2013). COPD is a progressive respiratory disease where inflammatory and structural cells in the lungs release reactive oxygen species (ROS) and reactive nitrogen species (RNS), inducing endogenous oxidative stress during the early stages of the disease. The imbalance between free radicals and antioxidants further exacerbates ROS release (Boukhenouna et al., 2018). Thus, the elevation of oxidative stress persists even after COPD patients stop smoking. This oxidative damage results in endogenous tissue and cellular damage, ultimately leading to chronic inflammation and aging (Barnes et al., 2019). A growing body of research has demonstrated the involvement of the PI3K/Akt/mTOR signaling pathway in promoting lung cell senescence and oxidative stress (Xiaofe et al., 2022), This suggests that blocking the PI3K/Akt pathway as a means to inhibit oxidative stress could hold promise as a therapeutic strategy for COPD patients.

Recent studies have reported a significant reduction of SIRT1 and SIRT6, which are anti-aging molecules, in the lungs of COPD patients (Zhang XY. et al., 2022). Oxidative stress serves as the primary regulator of these proteins' expression (Lakhdar et al., 2018). Inhibition of the PI3K signaling pathway, as demonstrated by Baker et al. (2016), significantly enhances the expression of SIRT1 and SIRT6 while reversing oxidative stress. Additionally, the knockout of PTEN, which inhibits PI3K signaling, resulted in reduced levels of SIRT1 and SIRT6. Studies by Xie S et al. (Xie and Wang, 2022) have shown that cigarette smoke extract downregulates the expression of CRYAB, a recognized anti-apoptotic protein, in the alveoli of COPD mice. Furthermore, overexpression of CRYAB inhibits oxidative stress, delays the activation of the PI3K/Akt signaling pathway, and reduces apoptosis. Oxidative stress also

affects histone deacetylase (HDAC) activity. HDAC, a glucocorticoid functional protein, is frequently associated with glucocorticoid resistance in COPD patients (Rossios et al., 2012), Marwick et al. (2009) found reduced HDAC activity in COPD mice, and knockout of PI3K restored the activity of this enzyme. Consequently, inhibiting the PI3K pathway reinstates histone activity in the presence of oxidative stress-induced glucocorticoid resistance. This restoration, in turn, revives the anti-inflammatory properties of glucocorticoids, leading to a positive inhibition of COPD progression.

4 Potential drug targeting PI3K/Akt for COPD

The pathogenesis of COPD is unclear and is commonly linked to inflammation, oxidative stress and reduced immune function. Current treatments include medication, oxygen therapy and rehabilitation therapy to improve symptoms of airflow restriction caused by reduced lung function (Wang et al., 2020). However, these methods have done little to prevent the progression of COPD disease. Common COPD drugs include beta 2 receptor agonists, anticholinergic drugs, and glucocorticoids. However, chronic inhalation of beta 2 receptor agonists may have adverse cardiovascular and metabolic effects (Vanfleteren et al., 2018). There are a number of side effects associated with anticholinergic drugs, including the dry mouth, blurred vision, and postural hypotension. Glucocorticoids negatively affect the hypothalamic-pituitary-adrenal axis and most COPD patients are insensitive to glucocorticoids. Therefore, there is an urgent need to find novel molecular targeted therapeutics for COPD.

4.1 Application of PI3K inhibitors in COPD treatment

PI3K is a signaling cascade component downstream of multiple cell receptors. Among the three subtypes of PI3K (α, γ and δ), PI3Kα is critical for airway inflammation and angiogenesis (Chen et al., 2021), while Pl3Kγ is pro-inflammatory and involved in inflammatory cell recruitment (Heit et al., 2008) and PI3Kδ contributes to corticosteroid resistance (To et al., 2010).

Wortmannin, a PI3K inhibitor with low substrate specificity. Significantly reduced the activity of neutrophils elastase (NE) and matrix metalloproteinase-9 (MMP-9) released by airway neutrophils in COPD mice and decreased neutrophils inflammation (Vlahos et al., 2012). In addition, Wortmannin induced differentiation of alveolar epithelial stem cells in COPD mice to repair the alveoli and restore respiratory function (Horiguchi et al., 2015). LY 294002 (a non-selective PI3K inhibitor) significantly restored sensitivity to corticosteroids in PBMC cells from COPD patients, but had no effect on the production of the inflammatory factor IL-8. In addition, LY-294002 inhibits the expression of intercellular adhesion molecule −1(ICAM-1) in COPD patients, mediating monocyte/macrophage adhesion and infiltrating inflammatory sites (Liu et al., 2018).

Bewley et al. (2016) found that NVS-PI3K-2 (PI3Kα specific inhibitor), NVS-PI3K-3 (PI3Kδ specific inhibitor) and NVS-PI3K-5 (PI3Kγ specific inhibitor) suppressed lung inflammation and bacterial colonization in COPD patients. Alveolar macrophages play a significant role in clearing bacteria and small apoptotic bodies. However, these inhibitors do not alter the phagocytosis of alveolar macrophages, i.e., do not negatively affect innate immunity of COPD macrophages. Similarly, lower concentrations of theophylline (PI3Kδ specific inhibitors) can target PI3K to reverse oxidative stress-induced corticosteroid resistance and suppress lung inflammation in COPD mice exposed to cigarette smoke (To et al., 2010). In addition, IC-87114, a PI3Kδ specific inhibitor, inhibits neutrophils recruitment and restores corticosteroid sensitivity impaired under oxidative stress by inhibiting PI3K signaling (Rossios et al., 2012). Wang et al. (2019) and others demonstrated that IL-1α and IL-1β expression in human bronchial epithelial cells (HBEC) were significantly upregulated in mucin protein Muc-5ac associated with high mucin secretion, and PIK-75 (PI3Kα specific inhibitor) significantly inhibited PM-induced inflammation and mucin hypersecretion in HBEC, while AS-252244 (PI3Kγ specific inhibitor) and IC-87114 did not. Small molecule inhibitors targeting the PI3K/Akt signaling pathway treat COPD through inhibiting oxidative stress and inflammation, as shown in Figure 2.

4.2 Application of Akt inhibitors in COPD treatment

It is thought that Akt is a central regulator of the molecular pathways involved in smoking-related diseases, particularly COPD. MK-2206 (Akt variant non-ATP competitive inhibitor), an anticancer agent that inhibits all Akt subtypes, is commonly used to synergistically enhance the anti-tumor efficacy of certain molecular targeted drugs (Yap et al., 2011). Jiang et al. (2018) found that MK-2206 reversed changes in markers involved with epithelial mesenchymal transition (EMT) in the lung epithelium of smoking mice. EMT is positively associated with an invasive or metastatic phenotype of COPD (Zhang et al., 2016), so MK-2206 inhibits COPD. In addition, MK-2206 pretreatment inhibited IL-1α and IL-1β and Muc-5ac expression (Wang J. et al., 2019), and also protected the diaphragm in COPD mice induced by hypoxia pretreatment (Chuang et al., 2018). In addition, GSK690693 (ATP-competitive pan-Akt inhibitor) significantly inhibited IL-8 induced apoptosis in inflammatory diaphragm cells (Wang L. et al., 2019). Akt inhibitors are currently understudied and underused in COPD diseases, and more recently, Akt-negative dominant mutant (Akt-DN) transfected cells from Lin CH et al. (Lin et al., 2020) have been shown to inhibit the Akt pathway and IL-8 secretion in human lung epithelial cells.

4.3 Others

In addition to some of these molecular targeted drugs, natural compounds, traditional Chinese medicine formulations, and some anti-inflammatory agents also inhibit the progression of COPD by inhibiting the PI3K/Akt pathway. It has been shown recently that

puerarin can relieve over-oxidation in cells (Zhang P. et al., 2022). Wang L. et al. (2022) found that puerarin reverses apoptosis in HBEC stimulated by cigarette smoke extract (CSE) via the PI3K/Akt signaling pathway. Icariin is one of the active components of Bufei Yishen formula, which can inhibit the mucus hypersecretion in COPD rats (Li J. et al., 2020). Icariin in combination with Nobilitin has a positive therapeutic effect on COPD by improving lung inflammation and emphysema and reducing lung pathological damage in COPD rats via the PI3K/Akt pathway (Lu et al., 2022). At the same time, Scutellaria has a reverse effect on lung pathologic injury induced by smoking in COPD rats (Xu et al., 2018). Crocin, the active ingredient in crocus, significantly inhibits the number of neutrophils and macrophages and the concentration of pro-inflammatory cytokines in COPD mice by modulating the PI3K/Akt-mediated inflammatory pathway (Xie et al., 2019).

As a vital component of complementary alternative medicine, TCM is considered to play its pharmacological role via its multi-component, multi-target, and multi-pathway properties (Ren et al., 2019). Notably, Bu-Shen-Fang-Fang-Chuan formula (BSFCF), a commonly used formula for treating COPD in China, attenuates the inflammatory response to COPD by inhibiting PI3K/Akt-Nrf2 and PI3K/Akt-NF-κB (Li Q. et al., 2020). Xuefu Zhuyu Decoction (XFZYD) is also widely used in the treatment of COPD, and (Hu et al., 2022) and others have found that XFZYD is effective in the treatment of COPD by interfering with the PI3K/Akt signaling pathway, improving oxidative stress and inflammatory responses, and relieving airway remodeling and ventilation disorders through web-based pharmacology and molecular docking experiments. In addition, Tiaobu Feishen (TBFS) was observed to reverse lung inflammation and airway remodeling (Li et al., 2012), Zhou et al. (2023) observed that TBFS has more effective in glucocorticoid-resistant COPD patients by modulating PI3K/Akt signaling to improve glucocorticoid resistance.

Anti-inflammatory agents that interfere with the PI3K/Akt pathway also have significant potential to improve COPD steroid resistance. Macrolides may reduce lung inflammation in COPD by modulating the PI3K/Akt pathway, such as erythromycin, which enhances corticosteroid sensitivity by inhibiting the activity of the PI3K/Akt pathway (Miao et al., 2015; Sun et al., 2015). Solithromycin (SOL, CEM-101), a macrolide/fluoronolactone that inhibits airway neutrophils in steroid-insensitive mice, is an effective anti-inflammatory agent for COPD treatment (Kobayashi et al., 2013). The statin simvastatin improves lung remodeling by reversing epithelial mesenchymal transition in alveolar epithelial cells. This effect is mediated by inhibition of the PI3K/Akt pathway (Milara et al., 2015). In addition, the tricyclic antidepressant nortriptyline can also increase corticosteroid sensitivity. Mercado et al. (2011) found that nortriptyline pretreatment inhibited Akt phosphorylation and PI3K activity, restoring oxidative stress-induced corticosteroid sensitivity as a potential treatment for respiratory diseases such as COPD that are corticosteroid insensitive.

5 Conclusion

The morbidity and mortality rates associated with COPD remain substantial, posing numerous challenges for healthcare professionals involved in COPD interventions. There is a growing body of evidence indicating the therapeutic potential of the PI3K/Akt signaling pathway, particularly different PI3K isoforms, in the treatment of COPD. Several non-specific PI3K inhibitors have demonstrated anti-inflammatory and antioxidant effects in COPD models. However, most broad-spectrum PI3K inhibitors exhibit greater genotoxicity, whereas PI3K subtype-specific inhibitors offer the desired therapeutic properties with reduced side effects. This may provide guidance for subsequent drug development targeting PI3K/Akt. In conclusion, there is an urgent need for further insights into the key regulatory mechanisms of the PI3K/Akt pathway in COPD development, as well as the exploration of safer and more effective therapeutic strategies derived from this approach.

Author contributions

YL designed and wrote this manuscript, HK wrote this manuscript, HeC designed this manuscript, GC checked this manuscript, HuC checked this manuscript, WR checked this manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Abeyrathna, P., and Su, Y. (2015). The critical role of Akt in cardiovascular function. *Vasc. Pharmacol.* 74, 38–48. doi:10.1016/j.vph.2015.05.008

Baker, J. R., Vuppusetty, C., Colley, T., Papaioannou, A. I., Fenwick, P., Donnelly, L., et al. (2016). Oxidative stress dependent microRNA-34a activation via PI3Kα reduces the expression of sirtuin-1 and sirtuin-6 in epithelial cells. *Sci. Rep.* 6, 35871. doi:10.1038/srep35871

Barnes, P. J., Baker, J., and Donnelly, L. E. (2019). Cellular senescence as a mechanism and target in chronic lung diseases. *Am. J. Respir. Crit. care Med.* 200 (5), 556–564. doi:10.1164/rccm.201810-1975TR

Barnes, P. J. (2016). Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. *J. allergy Clin. Immunol.* 138 (1), 16–27. doi:10.1016/j.jaci.2016.05.011

Barnes, P. J. (2022). Oxidative stress in chronic obstructive pulmonary disease. *Antioxidants (Basel, Switz.* 11 (5), 965. doi:10.3390/antiox11050965

Bewley, M. A., Belchamber, K. B., Chana, K. K., Budd, R. C., Donaldson, G., Wedzicha, J. A., et al. (2016). Differential effects of p38, MAPK, PI3K or rho kinase inhibitors on bacterial phagocytosis and efferocytosis by macrophages in COPD. *PloS one* 11 (9), e0163139. doi:10.1371/journal.pone.0163139

Biswas, S. K. (2016). Does the interdependence between oxidative stress and inflammation explain the antioxidant paradox? Oxidative medicine and cellular longevity. *Oxid. Med. Cell Longev.* 2016, 5698931. doi:10.1155/2016/5698931

Boukhenouna, S., Wilson, M. A., Bahmed, K., and Kosmider, B. (2018). Reactive oxygen species in chronic obstructive pulmonary disease. *Oxidative Med. Cell. Longev.* 2018, 5730395. doi:10.1155/2018/5730395

Brightling, C., and Greening, N. (2019). Airway inflammation in COPD: progress to precision medicine. *Eur. Respir. J.* 54 (2), 1900651. doi:10.1183/13993003.00651-2019

Byrne, A. L., Marais, B. J., Mitnick, C. D., Lecca, L., and Marks, G. B. (2015). Risk factors for and origins of COPD. *Lancet (London, Engl.* 385 (9979), 1723–1724. doi:10.1016/S0140-6736(15)60884-4

Cantley, L. C. (2002). The phosphoinositide 3-kinase pathway. *Sci. (New York, NY)* 296 (5573), 1655–1657. doi:10.1126/science.296.5573.1655

Carnero, A., Blanco-Aparicio, C., Renner, O., Link, W., and Leal, J. F. (2008). The PTEN/PI3K/AKT signalling pathway in cancer, therapeutic implications. *Curr. cancer drug targets* 8 (3), 187–198. doi:10.2174/156800908784293659

Chen, M., Nowak, D. G., and Trotman, L. C. (2014). Molecular pathways: PI3K pathway phosphatases as biomarkers for cancer prognosis and therapy. *Clin. cancer Res.* 20 (12), 3057–3063. doi:10.1158/1078-0432.CCR-12-3680

Chen, M., Pratt, C. P., Zeeman, M. E., Schultz, N., Taylor, B. S., O'Neill, A., et al. (2011). Identification of PHLPP1 as a tumor suppressor reveals the role of feedback activation in PTEN-mutant prostate cancer progression. *Cancer Cell* 20 (2), 173–186. doi:10.1016/j.ccr.2011.07.013

Chen, X., Zhabyeyev, P., Azad, A. K., Vanhaesebroeck, B., Grueter, C. E., Murray, A. G., et al. (2021). Pharmacological and cell-specific genetic PI3Kα inhibition worsens cardiac remodeling after myocardial infarction. *J. Mol. Cell. Cardiol.* 157, 17–30. doi:10.1016/j.yjmcc.2021.04.004

Cheng, Q., Chen, M., Liu, M., Chen, X., Zhu, L., Xu, J., et al. (2022). Semaphorin 5A suppresses ferroptosis through activation of PI3K-AKT-mTOR signaling in rheumatoid arthritis. *Cell Death Dis.* 13 (7), 608. doi:10.1038/s41419-022-05065-4

Chuang, C. C., Zhou, T., Olfert, I. M., and Zuo, L. (2018). Hypoxic preconditioning attenuates reoxygenation-induced skeletal muscle dysfunction in aged pulmonary TNF-α overexpressing mice. *Front. physiology* 9, 1720. doi:10.3389/fphys.2018.01720

Cole, P. A., Chu, N., Salguero, A. L., and Bae, H. (2019). AKTivation mechanisms. *Curr. Opin. Struct. Biol.* 59, 47–53. doi:10.1016/j.sbi.2019.02.004

Dandekar, A., Mendez, R., and Zhang, K. (2015). Cross talk between ER stress, oxidative stress, and inflammation in health and disease. *Methods Mol. Biol. Clift. NJ)* 1292, 205–214. doi:10.1007/978-1-4939-2522-3_15

Engelman, J. A., Luo, J., and Cantley, L. C. (2006). The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat. Rev. Genet.* 7 (8), 606–619. doi:10.1038/nrg1879

Fruman, D. A., Chiu, H., Hopkins, B. D., Bagrodia, S., Cantley, L. C., and Abraham, R. T. (2017). The PI3K pathway in human disease. *Cell* 170 (4), 605–635. doi:10.1016/j.cell.2017.07.029

Gewinner, C., Wang, Z. C., Richardson, A., Teruya-Feldstein, J., Etemadmoghadam, D., Bowtell, D., et al. (2009). Evidence that inositol polyphosphate 4-phosphatase type II is a tumor suppressor that inhibits PI3K signaling. *Cancer Cell* 16 (2), 115–125. doi:10.1016/j.ccr.2009.06.006

Glaviano, A., Foo, A. S. C., Lam, H. Y., Yap, K. C. H., Jacot, W., Jones, R. H., et al. (2023). PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer. *Mol. Cancer* 22 (1), 138. doi:10.1186/s12943-023-01827-6

Grabež, M., Škrbić, R., Stojiljković, M. P., Vučić, V., Rudić Grujić, V., Jakovljević, V., et al. (2022). A prospective, randomized, double-blind, placebo-controlled trial of polyphenols on the outcomes of inflammatory factors and oxidative stress in patients with type 2 diabetes mellitus. *Rev. Cardiovasc. Med.* 23 (2), 57. doi:10.31083/j.rcm2302057

Guo, P., Li, R., Piao, T. H., Wang, C. L., Wu, X. L., and Cai, H. Y. (2022). Pathological mechanism and targeted drugs of COPD. *Int. J. chronic Obstr. Pulm. Dis.* 17, 1565–1575. doi:10.2147/COPD.S366126

He, Y., Sun, M. M., Zhang, G. G., Yang, J., Chen, K. S., Xu, W. W., et al. (2021). Targeting PI3K/Akt signal transduction for cancer therapy. *Signal Transduct. Target. Ther.* 6 (1), 425. doi:10.1038/s41392-021-00828-5

Heit, B., Liu, L., Colarusso, P., Puri, K. D., and Kubes, P. (2008). PI3K accelerates, but is not required for, neutrophil chemotaxis to fMLP. *J. Cell Sci.* 121 (2), 205–214. doi:10.1242/jcs.020412

Hemmings, B. A., and Restuccia, D. F. (2015). The PI3K-PKB/Akt pathway. *Cold Spring Harb. Perspect. Biol.* 7 (4), a026609. doi:10.1101/cshperspect.a026609

Hoenderdos, K., Lodge, K. M., Hirst, R. A., Chen, C., Palazzo, S. G., Emerenciana, A., et al. (2016). Hypoxia upregulates neutrophil degranulation and potential for tissue injury. *Thorax* 71 (11), 1030–1038. doi:10.1136/thoraxjnl-2015-207604

Holloway, R. A., and Donnelly, L. E. (2013). Immunopathogenesis of chronic obstructive pulmonary disease. *Curr. Opin. Pulm. Med.* 19 (2), 95–102. doi:10.1097/MCP.0b013e32835cfff5

Horiguchi, M., Oiso, Y., Sakai, H., Motomura, T., and Yamashita, C. (2015). Pulmonary administration of phosphoinositide 3-kinase inhibitor is a curative treatment for chronic obstructive pulmonary disease by alveolar regeneration. *J. Control. release official J. Control. Release Soc.* 213, 112–119. doi:10.1016/j.jconrel.2015.07.004

Hu, Y., Lan, Y., Ran, Q., Gan, Q., and Huang, W. (2022). Analysis of the clinical efficacy and molecular mechanism of xuefu zhuyu decoction in the treatment of COPD based on meta-analysis and network pharmacology. *Comput. Math. methods Med.* 2022, 2615580. doi:10.1155/2022/2615580

Huang, X., Liu, G., Guo, J., and Su, Z. (2018). The PI3K/AKT pathway in obesity and type 2 diabetes. *Int. J. Biol. Sci.* 14 (11), 1483–1496. doi:10.7150/ijbs.27173

Hwang, J. H., Jiang, T., Kulkarni, S., Faure, N., and Schaffhausen, B. S. (2013). Protein phosphatase 2A isoforms utilizing Aβ scaffolds regulate differentiation through control of Akt protein. *J. Biol. Chem.* 288 (44), 32064–32073. doi:10.1074/jbc.M113.497644

Jean, S., and Kiger, A. A. (2014). Classes of phosphoinositide 3-kinases at a glance. *J. Cell Sci.* 127 (5), 923–928. doi:10.1242/jcs.093773

Jiang, B., Guan, Y., Shen, H. J., Zhang, L. H., Jiang, J. X., Dong, X. W., et al. (2018). Akt/PKB signaling regulates cigarette smoke-induced pulmonary epithelial-mesenchymal transition. *Lung cancer (Amsterdam, Neth.* 122, 44–53. doi:10.1016/j.lungcan.2018.05.019

Katso, R., Okkenhaug, K., Ahmadi, K., White, S., Timms, J., and Waterfield, M. D. (2001). Cellular function of phosphoinositide 3-kinases: implications for development, homeostasis, and cancer. *Annu. Rev. Cell Dev. Biol.* 17, 615–675. doi:10.1146/annurev.cellbio.17.1.615

Kobayashi, Y., Wada, H., Rossios, C., Takagi, D., Charron, C., Barnes, P. J., et al. (2013). A novel macrolide/fluoroketolide, solithromycin (CEM-101), reverses corticosteroid insensitivity via phosphoinositide 3-kinase pathway inhibition. *Br. J. Pharmacol.* 169 (5), 1024–1034. doi:10.1111/bph.12187

Lakhdar, R., McGuinness, D., Drost, E. M., Shiels, P. G., Bastos, R., MacNee, W., et al. (2018). Role of accelerated aging in limb muscle wasting of patients with COPD. *Int. J. chronic Obstr. Pulm. Dis.* 13, 1987–1998. doi:10.2147/COPD.S155952

Lareau, S. C., Fahy, B., Meek, P., and Wang, A. (2019). Chronic obstructive pulmonary disease (COPD). *Am. J. Respir. Crit. care Med.* 199 (1), P1–p2. doi:10.1164/rccm.1991P1

Lee, K. H., Lee, C. H., Woo, J., Jeong, J., Jang, A. H., and Yoo, C. G. (2018). Cigarette smoke extract enhances IL-17a-induced IL-8 production via up-regulation of IL-17r in human bronchial epithelial cells. *Mol. cells* 41 (4), 282–289. doi:10.14348/molcells.2018.2123

Li, J., Ma, J., Tian, Y., Zhao, P., Liu, X., Dong, H., et al. (2020a). Effective-component compatibility of Bufei Yishen formula II inhibits mucus hypersecretion of chronic obstructive pulmonary disease rats by regulating EGFR/PI3K/mTOR signaling. *J. Ethnopharmacol.* 257, 112796. doi:10.1016/j.jep.2020.112796

Li, J. S., Li, Y., Li, S. Y., Wang, Y. Y., Deng, L., Tian, Y. G., et al. (2012). Long-term effects of Tiaobu Feishen therapies on systemic and local inflammation responses in rats with stable chronic obstructive pulmonary disease. *Zhong xi yi jie he xue bao = J. Chin. Integr. Med.* 10 (9), 1039–1048. doi:10.3736/jcim20120913

Li, Q., Wang, G., Xiong, S. H., Cao, Y., Liu, B., Sun, J., et al. (2020b). Bu-Shen-Fang-Chuan formula attenuates cigarette smoke-induced inflammation by modulating the PI3K/Akt-Nrf2 and NF-κB signalling pathways. *J. Ethnopharmacol.* 261, 113095. doi:10.1016/j.jep.2020.113095

Lin, C. H., Shih, C. H., Jiang, C. P., Wen, H. C., Cheng, W. H., and Chen, B. C. (2020). Mammalian target of rapamycin and p70S6K mediate thrombin-induced nuclear factor-κB activation and IL-8/CXCL8 release in human lung epithelial cells. *Eur. J. Pharmacol.* 868, 172879. doi:10.1016/j.ejphar.2019.172879

Liu, C. W., Lee, T. L., Chen, Y. C., Liang, C. J., Wang, S. H., Lue, J. H., et al. (2018). PM(2.5)-induced oxidative stress increases intercellular adhesion molecule-1 expression in lung epithelial cells through the IL-6/AKT/STAT3/NF-κB-dependent pathway. *Part. fibre Toxicol.* 15 (1), 4. doi:10.1186/s12989-018-0240-x

Lu, J., Xie, L., Liu, C., Zhang, Q., and Sun, S. (2017). PTEN/PI3k/AKT regulates macrophage polarization in emphysematous mice. *Scand. J. Immunol.* 85 (6), 395–405. doi:10.1111/sji.12545

Lu, R., Xu, K., Qin, Y., Shao, X., Yan, M., Liao, Y., et al. (2022). Network pharmacology and experimental validation to reveal effects and mechanisms of icariin combined with nobiletin against chronic obstructive pulmonary diseases. *Evidence-based complementary Altern. Med. eCAM* 2022, 4838650. doi:10.1155/2022/4838650

Marwick, J. A., Caramori, G., Stevenson, C. S., Casolari, P., Jazrawi, E., Barnes, P. J., et al. (2009). Inhibition of PI3Kdelta restores glucocorticoid function in smoking-

induced airway inflammation in mice. *Am. J. Respir. Crit. care Med.* 179 (7), 542–548. doi:10.1164/rccm.200810-1570OC

Mercado, N., To, Y., Ito, K., and Barnes, P. J. (2011). Nortriptyline reverses corticosteroid insensitivity by inhibition of phosphoinositide-3-kinase-δ. *J. Pharmacol. Exp. Ther.* 337 (2), 465–470. doi:10.1124/jpet.110.175950

Miao, L., Gao, Z., Huang, F., Huang, S., Zhang, R., Ma, D., et al. (2015). Erythromycin enhances the anti-inflammatory activity of budesonide in COPD rat model. *Int. J. Clin. Exp. Med.* 8 (12), 22217–22226.

Milara, J., Peiró, T., Serrano, A., Artigues, E., Aparicio, J., Tenor, H., et al. (2015). Simvastatin increases the ability of roflumilast N-oxide to inhibit cigarette smoke-induced epithelial to mesenchymal transition in well-differentiated human bronchial epithelial cells *in vitro*. *Copd* 12 (3), 320–331. doi:10.3109/15412555.2014.948995

Neves, C. D. C., Lage, V. K. S., Lima, L. P., Matos, M. A., Vieira É, L. M., Teixeira, A. L., et al. (2021). Inflammatory and oxidative biomarkers as determinants of functional capacity in patients with COPD assessed by 6-min walk test-derived outcomes. *Exp. Gerontol.* 152, 111456. doi:10.1016/j.exger.2021.111456

Noorolyai, S., Shajari, N., Baghbani, E., Sadreddini, S., and Baradaran, B. (2019). The relation between PI3K/AKT signalling pathway and cancer. *Gene* 698, 120–128. doi:10.1016/j.gene.2019.02.076

Obeidat, M., Zhou, G., Li, X., Hansel, N. N., Rafaels, N., Mathias, R., et al. (2018). The genetics of smoking in individuals with chronic obstructive pulmonary disease. *Respir. Res.* 19 (1), 59. doi:10.1186/s12931-018-0762-7

Peterson, R. T., and Schreiber, S. L. (1999). Kinase phosphorylation: Keeping it all in the family. *Curr. Biol. CB* 9 (14), R521–R524. doi:10.1016/s0960-9822(99)80326-1

Qin, W., Cao, L., and Massey, I. Y. (2021). Role of PI3K/Akt signaling pathway in cardiac fibrosis. *Mol. Cell Biochem.* 476 (11), 4045–4059. doi:10.1007/s11010-021-04219-w

Rai, S. N., Dilnashin, H., Birla, H., Singh, S. S., Zahra, W., Rathore, A. S., et al. (2019). The role of PI3K/Akt and ERK in neurodegenerative disorders. *Neurotox. Res.* 35 (3), 775–795. doi:10.1007/s12640-019-0003-y

Rathinaswamy, M. K., Dalwadi, U., Fleming, K. D., Adams, C., Stariha, J. T. B., Pardon, E., et al. (2021). Structure of the phosphoinositide 3-kinase (PI3K) p110γ-p101 complex reveals molecular mechanism of GPCR activation. *Sci. Adv.* 7 (35), eabj4282. doi:10.1126/sciadv.abj4282

Ren, L., Guo, X. Y., Gao, F., Jin, M. L., and Song, X. N. (2019). Identification of the perturbed metabolic pathways associating with renal fibrosis and evaluating metabolome changes of pretreatment with Astragalus polysaccharide through liquid chromatography quadrupole time-of-flight mass spectrometry. *Front. Pharmacol.* 10, 1623. doi:10.3389/fphar.2019.01623

Risso, G., Blaustein, M., Pozzi, B., Mammi, P., and Srebrow, A. (2015). Akt/PKB: One kinase, many modifications. *Biochem. J.* 468 (2), 203–214. doi:10.1042/BJ20150041

Rossios, C., To, Y., Osoata, G., Ito, M., Barnes, P. J., and Ito, K. (2012). Corticosteroid insensitivity is reversed by formoterol via phosphoinositide-3-kinase inhibition. *Br. J. Pharmacol.* 167 (4), 775–786. doi:10.1111/j.1476-5381.2012.01864.x

Sandelowsky, H., Weinreich, U. M., Aarli, B. B., Sundh, J., Høines, K., Stratelis, G., et al. (2021). Copd - do the right thing. *BMC Fam. Pract.* 22 (1), 244. doi:10.1186/s12875-021-01583-w

Savova, M. S., Mihaylova, L. V., Tews, D., Wabitsch, M., and Georgiev, M. I. (2023). Targeting PI3K/AKT signaling pathway in obesity. *Biomed. Pharmacother.* 159, 114244. doi:10.1016/j.biopha.2023.114244

Sun, K., Luo, J., Guo, J., Yao, X., Jing, X., and Guo, F. (2020). The PI3K/AKT/mTOR signaling pathway in osteoarthritis: A narrative review. *Osteoarthr. Cartil.* 28 (4), 400–409. doi:10.1016/j.joca.2020.02.027

Sun, X., Chen, L., and He, Z. (2019). PI3K/Akt-Nrf2 and anti-inflammation effect of macrolides in chronic obstructive pulmonary disease. *Curr. drug Metab.* 20 (4), 301–304. doi:10.2174/1389200220666190227224748

Sun, X. J., Li, Z. H., Zhang, Y., Zhou, G., Zhang, J. Q., Deng, J. M., et al. (2015). Combination of erythromycin and dexamethasone improves corticosteroid sensitivity induced by CSE through inhibiting PI3K-δ/Akt pathway and increasing GR expression. *Am. J. physiology Lung Cell. Mol. physiology* 309 (2), L139–L146. doi:10.1152/ajplung.00292.2014

Tian, T., Wang, Z., and Zhang, J. (2017). Pathomechanisms of oxidative stress in inflammatory bowel disease and potential antioxidant therapies. *Oxidative Med. Cell. Longev.* 2017, 4535194. doi:10.1155/2017/4535194

To, Y., Ito, K., Kizawa, Y., Failla, M., Ito, M., Kusama, T., et al. (2010). Targeting phosphoinositide-3-kinase-delta with theophylline reverses corticosteroid insensitivity in chronic obstructive pulmonary disease. *Am. J. Respir. Crit. care Med.* 182 (7), 897–904. doi:10.1164/rccm.200906-0937OC

Valavanidis, A., Vlachogianni, T., Fiotakis, K., and Loridas, S. (2013). Pulmonary oxidative stress, inflammation and cancer: Respirable particulate matter, fibrous dusts and ozone as major causes of lung carcinogenesis through reactive oxygen species mechanisms. *Int. J. Environ. Res. public health* 10 (9), 3886–3907. doi:10.3390/ijerph10093886

Vanfleteren, L., Fabbri, L. M., Papi, A., Petruzzelli, S., and Celli, B. (2018). Triple therapy (ICS/LABA/LAMA) in COPD: Time for a reappraisal. *Int. J. chronic Obstr. Pulm. Dis.* 13, 3971–3981. doi:10.2147/COPD.S185975

Venkatesan, P. (2023). GOLD COPD report: 2023 update. *Lancet Respir. Med.* 11 (1), 18. doi:10.1016/S2213-2600(22)00494-5

Viennet, T., Rodriguez Ospina, S., Lu, Y., Cui, A., Arthanari, H., and Dempsey, D. R. (2023). Chemical and structural approaches to investigate PTEN function and regulation. *Methods Enzym.* 682, 289–318. doi:10.1016/bs.mie.2022.09.007

Vlahos, R., Wark, P. A., Anderson, G. P., and Bozinovski, S. (2012). Glucocorticosteroids differentially regulate MMP-9 and neutrophil elastase in COPD. *PloS one* 7 (3), e33277. doi:10.1371/journal.pone.0033277

Voigt, P., Dorner, M. B., and Schaefer, M. (2006). Characterization of p87PIKAP, a novel regulatory subunit of phosphoinositide 3-kinase gamma that is highly expressed in heart and interacts with PDE3B. *J. Biol. Chem.* 281 (15), 9977–9986. doi:10.1074/jbc.M512502200

Wang, C., Zhou, J., Wang, J., Li, S., Fukunaga, A., Yodoi, J., et al. (2020). Progress in the mechanism and targeted drug therapy for COPD. *Signal Transduct. Target. Ther.* 5 (1), 248. doi:10.1038/s41392-020-00345-x

Wang, J., Zhu, M., Wang, L., Chen, C., and Song, Y. (2019a). Amphiregulin potentiates airway inflammation and mucus hypersecretion induced by urban particulate matter via the EGFR-PI3Kα-AKT/ERK pathway. *Cell. Signal.* 53, 122–131. doi:10.1016/j.cellsig.2018.10.002

Wang, L., Gu, W., Shi, Y., Chen, Y., and Tan, Y. (2019b). Protective effects of astragaloside IV on IL-8-treated diaphragmatic muscle cells. *Exp. Ther. Med.* 17 (1), 519–524. doi:10.3892/etm.2018.6940

Wang, L., Jiang, W., Wang, J., Xie, Y., and Wang, W. (2022b). Puerarin inhibits FUNDC1-mediated mitochondrial autophagy and CSE-induced apoptosis of human bronchial epithelial cells by activating the PI3K/AKT/mTOR signaling pathway. *Aging* 14 (3), 1253–1264. doi:10.18632/aging.203317

Wang, Q., Shen, Z. N., Zhang, S. J., Sun, Y., Zheng, F. J., and Li, Y. H. (2022a). Protective effects and mechanism of puerarin targeting PI3K/Akt signal pathway on neurological diseases. *Front. Pharmacol.* 13, 1022053. doi:10.3389/fphar.2022.1022053

Xiaofei, Y., Tingting, L., Xuan, W., and Zhiyi, H. (2022). Erythromycin attenuates oxidative stress-induced cellular senescence via the PI3K-mTOR signaling pathway in chronic obstructive pulmonary disease. *Front. Pharmacol.* 13, 1043474. doi:10.3389/fphar.2022.1043474

Xie, S., and Wang, X. (2022). CRYAB reduces cigarette smoke-induced inflammation, apoptosis, and oxidative stress by retarding PI3K/Akt and NF-κB signaling pathways in human bronchial epithelial cells. *Allergologia Immunopathol.* 50 (5), 23–29. doi:10.15586/aei.v50i5.645

Xie, Y., He, Q., Chen, H., Lin, Z., Xu, Y., and Yang, C. (2019). Crocin ameliorates chronic obstructive pulmonary disease-induced depression via PI3K/Akt mediated suppression of inflammation. *Eur. J. Pharmacol.* 862, 172640. doi:10.1016/j.ejphar.2019.172640

Xu, C., Feng, C., Huang, P., Li, Y., Liu, R., Liu, C., et al. (2022). TNFα and IFNγ rapidly activate PI3K-AKT signaling to drive glycolysis that confers mesenchymal stem cells enhanced anti-inflammatory property. *Stem Cell Res. Ther.* 13 (1), 491. doi:10.1186/s13287-022-03178-3

Xu, F., Lin, J., Cui, W., Kong, Q., Li, Q., Li, L., et al. (2018). Scutellaria baicalensis attenuates airway remodeling via PI3K/Akt/NF-κB pathway in cigarette smoke mediated-COPD rats model. *Evidence-based complementary Altern. Med. eCAM* 2018, 1281420. doi:10.1155/2018/1281420

Xue, R., Wan, Y., Sun, X., Zhang, X., Gao, W., and Wu, W. (2019). Nicotinic mitigation of neuroinflammation and oxidative stress after chronic sleep deprivation. *Front. Immunol.* 10, 2546. doi:10.3389/fimmu.2019.02546

Yanagisawa, S., Baker, J. R., Vuppusetty, C., Fenwick, P., Donnelly, L. E., Ito, K., et al. (2017). Decreased phosphatase PTEN amplifies PI3K signaling and enhances proinflammatory cytokine release in COPD. *Am. J. physiology Lung Cell. Mol. physiology* 313 (2), L230–l9. doi:10.1152/ajplung.00382.2016

Yang, Y. M., Cho, Y. E., and Hwang, S. (2022). Crosstalk between oxidative stress and inflammatory liver injury in the pathogenesis of alcoholic liver disease. *Int. J. Mol. Sci.* 23 (2), 774. doi:10.3390/ijms23020774

Yap, T. A., Yan, L., Patnaik, A., Fearen, I., Olmos, D., Papadopoulos, K., et al. (2011). First-in-man clinical trial of the oral pan-AKT inhibitor MK-2206 in patients with advanced solid tumors. *J. Clin. Oncol.* 29 (35), 4688–4695. doi:10.1200/JCO.2011.35.5263

Ye, Q., Liu, Y., Zhang, G., Deng, H., Wang, X., Tuo, L., et al. (2023). Deficiency of gluconeogenic enzyme PCK1 promotes metabolic-associated fatty liver disease through PI3K/AKT/PDGF axis activation in male mice. *Nat. Commun.* 14 (1), 1402. doi:10.1038/s41467-023-37142-3

Zhang, A., Qian, F., Li, Y., Li, B., Yang, F., Hu, C., et al. (2023). Research progress of metformin in the treatment of liver fibrosis. *Int. Immunopharmacol.* 116, 109738. doi:10.1016/j.intimp.2023.109738

Zhang, C., Ding, X. P., Zhao, Q. N., Yang, X. J., An, S. M., Wang, H., et al. (2016). Role of α7-nicotinic acetylcholine receptor in nicotine-induced invasion and epithelial-to-

mesenchymal transition in human non-small cell lung cancer cells. *Oncotarget* 7 (37), 59199–59208. doi:10.18632/oncotarget.10498

Zhang, P., Xin, X., Fang, L., Jiang, H., Xu, X., Su, X., et al. (2017). HMGB1 mediates Aspergillus fumigatus-induced inflammatory response in alveolar macrophages of COPD mice via activating MyD88/NF-κB and syk/PI3K signalings. *Int. Immunopharmacol.* 53, 125–132. doi:10.1016/j.intimp.2017.10.007

Zhang, P., Zhang, Y., Wang, L., Wang, X., Xu, S., Zhai, Z., et al. (2022c). Reversal of NADPH oxidase-dependent early oxidative and inflammatory responses in chronic obstructive pulmonary disease by puerarin. *Oxidative Med. Cell. Longev.* 2022, 5595781. doi:10.1155/2022/5595781

Zhang, X. Y., Li, W., Zhang, J. R., Li, C. Y., Zhang, J., and Lv, X. J. (2022b). Roles of sirtuin family members in chronic obstructive pulmonary disease. *Respir. Res.* 23 (1), 66. doi:10.1186/s12931-022-01986-y

Zhang, Y., Wang, X., Li, A., Guan, Y., Shen, P., Ni, Y., et al. (2022a). PP2A regulates metastasis and vasculogenic mimicry formation via PI3K/AKT/ZEB1 axis in non-small cell lung cancers. *J. Pharmacol. Sci.* 150 (2), 56–66. doi:10.1016/j.jphs.2022.07.001

Zhou, P., Ma, J., Yu, W., Chen, K., Zhang, W., and Zhou, J. (2023). Tiao-bu-fei-shen formula improves glucocorticoid resistance of chronic obstructive pulmonary disease via downregulating the PI3K-Akt signaling pathway and promoting GR*α* expression. *eCAM* 2023, 4359616. doi:10.1155/2023/4359616

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Role and mechanisms of SGLT-2 inhibitors in the treatment of diabetic kidney disease

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Diabetic kidney disease (DKD) is a chronic inflammatory condition that affects approximately 20-40% of individuals with diabetes. Sodium-glucose co-transporter 2 (SGLT-2) inhibitors, emerging as novel hypoglycemic agents, have demonstrated significant cardiorenal protective effects in patients with DKD. Initially, it was believed that the efficacy of SGLT-2 inhibitors declined as the estimated glomerular filtration rate (eGFR) decreased, which led to their preferential use in DKD patients at G1-G3 stages. However, recent findings from the DAPA-CKD and EMPA-KIDNEY studies have revealed equally beneficial cardiorenal effects of SGLT-2 inhibitors in individuals at stage G4 DKD, although the underlying mechanism behind this phenomenon remains unclear. In this comprehensive analysis, we provide a systematic review of the mechanisms and functioning of SGLT-2 inhibitors, potential renal protection mechanisms, and the therapeutic efficacy and safety of SGLT-2 inhibitors in kidney diseases, with a particular focus on stage G4 DKD. Gaining a deeper understanding of the renal protective effect of SGLT-2 inhibitors and their underlying mechanisms is highly significance for the successful utilization of these inhibitors in the treatment of diverse kidney disorders.

KEYWORDS

SGLT-2 inhibitor, diabetic kidney disease, renal protection mechanism, efficacy, safety

Abbreviations: ABCG2, ATP-binding cassette subfamily G member 2; AGEs, Advanced glycosylation end products; AKI, Acute kidney injury; ATP, Adenosine triphosphate; CKD, Chronic kidney disease; DKD, Diabetic kidney disease; eGFR, Estimated glomerular filtration rate; EPO, Erythropoietin; ESRD, End-stage renal disease; GLUT9, Proteins glucose transporter protein 9; KDIGO, Kidney Disease: Improving Global Outcomes; NHE3, Na^+-H^+ exchanger 3; NO, Nitric oxide; RAAS, Renin-angiotensin-aldosterone system; RAGE, Receptors for advanced glycosylation end products; RAS, Renin-angiotensin system; SGLT-1, Sodium-glucose co-transporter protein 1; SGLT-2, Sodium-glucose co-transporter protein 2; SNS, Sympathetic nervous system; TGF, Tubuloglomerular feedback; TGF-1, Transforming growth factor 1; CANVAS, Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes ; CREDENCE , Canagliflozin and Renal Outcomes in Type 2 Diabetes and Nephropathy ; DAPA-CKD, Dapagliflozin in Patients with Chronic Kidney Disease; DECLARE-TIMI 58, Dapagliflozin and Cardiovascular Outcomes in Type 2 Diabetes; EMPA-KIDNEY, Empagliflozin in Patients with Chronic Kidney Disease; EMPA-REG OUTCOME, Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes; SCORED, Sotagliflozin in Patients with Diabetes and Chronic Kidney Disease.

1 Introduction

Diabetes, a prevalent illness worldwide, poses a significant threat to human health. As of 2019, approximately 463 million individuals were affected by diabetes, and this number is projected to reach 700 million by 2045 (1). Diabetic kidney disease (DKD), a common microvascular complication associated with diabetes, affects around 20-40% of diabetic patients and can progress to end-stage renal disease (ESRD) in some cases (2–4). While controlling blood glucose levels is crucial in slowing DKD progression, it becomes challenging to lower glycated hemoglobin in patients with advanced DKD, leading to difficulties in halting disease advancement due to patient or pharmaceutical factors (5). Consequently, the pursuit of effective therapeutic approaches that can safeguard kidney function and delay the onset of DKD has emerged as a prominent research area in DKD studies.

Currently, renin-angiotensin system inhibitors are recommended as the primary drugs per the guidelines for treating DKD. However, they are not entirely adequate for DKD treatment due to their potential increased risk of ESRD and acute renal failure (6, 7). The effectiveness and safety of SGLT-2 inhibitors, a novel hypoglycemic agent, have been validated for stage G1-G3 DKD therapy, resulting in the 2020 KDIGO guidelines recommending their use for DKD patients with an estimated glomerular filtration rate (eGFR) ≥ 30 mL/min/1.73 m^2 (8). Further research is still necessary to ascertain their efficacy and safety for individuals with stage G4-G5 DKD.

Recent findings from the DAPA-CKD and EMPA-KIDNEY trials have revealed that SGLT-2 inhibitors offer significant benefits in improving cardiovascular and renal function, as well as delaying the progression of renal disease in stage G4 DKD patients, irrespective of their diabetes status (9, 10). As a result, the 2022 KDIGO guidelines have also recommended a revision of the eGFR threshold to 20 mL/min/1.73 m^2 for DKD patients (11). Therefore, this paper aims to comprehensively review the research progress surrounding the mechanisms of action of SGLT-2 inhibitors, their potential renal protective effects, and their efficacy and safety in patients with stage G4 DKD.

2 The function of SGLT-2 inhibitors

2.1 SGLT-2 inhibitors and mechanism of action

SGLT-2 inhibitors are a relatively new class of antidiabetic medications that have gained attention in recent years. The use of non-selective SGLT inhibitors, extracted from apple tree root bark glycosides, was first reported in the 1830s and since then, SGLT-2 inhibitors have become a popular focus of research due to their unique mechanism of action, which does not rely on insulin (12). SGLT-1 and SGLT-2 are crucial molecules involved in glucose reabsorption in the kidney and are primarily located in the renal tubular epithelium. In normal physiological conditions, glucose filtered from the glomerulus enters the tubules and is reabsorbed by SGLT-1 and SGLT-2. Among these transporters, SGLT-2 is predominant in the S1 and S2 segments of the renal proximal tubule and functions as a high-capacity glucose transporter, responsible for approximately 90% of glucose reabsorption in the renal tubules. On the other hand, SGLT-1 functions as a low-capacity glucose transporter and is found not only in the kidneys but also in the gastrointestinal tract, where it plays a role in reabsorbing a smaller amount of glucose in the renal tubules (13) (Figure 1).

SGLT-2 inhibitors are a class of hypoglycemic agents that have gained popularity in recent years. They work by competing with the SGLT-2 protein for glucose binding in the renal tubules. This competition prevents SGLT-2 from binding to glucose, leading to reduced glucose reabsorption by the renal tubules (Figure 1). As a result, there is an increased excretion of glucose, sodium, and water in the urine. This leads to lower blood glucose and reduced volume load (14). Importantly, the mechanism of action of SGLT-2 inhibitors is independent of insulin regulation. It does not rely on the regulation of insulin secretion by pancreatic β-cells or insulin resistance in the body. Currently, there are several selective SGLT-2 inhibitors available on the market, such as empagliflozin, dapagliflozin, and luseogliflozin. These medications specifically inhibit SGLT-2. However, there are also SGLT-1 and SGLT-2 inhibitors, such as sotagliflozin and canagliflozin, which can inhibit both transporters (15). Overall, the discovery and development of SGLT-2 inhibitors have provided a novel approach for managing blood glucose levels in individuals with diabetes.

2.2 Hypoglycemic effects

The hypoglycemic effect of SGLT-2 inhibitors relies on renal tubular reabsorption, and thus the amount of glucose excretion in the urine is directly related to the glomerular filtration rate (16, 17). In individuals with advanced DKD, specifically those with stage G4-G5 DKD, the options for hypoglycemic medications are limited, and dosage is often restricted. Therefore, it is crucial to assess the effectiveness of treating patients with relatively advanced DKD using SGLT-2 inhibitors. Several clinical studies have focused on the glycemic efficacy of SGLT-2 inhibitors in individuals with type 2 diabetes and stage 3 chronic kidney disease (CKD). Their findings indicate that the glucose-lowering effect is attenuated compared to patients with normal renal function (18–20). In a study involving dapagliflozin treatment for patients with type 2 diabetes and stage 3b-4 CKD, glycated hemoglobin levels did not decrease over the 102-week treatment period (21). Similarly, another study focusing on DKD patients treated with luseogliflozin found that the increase in urinary glucose excretion was lower in those with stage G4 DKD compared to patients with stage G1-G3 DKD (22). These findings suggest that the hypoglycemic efficacy of SGLT-2 inhibitors is reduced in patients with stage G4 DKD when compared to those with stage G1-G3 DKD.

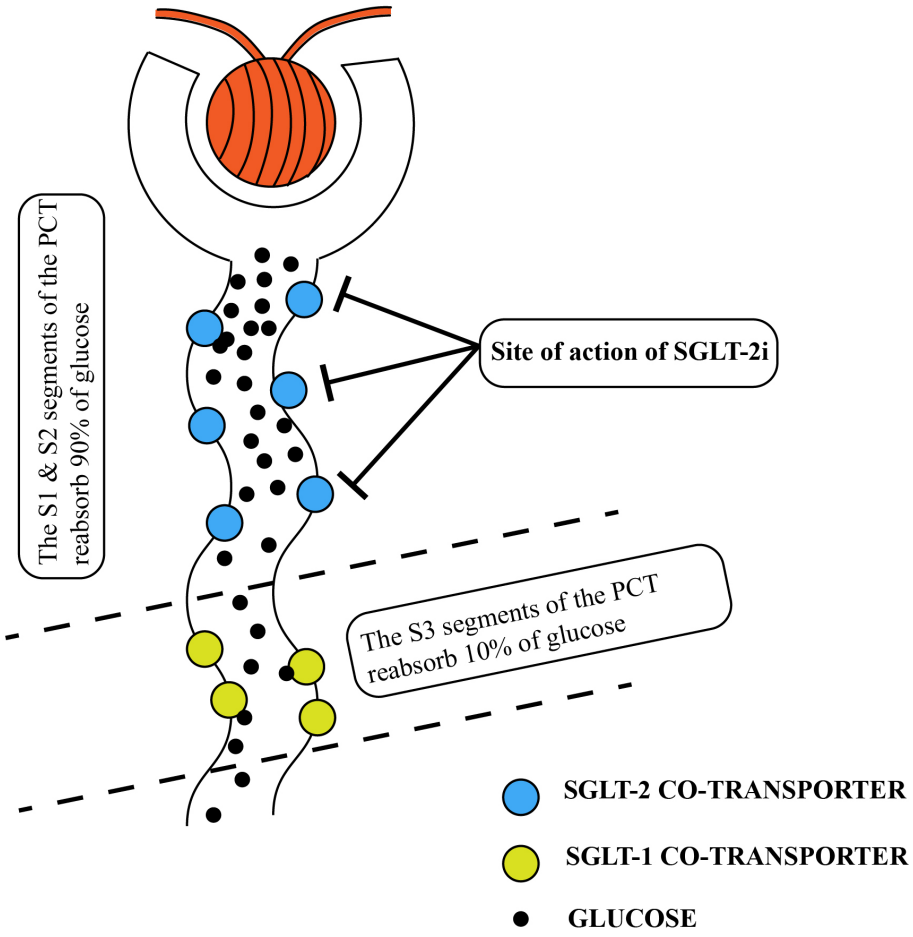

FIGURE 1
Normal renal tubular resorption of glucose. The location of the SGLT2-inhibitor's action is also shown in the diagram. PCT, proximal convoluted tubules; SGLT, sodium-glucose co-transporter.

2.3 Nephroprotective effects

2.3.1 Reduction of glomerular hyperfiltration

On one hand, SGLT-2 receptors are primarily found in the proximal tubule and are responsible for the reabsorption of glucose and sodium ions. Consequently, the decreased sodium concentration sensed at the macula densa location triggers a tubuloglomerular feedback (TGF) mechanism, which stimulates contraction of the efferent arteries and dilation of the afferent arteries. This results in increased blood flow into the glomerulus, raising the glomerular pressure and leading to glomerular hyperfiltration and subsequent damage (23). In contrast, SGLT-2 inhibitors can reduce Na^+ reabsorption in the renal tubules by competitively binding glucose. This restoration of the TGF mechanism promotes relaxation of the small arterial outflow, reducing intraglomerular pressure and alleviating glomerular hyperfiltration. These effects are crucial in preserving renal function and slowing the progression of nephropathy (24).

However, another important player in Na^+ reabsorption in the proximal tubule is the Na^+-H^+ exchanger 3 (NHE3), responsible for about 70% of Na^+ reabsorption through direct or indirect mechanisms (25). Activation of the TGF system leads to increased intraglomerular pressure and subsequent glomerular hyperfiltration. Recent research has demonstrated that NHE3 works synergistically with SGLT-2 in Na^+ reabsorption, with SGLT-2 tightly regulating NHE3 activity. Therefore, NHE3 is sensitive to the modulation by SGLT-2 inhibitors (26, 27). A study conducted on diabetic mice revealed that SGLT-2 inhibitors have the ability to inhibit NHE3 function by promoting NHE3 phosphorylation. This phosphorylation enhances Na^+ excretion, effectively reducing sodium levels within the body (28).This finding demonstrates the potential of SGLT-2 inhibitors as therapeutic agents in regulating NHE3 activity and mitigating glomerular hyperfiltration.

Hence, the underlying mechanism behind the natriuretic effect of SGLT-2 inhibitors is to combat sodium-water retention and repair TGF system, thereby alleviating the condition of glomerular hyperfiltration for renal protection. This effect could be attributed to the competitive inhibition of SGLT receptors and the subsequent inhibition of NHE3. Notably, NHE3 seems to play a pivotal role in the overall natriuretic effect of SGLT-2 inhibitors.

2.3.2 Regulation of the renin-angiotensin-aldosterone system (RAAS)

The activation of RAAS, particularly the increased secretion of angiotensin II (Ang II) and aldosterone, has been established as a crucial factor in the progression of DKD (29–31). In response to elevated blood glucose levels, SGLT-2 in the proximal renal tubule enhances Na^+ and glucose reabsorption. This leads to reduced sensing of Na^+ by the macula densa, resulting in decreased adenosine production, which further activates the RAAS and causes constriction of the small afferent arteries. Moreover, the enhanced production of Ang II, stimulated by RAAS activation, also triggers the release of aldosterone. The resulting oxidative stress, inflammation, and renal fibrosis contribute to the accelerated progression of DKD (32). With the usage of SGLT2 inhibitors, the macula densa becomes more sensitive to changes in Na^+ concentration. This results in increased adenosine production and suppression of RAAS activation. Consequently, there is vasoconstriction in the afferent arteries, while the inhibition of RAAS also reduces the release of aldosterone release. These combined effects help to alleviate oxidative stress, inflammation, and fibrosis in the kidney, thereby mitigating the progression of DKD (33).

In the past few decades, RAAS inhibitors have been widely used as the primary approach to treat DKD (34). However, the distinction between SGLT-2 inhibitors and RAAS inhibitors lies in their differential effects on the small afferent and efferent glomerular arteries. RAAS inhibitors dilate both the afferent and efferent arterioles, but the efferent arterioles are dilated to a greater extent, resulting in continued glomerular ultrafiltration and subsequent damage to kidney units and podocytes (35). Consequently, RAAS inhibitors alone may not effectively halt the progression of early-stage DKD or provide optimal treatment for individuals with advanced DKD (29, 36). However, SGLT-2 inhibition provides a greater degree of renal protection compared to RAAS inhibition as it not only relieves glomerular hyperfiltration but also reduces intra-glomerular pressure by dilating small outgoing glomerular arteries and constricting small incoming glomerular arteries. This unique mechanism of action suggests that SGLT-2 inhibitors may offer enhanced efficacy in delaying the progression of DKD.

3 Potential renal protection mechanisms

Although the exact mechanisms by which SGLT-2 inhibitors protect the kidneys are not fully understood, recent research has shown that these medications have numerous potential renoprotective effects in addition to their traditional hypoglycemic effects. These effects include reducingglomerular hyperfiltration and volume load, inhibiting inflammation and fibrosis, improving oxidative stress, enhancing erythropoietin (EPO) production, enhancing mitochondrial energy supply, inhibiting the sympathetic nervous system, protecting vascular endothelial cells, and reducing blood uric acid levels, among others (37–42) (Figure 2).

3.1 Anti-inflammatory, anti-oxidative stress and anti-fibrotic effects

Mild systemic inflammation is commonly observed in patients with DKD. Prolonged hyperglycemia leads to the production of advanced glycosylation end products (AGEs) through the glycosylation of non-enzymatic proteins. These AGEs promote the formation of receptors for advanced glycosylation end products (RAGE), resulting in oxidative stress and increased levels of reactive oxygen species. This oxidative stress contributes to the progression of kidney fibrosis and further decline in renal function, marking a crucial phase in the development of DKD (43, 44). One of the key pro-fibrotic factors involved in this process is transforming growth factor 1 (TGF-1). TGF-1, along with other pro-fibrotic factors, intensifies inflammation and exerts strong pro-fibrotic effects. It promotes the proliferation of mesangial cells and the deposition of extra-mesenchymal matrix, leading to glomerulosclerosis and interstitial fibrosis (45).

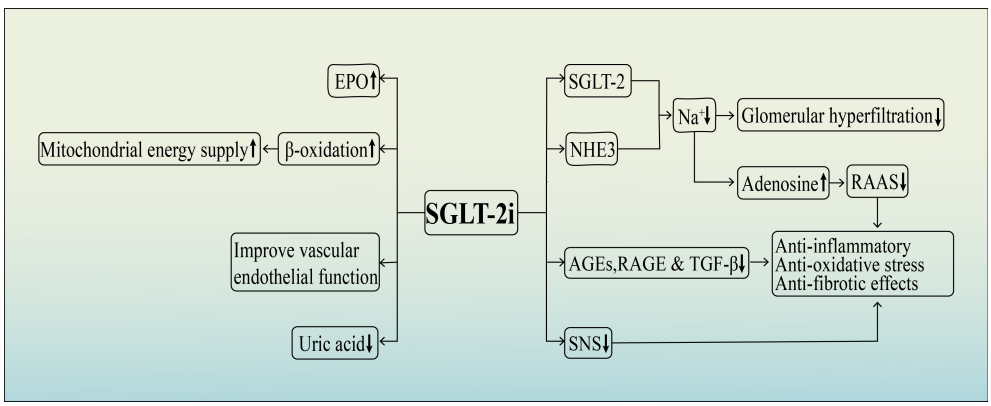

FIGURE 2
The diagram shows the renal protection mechanism of SGLT-2 inhibitors.

In a study by Ojima et al., diabetic rats were used to investigate the effects of empagliflozin on renal tissues. After 4 weeks of empagliflozin treatment, there was a significant reduction in the expression of AGEs, RAGE, and other proteins in renal tissues. This resulted in the inhibition of the AGE-RAGE oxidative stress axis, demonstrating empagliflozin's potential as an anti-inflammatory, anti-fibrotic, and tubular protective agent (46). Further experiments revealed that empagliflozin effectively mitigated oxidative stress and attenuated damage to the renal tissues. It is believed that this protective effect may be attributed to empagliflozin's ability to downregulate the TGF-β-Smad pathway and upregulate the Nrf2/ARE pathway (47). Consequently, SGLT-2 inhibitors such as empagliflozin have the potential to safeguard kidney health by suppressing the expression of AGEs, RAGE, and TGF-β1, thereby exerting anti-inflammatory, anti-oxidative stress, and anti-fibrotic effects.

3.2 Promotion of EPO production

Renal anemia is a prevalent complication in advanced DKD, and its management has been shown to slow down the progression of renal failure (48, 49). In diabetic patients, increased glucose reabsorption through SGLT-2 by proximal tubular epithelial cells results in excessive consumption of adenosine triphosphate (ATP) produced by the Na^+/K^+ pump. This leads to heightened oxygen consumption by tubular epithelial cells and subsequently reduced oxygen partial pressure in the kidney's cortical tissue. The hypoxic microenvironment of renal tubular epithelial cells triggers the transformation of EPO-producing fibroblasts into myofibroblasts, consequently reducing EPO production (50, 51). By diminishing glucose reabsorption and lowering the demand on the Na^+/K^+ pump ATP, SGLT-2 inhibitors alleviate metabolic stress on proximal tubules, thereby improving the microenvironment within renal tubules.

This leads to the transformation of myofibroblasts to fibroblasts, which partially increases EPO production and contributes to the correction of anemia (52). The EMPA-HEART CardioLink-6 trial demonstrated that empagliflozin-induced early elevation of EPO and subsequent increments in hematocrit correlated with reductions in ferritin and hemoglobin levels (53). An analysis of data from the EMPA-REG outcome trial suggests that approximately 50% of the mortality benefits associated with increased hematocrit in SGLT-2 inhibitor-treated patients may be attributed to enhanced erythropoietin production. This gain might be due to the protective effects of EPO as a circulating pleiotropic cytokine, including promoting angiogenesis, improvingmitochondrial function, and suppressing inflammation. Moreover, the elevated hematocrit resulting from EPO also directly enhances the oxygen-carrying capacity of systemic tissues (54). Hence, by stimulating the production of EPO,SGLT-2 inhibitors have the potential to safeguard renal function and slow down the advancement of renal failure.

3.3 Improved mitochondrial energy supply

The renal tubule's epithelial cells contain abundant mitochondria, which play a crucial role in supplying energy for the reabsorption of metabolic substances like glucose and Na^+ in the renal tubules. ATP, produced by mitochondria, is essential for glucose uptake by tubular epithelial cells. Glucose and fatty acids are the primary sources of substrates for ATP production, with β-oxidation of fatty acids generating significantly more ATP than aerobic fermentation of glucose (55, 56). In a hyperglycemic environment, there is an increase in glycolysis, tricarboxylic acid cycle, and β-oxidation, along with an elevation in oxygen free radical production, leading to tubulointerstitial fibrosis and hastening the progression of renal failure (57, 58). Research indicatesthat impaired fatty acid oxidation occurs in parallel with the advancement of DKD (59, 60). Hence, enhancing mitochondrial fatty acid oxidation to ensure a more efficient energy supply could potentially delay the progression of DKD.

SGLT-2 inhibitors have been found to increase urinary glucose excretion, leading to a decrease in glucose levels in the body. This causes a shift in energy utilization, where fatty acid β-oxidation is employed as the main source of energy. This shift in energy supply is primarily responsible for the weight loss observed with SGLT-2 inhibitors (61). Furthermore, increased β-oxidation results in the production of excess acetyl coenzyme A, which generates ketone bodies (β-hydroxybutyrate, acetoacetate, and acetone). These ketone bodies serve as a fuel source for ATP production in the mitochondria, thus improving mitochondrial energy supply (62, 63). Moreover, SGLT-2 inhibitors can enhance mitochondrial energy supply through various mechanisms. They promote EPO production, enhance renal tissue oxygenation, and facilitate the conversion of synthetic ATP fuel from glucose to ketone bodies (64). Numerous clinical studies have demonstrated that the application of SGLT-2 inhibitors promotes the synthesis of ketone body and decreases the insulin-to-glucagon ratio (65–67). Hence, it is believed that SGLT-2 inhibitors have the potential to enhance kidney function by augmenting ketone body production through β-oxidation, thereby improving the mitochondrial energy supply to the renal tubular epithelial cells.

3.4 Inhibition of the sympathetic nervous system (SNS)

Persistent activation of the SNS has been strongly linked to the onset of type 2 diabetes (68). Moreover, its excessive activation is associated with a negative prognosis in patients suffering from advanced DKD (69). SNS hyperactivity has been correlated with glomerulosclerosis, protein loss, and microalbuminuria. Additionally, it promotes the development of renal fibrosis by triggering pro-inflammatory and pro-fibrotic markers such as tumor necrosis factor-β, it also hastens the development of renal fibrosis. This, in turn, leads to a decline in glomerular filtration rate and further exacerbates SNS activation, creating a harmful cycle that accelerates the progression of DKD (70, 71). Sano has proposed that the kidneys play a central role in sympathetic overactivation and has suggested that SGLT-2 inhibitors could potentially provide cardiovascular and renal benefits by reducing afferent renal nerve activity and inhibiting reflex mechanisms within the central nervous system that activate the systemic sympathetic nerves (72).

Herat et al. proposed that the neurotransmitter norepinephrine, released by the sympathetic nervous system, may enhance the expression of SGLT-2. This, in turn, leads to an increase in the reabsorption of glucose and sodium in the renal tubules, as well as elevated levels of blood sugar and blood pressure (73). It has also been demonstrated that dapagliflozin can reduce the expression of tyrosine hydroxylase and norepinephrine in hypertensive mice. These findings suggest that the effect of SGLT-2 inhibitors in protecting renal function could be attributed to a decrease in renal sympathetic nerve activity.

3.5 Improve vascular endothelial function

DKD is a complication of diabetes mellitus characterized by damage to the small blood vessels. The dysfunction of the vascular endothelium is considered the initial factor contributing to the development of diabetic microangiopathy. Impaired function of endothelial cell often results in reduced production of nitric oxide (NO) (74, 75). However, extended administration of SGLT-2 inhibitors has been shown to effectively improve vascular endothelial dysfunction in diabetic rats by enhancing NO diastolic function, reducing oxidative stress, and alleviating glucose toxicity in the aortic rings (76, 77). In a study investigating endothelial dysfunction in diabetes, dapagliflozin was found to potentially facilitate the repair of vascular endothelial by decreasing the expression of vascular adhesion molecules, phosphorylated IκB expression, and infiltration of inflammatory macrophages *in vivo* (41).

3.6 Reduce uric acid

Chronic hyperuricemia has been consistently identified as a risk factor for the progression of CKD, particularly in individuals with coexisting type 2 diabetes (78). The kidneys play a crucial role in eliminating uric acid from the body, primarily through the renal tubules (79). Within the renal tubules, specific proteins, namely glucose transporter protein 9 (GLUT9) and ATP-binding cassette subfamily G member 2 (ABCG2), are responsible for the reabsorption and excretion of urate, respectively. Of these proteins, GLUT9 plays a central role in the handling of urate (78). The hypouric acid effect of SGLT-2 inhibitors has been linked to the excretion of sugar in the urine (80). These inhibitors work by blocking SGLT receptors, leading to increased glucose excretion in the urine. The excess urinary glucose then competes with GLUT9, reducing the reabsorption of urate and increasing the excretion of uric acid (78). Research has demonstrated that the ability of empagliflozin to lower uric acid levels is associated with the upregulation of ABCG2 expression, which is mediated by the AMPK/Akt/CREB signaling pathway (81).

4 Efficacy and safety of SGLT-2 inhibitors in stage G4 DKD

In recent years, the effectiveness and safety of SGLT-2 inhibitors in treating patients with type 2 diabetes and mild to moderate renal insufficiency (eGFR ≥ 30 mL/min/1.73 m^2) have been confirmed through large clinical trials such as EMPA-REG OUTCOMES (82), DECLARE-TIME 58 (83), CANVAS-R (84), and CREDENCE (85). However, the efficacy and safety of SGLT-2 inhibitors in patients with stage G4 and even G5 DKD have remained unclear until the release of the results from DAPA-CKD (9) and EMPA-KIDNEY (10). These studies have provided a significant basis for establishing the effectiveness and safety of SGLT-2 inhibitors in patients with stage G4 DKD.

4.1 Effect on cardiac and renal outcomes

The DAPA-CKD clinical trial enrolled patients with CKD and an eGFR ranging from 25 to 75 mL/min/1.73 m^2, with up to 67.5% of them having DKD. During the study, which had a median follow-up duration of 2.4 years, the results demonstrated a significant reduction in the risk of the primary endpoint event, which included sustained reduction in eGFR exceeding 50%, ESRD, and a composite endpoint of death resulting from renal or cardiovascular causes in the dapagliflozin treatment group (9). In the subgroup analysis of patients with stage 4 CKD, the use of dapagliflozin group demonstrated significant benefits. The dapagliflozin group exhibited a 27% reduction in the risk of experiencing the main composite endpoint event, which included sustained decline in eGFR exceeding 50%, ESRD, and death from kidney disease. Additionally, there was a 29% decrease in the risk of experiencing sustained decline in eGFR > 50%, ESRD, and death from kidney disease.The risk of hospitalization for heart failure or cardiovascular death was lowered by 17%, and the risk of all-cause mortality decreased by 32%. Furthermore, dapagliflozin treatment also resulted in a slower rate of decline in eGFR and significantly reduced proteinuria. Remarkably, there were no significant differences in therapeutic benefits and safety observed between stage 2-3 CKD and stage 4 CKD groups (86).

The EMPA-KIDNEY trial, with a median follow-up of 2 years, included 46% of patients with DKD and 34.5% of patients with stage 4 CKD and an eGFR of 20–30 mL/min/1.73 m^2. The primary endpoint events were cardiovascular death or progression of kidney disease. Progression of kidney disease was defined as a > 40% reduction in eGFR from baseline values, a sustained decrease in eGFR to < 10 mL/min/1.73 m^2, ESRD, or death due to kidney disease causes. The results of the trial showed that the group receiving empagliflozin had a 28% lower risk of cardiac and kidney endpoints compared to the placebo group. Furthermore, there was a 14% lower risk of hospitalization for any reason in the empagliflozin group. Additionally, empagliflozin treatment delayed the decline in eGFR and reduced albuminuria, particularly in patients with a higher urine protein to creatinine ratio at baseline.These beneficial outcomes were consistent across subgroups defined by different eGFR ranges (10). Overall, the EMPA-KIDNEY trial demonstrates that empagliflozin is effective in reducing the risk of cardiovascular and kidney events, hospitalization, and progression of kidney disease in patients with DKD and CKD stage 4. These findings highlight the potential benefits of empagliflozin in managing kidney disease in these patient populations.

In the CREDENCE trial, which focused on patients with DKD, the primary endpoint was defined as doubling of blood creatinine levels, development of ESRD, or death from cardiovascular or renal causes. After 2.6 years of follow-up, the results indicated that canagliflozin reduced the risk of the primary outcome by 30% and the risk of progressing to ESRD by 32% (85). Interestingly, in the subgroup of patients with stage G4 DKD, canagliflozin did not significantly improve glycated hemoglobin levels. However, it did lead to a significant 33% reduction in urinary albumin levels and a slower decline in eGFR compared to the placebo group. These findings were consistent with the DAPA-CKD and EMPA-KIDNEY trials, where the risk of major outcomes in the stage G4 DKD subgroup was similar to that of other subgroups defined by eGFR (87). Overall, these results highlight the efficacy of canagliflozin in reducing the risk of renal and cardiovascular events, as well as slowing the progression of kidney disease in patients with DKD, including those with stage G4 DKD.

The SCORED trial aimed to evaluate the effects of sotagliflozin in patients with DKD, specifically those with an eGFR of 25-65 mL/min/1.73 m^2. The trial had a median follow-up period of 16 months. The primary endpoint was the incidence of serious cardiovascular adverse events. Additionally, the advancement of renal disease was assessed as a secondary endpoint, which included a decline in eGFR of more than 50% from baseline, ESRD, dialysis, or renal transplantation. However, the results of the trial did not demonstrate statistically significant differences in either all-cause mortality or the renal composite endpoint outcomes between the sotagliflozin group and the control group (88). Surprisingly, the subgroup analysis focusing on patients with stage G4 DKD did not show substantial reductions in the primary endpoints of cardiovascular death and heart failure (89).

A separate study investigating the effects of sotagliflozin over a 52-week period showed that it did not significantly improve glycated hemoglobin levels after 26 weeks. Furthermore, its cardiorenal outcomes were consistent with the findings from the SCORED trial (90). Notably, sotagliflozin did not demonstrate a significant reduction in the risk of cardio-renal outcomes specifically in patients with stage G4 DKD, which is in contrast to the positive results observed in other trials such as DAPA-CKD, EMPA-KIDNEY, and CREDENCE studies. To summarize, SGLT-2 inhibitors have been found to offer significant cardiac and renal benefits to patients with stage G4 DKD, which were comparable to those observed in patients with stage G1-G3 DKD. It is widely acknowledged that as the eGFR decreases, patients experience a decline in glomerular filtration function and a reduced ability to reabsorb glucose and Na^+ in the renal tubules.

Therefore, their ability to lower blood sugar, improve glomerular hyperfiltration, and reduce volume load is diminished. However, even in the presence of a significant decrease in eGFR, SGLT-2 inhibitors still provide notable cardiorenal benefits. These benefits may be attributed to potential renoprotective effects, such as reducing inflammation and fibrosis, improving oxidative stress, enhancing mitochondrial energy supply, inhibiting the SNS, promoting EPO production, and improving vascular endothelial function. Additionally, considering that sotagliflozin is less effective in improving cardiovascular and renal outcomes in patients with more advanced DKD, dapagliflozin, empagliflozin, and canagliflozin are recommended for the treatment of patients with relatively advanced DKD (Table 1).

4.2 Adverse reactions

When evaluating the safety of SGLT-2 inhibitors in patients with stage G4 DKD, it is crucial to consider both their positive impact on the heart and kidneys, as well as any potential negative side effects they may cause. Various types of SGLT-2 inhibitors exhibit different adverse events, particularly hypoglycemia, genitourinary infection, ketoacidosis, acute kidney injury (AKI), fractures or amputations, and other adverse events. Therefore, when utilizing SGLT-2 inhibitors, a thorough assessment of the patient's condition should be conducted.

TABLE 1 Effect of SGLT-2 inhibitors on cardiac and renal outcomes.

Drug	DAPA-CKD	EMPA-KIDNEY	CREDENCE	SCORED
	Dapagliflozin	Empagliflozin	Canagliflozin	Sotagliflozin
Median follow-up(years)	2.4	2.0	2.6	1.3
eGFR(ml/min/1.73m^2)	25-75	20-90	30-90	25-60
Stage 4 CKD rate	14%(n=624)	34.2%(n=1131)	4%(n=174)	7.7%(n=813)
Renal outcomes Hazard Ratio(95%CI) P Value	 0.56(0.45-0.68) p<0.001	 0.72(0.64-0.82) P<0.001	 0.66(0.53-0.81) P<0.001	 0.71(0.46-1.08) p>0.05
Cardiovascular outcomes Hazard Ratio(95%CI) P Value	 0.71(0.55-0.92) P=0.009	 0.84 (0.64-0.82) P=0.15	 0.69(0.57-0.83) P<0.001	 0.74(0.63-0.88) P<0.001
Deaths from any cause Hazard Ratio(95%CI) P Value	 0.69(0.53-0.88) P=0.004	 0.87(0.70-1.08) P=0.21	 0.83(0.68-1.02) p>0.05	 0.99(0.83-1.18) p>0.05

4.2.1 Hypoglycemia

Since the primary mechanism of SGLT-2 inhibitors involves inhibiting renal glucose excretion, which is independent of insulin secretion, the risk of hypoglycemia is low (91). Clinical trials such as DAPA-CKD, EMPA-KIDNEY, SOCRED, and CREDENCE have demonstrated no increased risk of hypoglycemia in patients with stage G4 DKD (9, 10, 85, 88). Additionally, a study involving type 2 diabetic patients with CKD stages 3b-4 also found no severe hypoglycemic events in the dapagliflozin group, while three (4.3%) severe hypoglycemic events occurred in the placebo group (21). Therefore, SGLT-2 inhibitors have not been shown to raise the incidence of hypoglycemia in patients with advanced DKD.

4.2.2 Ketoacidosis

Ketoacidosis, which is often characterized by gastrointestinal symptoms such as nausea, vomiting, and diarrhea, is a serious adverse effect of SGLT-2 inhibitors (92). The occurrence of ketoacidosis may have a multifaceted mechanism. On one hand, the reduction in blood glucose induced by SGLT-2 inhibitor inhibits insulin secretion. On the other hand, the mechanisms associated with SGLT-2 inhibitors also lead to an increase in glucagon secretion, resulting in a high glucagon/insulin ratio. This elevated ratio plays a significant role in promoting hepatic fatty acid oxidation and ketone bodies production (93). Additionally, the loss of glucose in the kidneys promotes the secondary reabsorption of ketone bodies, further contributing to ketoacidosis (94).

In a recent multicenter study, the risk ratios for ketoacidosis were found to be 3.58, 2.52, and 1.86 for canagliflozin, empagliflozin, and dapagliflozin respectively. The high incidence of ketoacidosis with canagliflozin is primarily attributed to its greater selectivity for SGLT-1 over SGLT-2. Inhibition of SGLT-1 contributes to the development of diarrhea and volume deficit, which can serve as important trigger for ketoacidosis (95). In both the EMPA-KIDNEY and SOCRED trials, ketoacidosis occurred more frequently in the empagliflozin and sotagliflozin groups. Adverse events of diarrhea were also more common in the sotagliflozin group, further increasing the risk of ketoacidosis (10, 88). Therefore, individuals taking SGLT-2 inhibitors should be vigilant for potential ketoacidosis if they experience gastrointestinal symptoms.

4.2.3 Genitourinary system infections

SGLT-2 inhibitors, by promoting the excretion of high amounts of sugar in urine through the kidneys, can increase the risk of urinary tract infections, particularly Candida infections. These infections are more common in females than males and can typically be alleviated with conventional drug therapy (96). Findings from the SCORED trial indicate a higher incidence of genital fungal infections in the sotagliflozin group compared to the placebo group, and this difference was statistically significant (88). Furthermore, other related studies have also suggested a higher likelihood of genital fungal infections in patients with type 2 diabetic CKD stages 3b-4 who use dapagliflozin (21).

4.2.4 Acute kidney injury

Previous explanations for SGLT-2 inhibitor-induced AKI have often focused on the diuretic effect of these inhibitors, which can lead to a decrease in eGFR due to constriction of the small incoming glomerular arteries through tubular feedback (97). However, as research progressed, a more plausible mechanism for the development of AKI was proposed. SGLT-2 inhibitors cause uric aciduria by enhancing the excretion of uric acid via GLUT9 in the renal proximal tubules. This, in turn, triggers an immune response through the activation of inflammatory vesicles and localized inflammatory reactions, ultimately resulting in AKI (98). Additionally, when SGLT-2 inhibitors inhibit glucose reabsorption in the S1 and S2 segments of the renal tubules, there is an increased influx of glucose into the S3 segment. Conversely, the uninhibited SGLT1 receptors also facilitate greater glucose uptake, which may activate aldose reductase and lead to the conversion of glucose in the S3 segment into fructose and sorbitol.

The conversion of glucose in the S3 segment into fructose through fructose kinase activity contributes to the local production of uric acid, oxidative stress, and the release of chemokines, which promote AKI (99). Moreover, the accumulation of sorbitol and fructose due to decreased inositol levels in hyperglycemia may also predispose individuals to the development of AKI (100). Although some studies suggest a potential risk of AKI with SGLT-2 inhibitors, a larger body of research, including studies involving patients with advanced diabetic nephropathy, has failed to demonstrate an increased risk of AKI. The DAPA-CKD trial has indicated that the decrease in serious adverse events related to AKI may reflect the potential protective effect of dapagliflozin beyond the initial decline in eGFR (9). Similarly, the application of empagliflozin, sotagliflozin, and canagliflozin in patients with relatively advanced CKD has also shown no elevated risk of AKI (10, 85, 88). Hence, further studies are needed to confirm whether SGLT-2 inhibitors actually increase the incidence of AKI.

4.2.5 Fracture and amputation

According to the findings from the CANVAS clinical study, the group treated with canagliflozin exhibited a significantly higher incidence of fracture and amputation compared to the placebo group, with incidences that were 97% and 26% higher, respectively. However, the exact mechanism by which SGLT-2 inhibitors contribute to fractures and amputations remains unclear. There have been suggestions that hypovolemia caused by osmotic diuresis and altered bone metabolism due to increased phosphate uptake may be the primary factors responsible for fractures (101, 102). Similarly, hypovolemia and reduced blood flow to the lower extremities resulting from diuresis are thought to be the main causes of amputation (103). In contrast, no increased risk of fracture or amputation was observed in the CREDENCE trial involving canagliflozin (85). Likewise, the DAPA-CKD, EMPA-KIDNEY, and SOCRED trials also found no elevated risk of fracture or amputation (9, 10, 88). After evaluating the potential risks of amputation linked to the use of SGLT-2 inhibitors, the panel reached the conclusion that canagliflozin was the only specific medication associated with

an increased risk. They found no evidence suggesting that other SGLT-2 inhibitors posed a similar risk of amputation (104).

Following a comprehensive assessment of the extended effectivenes and adverse events of four SGLT-2 inhibitors in patients with stage G4 DKD, over a follow-up period ranging from 1.3 to 2.6 years, it has been observed that these medications exhibit reduced efficacy in lowering blood glucose levels in such individuals. Additionally, they are associated with adverse events, including urinary tract infections, as well as potential serious risks such as ketoacidosis, fractures, and amputations. However, instances of hypoglycemia and AKI are infrequent. Moreover, most SGLT-2 inhibitors significantly decrease the likelihood of cardiovascular and renal complications in both diabetic and non-diabetic patients with kidney disease over long-term treatment. Consequently, SGLT-2 inhibitors represent a valuable drug option for managing stage G4 DKD in the long run. Furthermore, findings from a 4-year study focusing on patients with diabetes indicate that dapagliflozin demonstrates favorable safety and tolerability profile when used over an extended duration (105). In general, the long-term safety and tolerability of SGLT-2 inhibitors in patients with G4 DKD were found to be relatively favorable. However, it is important to note that the four primary clinical study populations may not fully represent patients with DKD, particularly those in stages G4-5. Therefore, further studies are required to evaluated the effectiveness and safety of SGLT-2 inhibitors specifically in this patient population.

4.3 Advantages and disadvantages of individual SGLT-2 inhibitors

4.3.1 Dapagliflozin

Dapagliflozin, the first SGLT-2 inhibitor approved for the treatment of type 2 diabetes (106), continues to demonstrate significant benefits in reducing renal and cardiovascular outcomes, as well as the risk of mortality in patients with stage G4 DKD. Furthermore, its long-term use does not appear to increase the incidence of adverse events, even when patients have an eGFR as low as 15 mL/min/1.73 m^2. Regarding adverse events, the incidence of serious adverse events was found to be 34.5%. However, the dapagliflozin group did not show an increased risk of adverse events such as hypoglycemia, ketoacidosis, AKI, fracture, and amputation compared to the DKD dapagliflozin group in stages G2-3 or the placebo group in stage G4 DKD. It is worth noting, however, that the incidence of renal-related adverse events was higher in patients with stage G4 DKD.

4.3.2 Empagliflozin

In the EMPA-KIDNEY trial, it was observed that empagliflozin had a beneficial effect in reducing the risk of renal outcomes. However, it did not show a significant reduction in the risk of cardiovascular outcomes or death. Regarding adverse events, the incidence of serious adverse events was found to be 35.2%. It is worth noting that while there is a potential risk of genitourinary infection and ketoacidosis, there was no increased risk of fracture or amputation.

4.3.3 Canagliflozin

In the CREDENCE trial, canagliflozin demonstrated a reduction in the risk of both renal outcomes and cardiovascular outcomes. However, it did not show a significant reduction in the risk of all-cause mortality. With regard to adverse events, the incidence of serious adverse events was found to be 33.5%. The most common serious adverse events were urinary tract infections (13.4%) and ketoacidosis (0.5%). Importantly, there was no increased risk of fracture or amputation associated with canagliflozin usage.

4.3.4 Sotagliflozin

In the SOCRED trial, sotagliflozin showed a reduction in cardiovascular outcomes risk. However, it did not demonstrate a reduction in renal or cardiovascular risk specifically in the stage G4 DKD subgroup. In terms of adverse events, the incidence of serious adverse events was 23.4%. Diarrhea (8.5%), ketoacidosis (0.6%), and urinary tract infections (11.5%) were more frequently reported with sotagliflozin compared to placebo. Importantly, there was no increased risk of fracture or amputation observed.

In summary, all four SGLT-2 inhibitors have shown the ability to reduce renal, cardiovascular, and mortality risks in patients with stage G4 DKD, with generally favorable safety profiles. Among them, dapagliflozin stands out as it effectively reduces renal, cardiovascular, and mortality risks without the occurrence of ketoacidosis, and it also lowers the incidence of AKI. However, sotagliflozin appears to be less effective in improving cardiac and renal outcomes in advanced DKD patients and should be used with caution in this population. In terms of adverse events, sotagliflozin has the lowest incidence of serious adverse events, but is more associated with diarrhea and urinary tract infections. It is worth noting that, except for dapagliflozin, the other three SGLT-2 inhibitors carry a potential risk of ketoacidosis. Therefore, dapagliflozin is recommended as a preferable option for patients with advanced DKD.

5 Conclusion and Prospect

Delaying the progression of diabetic nephropathy, a leading cause of ESRD, is of utmost importance. SGLT-2 inhibitors, a novel class of glucose-lowering drugs, have shown substantial cardiovascular and renal protective effects in patients with stage 1-4 CKD, regardless of the presence of diabetes. However, as eGFR declines, the hypoglycemic effects, improvement of glomerular hyperfiltration, and suppression of the RAAS by SGLT-2 inhibitors become less effective. Despite this, SGLT-2 inhibitors continue to demonstrate significant cardiorenal benefits in advanced DKD, potentially due to their ability to suppress inflammation and fibrosis, improve oxidative stress, enhance EPO production, optimize mitochondrial energy supply, inhibit the SNS, and protect vascular endothelial cells. These mechanisms likely contribute to the observed renal protective effects of SGLT-2 inhibitors in advanced DKD.

Further investigation and clarification are needed to fully understand the linked mechanisms and the renal protective effects of SGLT-2 inhibitors from multiple perspectives. Currently, the efficacy and safety of SGLT-2 inhibitors in treating patients with advanced DKD have been increasingly supported. The threshold for using SGLT-2 inhibitor has been lowered to an eGFR of 20 mL/min/1.73 m^2, and they can be continued until the initiation of dialysis or renal transplantation, as long as they are well-tolerated by the patient. It is exciting to anticipate whether future advancements will relax the restrictions on the use of SGLT-2 inhibitors for patients with all stages of CKD.

Author contributions

J-XT and C-WY formulated and conceived of this study. Z-CD, J-XC, RZ, X-BL, J-XT, and C-WY wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res Clin Pract* (2019) 157:107843. doi: 10.1016/j.diabres.2019.107843

2. de Boer IH, Rue TC, Hall YN, Heagerty PJ, Weiss NS, Himmelfarb J. Temporal trends in the prevalence of diabetic kidney disease in the United States. *JAMA* (2011) 305(24):2532–9. doi: 10.1001/jama.2011.861

3. Afkarian M, Zelnick LR, Hall YN, Heagerty PJ, Tuttle K, Weiss NS, et al. Clinical manifestations of kidney disease among US adults with diabetes, 1988-2014. *JAMA* (2016) 316(6):602–10. doi: 10.1001/jama.2016.10924

4. Luk AOY, Hui EMT, Sin M-C, Yeung C-Y, Chow W-S, Ho AYY, et al. Declining trends of cardiovascular-renal complications and mortality in type 2 diabetes: the hong kong diabetes database. *Diabetes Care* (2017) 40(7):928–35. doi: 10.2337/dc16-2354

5. Wong MG, Perkovic V, Chalmers J, Woodward M, Li Q, Cooper ME, et al. Long-term benefits of intensive glucose control for preventing end-stage kidney disease: ADVANCE-ON. *Diabetes Care* (2016) 39(5):694–700. doi: 10.2337/dc15-2322

6. Onuigbo MA. Analytical review of the evidence for renoprotection by renin-angiotensin-aldosterone system blockade in chronic kidney disease - a call for caution. *Nephron Clin Pract* (2009) 113(2):c63–c70. doi: 10.1159/000228536

7. Suissa S, Hutchinson T, Brophy JM, Kezouh A. ACE-inhibitor use and the long-term risk of renal failure in diabetes. *Kidney Int* (2006) 69(5):913–9. doi: 10.1038/sj.ki.5000159

8. KDIGO 2020 clinical practice guideline for diabetes management in chronic kidney disease. *Kidney Int* (2020) 98(4S):S1–S115. doi: 10.1016/j.kint.2020.06.019

9. Heerspink HJL, Stefánsson BV, Correa-Rotter R, Chertow GM, Greene T, Hou F-F, et al. Dapagliflozin in patients with chronic kidney disease. *N Engl J Med* (2020) 383 (15):1436–46. doi: 10.1056/NEJMoa2024816

10. Herrington WG, Staplin N, Wanner C, Green JB, Hauske SJ, Emberson JR, et al. Empagliflozin in patients with chronic kidney disease. *N Engl J Med* (2023) 388(2):117–27. doi: 10.1056/NEJMoa2204233

11. KDIGO 2022 clinical practice guideline for diabetes management in chronic kidney disease. *Kidney Int* (2022) 102(5S):S1–S127. doi: 10.1016/j.kint.2022.06.008

12. Ehrenkranz JRL, Lewis NG, Kahn CR, Roth J. Phlorizin: a review. *Diabetes Metab Res Rev* (2005) 21(1):31–8. doi: 10.1002/dmrr.532

13. Lee YJ, Lee YJ, Han HJ. Regulatory mechanisms of Na(+)/glucose cotransporters in renal proximal tubule cells. *Kidney Int Suppl* (2007) 106):S27–35. doi: 10.1038/sj.ki.5002383

14. Vivian EM. Sodium-glucose co-transporter 2 (SGLT2) inhibitors: a growing class of antidiabetic agents. *Drugs Context.* (2014) 3:212264. doi: 10.7573/dic.212264

15. Cefalo CMA, Cinti F, Moffa S, Impronta F, Sorice GP, Mezza T, et al. Sotagliflozin, the first dual SGLT inhibitor: current outlook and perspectives. *Cardiovasc Diabetol* (2019) 18(1):20. doi: 10.1186/s12933-019-0828-y

16. Sha S, Devineni D, Ghosh A, Polidori D, Chien S, Wexler D, et al. Canagliflozin, a novel inhibitor of sodium glucose co-transporter 2, dose dependently reduces calculated renal threshold for glucose excretion and increases urinary glucose excretion in healthy subjects. *Diabetes Obes Metab* (2011) 13(7):669–72. doi: 10.1111/j.1463-1326.2011.01406.x

17. Liang Y, Arakawa K, Ueta K, Matsushita Y, Kuriyama C, Martin T, et al. Effect of canagliflozin on renal threshold for glucose, glycemia, and body weight in normal and diabetic animal models. *PloS One* (2012) 7(2):e30555. doi: 10.1371/journal.pone.0030555

18. Yale JF, Bakris G, Cariou B, Nieto J, David-Neto E, Yue D, et al. Efficacy and safety of canagliflozin over 52 weeks in patients with type 2 diabetes mellitus and chronic kidney disease. *Diabetes Obes Metab* (2014) 16(10):1016–27. doi: 10.1111/dom.12348

19. Kohan DE, Fioretto P, Tang W, List JF. Long-term study of patients with type 2 diabetes and moderate renal impairment shows that dapagliflozin reduces weight and blood pressure but does not improve glycemic control. *Kidney Int* (2014) 85(4):962–71. doi: 10.1038/ki.2013.356

20. Barnett AH, Mithal A, Manassie J, Jones R, Rattunde H, Woerle HJ, et al. Efficacy and safety of empagliflozin added to existing antidiabetes treatment in patients with type 2 diabetes and chronic kidney disease: a randomised, double-blind, placebo-controlled trial. *Lancet Diabetes Endocrinol* (2014) 2(5):369–84. doi: 10.1016/S2213-8587(13)70208-0

21. Dekkers CCJ, Wheeler DC, Sjöström CD, Stefansson BV, Cain V, Heerspink HJL. Effects of the sodium-glucose co-transporter 2 inhibitor dapagliflozin in patients with type 2 diabetes and Stages 3b-4 chronic kidney disease. *Nephrol Dial Transplant.* (2018) 33(11):2005–11. doi: 10.1093/ndt/gfx350

22. Samukawa Y, Haneda M, Seino Y, Sasaki T, Fukatsu A, Kubo Y, et al. Pharmacokinetics and pharmacodynamics of luseogliflozin, a selective SGLT2 inhibitor, in Japanese patients with type 2 diabetes with mild to severe renal impairment. *Clin Pharmacol Drug Dev* (2018) 7(8):820–8. doi: 10.1002/cpdd.456

23. Cherney DZI, Perkins BA, Soleymanlou N, Maione M, Lai V, Lee A, et al. Renal hemodynamic effect of sodium-glucose cotransporter 2 inhibition in patients with type

1 diabetes mellitus. *Circulation* (2014) 129(5):587–97. doi: 10.1161/CIRCULATIONAHA.113.005081

24. van Bommel EJM, Muskiet MHA, van Baar MJB, Tonneijck L, Smits MM, Emanuel AL, et al. The renal hemodynamic effects of the SGLT2 inhibitor dapagliflozin are caused by post-glomerular vasodilatation rather than pre-glomerular vasoconstriction in metformin-treated patients with type 2 diabetes in the randomized, double-blind RED trial. *Kidney Int* (2020) 97(1):202–12. doi: 10.1016/j.kint.2019.09.013

25. Godinho AN, Costa GT, Oliveira NO, Cardi BA, Uchoa DEA, Silveira ER, et al. Effects of cardiotonic steroids on isolated perfused kidney and NHE3 activity in renal proximal tubules. *Biochim Biophys Acta Gen Subj.* (2017) 1861(8):1943–50. doi: 10.1016/j.bbagen.2017.05.012

26. Onishi A, Fu Y, Patel R, Darshi M, Crespo-Masip M, Huang W, et al. A role for tubular Na+/H+ exchanger NHE3 in the natriuretic effect of the SGLT2 inhibitor empagliflozin. *Am J Physiol Renal Physiol* (2020) 319(4):F712–F28. doi: 10.1152/ajprenal.00264.2020

27. Pessoa TD, Campos LCG, Carraro-Lacroix L, Girardi ACC, Malnic G. Functional role of glucose metabolism, osmotic stress, and sodium-glucose cotransporter isoform-mediated transport on Na+/H+ exchanger isoform 3 activity in the renal proximal tubule. *J Am Soc Nephrol.* (2014) 25(9):2028–39. doi: 10.1681/ASN.2013060588

28. Silva Dos Santos D, Polidoro JZ, Borges-Júnior FA, Girardi ACC. Cardioprotection conferred by sodium-glucose cotransporter 2 inhibitors: a renal proximal tubule perspective. *Am J Physiol Cell Physiol* (2020) 318(2):C328–6s. doi: 10.1152/ajpcell.00275.2019

29. Chawla T, Sharma D, Singh A. Role of the renin angiotensin system in diabetic nephropathy. *World J Diabetes.* (2010) 1(5):141–5. doi: 10.4239/wjd.v1.i5.141

30. Jardine MJ, Mahaffey KW, Neal B, Agarwal R, Bakris GL, Brenner BM, et al. The canagliflozin and renal endpoints in diabetes with established nephropathy clinical evaluation (CREDENCE) study rationale, design, and baseline characteristics. *Am J Nephrol.* (2017) 46(6):462–72. doi: 10.1159/000484633

31. Bakris GL, Agarwal R, Anker SD, Pitt B, Ruilope LM, Rossing P, et al. Effect of finerenone on chronic kidney disease outcomes in type 2 diabetes. *N Engl J Med* (2020) 383(23):2219–29. doi: 10.1056/NEJMoa2025845

32. Ravindran S, Munusamy S. Renoprotective mechanisms of sodium-glucose co-transporter 2 (SGLT2) inhibitors against the progression of diabetic kidney disease. *J Cell Physiol* (2022) 237(2):1182–205. doi: 10.1002/jcp.30621

33. Vallon V, Osswald H. Adenosine receptors and the kidney. *Handb Exp Pharmacol* (2009) 193):443–70. doi: 10.1007/978-3-540-89615-9_15

34. Vergara A, Jacobs-Cachá C, Soler MJ. Sodium-glucose cotransporter inhibitors: beyond glycaemic control. *Clin Kidney J* (2019) 12(3):322–5. doi: 10.1093/ckj/sfz019

35. Anders H-J, Davis JM, Thurau K. Nephron protection in diabetic kidney disease. *N Engl J Med* (2016) 375(21):2096–8. doi: 10.1056/NEJMcibr1608564

36. Burns KD, Cherney D. Renal angiotensinogen and sodium-glucose cotransporter-2 inhibition: insights from experimental diabetic kidney disease. *Am J Nephrol.* (2019) 49(4):328–30. doi: 10.1159/000499598

37. Heerspink HJL, Perco P, Mulder S, Leierer J, Hansen MK, Heinzel A, et al. Canagliflozin reduces inflammation and fibrosis biomarkers: a potential mechanism of action for beneficial effects of SGLT2 inhibitors in diabetic kidney disease. *Diabetologia* (2019) 62(7):1154–66. doi: 10.1007/s00125-019-4859-4

38. Packer M. Role of impaired nutrient and oxygen deprivation signaling and deficient autophagic flux in diabetic CKD development: implications for understanding the effects of sodium-glucose cotransporter 2-inhibitors. *J Am Soc Nephrol.* (2020) 31(5):907–19. doi: 10.1681/ASN.2020010010

39. Wan N, Rahman A, Hitomi H, Nishiyama A. The effects of sodium-glucose cotransporter 2 inhibitors on sympathetic nervous activity. *Front Endocrinol (Lausanne).* (2018) 9:421. doi: 10.3389/fendo.2018.00421

40. Hussain M, Elahi A, Hussain A, Iqbal J, Akhtar L, Majid A. Sodium-glucose cotransporter-2 (SGLT-2) attenuates serum uric acid (SUA) level in patients with type 2 diabetes. *J Diabetes Res* (2021) 2021:9973862. doi: 10.1155/2021/9973862

41. Gaspari T, Spizzo I, Liu H, Hu Y, Simpson RW, Widdop RE, et al. Dapagliflozin attenuates human vascular endothelial cell activation and induces vasorelaxation: A potential mechanism for inhibition of atherogenesis. *Diabetes Vasc Dis Res* (2018) 15(1):64–73. doi: 10.1177/1479164117733626

42. Vargas-Delgado AP, Arteaga Herrera E, Tumbaco Mite C, Delgado Cedeno P, Van Loon MC, Badimon JJ. Renal and cardiovascular metabolic impact caused by ketogenesis of the SGLT2 inhibitors. *Int J Mol Sci* (2023) 24(4):4144. doi: 10.3390/ijms24044144

43. Papadopoulou-Marketou N, Chrousos GP, Kanaka-Gantenbein C. Diabetic nephropathy in type 1 diabetes: a review of early natural history, pathogenesis, and diagnosis. *Diabetes Metab Res Rev* (2017) 33(2):e2841. doi: 10.1002/dmrr.2841

44. Sagoo MK, Gnudi L. Diabetic nephropathy: Is there a role for oxidative stress? *Free Radic Biol Med* (2018) 116:50–63. doi: 10.1016/j.freeradbiomed.2017.12.040

45. Lan HY. Diverse roles of TGF-β/Smads in renal fibrosis and inflammation. *Int J Biol Sci* (2011) 7(7):1056–67. doi: 10.7150/ijbs.7.1056

46. Ojima A, Matsui T, Nishino Y, Nakamura N, Yamagishi S. Empagliflozin, an inhibitor of sodium-glucose cotransporter 2 exerts anti-inflammatory and antifibrotic effects on experimental diabetic nephropathy partly by suppressing AGEs-receptor axis. *Horm Metab Res* (2015) 47(9):686–92. doi: 10.1055/s-0034-1395609

47. Li C, Zhang J, Xue M, Li X, Han F, Liu X, et al. SGLT2 inhibition with empagliflozin attenuates myocardial oxidative stress and fibrosis in diabetic mice heart. *Cardiovasc Diabetol* (2019) 18(1):15. doi: 10.1186/s12933-019-0816-2

48. Akizawa T, Saito A, Gejyo F, Suzuki M, Nishizawa Y, Tomino Y, et al. Impacts of recombinant human erythropoietin treatment during predialysis periods on the progression of chronic kidney disease in a large-scale cohort study (Co-JET study). *Ther Apher Dial.* (2014) 18(2):140–8. doi: 10.1111/1744-9987.12066

49. Tsuruya K, Yoshida H, Suehiro T, Fujisaki K, Masutani K, Kitazono T. Erythropoiesis-stimulating agent slows the progression of chronic kidney disease: a possibility of a direct action of erythropoietin. *Ren Fail* (2016) 38(3):390–6. doi: 10.3109/0886022X.2015.1136874

50. O'Neill J, Fasching A, Pihl L, Patinha D, Franzén S, Palm F. Acute SGLT inhibition normalizes O2 tension in the renal cortex but causes hypoxia in the renal medulla in anaesthetized control and diabetic rats. *Am J Physiol Renal Physiol* (2015) 309(3):F227–F34. doi: 10.1152/ajprenal.00689.2014

51. Takaori K, Nakamura J, Yamamoto S, Nakata H, Sato Y, Takase M, et al. Severity and frequency of proximal tubule injury determines renal prognosis. *J Am Soc Nephrol.* (2016) 27(8):2393–406. doi: 10.1681/ASN.2015060647

52. Sano M, Goto S. Possible mechanism of hematocrit elevation by sodium glucose cotransporter 2 inhibitors and associated beneficial renal and cardiovascular effects. *Circulation* (2019) 139(17):1985–7. doi: 10.1161/CIRCULATIONAHA.118.038881

53. Verma S, Mazer CD, Yan AT, Mason T, Garg V, Teoh H, et al. Effect of empagliflozin on left ventricular mass in patients with type 2 diabetes mellitus and coronary artery disease: the EMPA-HEART cardioLink-6 randomized clinical trial. *Circulation* (2019) 140(21):1693–702. doi: 10.1161/CIRCULATIONAHA.119.042375

54. Mazer CD, Hare GMT, Connelly PW, Gilbert RE, Shehata N, Quan A, et al. Effect of empagliflozin on erythropoietin levels, iron stores, and red blood cell morphology in patients with type 2 diabetes mellitus and coronary artery disease. *Circulation* (2020) 141(8):704–7. doi: 10.1161/CIRCULATIONAHA.119.044235

55. Corcoran SE, O'Neill LAJ. HIF1α and metabolic reprogramming in inflammation. *J Clin Invest.* (2016) 126(10):3699–707. doi: 10.1172/JCI84431

56. Forbes JM, Thorburn DR. Mitochondrial dysfunction in diabetic kidney disease. *Nat Rev Nephrol.* (2018) 14(5):291–312. doi: 10.1038/nrneph.2018.9

57. Sas KM, Kayampilly P, Byun J, Nair V, Hinder LM, Hur J, et al. Tissue-specific metabolic reprogramming drives nutrient flux in diabetic complications. *JCI Insight* (2016) 1(15):e86976. doi: 10.1172/jci.insight.86976

58. Jang H-S, Noh MR, Kim J, Padanilam BJ. Defective mitochondrial fatty acid oxidation and lipotoxicity in kidney diseases. *Front Med (Lausanne).* (2020) 7:65. doi: 10.3389/fmed.2020.00065

59. Afshinnia F, Nair V, Lin J, Rajendiran TM, Soni T, Byun J, et al. Increased lipogenesis and impaired β-oxidation predict type 2 diabetic kidney disease progression in American Indians. *JCI Insight* (2019) 4(21):e130317. doi: 10.1172/jci.insight.130317

60. Stadler K, Goldberg IJ, Susztak K. The evolving understanding of the contribution of lipid metabolism to diabetic kidney disease. *Curr Diabetes Rep* (2015) 15(7):40. doi: 10.1007/s11892-015-0611-8

61. Ferrannini E, Baldi S, Frascerra S, Astiarraga B, Heise T, Bizzotto R, et al. Shift to fatty substrate utilization in response to sodium-glucose cotransporter 2 inhibition in subjects without diabetes and patients with type 2 diabetes. *Diabetes* (2016) 65(5):1190–5. doi: 10.2337/db15-1356

62. Ferrannini E. Sodium-glucose co-transporters and their inhibition: clinical physiology. *Cell Metab* (2017) 26(1):27–38. doi: 10.1016/j.cmet.2017.04.011

63. Kalra S, Jain A, Ved J, Unnikrishnan AG. Sodium-glucose cotransporter 2 inhibition and health benefits: The Robin Hood effect. *Indian J Endocrinol Metab* (2016) 20(5):725–9. doi: 10.4103/2230-8210.183826

64. Lambers Heerspink HJ, de Zeeuw D, Wie L, Leslie B, List J. Dapagliflozin a glucose-regulating drug with diuretic properties in subjects with type 2 diabetes. *Diabetes Obes Metab* (2013) 15(9):853–62. doi: 10.1111/dom.12127

65. Min SH, Oh TJ, Baek SI, Lee DH, Kim KM, Moon JH, et al. Degree of ketonaemia and its association with insulin resistance after dapagliflozin treatment in type 2 diabetes. *Diabetes Metab* (2018) 44(1):73–6. doi: 10.1016/j.diabet.2017.09.006

66. Kimura Y, Kuno A, Tanno M, Sato T, Ohno K, Shibata S, et al. Canagliflozin, a sodium-glucose cotransporter 2 inhibitor, normalizes renal susceptibility to type 1 cardiorenal syndrome through reduction of renal oxidative stress in diabetic rats. *J Diabetes Investig* (2019) 10(4):933–46. doi: 10.1111/jdi.13009

67. Mulder S, Heerspink HJL, Darshi M, Kim JJ, Laverman GD, Sharma K, et al. Effects of dapagliflozin on urinary metabolites in people with type 2 diabetes. *Diabetes Obes Metab* (2019) 21(11):2422–8. doi: 10.1111/dom.13823

68. Huggett RJ, Scott EM, Gilbey SG, Stoker JB, Mackintosh AF, Mary DASG. Impact of type 2 diabetes mellitus on sympathetic neural mechanisms in hypertension. *Circulation* (2003) 108(25):3097–101. doi: 10.1161/01.CIR.0000103123.66264.FE

69. Masuo K, Lambert GW, Esler MD, Rakugi H, Ogihara T, Schlaich MP. The role of sympathetic nervous activity in renal injury and end-stage renal disease. *Hypertens Res* (2010) 33(6):521–8. doi: 10.1038/hr.2010.35

70. Pop-Busui R, Kirkwood I, Schmid H, Marinescu V, Schroeder J, Larkin D, et al. Sympathetic dysfunction in type 1 diabetes: association with impaired myocardial

blood flow reserve and diastolic dysfunction. *J Am Coll Cardiol* (2004) 44(12):2368–74. doi: 10.1016/j.jacc.2004.09.033

71. Komici K, Femminella GD, de Lucia C, Cannavo A, Bencivenga L, Corbi G, et al. Predisposing factors to heart failure in diabetic nephropathy: a look at the sympathetic nervous system hyperactivity. *Aging Clin Exp Res* (2019) 31(3):321–30. doi: 10.1007/s40520-018-0973-2

72. Sano M. A new class of drugs for heart failure: SGLT2 inhibitors reduce sympathetic overactivity. *J Cardiol* (2018) 71(5):471–6. doi: 10.1016/j.jjcc.2017.12.004

73. Herat LY, Magno AL, Rudnicka C, Hricova J, Carnagarin R, Ward NC, et al. SGLT2 inhibitor-induced sympathoinhibition: A novel mechanism for cardiorenal protection. *JACC Basic Transl Sci* (2020) 5(2):169–79. doi: 10.1016/j.jacbts.2019.11.007

74. Bruno RM, Penno G, Daniele G, Pucci L, Lucchesi D, Stea F, et al. Type 2 diabetes mellitus worsens arterial stiffness in hypertensive patients through endothelial dysfunction. *Diabetologia* (2012) 55(6):1847–55. doi: 10.1007/s00125-012-2517-1

75. 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

76. Lin B, Koibuchi N, Hasegawa Y, Sueta D, Toyama K, Uekawa K, et al. Glycemic control with empagliflozin, a novel selective SGLT2 inhibitor, ameliorates cardiovascular injury and cognitive dysfunction in obese and type 2 diabetic mice. *Cardiovasc Diabetol* (2014) 13:148. doi: 10.1186/s12933-014-0148-1

77. Oelze M, Kröller-Schön S, Welschof P, Jansen T, Hausding M, Mikhed Y, et al. The sodium-glucose co-transporter 2 inhibitor empagliflozin improves diabetes-induced vascular dysfunction in the streptozotocin diabetes rat model by interfering with oxidative stress and glucotoxicity. *PloS One* (2014) 9(11):e112394. doi: 10.1371/journal.pone.0112394

78. Bailey CJ. Uric acid and the cardio-renal effects of SGLT2 inhibitors. *Diabetes Obes Metab* (2019) 21(6):1291–8. doi: 10.1111/dom.13670

79. Chen M, Lu X, Lu C, Shen N, Jiang Y, Chen M, et al. Soluble uric acid increases PDZK1 and ABCG2 expression in human intestinal cell lines via the TLR4-NLRP3 inflammasome and PI3K/Akt signaling pathway. *Arthritis Res Ther* (2018) 20(1):20. doi: 10.1186/s13075-018-1512-4

80. Ahmadieh H, Azar S. Effects of sodium glucose cotransporter-2 inhibitors on serum uric acid in type 2 diabetes mellitus. *Diabetes Technol Ther* (2017) 19(9):507–12. doi: 10.1089/dia.2017.0070

81. Lu Y-H, Chang Y-P, Li T, Han F, Li C-J, Li X-Y, et al. Empagliflozin attenuates hyperuricemia by upregulation of ABCG2 via AMPK/AKT/CREB signaling pathway in type 2 diabetic mice. *Int J Biol Sci* (2020) 16(3):529–42. doi: 10.7150/ijbs.33007

82. Parving H-H, Lambers-Heerspink H, de Zeeuw D. Empagliflozin and progression of kidney disease in type 2 diabetes. *N Engl J Med* (2016) 375(18):1800–1. doi: 10.1056/NEJMc1611290

83. Wiviott SD, Raz I, Bonaca MP, Mosenzon O, Kato ET, Cahn A, et al. Dapagliflozin and cardiovascular outcomes in type 2 diabetes. *N Engl J Med* (2019) 380(4):347–57. doi: 10.1056/NEJMoa1812389

84. Neal B, Perkovic V, Matthews DR. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *N Engl J Med* (2017) 377(21):2099. doi: 10.1056/NEJMoa1611925

85. Perkovic V, Jardine MJ, Neal B, Bompoint S, Heerspink HJL, Charytan DM, et al. Canagliflozin and renal outcomes in type 2 diabetes and nephropathy. *N Engl J Med* (2019) 380(24):2295–306. doi: 10.1056/NEJMoa1811744

86. Chertow GM, Vart P, Jongs N, Toto RD, Gorriz JL, Hou FF, et al. Effects of dapagliflozin in stage 4 chronic kidney disease. *J Am Soc Nephrol.* (2021) 32(9):2352–61. doi: 10.1681/ASN.2021020167

87. Bakris G, Oshima M, Mahaffey KW, Agarwal R, Cannon CP, Capuano G, et al. Effects of Canagliflozin in Patients with Baseline eGFR <30 ml/min per 1.73 m2: Subgroup Analysis of the Randomized CREDENCE Trial. *Clin J Am Soc Nephrol* (2020) 15(12):1705–14. doi: 10.2215/CJN.10140620

88. Bhatt DL, Szarek M, Pitt B, Cannon CP, Leiter LA, McGuire DK, et al. Sotagliflozin in patients with diabetes and chronic kidney disease. *N Engl J Med* (2021) 384(2):129–39. doi: 10.1056/NEJMoa2030186

89. Bhatt DL, Szarek M, Steg PG, Cannon CP, Leiter LA, McGuire DK, et al. Sotagliflozin in patients with diabetes and recent worsening heart failure. *N Engl J Med* (2021) 384(2):117–28. doi: 10.1056/NEJMoa2030183

90. Cherney DZI, Ferrannini E, Umpierrez GE, Peters AL, Rosenstock J, Carroll AK, et al. Efficacy and safety of sotagliflozin in patients with type 2 diabetes and severe renal impairment. *Diabetes Obes Metab* (2021) 23(12):2632–42. doi: 10.1111/dom.14513

91. McGill JB, Subramanian S. Safety of sodium-glucose co-transporter 2 inhibitors. *Am J Cardiol* (2019) 124 Suppl 1:S45–52. doi: 10.1016/j.amjcard.2019.10.029

92. Umpierrez GE. Diabetes: SGLT2 inhibitors and diabetic ketoacidosis - a growing concern. *Nat Rev Endocrinol* (2017) 13(8):441–2. doi: 10.1038/nrendo.2017.77

93. Blau JE, Tella SH, Taylor SI, Rother KI. Ketoacidosis associated with SGLT2 inhibitor treatment: Analysis of FAERS data. *Diabetes Metab Res Rev* (2017) 33(8):471–479.e1. doi: 10.1002/dmrr.2924

94. Brown E, Wilding JPH, Alam U, Barber TM, Karalliedde J, Cuthbertson DJ. The expanding role of SGLT2 inhibitors beyond glucose-lowering to cardiorenal protection. *Ann Med* (2021) 53(1):2072–89. doi: 10.1080/07853890.2020.1841281

95. Douros A, Lix LM, Fralick M, Dell'Aniello S, Shah BR, Ronksley PE, et al. Sodium-glucose cotransporter-2 inhibitors and the risk for diabetic ketoacidosis: A multicenter cohort study. *Ann Intern Med* (2020) 173(6):417–25. doi: 10.7326/M20-0289

96. Vasilakou D, Karagiannis T, Athanasiadou E, Mainou M, Liakos A, Bekiari E, et al. Sodium-glucose cotransporter 2 inhibitors for type 2 diabetes: a systematic review and meta-analysis. *Ann Intern Med* (2013) 159(4):262–74. doi: 10.7326/0003-4819-159-4-201308200-00007

97. Rampersad C, Kraut E, Whitlock RH, Komenda P, Woo V, Rigatto C, et al. Acute kidney injury events in patients with type 2 diabetes using SGLT2 inhibitors versus other glucose-lowering drugs: A retrospective cohort study. *Am J Kidney Dis* (2020) 76 (4). doi: 10.1053/j.ajkd.2020.03.019

98. Hahn K, Kanbay M, Lanaspa MA, Johnson RJ, Ejaz AA. Serum uric acid and acute kidney injury: A mini review. *J Adv Res* (2017) 8(5):529–36. doi: 10.1016/j.jare.2016.09.006

99. Jain R, Bhavatharini N, Saravanan T, Seshiah V, Jain N. Use of sodium-glucose transport protein 2 (SGLT2) inhibitor remogliflozin and possibility of acute kidney injury in type-2 diabetes. *Cureus* (2022) 14(12):e32573. doi: 10.7759/cureus.32573

100. Kitamura H, Yamauchi A, Sugiura T, Matsuoka Y, Horio M, Tohyama M, et al. Inhibition of myo-inositol transport causes acute renal failure with selective medullary injury in the rat. *Kidney Int* (1998) 53(1):146–53. doi: 10.1046/j.1523-1755.1998.00747.x

101. Neal B, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondu N, et al. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *N Engl J Med* (2017) 377(7):644–57. doi: 10.1056/NEJMoa1611925

102. Adimadhyam S, Lee TA, Calip GS, Smith Marsh DE, Layden BT, Schumock GT. Sodium-glucose co-transporter 2 inhibitors and the risk of fractures: A propensity score-matched cohort study. *Pharmacoepidemiol Drug Saf.* (2019) 28(12):1629–39. doi: 10.1002/pds.4900

103. Zerovnik S, Kos M, Locatelli I. Risk of lower extremity amputations in patients with type 2 diabetes using sodium-glucose co-transporter 2 inhibitors. *Acta Diabetol* (2022) 59(2):233–41. doi: 10.1007/s00592-021-01805-8

104. Katsiki N, Dimitriadis G, Hahalis G, Papanas N, Tentolouris N, Triposkiadis F, et al. Sodium-glucose co-transporter-2 inhibitors (SGLT2i) use and risk of amputation: an expert panel overview of the evidence. *Metabolism* (2019) 96:92–100. doi: 10.1016/j.metabol.2019.04.008

105. Del Prato S, Nauck M, Durán-Garcia S, Maffei L, Rohwedder K, Theuerkauf A, et al. Long-term glycaemic response and tolerability of dapagliflozin versus a sulphonylurea as add-on therapy to metformin in patients with type 2 diabetes: 4-year data. *Diabetes Obes Metab* (2015) 17(6):581–90. doi: 10.1111/dom.12459

106. Albarrán OG, Ampudia-Blasco FJ. [Dapagliflozin, the first SGLT-2 inhibitor in the treatment of type 2 diabetes]. *Med Clin (Barc).* (2013) 141 Suppl 2:36–43. doi: 10.1016/S0025-7753(13)70062-9

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Research progress of NF-κB signaling pathway and thrombosis

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Venous thromboembolism is a very common and costly health problem. Deep-vein thrombosis (DVT) can cause permanent damage to the venous system and lead to swelling, ulceration, gangrene, and other symptoms in the affected limb. In addition, more than half of the embolus of pulmonary embolism comes from venous thrombosis, which is the most serious cause of death, second only to ischemic heart disease and stroke patients. It can be seen that deep-vein thrombosis has become a serious disease affecting human health. In recent years, with the deepening of research, inflammatory response is considered to be an important pathway to trigger venous thromboembolism, in which the transcription factor NF-κB is the central medium of inflammation, and the NF-κB signaling pathway can regulate the pro-inflammatory and coagulation response. Thus, to explore the mechanism and make use of it may provide new solutions for the prevention and treatment of thrombosis.

KEYWORDS

thrombosis, NF-κB signal pathway, inflammation, miRNA, TCM, natural compounds, drugs

1 Introduction

Deep-vein thrombosis (DVT) is a serious disease threatening human life. The incidence of DVT is 10-40% after general surgery and 40-60% after major orthopedic surgery, and in the absence of preventive measures, DVT can lead to further diseases such as pulmonary hypertension, recurrent thrombosis, post-thrombotic syndrome, and even fatal pulmonary embolism (1, 2). Therefore, patients with acute DVT face a high risk of death. The treatment of DVT in modern medicine can be roughly divided into anticoagulation therapy and thrombectomy (3). Anticoagulation therapy and surgery have made great progress in the treatment of DVT and are widely used. If more methods can continue to be found to treat thrombosis, then the recovery probability of related patients will be increased. Therefore, research on the prevention and treatment of thrombosis has never stopped.

Blood flow retardation, blood hypercoagulability, and endothelial cell injury are the three major factors of venous thrombosis (4, 5). They play different roles in the mechanism of venous thrombosis but are related to each other. Vascular endothelial cells divide blood from subendothelial tissue, store and secrete factors that affect platelet function, prevent platelet adhesion, and allow blood to flow normally. However, when the endothelium is disturbed by physical or chemical factors, the endothelial cells will undergo programmed biochemical changes, transform into the front surface of the thrombus, express TF, and accelerate the activation of factor X and factor IX, thereby activating the coagulation system (6). Changes in hemodynamics promote changes in the state of the vascular endothelium, and the flow of blood through the vasculature generates wall shear stress, resulting in structural and functional changes in the vessel wall (7). Shear stress also strongly affects endothelial cell gene expression (8, 9). There are "shear-stress response elements" in the promoters of related genes (10, 11), various "mechanical transducers" and downstream signal pathways, which associate external mechanical stimuli with intracellular and nuclear events (12–14). The hypercoagulable state is one of the important factors of venous thrombosis (15). When too many clotting proteins are produced in the blood, abnormal clotting proteins are produced to resist decomposition, and too few proteins that prevent thrombosis are produced, which will cause the blood to become hypercoagulable (16). The combination of the hypercoagulable state and acquired risk factors (surgery, braking, or hormone therapy) increases the risk of thrombosis (17, 18). Proper thrombus prevention can prevent the risk of thrombosis from exceeding this critical threshold, but thrombosis occurs when internal and external forces exceed the critical threshold (19).

In recent years, studies have found that inflammation is closely related to the formation and development of deep venous thrombosis. Inflammation mediates vascular endothelial cell injury (20, 21), releases vascular cell adhesion molecules (VCAM) and intercellular adhesion molecules (ICAM), stagnates blood flow, and accelerates venous thrombosis (22–25). The formation of blood clots exacerbates the inflammatory response, and the two affect each other. Therefore, study of the occurrence of venous thrombosis and the discovery of related proteins regulating inflammatory factors are of great significance for delaying and alleviating the formation of venous thrombosis and judging the prognosis.

2 Overview of the NF-κB signaling pathway

The NF-κB family consists of a group of structurally related and evolutionarily conserved transcription factors that play a key role in inflammatory response, immune function, cell survival, and prevention of apoptosis (26). There are currently five members of the mammalian NF-κB family, known as RelA (also known as p65), RelB, c-Rel, NF-κB1 (p50 and its precursor p105), and NF-κB2 (p52 and its precursor p100) (27).

Despite the expanding complexity of NF-κB signaling, the two most recognized pathways in mammalian cells are the so-called classical and atypical pathways (28–30), both of which are important in inflammatory response and immune regulation, despite their differences in signaling composition and biological function. The classical NF-κB pathway is induced by pro-inflammatory cytokines and depends on the induced degradation of IκB, specifically Iκbα, to activate the NF-κB1 p50, RelA, or c-Rel complex (28). The non-classical NF-κB pathway is triggered by certain members of the TNF family of cytokines rather than by TNF-A itself and depends on the induction process of p100 rather than the degradation of IκBα, leading to the activation of the NF-κB2 P52 or RelB complex (31–33). Activated NF-κB is transferred from the cytoplasm to the nucleus, where it causes the expression of target genes associated with inflammation. The NF-κB family has been shown to activate more than 500 inflammation-related genes (34, 35) and can initiate the expression of cytokines necessary for inflammation. Some of these cytokines, such as IL-1 and TNF-α, activate NF-κB itself, leading to the formation of a positive feedback loop that has the potential to produce chronic and excessive inflammation when NF-κB becomes abnormally or persistently active.

3 Relationship between NF-κB, inflammation, and thrombosis

3.1 The inflammatory response promotes thrombosis

In general, DVT can be caused by a variety of risk factors, including genetics, dietary habits, obesity, aging, trauma, and cancer (2). In recent years, with the deepening of research, inflammation is considered to be an important way for various risk factors to trigger the formation of VTE (36–39). In our recent case, the novel coronavirus infection (COVID-19) is caused by the novel coronavirus SARS-CoV-2, which is characterized by an excessive inflammatory response. It has been reported that about half of the hospitalized patients with COVID-19 have serious symptoms, such as deep-vein thrombosis and coagulation dysfunction in the lower extremities, and some patients may die (40–44). It has also been reported that the injection of the COVID-19 vaccine can cause an immune inflammatory response, thus promoting thrombosis and thrombocytopenia (45–50). When studying the changes of inflammatory factors in the plasma of DVT patients, it was found that the expression level of IL-17A was up-regulated, and the level of platelet aggregation was increased, which promoted platelet activation and aggregation, thus playing a role in promoting the formation of DVT (51). In addition, pro-inflammatory factors represented by interleukin-1 (IL-1), IL-6, IL-1β, IL-18, cox-2, TNF-α (52–55), and other inflammatory factors can induce inflammatory response, accelerate tissue injury, and stimulate the release of inflammatory mediators, leading to vascular endothelial injury and apoptosis (20, 21). This indicates that inflammation has become a factor that cannot be ignored in the mechanism of thrombosis.

3.2 NF-κB induces an inflammatory response

Transcription factor NF-κB is the central mediator of inflammatory response, mainly in the form of p65 and p50 binding and in the form of dimer; when stimulated, NF-κB p65/p50 dissociates with IκBα and enters the nucleus to activate the corresponding gene transcription (56). The NF-κB signaling pathway is the central link of various inflammatory responses, which can up-regulate the expression of pro-inflammatory factors in the activated state, and inflammatory response can release inflammatory factor IL-1β through the NF-κB pathway, resulting in increased expression of monocyte chemotactic protein-1 (MCP-1) and activation of endothelial cells (57, 58). Releasing intercellular adhesion molecule-1 (ICAM-1), platelet endothelial cell adhesion molecule-1 (PECAM-1), and vascular cell adhesion molecule-1 (VCAM-1), etc. (59, 60), further activates NF-κB, amplifies inflammatory response, and releases more inflammatory factors. Increased platelet reactivity, activation of the plasma coagulation cascade, and impaired function of physiological anticoagulants result in the hypercoagulability of blood (61, 62).

3.3 NF-κB is involved in thrombosis

NF-κB signaling plays an important role in the vascular system and in the cell types involved in thromboinflammatory processes. By mediating the interaction between endothelial cells, platelets, and inflammatory response, NF-κB disrupts the coagulation–fibrinolysis balance and induces thrombosis (63).

3.3.1 Platelets

Platelets are not only involved in primary hemostasis but also in the formation of thrombus induced by inflammation. Platelet activators include not only thrombin and ADP but also molecules involved in inflammation (64). Platelets as non-nucleated cells also contain members of the NF-κB family and their corresponding signaling molecules, which are involved in platelet activation and secondary feedback loops (56). Activated platelets express or secrete pro-inflammatory and pro-coagulant substances on their surfaces, such as adhesion molecules, growth factors, cytokines, and the fibrinolytic inhibitor PAI-1, inducing surface aggregation of coagulation factors (65).

3.3.2 Endothelial cell

The injury of the vein wall or vein endothelial cells caused by various factors is one of the factors that cause DVT. Studies have confirmed that NF-κB signaling molecules exist in endothelial cells (63). When injured, endothelial cells are activated, which can inhibit the expression of thrombomodulin (TM) and activate the expression of endothelial tissue factor (TF), resulting in the activation of adhesion molecules such as P-selectin and clotting factor vWF (66), and the endothelial cells change from anticoagulant, anti-inflammatory, and vasodilator functions to proinflammatory and pre-thrombotic states.

3.3.3 *NETs*

Inflammatory cells represented by neutrophils and monocytes were able to rapidly aggregate and adhere to the venous endothelium (67). Among them, neutrophils can activate coagulation factors XII, initiate endogenous coagulation, and also form neutrophil extracellular traps (NETs) after apoptosis. NETs, as part of the body's innate immunity, are an extracellular network of fibers made up of disaggregated chromatin (DNA fibers and histones) released by neutrophils and more than 30 granule proteins with antimicrobial properties (68, 69), providing a supporting basis for thrombosis through their fiber network structure (70). They interact with platelets to further stimulate platelet aggregation, activate thrombin, and accelerate the DVT process (71, 72) (Figure 1).

4 Interference with NF-κB signaling pathway and deep-vein thrombosis

The NF-κB signaling pathway is closely related to thrombosis. Activation of the NF-κB signaling pathway can significantly increase the levels of thromboxane B2 (TXB2), interleukin6 (IL-6), tumor necrosis factor-α (TNF-α), and PAI and significantly decrease the levels of 6-keto-PGF1α and t-PA, exacerbating inflammation and thrombosis (73). Therefore, we may think that thrombosis can be prevented and that it can be reduced by inhibiting the NF-κB signaling pathway.

4.1 microRNA regulates NF-κB signaling pathway to interfere with deep-vein thrombosis

MiRNAs are key regulators of many biological processes (cell differentiation, proliferation, apoptosis, and metabolism), and abnormal expression of miRNAs is known to be associated with a variety of human diseases. Hugo's (2016) study demonstrated the abnormal expression of miRNA in venous thrombosis and suggested that miRNA may be related to the molecular mechanism of DVT. There is evidence that miRNA plays an important role in hemostasis (74), and some foreign scholars (75) have confirmed that miR-181a-5p can inhibit the expression of F11 mRNA and coagulation factor XI. Some members of the miRNA family (miR-126 and miR-145) can promote the dissolution and recanalization of thrombus (76, 77), suggesting that miRNA may be involved in the formation of DVT.

4.1.1 miRNA-181b

DVT group and miR-181b overexpression and inhibition of rat models were constructed by producing a deep-vein thrombosis model and injecting normal saline, miR-181b mimics, and inhibitors into the tail vein. The changes of NF-κB (P65) in the venous endothelium of rats in each group were analyzed, and the expression level of NF-κB (P65) in the venous endothelium of rats in the Normal group was used as a reference. The results showed

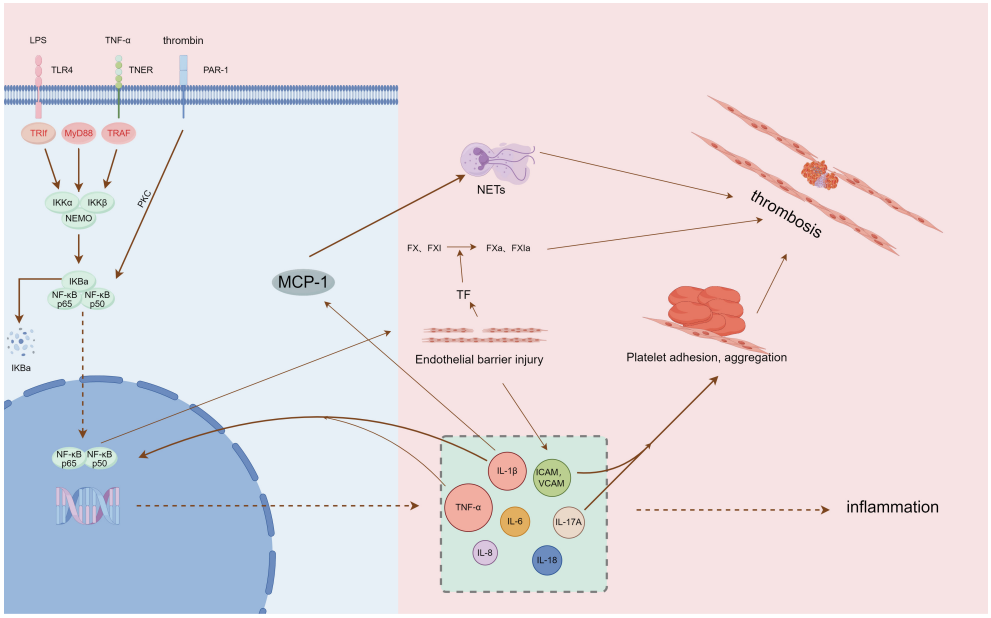

FIGURE 1
Summary of NF-κB pathway mediating inflammation and thrombosis (by Figdraw, authorization ID: PIWYI6b557).

that the expression of NF-κB (P65) in the venous endothelium of rats in the 181b-i group was the highest, followed by the DVT group, and the 181b-m group was lower than the DVT group but higher than the Normal group; moreover, the difference was statistically significant ($P<0.05$). These results indicate that the expression of NF-κB (P65) is increased in the rat vena cava DVT model, and miR-181b can inhibit the expression of NF-κB (P65). By comparing the length and wet weight of the thrombus in each model group, no thrombus formation was observed in the Normal group. Overexpression of miR-181b can shorten the length of the thrombus and lighten the wet weight after thrombus formation in rats; inhibition of miR-181b expression can lengthen the length of thrombus and increase the wet weight after thrombus formation. These results suggest that miR-181b can reduce the formation of DVT by inhibiting the NF-κB signaling pathway to a certain extent (78).

4.1.2 miRNA-150

Up-regulating the expression of miR-150 inhibited thrombosis in DVT rats. The effect of miR-150 on inflammation was studied *in vivo* and *in vitro*. The rats were injected with LV-NC, and it was found that the administration of LV-miR-150 inhibited the platelet aggregation inhibition rate and TXB2 content in the rat model and significantly inhibited thrombosis. Transfected into *in vivo* skin cells, PAI-1, TNF-α, IL-6, and IL-8 levels were significantly reduced, indicating that miR-150 alleviated inflammation and inhibited apoptosis of vascular endothelial cells. The expression level of NF-κB p50 in vascular endothelial cells transfected with miR-150 mimics was significantly decreased, while the expression level of NF-κB p50 was significantly increased by miR-150 inhibitors. These results suggest that miR-150 may have a negative regulatory effect on NF-κB p50. Therefore, overexpression of miR-150 can be used as a potential therapeutic target for future DVT (79).

4.1.3 miRNA-141

This study found that by overexpression of miRNA-141, the expression of TLR4 and its signaling pathway-related proteins NF-κB, Rac1, and IL-1β in vascular tissues of thrombotic rats was significantly down-regulated, and by restoring the expression of TLR4 and NF-κB, the expression of Rac1 and IL-1β was restored at the same time, and multiple related indexes of miRNA-141 on thrombus were significantly reversed (80). Activated TLR4 induces late activation of NF-κB, which significantly increases TNF-α expression and causes widespread inflammation (53). Furthermore, TNF-α stimulates the TNF receptor (TNFR) and induces phosphorylation of IκB kinase (IKK), which in turn enhances NF-κB activity (81). NF-κB is the end point of the TLR4/NF-κB pathway and the regulatory hub of an inflammatory response, and its activation can enhance the inflammatory response and promote the formation of a thrombus. This may be a key mechanism by which miRNA-141 inhibits thrombus formation by regulating the TLR4/NF-κB signaling pathway (Table 1).

4.2 TCM preparations alleviate thrombosis by modulating NF-κB signaling

4.2.1 *Qihong Tongluo* prescription

Qihong Tongluo prescription is mainly composed of Astragalus and safflower. Astragalus has a variety of biological functions, including potent immunomodulatory, antioxidant, anti-inflammatory, and antitumor activities (82). Isoflavones, saponins,

TABLE 1 The effect of miRNA on NF-κB-mediated inflammatory response.

MiRNA	Anti-inflammatory effect	Action target	reference
miRNA-181b	e-selectin、VCAM-1、ICAM-1	NF-κB p65	(78)
	Thrombus length and wet weight		
miRNA-150	PAI-1、TNFα、IL-8、IL-6	NF-κB p50	(79)
	Reduce the degree of vascular obstruction; Endothelial cell proliferation was enhanced		
miRNA-141	Rac1、IL-1β	TLR4、NF-κB	(80)
	Inhibit thrombosis and platelet aggregation		

and polysaccharides are three types of beneficial compounds for their pharmacological activity and therapeutic efficacy (82–84). Astragalus polysaccharides decreased the expression of IL-1β, IL-6, TNF-α, and INF-γ by regulating the toll-like receptor 4 (TLR4)/NF-κB signaling pathway (85, 86). In addition, safflower can inhibit platelet activation, adhesion, and aggregation (87, 88). In summary, we believe that Qihong Tongluo prescription's inhibition of thrombosis is the result of the joint action of its active components.

4.2.2 Gegen Qinlian pills

The high incidence of thrombotic events is one of the clinical features of coronavirus disease (COVID-19) due to the high inflammatory response caused by the virus. Gegen Qinlian pill (GQP) is a traditional Chinese medicine, which can inhibit toll-like receptor 4 (TLR4)/nuclear factor κB (NF-κB) signaling (89–91), has good anti-inflammatory activity, has a good effect on the treatment of COVID-19, and has shown anti-thrombotic potential. In our study, GQP treatment significantly reduced the expression of TNF-α, NLRP3, and NF-κB, reduced lung, liver, and tail thrombosis, and increased tail blood flow in mice (92). This at least partially supports the hypothesis that GQP can inhibit inflammation-induced thrombosis by inhibiting NF-κB/NLRP3 signaling.

4.2.3 Huanglianjiedu Decoction

HLJJD is a famous prescription in China, and its main compounds have been studied as baicalin and berberine (93). Baicalin, a flavonoid compound extracted from the root of Scutellaria baicalensis, has significant anti-inflammatory and antibacterial effects, scavenging oxygen free radicals and anti-allergic reactions (94, 95). Baicalin can inhibit the NF-κB signaling pathway, reduce the expression level of the p-NF-κB p65 protein, and reduce inflammatory response (96, 97). Berberine is an isoquinoline alkaloid isolated from Coptis chinensis, a Chinese medicinal plant, and has significant anti-inflammatory effects (98, 99). For the infection-induced tissue injury model, the gene and protein expression levels of TNF-α, TLRs, and NF-κB p65 were significantly reduced in the berberine treatment group (100–102). TLR4 is a pattern recognition receptor, and activated TLR4 induces late activation of NF-κB, which significantly increases TNF-α expression and causes widespread inflammation (53). TNF-α stimulates the phosphorylation of IκB kinase (IKK), which in turn enhances the activity of NF-κB (81). As the endpoint of the TLR4/NF-κB pathway and the regulatory hub of the inflammatory response, the activation of NF-κB can enhance the inflammatory response and promote the formation of thrombosis.

Through intravenous injection of the effective components of Huanglian Jidutang into the thrombus model, the results showed that the contents of IL-1β, IL-6, and TNF-α and the expression levels of TLR4, NF-κB, NLRP3, and Caspase-1 were decreased (103), and they also showed significantly reduced thrombus dry weight, Block platelet aggregation, and adhesion induced by collagen (104). In conclusion, the inhibitory effect of the active components of Huanglian Jiedu Decoction on the NF-κB pathway is the cause of alleviating inflammation and reducing thrombosis.

4.2.4 Liu Shen Wan

LSW is a classic proprietary Chinese medicine with anti-inflammatory and analgesic effects (105). Its main components are cow gallstone, musk secretion, toad secretion, pearl shell, realgar, and borneol (106). In PR8-infected cells, LSW significantly down-regulated the expression levels of IL-1β, TNF-α, IL-6, and IFN-γ. In mice infected with PR8, LSW reduced the secretion of TNF-α, IL-1β, IL-6, and IFN-γ in lung tissue, significantly improving survival. In addition, LSW significantly reduced the expression levels of TLR4, phosphor-NF-κB p65, and phosphor-iκBα (107). It is suggested that LSW exerts anti-inflammatory effects by regulating the TLR4/NF-κB signaling pathway.

4.2.5 *Rhein*

Rhein is widely found in a variety of Chinese herbs, including Rhubarb palmatum, aloe curacao, cassia stenophyllum, and polygonum multiflorum. It has antioxidant, antiviral, anti-inflammatory, anti-tumor, and immunomodulatory activities (108). In mice infected with PR8, rhein significantly improved survival and reduced the lung index and lung expression levels of IL-1β, IL-6, IL-8, and TNF-α. In addition, rhein significantly reduced the protein levels of TLR2, TLR3, and TLR4 and the phosphorylation of NF-κB p65 in PR8-infected cells. The addition of TLR4 and NF-κB activators can antagonize the inhibitory effect of rhein on viral replication (109), so the anti-inflammatory effect of rhein may be related to inhibiting the activation of the TLRs/NF-κB signaling pathway.

4.2.6 Flavonoids

H. cordatum Thunb. is an important plant medicine with antiviral, antibacterial, anti-inflammatory, and antioxidant activities (110–112). Flavonoids, one of the effective components of this phytomedicine, can reduce lung and intestinal damage in mice, inhibit the over-release of tumor necrosis factor-α, IL-1, IL-8, and MCP-1 in the lung tissue of infected mice, and inhibit the up-regulated expression of TLR and NF-κB p65 proteins (113). HCP may play an anti-inflammatory role by inhibiting the activation of the TLR/NF-κB signaling pathway (Table 2).

There are studies that Xiaoshuantongmai decoction can mediate microRNA-181b intervention in deep-vein thrombosis. According to the equivalent dose ratio of kg body weight of human and animal, the drug dose was converted, and based on the deep-vein thrombosis model, normal saline and Xiaoshuantongmai decoction were administered. The results showed that the expression level of miR-181b in the venous endothelium of the Xiaoshuantongmai Tang group was the highest, followed by the blank control group and the sham operation group, and the lowest was in the model control group, with a statistical difference. It was proved that Xiaoshuantongmai decoction can up-regulate the expression of miR-181b in the venous endothelium of DVT model rats (78).

4.3 Anticoagulant and antiplatelet agents inhibit thrombosis by regulating the NF-κB signaling pathway

4.3.1 Aspirin and salicylate

Acute pulmonary thromboembolism (APE) is a disorder of pulmonary circulation caused by a blockage of the pulmonary artery. Extensive inflammatory responses have been demonstrated in the lung tissue of APE rats, accompanied by significantly elevated levels of tumor necrosis factor-α, interleukin-1-β, and IL-8 (114, 115). High levels of NF-κB were also observed in rats after APE induction (116, 117). Aspirin and salicylate have been reported to inhibit NF-κB equally (118, 119). Healthy SD rats were randomly divided into a control group, sham operation group, APE model group, and aspirin low-dose, medium-dose, and high-dose groups. After APE induction for 6, 24, and 72 h, rats in the low-, medium-, and high-dose aspirin groups were given daily doses of aspirin at 150, 300, and 600 mg/kg, respectively, for 3 consecutive days. The other groups were given the same amount of normal saline. In the APE model group, thrombus formation, alveolar wall injury, pulmonary hemorrhage, and inflammatory cell infiltration occurred at all time points. After aspirin treatment, pathological changes such as pulmonary hemorrhage and inflammatory cell infiltration were reduced. Compared with the APE model group, the expression of the NF-κB protein measured by Western blotting was significantly decreased in other groups at each time point ($P<0.05$, $P<0.001$). The highest expression of the NF-κB protein was observed in the APE model group, and NF-κB protein expression decreased gradually in a dose-dependent manner in rats receiving aspirin (120). In summary, aspirin can significantly inhibit the NF-κB pathway in a dose-dependent manner to reduce inflammation and alleviate lung injury after APE.

4.3.2 Platelet P2Y12 receptor antagonist

Clinically, ticagrelor and clopidogrel (antiplatelet coagulants) are often combined with PCI for acute coronary syndromes (ACS) (121). They cure ACS12 adenosine diphosphate (ADP) receptors by targeting the platelet P2Y to inhibit platelet aggregation and reduce

TABLE 2 The effect of traditional Chinese medicine on NF-κB-mediated inflammatory response.

TCM	Single active component	Anti-inflammatory effect	Molecular target	reference
Qihong Tongluo prescription	Astragalus polysaccharide	IL-1β, IL-6, TNF-α, INF-γ, MCP-1	TLR4, NF-κB p65	(85), (86)
	Safflower flower	Inhibited platelet aggregation		(87), (88)
Gegen Qinlian Pills (GQP)	——	TNF-α, IL-6, IL-1β, IL-4	HMGB1, TLR4, NFκB, NLRP3	(89), (92)
		Reduced lung, liver, and tail thrombus formation in mice; increased tail blood flow. The adhesion of platelet to HUVEC was decreased		
Huanglianjiedu Decoction(HLJJD)	baicalin	IL-1β, TNF-α, PEG2	TLR4,NF-κB p65, CD14,p-IKBα/IKBα	(94), (96)
	berberine	TNF – α,ICAM – 1,MCP – 1,IL-1β,IL-6,NLRP3	AMPK,MyD88,NF-κB-p65,TLR4	(98), (100), (103)
Liu Shen Wan (LSW)	——	TNF-α,IL-1β,IL-6,IFN-γ	TLR4,p-NF-κB p65, NF-κB p65,p -κB α	(107)
		The infiltration of inflammatory cells in the lung was reduced		
——	Rhein	It improved the survival rate of mice and reduced lung inflammation	TLR4,Akt,p38,JNK, MAPK,NF-κB	(109)
——	Flavonoids	MCP-1,IL-8,TNF-α,MDA	TLR3/4/7,NF-κB p65	(113)
Xiao shuan jing mai decoction		The expression of miR-181b in venous endothelium of DVT model rats was significantly up-regulated	miRNA-181	(78)

thrombosis (122). In this study, human umbilical vein endothelial cells (HUVECs) were cultured with ticagrelor or clopidogrel and given lipopolysaccharide (LPS) and CD14. Human umbilical vein endothelial cells (HUVECs) were cultured with ticagrelor or clopidogrel and given lipopolysaccharide (LPS) and CD14. Ticagrelor and clopidogrel reduce the expression of TNF-α, IL-1, IL-6, IL-8, and IL-2, inhibit p65 phosphorylation and IKB-α degradation, and significantly reduce the amount of nuclear translocation p65 (123, 124). These findings suggest that ticagrelor and clopidogrel inhibit the production of inflammatory cytokines by inhibiting the NF-KB pathway.

4.3.3 The PAR-1 antagonist

PAR-1 can be activated by thrombin to regulate platelet aggregation and endothelial permeability, so it is clinically used as a target for anti-platelet drugs to prevent thrombosis (125–127). Vorapaxar is a representative drug (128). IR induction was performed in rat lung models by perfusion *in vitro*. Male rats were treated with the specific PAR-1 antagonist vorapaxar or the control agent with 40 min of ischemia and 60 min of reperfusion. *In vitro*, mouse lung epithelial cells (MLE-12) were treated with vorapaxar and subjected to hypoxic reoxidation (HR). We found that vorapaxar reduced the production of thrombin, inflammatory factors, cytokine-induced neutrophil chemokine-1, interleukin-6, and tumor necrosis factor-α, pulmonary edema and neutrophil infiltration, and it alleviated lung cell apoptosis and down-regulated the nuclear factor-κB (NF-κB) pathway. It also blocked HR-induced NF-κB activation and the production of inflammatory chemokines in MLE12 cells. The results suggest that vorapaxar acts by blocking PAR-1 expression and modulating the NF-κB pathway (129) (Table 3).

5 Limitations in inhibiting NF-κB signaling pathways

At present, the targeted inhibition of the NF-κB signaling pathway for the treatment of thrombus has not reached the most perfect degree. On the one hand, these drugs/compounds can inhibit the inflammatory response and inhibit the expression of NF-κB, but there is no clear evidence of a targeted relationship, and studies have shown that combination drugs work better (130) and are a more clinically promising therapeutic strategy.

On the other hand, inhibition of the NF-κB pathway is a double-edged sword due to its broad effects. NF-κB mediates cell survival, cell differentiation, and cell proliferation (131), and inhibition of NF-κB has also been shown to play an important role in cancer treatment (132–135). However, long-term use of NF-κB inhibitors can cause side effects such as immune deficiency (136, 137) and intestinal homeostasis imbalance (138), and NF-κB inhibitors should be treated with for a short period. An ideal NF-κB inhibitor would only target the NF-κB pathway without affecting other signaling pathways. However, NF-κB inhibitors interfere with the NF-κB pathway by interfering with other pathways, such as PI3K/Akt and MAPK signaling (87, 115, 117).

6 Conclusion

In conclusion, the NF-κB signaling pathway plays an important role in inflammation and deep-vein thrombosis, and the regulation of the NF-κB pathway may bring new strategies for the treatment of thrombosis. Although the role of NF-κB signaling in venous thrombosis has been extensively studied in recent years, the application of NF-κB inhibitors in the treatment of thrombosis has a long way to go, regulating miRNAs or using drugs to interfere with the NF-κB signaling pathway. It may be a potential therapeutic option to improve thrombosis, but the dose and side effects of medication and whether regulating miRNAs will improve other downstream pathways of NF-κB signaling remain to be explored. Given the relationship between inflammation and blood clots, preventing inflammation is a better way to reduce blood clots. Therefore, in-depth study of the mechanism of the NF-κB signaling pathway inducing inflammation will help to elucidate the pathogenesis of venous thrombosis and will also have a far-reaching impact on the development of safer and more effective drugs and the prevention and treatment of thrombosis.

TABLE 3 The effect of common antithrombotic drugs on NF-κB-mediated inflammatory response.

Drugs	Representative drug	Anti-inflammatory effect	Action target	reference
anticoagulant	Aspirin and salicylate	Inhibited inflammation and relieved lung injury after APE	NF-κB	(120)
Platelet P2Y12 receptor antagonist	Ticagrelor and clopidogrel	TNFα,IL-1,IL-8,IL-6,IL-2,IL-7,TNF-α,CRP	NF-κB	(123), (124)
		Alleviated LPS-induced cell viability, cell migration and angiogenesis, cell cycle changes, and apoptosis and reduced myocardial ischemia-reperfusion injury (IRI) in ischemic myocardism		
The PAR-1 antagonist	Vorapaxar	CINC-1,IL-6,TNF-α,PAR-1,MPO	PI3K、NF-κB MAPK IκB-α	(129)
		Improved pulmonary edema, pulmonary histopathological changes.		

Author contributions

ZW: Writing – original draft, Writing – review & editing. CF: Investigation, Writing – review & editing. MY: Conceptualization, Investigation, Writing – review & editing. DW: Supervision, Writing – review & editing. MC: Supervision, Writing – review & editing. TG: Supervision, Writing – review & editing, Investigation. JM: Investigation, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Di Nisio M, van Es N, Büller HR. Deep vein thrombosis and pulmonary embolism. *Lancet* (2016) 388:3060–73. doi: 10.1016/S0140-6736(16)30514-1

2. Áinle FN, Kevane B. Which patients are at high risk of recurrent venous thromboembolism (deep vein thrombosis and pulmonary embolism)? *Hematol Am Soc Hematol Educ Program* (2020) 2020:201–12. doi: 10.1182/hematology.2020002268

3. Tritschler T, Kraaijpoel N, Le Gal G, Wells PS. Venous thromboembolism: advances in diagnosis and treatment. *JAMA* (2018) 320:1583–94. doi: 10.1001/jama.2018.14346

4. Blann AD, Lip GY. Virchow's triad revisited: the importance of soluble coagulation factors, the endothelium, and platelets. *Thromb Res* (2001) 101:321–7. doi: 10.1016/s0049-3848(00)00419-9

5. Gimbrone MA, García-Cardeña G. Vascular endothelium, hemodynamics, and the pathobiology of atherosclerosis. *Cardiovasc Pathol* (2013) 22:9–15. doi: 10.1016/j.carpath.2012.06.006

6. Cines DB, Pollak ES, Buck CA, Loscalzo J, Zimmerman GA, McEver RP, et al. Endothelial cells in physiology and in the pathophysiology of vascular disorders. *Blood* (1998) 91:3527–61.

7. Resnick N, Yahav H, Shay-Salit A, Shushy M, Schubert S, Zilberman LCM, et al. Fluid shear stress and the vascular endothelium: for better and for worse. *Prog Biophys Mol Biol* (2003) 81:177–99. doi: 10.1016/s0079-6107(02)00052-4

8. Lehoux S, Tedgui A. Cellular mechanics and gene expression in blood vessels. *J Biomech* (2003) 36:631–43. doi: 10.1016/s0021-9290(02)00441-4

9. Chien S, Li S, Shyy YJ. Effects of mechanical forces on signal transduction and gene expression in endothelial cells. *Hypertension* (1998) 31:162–9. doi: 10.1161/01.hyp.31.1.162

10. Resnick N, Collins T, Atkinson W, Bonthron DT, Dewey CF, Gimbron MA. Platelet-derived growth factor B chain promoter contains a cis-acting fluid shear-stress-responsive element. *Proc Natl Acad Sci U.S.A.* (1993) 90:7908. doi: 10.1073/pnas.90.16.7908-d

11. Korenaga R, Ando J, Kosaki K, Isshiki M, Takada Y, Kamiya A. Negative transcriptional regulation of the VCAM-1 gene by fluid shear stress in murine endothelial cells. *Am J Physiol* (1997) 273:C1506–1515. doi: 10.1152/ajpcell.1997.273.5.c1506

12. Davies PF. Flow-mediated endothelial mechanotransduction. *Physiol Rev* (1995) 75:519–60. doi: 10.1152/physrev.1995.75.3.519

13. Hahn C, Schwartz MA. Mechanotransduction in vascular physiology and atherogenesis. *Nat Rev Mol Cell Biol* (2009) 10:53–62. doi: 10.1038/nrm2596

14. Nigro P, Abe J-I, Berk BC. Flow shear stress and atherosclerosis: a matter of site specificity. *Antioxid Redox Signal* (2011) 15:1405–14. doi: 10.1089/ars.2010.3679

15. Chan N, Eikelboom J. Hypercoagulability and thrombosis in COVID-19: a modifiable cause for mortality? *Eur Heart J* (2021) 42:3143–5. doi: 10.1093/eurheartj/ehab417

16. Piazza G. Cardiology patient page. Thrombophilia and hypercoagulability. *Circulation* (2014) 130:e9–10. doi: 10.1161/CIRCULATIONAHA.113.007665

17. Salomon O, Steinberg DM, Zivelin A, Gitel S, Dardik R, Rosenberg N, et al. Single and combined prothrombotic factors in patients with idiopathic venous thromboembolism: prevalence and risk assessment. *Arterioscler Thromb Vasc Biol* (1999) 19:511–8. doi: 10.1161/01.atv.19.3.511

18. Vandenbroucke JP, Koster T, Briët E, Reitsma PH, Bertina RM, Rosendaal FR. Increased risk of venous thrombosis in oral-contraceptive users who are carriers of factor V Leiden mutation. *Lancet* (1994) 344:1453–7. doi: 10.1016/s0140-6736(94)90286-0

19. Anderson JAM, Weitz JI. Hypercoagulable states. *Crit Care Clin* (2011) 27:933–52. doi: 10.1016/j.ccc.2011.09.007

20. Iba T, Helms J, Levi M, Levy JH. Thromboinflammation in acute injury: infections, heatstroke, and trauma. *J Thromb Haemost* (2023) 21:S1538-7836(23)00583-4. doi: 10.1016/j.jtha.2023.07.020

21. Fischetti F, Tedesco F. Cross-talk between the complement system and endothelial cells in physiologic conditions and in vascular diseases. *Autoimmunity* (2006) 39:417–28. doi: 10.1080/08916930600739712

22. Pilard M, Ollivier EL, Gourdou-Latyszenok V, Couturaud F, Lemarié CA. Endothelial cell phenotype, a major determinant of venous thrombo-inflammation. *Front Cardiovasc Med* (2022) 9:864735. doi: 10.3389/fcvm.2022.864735

23. Lopatko Fagerström I, Ståhl A-L, Mossberg M, Tati R, Kristoffersson A-C, Kahn R, et al. Blockade of the kallikrein-kinin system reduces endothelial complement activation in vascular inflammation. *EBioMedicine* (2019) 47:319–28. doi: 10.1016/j.ebiom.2019.08.020

24. Sano M, Takahashi R, Ijichi H, Ishigaki K, Yamada T, Miyabayashi K, et al. Blocking VCAM-1 inhibits pancreatic tumour progression and cancer-associated thrombosis/thromboembolism. *Gut* (2021) 70:1713–23. doi: 10.1136/gutjnl-2020-320608

25. Kong D-H, Kim YK, Kim MR, Jang JH, Lee S. Emerging roles of vascular cell adhesion molecule-1 (VCAM-1) in immunological disorders and cancer. *Int J Mol Sci* (2018) 19:1057. doi: 10.3390/ijms19041057

26. Gilmore TD. Introduction to NF-kappaB: players, pathways, perspectives. *Oncogene* (2006) 25:6680–4. doi: 10.1038/sj.onc.1209954

27. Hayden MS, Ghosh S. Shared principles in NF-kappaB signaling. *Cell* (2008) 132:344–62. doi: 10.1016/j.cell.2008.01.020

28. Vallabhapurapu S, Karin M. Regulation and function of NF-kappaB transcription factors in the immune system. *Annu Rev Immunol* (2009) 27:693–733. doi: 10.1146/annurev.immunol.021908.132641

29. Bonizzi G, Karin M. The two NF-kappaB activation pathways and their role in innate and adaptive immunity. *Trends Immunol* (2004) 25:280–8. doi: 10.1016/j.it.2004.03.008

30. Ghosh S, Hayden MS. New regulators of NF-kappaB in inflammation. *Nat Rev Immunol* (2008) 8:837–48. doi: 10.1038/nri2423

31. Sun S-C. The non-canonical NF-κB pathway in immunity and inflammation. *Nat Rev Immunol* (2017) 17:545–58. doi: 10.1038/nri.2017.52

32. Sun S-C. Non-canonical NF-κB signaling pathway. *Cell Res* (2011) 21:71–85. doi: 10.1038/cr.2010.177

33. Xiao G, Harhaj EW, Sun SC. NF-kappaB-inducing kinase regulates the processing of NF-kappaB2 p100. *Mol Cell* (2001) 7:401–9. doi: 10.1016/s1097-2765(01)00187-3

34. Gupta SC, Kim JH, Prasad S, Aggarwal BB. Regulation of survival, proliferation, invasion, angiogenesis, and metastasis of tumor cells through modulation of inflammatory pathways by nutraceuticals. *Cancer Metastasis Rev* (2010) 29:405–34. doi: 10.1007/s10555-010-9235-2

35. Gupta SC, Sundaram C, Reuter S, Aggarwal BB. Inhibiting NF-κB activation by small molecules as a therapeutic strategy. *Biochim Biophys Acta* (2010) 1799:775–87. doi: 10.1016/j.bbagrm.2010.05.004

36. Luo W, Li Y-X, Jiang L-J, Chen Q, Wang T, Ye D-W. Targeting JAK-STAT signaling to control cytokine release syndrome in COVID-19. *Trends Pharmacol Sci* (2020) 41:531–43. doi: 10.1016/j.tips.2020.06.007

37. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in covid-19. *N Engl J Med* (2020) 383:120–8. doi: 10.1056/NEJMoa2015432

38. Mo J, Huang H, Zhang C, Wang B, He F, Yin L, et al. To study influence of JAK-STAT signaling pathway in traumatic deep vein thrombosis. *ZHONG GUO WU ZHEN XUE ZA ZHI* (2007) 7:6212–4.

39. Zhuang S. *The Study of Inflammation Factors in the Mechanism of Deep Venous Thrombosis* (2005). Naval Medical University. Available at: https://kns.cnki.net/kcms2/article/abstract?v=3uoqIhG8C447WN1SO36whBaOoOkzJ23ELn_-3AAgJ5enmUaXDTPHrESUbr8kKkBIzUTQYESjzuMjzMXdnFBjhD9MuVtV0mqx&uniplatform=NZKPT (Accessed August 15, 2023). A dissertation submitted for the Degree of Doctor of Medicine.

40. Ren B, Yan F, Deng Z, Zhang S, Xiao L, Wu M, et al. Extremely high incidence of lower extremity deep venous thrombosis in 48 patients with severe COVID-19 in Wuhan. *Circulation* (2020) 142:181–3. doi: 10.1161/CIRCULATIONAHA.120.047407

41. Zhang L, Feng X, Zhang D, Jiang C, Mei H, Wang J, et al. Deep vein thrombosis in hospitalized patients with COVID-19 in Wuhan, China: prevalence, risk factors, and outcome. *Circulation* (2020) 142:114–28. doi: 10.1161/CIRCULATIONAHA.120.046702

42. Gorog DA, Storey RF, Gurbel PA, Tantry US, Berger JS, Chan MY, et al. Current and novel biomarkers of thrombotic risk in COVID-19: a Consensus Statement from the International COVID-19 Thrombosis Biomarkers Colloquium. *Nat Rev Cardiol* (2022) 19:475–95. doi: 10.1038/s41569-021-00665-7

43. Miesbach W, Makris M. COVID-19: coagulopathy, risk of thrombosis, and the rationale for anticoagulation. *Clin Appl Thromb Hemost* (2020) 26:1076029620938149. doi: 10.1177/1076029620938149

44. Flaumenhaft R, Enjyoji K, Schmaier AA. Vasculopathy in COVID-19. *Blood* (2022) 140:222–35. doi: 10.1182/blood.2021012250

45. Scully M, Singh D, Lown R, Poles A, Solomon T, Levi M, et al. Pathologic Antibodies to Platelet Factor 4 after ChAdOx1 nCoV-19 Vaccination. *N Engl J Med* (2021) 384:2202–11. doi: 10.1056/NEJMoa2105385

46. Hunter PR. Thrombosis after covid-19 vaccination. *BMJ* (2021) 373:n958. doi: 10.1136/bmj.n958

47. Gupta A, Sardar P, Cash ME, Milani RV, Lavie CJ. Covid-19 vaccine- induced thrombosis and thrombocytopenia-a commentary on an important and practical clinical dilemma. *Prog Cardiovasc Dis* (2021) 67:105–7. doi: 10.1016/j.pcad.2021.05.001

48. Hippisley-Cox J, Patone M, Mei XW, Saatci D, Dixon S, Khunti K, et al. Risk of thrombocytopenia and thromboembolism after covid-19 vaccination and SARS-CoV-2 positive testing: self-controlled case series study. *BMJ* (2021) 374:n1931. doi: 10.1136/bmj.n1931

49. Kim AY, Woo W, Yon DK, Lee SW, Yang JW, Kim JH, et al. Corrigendum to "Thrombosis patterns and clinical outcome of COVID-19 vaccine-induced immune thrombotic thrombocytopenia: A Systematic Review and Meta-Analysis" International Journal of Infectious Diseases, Volume 119, June 2022, Page 130-139. *Int J Infect Dis* (2022) 123:166. doi: 10.1016/j.ijid.2022.08.025

50. Hwang J, Lee SB, Lee SW, Lee MH, Koyanagi A, Jacob L, et al. Comparison of vaccine-induced thrombotic events between ChAdOx1 nCoV-19 and Ad26.COV.2.S vaccines. *J Autoimmun* (2021) 122:102681. doi: 10.1016/j.jaut.2021.102681

51. Ding P. *The role and mechanism of IL-17A in the formation of deep vein thrombosis* (2018). Huazhong University of Science and Technology. Available at: https://kns.cnki.net/kcms2/article/abstract?v=3uoqIhG8C447WN1SO36whLpCgh0R0Z-iDdIt-WSAdV5IJ_Uy2HKRAR6bP3qoZUcgSakIAbnlVhhMH0B_SQEPiC6IXYLGCRSz&uniplatform=NZKPT (Accessed August 15, 2023). A dissertation submitted for the Degree of Doctor of Medicine.

52. Zhang Y, Zhang Z, Wei R, Miao X, Sun S, Liang G, et al. IL (Interleukin)-6 contributes to deep vein thrombosis and is negatively regulated by miR-338-5p. *Arterioscler Thromb Vasc Biol* (2020) 40:323–34. doi: 10.1161/ATVBAHA.119.313137

53. Sierra-Mondragón E. Low expression of IL-6 and TNF-α correlates with the presence of the nuclear regulators of NF-κB, IκBNS and BCL-3, in the uterus of mice. *Mol Immunol* (2015) 68:333–40. doi: 10.1016/j.molimm.2015.09.020

54. Zheng X, Liu H, Ma M, Ji J, Zhu F, Sun L. Anti-thrombotic activity of phenolic acids obtained from Salvia miltiorrhiza f. alba in TNF-α-stimulated endothelial cells via the NF-κB/JNK/p38 MAPK signaling pathway. *Arch Pharm Res* (2021) 44:427–38. doi: 10.1007/s12272-021-01325-7

55. Guo T, Shi J, Chen X, Zeng J, Zhang F, Dai P, et al. The effect of COX-2 targeting interference on TDVT in rats. *Orthopaedic Biomechanics Materials Clin Study* (2022) 19:11–14+19.

56. Mussbacher M, Salzmann M, Brostjan C, Hoesel B, Schoegenhofer C, Datler H, et al. Cell type-specific roles of NF-κB linking inflammation and thrombosis. *Front Immunol* (2019) 10:85. doi: 10.3389/fimmu.2019.00085

57. Li YS, Shyy YJ, Wright JG, Valente AJ, Cornhill JF, Kolattukudy PE. The expression of monocyte chemotactic protein (MCP-1) in human vascular endothelium in *vitro* and in *vivo*. *Mol Cell Biochem* (1993) 126:61–8. doi: 10.1007/BF01772208

58. Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. *J Interferon Cytokine Res* (2009) 29:313–26. doi: 10.1089/jir.2008.0027

59. Albayati MA, Grover SP, Saha P, Lwaleed BA, Modarai B, Smith A. Postsurgical inflammation as a causative mechanism of venous thromboembolism. *Semin Thromb Hemost* (2015) 41:615–20. doi: 10.1055/s-0035-1556726

60. Delekta PC, Apel IJ, Gu S, Siu K, Hattori Y, McAllister-Lucas LM, et al. Thrombin-dependent NF-κB activation and monocyte/endothelial adhesion are mediated by the CARMA3·Bcl10·MALT1 signalosome. *J Biol Chem* (2010) 285:41432–42. doi: 10.1074/jbc.M110.158949

61. Margetic S. Inflammation and haemostasis. *Biochem Med (Zagreb)* (2012) 22:49–62. doi: 10.11613/BM.2012.006

62. Stakos DA, Kambas K, Konstantinidis T, Mitroulis I, Apostolidou E, Arelaki S, et al. Expression of functional tissue factor by neutrophil extracellular traps in culprit artery of acute myocardial infarction. *Eur Heart J* (2015) 36:1405–14. doi: 10.1093/eurheartj/ehv007

63. Rahman A, Fazal F. Blocking NF-κB: an inflammatory issue. *Proc Am Thorac Soc* (2011) 8:497–503. doi: 10.1513/pats.201101-009MW

64. Verhamme P, Hoylaerts MF. The pivotal role of the endothelium in haemostasis and thrombosis. *Acta Clin Belg* (2006) 61:213–9. doi: 10.1179/acb.2006.036

65. Chang M, Guo F, Zhou Z, Huang X, Yi L, Dou Y, et al. HBP induces the expression of monocyte chemoattractant protein-1 via the FAK/PI3K/AKT and p38 MAPK/NF-κB pathways in vascular endothelial cells. *Cell Signal* (2018) 43:85–94. doi: 10.1016/j.cellsig.2017.12.008

66. Xu XR, Zhang D, Oswald BE, Carrim N, Wang X, Hou Y, et al. Platelets are versatile cells: New discoveries in hemostasis, thrombosis, immune responses, tumor metastasis and beyond. *Crit Rev Clin Lab Sci* (2016) 53:409–30. doi: 10.1080/10408363.2016.1200008

67. Yau JW, Teoh H, Verma S. Endothelial cell control of thrombosis. *BMC Cardiovasc Disord* (2015) 15:130. doi: 10.1186/s12872-015-0124-z

68. Urban CF, Ermert D, Schmid M, Abu-Abed U, Goosmann C, Nacken W, et al. Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against Candida albicans. *PloS Pathog* (2009) 5:e1000639. doi: 10.1371/journal.ppat.1000639

69. Cao W, Shao Z, Duan M, Gao L, Zhang Y, Xu X. Research progress of neutrophil extracellular trap (NETs) in related inflammatory diseases. *ZHONG GUO MIAN YI XUE ZA ZHI* (2019) 35:635–8.

70. Thailin C, Hisada Y, Lundstrom S, Mackman N, Wallen H. Neutrophil extracellular traps: villains and targets in arterial, venous, and cancer-associated thrombosis. *Arteriosclerosis thrombosis Vasc Biol* (2019) 39:1724–38. doi: 10.1161/ATVBAHA.119.312463

71. Klier M, Gowert NS, Jäckel S, Reinhardt C, Elvers M. Phospholipase D1 is a regulator of platelet-mediated inflammation. *Cell Signal* (2017) 38:171–81. doi: 10.1016/j.cellsig.2017.07.007

72. Mackman N. New insights into the mechanisms of venous thrombosis. *J Clin Invest* (2012) 122:2331–6. doi: 10.1172/JCI60229

73. Cheng Z, Jia W, Tian X, Jiang P, Zhang Y, Li J, et al. Cotinine inhibits TLR4/NF-κB signaling pathway and improves deep vein thrombosis in rats. *Biosci Rep* (2020) 40:BSR20201293. doi: 10.1042/BSR20201293

74. Starikova I, Jamaly S, Sorrentino A, Blondal T, Latysheva N, Sovershaev M, et al. Differential expression of plasma miRNAs in patients with unprovoked venous thromboembolism and healthy control individuals. *Thromb Res* (2015) 136:566–72. doi: 10.1016/j.thromres.2015.07.005

75. Salloum-Asfar S, Teruel-Montoya R, Arroyo AB, García-Barberá N, Chaudhry A, Schuetz E, et al. Regulation of coagulation factor XI expression by microRNAs in the human liver. *PloS One* (2014) 9:e111713. doi: 10.1371/journal.pone.0111713

76. Chen R, Chen S, Liao J, Chen X, Xu X. MiR-145 facilitates proliferation and migration of endothelial progenitor cells and recanalization of arterial thrombosis in cerebral infarction mice via JNK signal pathway. *Int J Clin Exp Pathol* (2015) 8:13770–6.

77. Cz C LL, Hf L, Dp B. MicroRNAs modulate hematopoietic lineage differentiation. *Sci (New York NY)* (2004) 303:83–6. doi: 10.1126/science.1091903

78. Pang X. *Study on the Mechanism of Xiao Shuan Tong Mai Decoction Intervening Deep Vein Thrombosis by Mediating microRNA-181b Regulating NF-κB Signal Pathway*. Shandong University of Traditional Chinese Medicine (2020). A dissertation submitted for the Degree of Doctor of Medicine. doi: 10.27282/d.cnki.gsdzu.2020.000031

79. Tian Y, Li X, Bai C, Yang Z, Zhang L, Luo J. Regulation of microRNA-150 on inflammatory response in rat models of deep vein thrombosis. *Genomics Appl Biol* (2020) 39:4753–60. doi: 10.13417/j.gab.039.004753

80. Feng Q, Xu C, Sun M. miRNA-141 inhibits thrombosis in vascular pathways through the TLR4 signaling pathway. *XIBU YIXUE* (2021) 33:186–90.

81. Zhang Q, Lenardo MJ, Baltimore D. 30 years of NF-κB: A blossoming of relevance to human pathobiology. *Cell* (2017) 168:37–57. doi: 10.1016/j.cell.2016.12.012

82. Guo Z, Lou Y, Kong M, Luo Q, Liu Z, Wu J. A systematic review of phytochemistry, pharmacology and pharmacokinetics on astragali radix: implications for astragali radix as a personalized medicine. *Int J Mol Sci* (2019) 20:1463. doi: 10.3390/ijms20061463

83. Li X, Qu L, Dong Y, Han L, Liu E, Fang S, et al. A review of recent research progress on the astragalus genus. *Molecules* (2014) 19:18850–80. doi: 10.3390/molecules191118850

84. Zhang K, Pugliese M, Pugliese A, Passantino A. Biological active ingredients of traditional Chinese herb Astragalus membranaceus on treatment of diabetes: a systematic review. *Mini Rev Med Chem* (2015) 15:315–29. doi: 10.2174/1389557515666150227113431

85. Liu T, Zhang M, Niu H, Liu J, Ruilian M, Wang Y, et al. Astragalus polysaccharide from Astragalus Melittin ameliorates inflammation via suppressing the activation of TLR-4/NF-κB p65 signal pathway and protects mice from CVB3-induced virus myocarditis. *Int J Biol Macromol* (2019) 126:179–86. doi: 10.1016/j.ijbiomac.2018.12.207

86. Chu X, Zhu Y, Su K, Zhang T, Ma Y. Protective effect of Qihong Tongluo prescription on vascular endothelial cells in rats with deep venous thrombosis based on NF-κB pathway. *Chin J Exp Traditional Med Formulae* (2023) 29:60–8. doi: 10.13422/j.cnki.syfjx.20230495

87. Yu G, Luo Z, Zhou Y, Zhang L, Wu Y, Ding L, et al. Uncovering the pharmacological mechanism of Carthamus tinctorius L. @ on cardiovascular disease by a systems pharmacology approach. *BioMed Pharmacother* (2019) 117:109094. doi: 10.1016/j.biopha.2019.109094

88. Cao J, Chen Z, Zhu Y, Li Y, Guo C, Gao K, et al. Huangqi-Honghua combination and its main components ameliorate cerebral infarction with Qi deficiency and blood stasis syndrome by antioxidant action in rats. *J Ethnopharmacol* (2014) 155:1053–60. doi: 10.1016/j.jep.2014.05.061

89. Li R, Chen Y, Shi M, Xu X, Zhao Y, Wu X, et al. Gegen Qinlian decoction alleviates experimental colitis via suppressing TLR4/NF-κB signaling and enhancing antioxidant effect. *Phytomedicine* (2016) 23:1012–20. doi: 10.1016/j.phymed.2016.06.010

90. Choo M-K, Park E-K, Yoon H-K, Kim D-H. Antithrombotic and antiallergic activities of daidzein, a metabolite of puerarin and daidzin produced by human intestinal microflora. *Biol Pharm Bull* (2002) 25:1328–32. doi: 10.1248/bpb.25.1328

91. Zhang Y, Ma X, Guo C, Wang M, Kou N, Qu H, et al. Pretreatment with a combination of ligustrazine and berberine improves cardiac function in rats with coronary microembolization. *Acta Pharmacol Sin* (2016) 37:463–72. doi: 10.1038/aps.2015.147

92. Wei X, Zhang B, Wei F, Ding M, Luo Z, Han X, et al. Gegen Qinlian pills alleviate carrageenan-induced thrombosis in mice model by regulating the HMGB1/NF-κB/NLRP3 signaling. *Phytomedicine* (2022) 100:154083. doi: 10.1016/j.phymed.2022.154083

93. He T, Wang M, Kong J, Wang Q, Tian Y, Li C, et al. Integrating network pharmacology and non-targeted metabolomics to explore the common mechanism of Coptis Categorized Formula improving T2DM zebrafish. *J Ethnopharmacol* (2022) 284:114784. doi: 10.1016/j.jep.2021.114784

94. Fu Y-J, Xu B, Huang S-W, Luo X, Deng X-L, Luo S, et al. Baicalin prevents LPS-induced activation of TLR4/NF-κB p65 pathway and inflammation in mice via inhibiting the expression of CD14. *Acta Pharmacol Sin* (2021) 42:88–96. doi: 10.1038/s41401-020-0411-9

95. Jiang M, Li Z, Zhu G. Immunological regulatory effect of flavonoid baicalin on innate immune toll-like receptors. *Pharmacol Res* (2020) 158:104890. doi: 10.1016/j.phrs.2020.104890

96. Shen J, Cheng J, Zhu S, Zhao J, Ye Q, Xu Y, et al. Regulating effect of baicalin on IKK/IKB/NF-kB signaling pathway and apoptosis-related proteins in rats with ulcerative colitis. *Int Immunopharmacol* (2019) 73:193–200. doi: 10.1016/j.intimp.2019.04.052

97. Zeng A, Liang X, Zhu S, Liu C, Luo X, Zhang Q, et al. Baicalin, a potent inhibitor of NF-κB signaling pathway, enhances chemosensitivity of breast cancer cells to docetaxel and inhibits tumor growth and metastasis both *in vitro* and *in vivo*. *Front Pharmacol* (2020) 11:879. doi: 10.3389/fphar.2020.00879

98. Liu S-J, Yin C-X, Ding M-C, Wang Y-Z, Wang H. Berberine inhibits tumor necrosis factor-α-induced expression of inflammatory molecules and activation of nuclear factor-κB via the activation of AMPK in vascular endothelial cells. *Mol Med Rep* (2015) 12:5580–6. doi: 10.3892/mmr.2015.4061

99. Kuo C-L, Chi C-W, Liu T-Y. The anti-inflammatory potential of berberine in *vitro* and in *vivo*. *Cancer Lett* (2004) 203:127–37. doi: 10.1016/j.canlet.2003.09.002

100. Li C, Ai G, Wang Y, Lu Q, Luo C, Tan L, et al. Oxyberberine, a novel gut microbiota-mediated metabolite of berberine, possesses superior anti-colitis effect: Impact on intestinal epithelial barrier, gut microbiota profile and TLR4-MyD88-NF-κB pathway. *Pharmacol Res* (2020) 152:104603. doi: 10.1016/j.phrs.2019.104603

101. Wu Y, Li J, Kim Y, Wu J, Wang Q, Hao Y. *In vivo* and in *vitro* antiviral effects of berberine on influenza virus. *Chin J Integr Med* (2011) 17:444–52. doi: 10.1007/s11655-011-0640-3

102. Yan Y-Q, Fu Y-J, Wu S, Qin H-Q, Zhen X, Song B-M, et al. Anti-influenza activity of berberine improves prognosis by reducing viral replication in mice. *Phytother Res* (2018) 32:2560–7. doi: 10.1002/ptr.6196

103. Liu J JIES, Chen B, Zeng H, MA Y. Mechanisms of Huanglian Jiedu decoction in treating acute gouty arthritis based on NLRP3 inflammasome and TLR4/NF-κB signal pathway. *Chin J Exp Traditional Med Formulae* (2023), 1–9. doi: 10.13422/j.cnki.syfjx.20230802

104. Liu H, Chen X, Liu Y, Fang C, Chen S. Antithrombotic effects of Huanglian Jiedu decoction in a rat model of ischaemia-reperfusion-induced cerebral stroke. *Pharm Biol* (2021) 59:823–7. doi: 10.1080/13880209.2021.1942505

105. Ma H-Y, Kou J-P, Wang J-R, Yu B-Y. Evaluation of the anti-inflammatory and analgesic activities of Liu-Shen-Wan and its individual fractions. *J Ethnopharmacol* (2007) 112:108–14. doi: 10.1016/j.jep.2007.02.008

106. Zhang L, Ye X, Liu Y, Zhang Z, Xia X, Dong S. Research progress on the effect of traditional Chinese medicine on the activation of PRRs-mediated NF-κB signaling pathway to inhibit influenza pneumonia. *Front Pharmacol* (2023) 14:1132388. doi: 10.3389/fphar.2023.1132388

107. Ma Q, Huang W, Zhao J, Yang Z. Liu Shen Wan inhibits influenza a virus and excessive virus-induced inflammatory response via suppression of TLR4/NF-κB signaling pathway in *vitro* and in *vivo*. *J Ethnopharmacol* (2020) 252:112584. doi: 10.1016/j.jep.2020.112584

108. Sun H, Luo G, Chen D, Xiang Z. A comprehensive and system review for the pharmacological mechanism of action of rhein, an active anthraquinone ingredient. *Front Pharmacol* (2016) 7:247. doi: 10.3389/fphar.2016.00247

109. Wang Q-W, Su Y, Sheng J-T, Gu L-M, Zhao Y, Chen X-X, et al. Anti-influenza A virus activity of rhein through regulating oxidative stress, TLR4, Akt, MAPK, and NF-κB signal pathways. *PloS One* (2018) 13:e0191793. doi: 10.1371/journal.pone.0191793

110. Li W, Fan T, Zhang Y, Fan T, Zhou P, Niu X, et al. Houttuynia cordata Thunb. volatile oil exhibited anti-inflammatory effects in *vivo* and inhibited nitric oxide and tumor necrosis factor-α production in LPS-stimulated mouse peritoneal macrophages in *vitro*. *Phytother Res* (2013) 27:1629–39. doi: 10.1002/ptr.4905

111. Cheng B-H, Chan JY-W, Chan BC-L, Lin H-Q, Han X-Q, Zhou X, et al. Structural characterization and immunomodulatory effect of a polysaccharide HCP-2 from Houttuynia cordata. *Carbohydr Polym* (2014) 103:244–9. doi: 10.1016/j.carbpol.2013.12.048

112. Ling L, Ren A, Lu Y, Zhang Y, Zhu H, Tu P, et al. The synergistic effect and mechanisms of flavonoids and polysaccharides from Houttuynia cordata on H1N1-induced pneumonia in mice. *J Ethnopharmacol* (2023) 302:115761. doi: 10.1016/j.jep.2022.115761

113. Ling L-J, Lu Y, Zhang Y-Y, Zhu H-Y, Tu P, Li H, et al. Flavonoids from Houttuynia cordata attenuate H1N1-induced acute lung injury in mice via inhibition of influenza virus and Toll-like receptor signalling. *Phytomedicine* (2020) 67:153150. doi: 10.1016/j.phymed.2019.153150

114. Sun C, Wang L, Jiang H, Yang R. [Effects of aspirin on CX3CL1 and CX3CR1 in acute pulmonary embolism rats]. *Zhonghua Yi Xue Za Zhi* (2013) 93:69–72.

115. Wang L, Wu J, Zhang W, Zhi Y, Wu Y, Jiang R, et al. Effects of aspirin on the ERK and PI3K/Akt signaling pathways in rats with acute pulmonary embolism. *Mol Med Rep* (2013) 8:1465–71. doi: 10.3892/mmr.2013.1676

116. Lu W-J, Lee J-J, Chou D-S, Jayakumar T, Fong T-H, Hsiao G, et al. A novel role of andrographolide, an NF-kappa B inhibitor, on inhibition of platelet activation: the pivotal mechanisms of endothelial nitric oxide synthase/cyclic GMP. *J Mol Med (Berl)* (2011) 89:1261–73. doi: 10.1007/s00109-011-0800-0

117. Lu WJ, Lin KH, Hsu MJ, Chou DS, Hsiao G, Sheu JR. Suppression of NF-κB signaling by andrographolide with a novel mechanism in human platelets: regulatory roles of the p38 MAPK-hydroxyl radical-ERK2 cascade. *Biochem Pharmacol* (2012) 84:914–24. doi: 10.1016/j.bcp.2012.06.030

118. Frantz B, O'Neill EA. The effect of sodium salicylate and aspirin on NF-kappa B. *Science* (1995) 270:2017–9. doi: 10.1126/science.270.5244.2017

119. Kopp E, Ghosh S. Inhibition of NF-kappa B by sodium salicylate and aspirin. *Science* (1994) 265:956–9. doi: 10.1126/science.8052854

120. Wang L-C, Jiang R-L, Zhang W, Wei L-L, Yang R-H. Effects of aspirin on the expression of nuclear factor-κB in a rat model of acute pulmonary embolism. *World J Emerg Med* (2014) 5:229–33. doi: 10.5847/wjem.j.issn.1920-8642.2014.03.013

121. Pels K, Schwimmbeck PL, Rosenthal P, Loddenkemper C, Dang-Heine C, Rauch U, et al. Long-term clopidogrel administration following severe coronary injury reduces proliferation and inflammation via inhibition of nuclear factor-kappaB and activator protein 1 activation in pigs. *Eur J Clin Invest* (2009) 39:174–82. doi: 10.1111/j.1365-2362.2009.02089.x

122. Schneider DJ. Mechanisms potentially contributing to the reduction in mortality associated with ticagrelor therapy. *J Am Coll Cardiol* (2011) 57:685–7. doi: 10.1016/j.jacc.2010.11.016

123. Jia Z, Huang Y, Ji X, Sun J, Fu G. Ticagrelor and clopidogrel suppress NF-κB signaling pathway to alleviate LPS-induced dysfunction in vein endothelial cells. *BMC Cardiovasc Disord* (2019) 19:318. doi: 10.1186/s12872-019-01287-1

124. Liu X, Wang Y, Zhang M, Liu Y, Hu L, Gu Y. Ticagrelor reduces ischemia-reperfusion injury through the NF-κB-dependent pathway in rats. *J Cardiovasc Pharmacol* (2019) 74:13–9. doi: 10.1097/FJC.0000000000000675

125. Ramachandran R, Noorbakhsh F, Defea K, Hollenberg MD. Targeting proteinase-activated receptors: therapeutic potential and challenges. *Nat Rev Drug Discovery* (2012) 11:69–86. doi: 10.1038/nrd3615

126. Chambers RC, Scotton CJ. Coagulation cascade proteinases in lung injury and fibrosis. *Proc Am Thorac Soc* (2012) 9:96–101. doi: 10.1513/pats.201201-006AW

127. De Ceunynck K, Peters CG, Jain A, Higgins SJ, Aisiku O, Fitch-Tewfik JL, et al. PAR1 agonists stimulate APC-like endothelial cytoprotection and confer resistance to thromboinflammatory injury. *Proc Natl Acad Sci U.S.A.* (2018) 115:E982–91. doi: 10.1073/pnas.1718600115

128. Gupta R, Lin M, Mehta A, Aedma SK, Shah R, Ranchal P, et al. Protease-activated receptor antagonist for reducing cardiovascular events - A review on vorapaxar. *Curr Probl Cardiol* (2023) 48:101035. doi: 10.1016/j.cpcardiol.2021.101035

129. Chu S-J, Tang S-E, Pao H-P, Wu S-Y, Liao W-I. Protease-activated receptor-1 antagonist protects against lung ischemia/reperfusion injury. *Front Pharmacol* (2021) 12:752507. doi: 10.3389/fphar.2021.752507

130. Li F, Xu D, Hou K, Gou X, Lv N, Fang W, et al. Pretreatment of Indobufen and Aspirin and their Combinations with Clopidogrel or Ticagrelor Alleviates Inflammasome Mediated Pyroptosis via Inhibiting NF-κB/NLRP3 Pathway in Ischemic Stroke. *J Neuroimmune Pharmacol* (2021) 16:835–53. doi: 10.1007/s11481-020-09978-9

131. Cheng W, Cui C, Liu G, Ye C, Shao F, Bagchi AK, et al. NF-κB, A potential therapeutic target in cardiovascular diseases. *Cardiovasc Drugs Ther* (2023) 37:571–84. doi: 10.1007/s10557-022-07362-8

132. Yu H, Lin L, Zhang Z, Zhang H, Hu H. Targeting NF-κB pathway for the therapy of diseases: mechanism and clinical study. *Signal Transduct Target Ther* (2020) 5:209. doi: 10.1038/s41392-020-00312-6

133. Taniguchi K, Karin M. NF-κB, inflammation, immunity and cancer: coming of age. *Nat Rev Immunol* (2018) 18:309–24. doi: 10.1038/nri.2017.142

134. Rasmi RR, Sakthivel KM, Guruvayoorappan C. NF-κB inhibitors in treatment and prevention of lung cancer. *BioMed Pharmacother* (2020) 130:110569. doi: 10.1016/j.biopha.2020.110569

135. Patel M, Horgan PG, McMillan DC, Edwards J. NF-κB pathways in the development and progression of colorectal cancer. *Transl Res* (2018) 197:43–56. doi: 10.1016/j.trsl.2018.02.002

136. Pflug KM, Sitcheran R. Targeting NF-κB-inducing kinase (NIK) in immunity, inflammation, and cancer. *Int J Mol Sci* (2020) 21:8470. doi: 10.3390/ijms21228470

137. Barnabei L, Laplantine E, Mbongo W, Rieux-Laucat F, Weil R. NF-κB: at the borders of autoimmunity and inflammation. *Front Immunol* (2021) 12:716469. doi: 10.3389/fimmu.2021.716469

138. Wang B, Shen J. NF-κB inducing kinase regulates intestinal immunity and homeostasis. *Front Immunol* (2022) 13:895636. doi: 10.3389/fimmu.2022.895636

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Whether MTHFD2 plays a new role: from anticancer targets to anti-inflammatory disease

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KEYWORDS

methylenetetrahydrofolate dehydrogenase 2, T cells, inflammatory disease, therapeutic target, one-carbon metabolism enzyme

1 Introduction

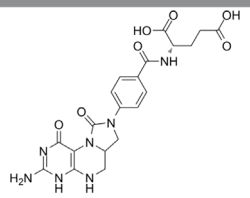
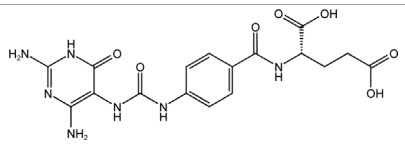
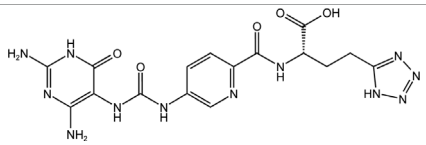
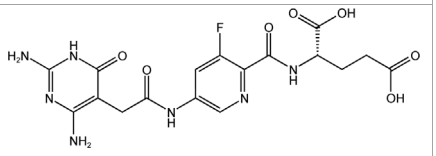
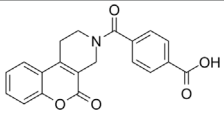
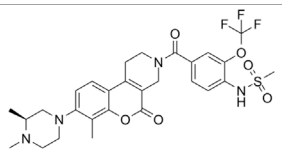
Methylenetetrahydrofolate dehydrogenase 2 (MTHFD2) is a mitochondrial one-carbon (1C) metabolism enzyme that is overexpressed in cancer cells and barely expressed in most healthy adult tissues (Nilsson et al., 2014; Jha et al., 2023). The overexpression of MTHFD2 could provide the basis for biosynthesis of pyrimidine and purine during rapid proliferation of cancer cells which is widely needed for the growth of all tumors (Kim et al., 2016; Zhao et al., 2021; Bonagas et al., 2022; Zhao et al., 2022). Inhibition of MTHFD2 leads to imbalance of NADPH and redox homeostasis, which inhibits tumorigenic proliferation and growth, and increases cancer cell death under hypoxia (Ju et al., 2019). The knockdown of MTHFD2 leads to decreased expression of cell cycle genes suggesting interference with cell cycle progression (Yu et al., 2020). Because of the low expression of MTHFD2 in most adult tissues, targeting MTHFD2 is unlikely to produce significant side effects and MTHFD2 could be as a novel target for cancer therapy (Nishimura et al., 2019; Cuthbertson et al., 2021; Yang et al., 2021).

Recent research found that MTHFD2 was consistently overexpressed in many diseases, including ulcerative colitis, Celiac's disease, systemic lupus erythematosus (SLE), psoriatic arthritis, Sjogren's syndrome, multiple sclerosis (MS) and so on (Sugiura et al., 2022). Inhibition of MTHFD2 promotes regulatory CD4 T cell (Treg) activity, which suppresses the immune response. Does MTHFD2 play a new role from anticancer targets to inti-inflammatory disease?

2 A new role on anti-inflammatory disease and proposed mechanisms

In fact, what we are more interested in is that MTHFD2 deficiency can reduce disease degree in various inflammatory condition models. T-cell dependent Delayed Type Hypersensitivity (DTH) mouse models trials showed that MTHFD2 inhibitors did not increase inflammatory symptoms in mice, and increase animal weight, suggesting that the inhibitor has a protective effect on inflammation extending to B cell function (Sugiura et al., 2022). MS is an inflammatory demyelinating disease originating in the central nervous system. Compared to control group, Experimental Autoimmune Encephalomyelitis (EAE) model using with MTHFD2 inhibitors (MTHFD2i) resulted in significantly lower disease degree and cumulative clinical score. The infiltration of CD4st, $CD4^+$ and $CD8^+$ cells in the spinal cord of mice was significantly reduced after MTHFD2i treatment (Sugiura et al., 2022). In two other different inflammatory models-inflammatory bowel disease (IBD) and

TABLE 1 Novel MTHFD2 inhibitors.

Name	Target	Structure	Pathology
LY345899	Dual MTHFD1/ 2 inhibitor		LY345899 treatment statistically significantly suppresses tumor growth and decreases the tumor weight in CRC patient-derived xenograft models
TH7299	Dual MTHFD1/ 2 inhibitor		(–)
TH9028	Dual MTHFD1/ 2 inhibitor		TH9028 and TH9619 showed overall strong antiproliferative efficacy in acute myeloid leukemia (AML) cells and T-ALL Jurkat cells comparable to standard-of-care compounds, with reduced effect on lymphoblastoid cell line (LCL) viability
TH9619	Dual MTHFD1/ 2 inhibitor		
DS44960156	MTHFD2 inhibitor		DS44960156has > 18-fold selectivity for MTHFD2 over MTHFD1, with a low molecular weight and a good ligand efficiency
DS18561882	MTHFD2 inhibitor		DS18561882, combined with enzalutamide can signifcantly inhibit CRPC cell proliferation *in vitro* and tumor growth *in vivo*. DS18561882 has also been shown to reduce disease degree in variety of inflammatory disease models *in vivo*

allergic airway disease, mice receptor of $CD4^{\Delta Mthfd2}$ T cells continued gaining body weight, the number and frequency of $CD4^{\Delta Mthfd2}$ T cells in spleen and mesenteric lymph nodes (MLNs) were significantly reduced. Meanwhile, the neutrophil richness in the bronchioalveolar lavage fluid (BALF) of $CD4^{\Delta Mthfd2}$ mice affected by Alternaria-induced allergic airway disease showed a decreasing tendency (Sugiura et al., 2022). The sensitivity of T-cell to MTHFD2i might provide an efficacious strategy of immunotherapy for $CD4^+$ T-cell-driven inflammation, and produce fewer adverse reactions than presently usable therapeutics. It should be worth studying whether T cell nucleus carries MTHFD2 and whether MTHFD2 is a therapeutic target for inflammatory disease.

$CD4^+$ T cells are the key mediators and adaptive immunity which play a crucial role in host defense against pathogens (Candia and Matarese, 2022). $CD4^+$ T cell subpopulations need MTHFD2 to varying degrees for activation, proliferation, survival, and cytokine production (Sugiura et al., 2022). Sugiura et al. (2022) have found that MTHFD2 in patients with inflammatory disease continues to upregulate combined with cell CRISPR-based screening and genetic test. The research showed that MTHFD2 may function as a metabolic checking point for the Th17/Treg cell axis and highlight its potential as a target for anti-inflammatory immunotherapeutic treatment. Meanwhile, MTHFD2i raised the basal and maximal oxygen consumption rate (OCR) of Th17 cells and decreased the expression of interferon-gamma (IFN-g) and interleukin (IL)-17 in Th1 and Th17 cells, which appears to alter the counterbalance between the pathogenic and anti-inflammatory state.

MTHFD2 has been shown to regulate *de novo* purine synthesis and signal transduction in activated T cells, promoting proliferation and the production of inflammatory cytokine (Ducker et al., 2016). MTHFD2 has been reported to transport to the nucleus and is presumed regulate gene expression (Gustafsson Sheppard et al., 2015). The lack of MTHFD2 could lead to the accumulation of intermediates in the purine synthesis pathway, which activates AMP-activated protein kinase to inhibit the mechanistic target of rapamycin (mTORC)1 (Su et al., 2019). The mTORC1 pathway plays a crucial role in promoting synthetic metabolism, driving a

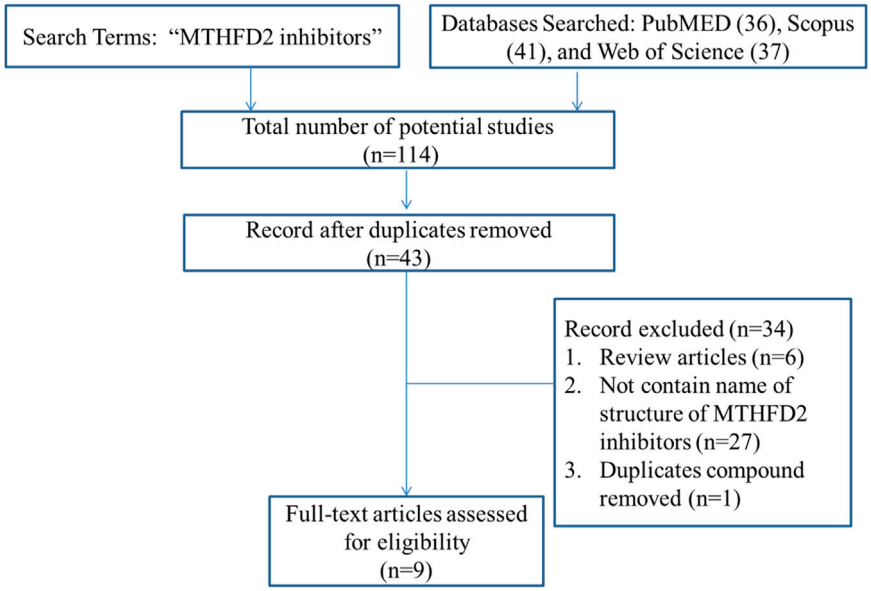


FIGURE 1
Study flow diagram of literature search.

mass of the transcription factor ATF4 and inducing the expression of MTHFD2 (Ben-Sahra et al., 2016). Inhibition of mTORC1 signaling transduction might lead to changes in the metabolic process from glycolysis to mitochondrial respiration, and alter T cell receptor cycle metabolites (Shang et al., 2021).

3 Novel MTHFD2 inhibitors

One possible mechanism is that MTHFD2i damages T cell expansion through inadequate nucleotide production. Scientists have been working on the design and development of MTHFD2i as anticancer drugs (Table 1). Tricyclic coumarins and xanthine compounds are the only selective inhibitors of MTHFD2 reported to date (Jha et al., 2023). Comprehensive searches of English databases, including PubMed, Scopus, and Web of Science, and the time of index was from inception to 30 April 2023 for each database. Full-text searches were performed using "MTHFD2 inhibitors" in all fields (Figure 1). The dual MTHFD1/2 inhibitor LY345899 synthesized in 2017 has been demonstrated efficacy in improving disease conditions in the EAE model (Gustafsson et al., 2017; Ju et al., 2019). A simplification of the tricyclic core of LY345899 shows that TH9028, TH9619 and TH7299 are actually more active against MTHFD1 and MTHFD2L (Bonagas et al., 2022; Scaletti et al., 2022; Green et al., 2023). A novel isozyme-selective MTHFD2 inhibitor DS44960156 might provide further optimization options due to its >18-fold selectivity for MTHFD2 over MTHFD1, with a smaller molecular weight and favorable ligand efficiency (Kawai et al., 2019a). Subsequently, the same team developed an effective, selective, and oral MTHFD2i (DS18561882) which has favorable oral pharmacokinetic characteristics with the strongest cell activity and tumor growth inhibition (Kawai et al., 2019b; Lee et al., 2021). Most importantly, DS18561882 has been shown to reduce disease degree in variety of inflammatory disease models *in vivo* (Sugiura et al., 2022), which leads us to believe that MTHFD2 may be an anti-inflammatory and autoimmune target *in vivo* in the future.

4 Conclusion

MTHFD2 is a mitochondrial one-carbon metabolism enzyme highly expressed in several human tumors, and targeting MTHFD2 has been used as the target of tumor therapy. Recent research suggests that MTHFD2 inhibitors appear to reduce inflammatory disease severity and alter the counterbalance between the pathogenic and anti-inflammatory state, which may serve as an anti-inflammatory and autoimmune target *in vivo* in the future. The research of anti-inflammatory drugs is expected to be promoted and developed.

Author contributions

HT: Conceptualization, Writing–original draft. NH: Conceptualization, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Ben-Sahra, I., Hoxhaj, G., Ricoult, S. J. H., Asara, J. M., and Manning, B. D. (2016). mTORC1 induces purine synthesis through control of the mitochondrial tetrahydrofolate cycle. *Science* 351, 728–733. doi:10.1126/science.aad0489

Bonagas, N., Gustafsson, N. M. S., Henriksson, M., Marttila, P., Gustafsson, R., Wiita, E., et al. (2022). Pharmacological targeting of MTHFD2 suppresses acute myeloid leukemia by inducing thymidine depletion and replication stress. *Nat. Cancer* 3, 156–172. doi:10.1038/s43018-022-00331-y

Candia, P. D., and Matarese, G. (2022). The folate way to T cell fate. *Immunity* 55, 1–3. doi:10.1016/j.immuni.2021.12.009

Cuthbertson, C. R., Arabzada, Z., Bankhead, A., Kyani, A., and Neamati, N. (2021). A review of small-molecule inhibitors of one-carbon enzymes: SHMT2 and MTHFD2 in the spotlight. *ACS Pharmacol. Transl. Sci.* 4, 624–646. doi:10.1021/acsptsci.0c00223

Ducker, G. S., Chen, L., Morscher, R. J., Ghergurovich, J. M., Esposito, M., Teng, X., et al. (2016). Reversal of cytosolic one-carbon flux compensates for loss of the mitochondrial folate pathway. *Cell Metab.* 23, 1140–1153. doi:10.1016/j.cmet.2016.04.016

Green, A. C., Marttila, P., Kiweler, N., Chalkiadaki, C., Wiita, E., Cookson, V., et al. (2023). Formate overflow drives toxic folate trapping in MTHFD1 inhibited cancer cells. *Nat. Metab.* 5, 642–659. doi:10.1038/s42255-023-00771-5

Gustafsson, R., Jemth, A.-S., Gustafsson, N. M. S., Färnegårdh, K., Loseva, O., Wiita, E., et al. (2017). Crystal structure of the emerging cancer target MTHFD2 in complex with a substrate-based inhibitor. *Cancer Res.* 77, 937–948. doi:10.1158/0008-5472.CAN-16-1476

Gustafsson Sheppard, N., Jarl, L., Mahadessian, D., Strittmatter, L., Schmidt, A., Madhusudan, N., et al. (2015). The folate-coupled enzyme MTHFD2 is a nuclear protein and promotes cell proliferation. *Sci. Rep.* 5, 15029. doi:10.1038/srep15029

Jha, V., Holmelin, F. L., and Eriksson, L. A. (2023). Binding analysis and structure-based design of tricyclic coumarin-derived MTHFD2 inhibitors as anticancer agents: insights from computational modeling. *ACS Omega* 8, 14440–14458. doi:10.1021/acsomega.2c08025

Ju, H.-Q., Lu, Y.-X., Chen, D.-L., Zuo, Z.-X., Liu, Z.-X., Wu, Q.-N., et al. (2019). Modulation of redox homeostasis by inhibition of MTHFD2 in colorectal cancer: mechanisms and therapeutic implications. *J. Natl. Cancer Inst.* 111, 584–596. doi:10.1093/jnci/djy160

Kawai, J., Ota, M., Ohki, H., Toki, T., Suzuki, M., Shimada, T., et al. (2019a). Structure-based design and synthesis of an isozyme-selective MTHFD2 inhibitor with a tricyclic coumarin scaffold. *ACS Med. Chem. Lett.* 10, 893–898. doi:10.1021/acsmedchemlett.9b00069

Kawai, J., Toki, T., Ota, M., Inoue, H., Takata, Y., Asahi, T., et al. (2019b). Discovery of a potent, selective, and orally available MTHFD2 inhibitor (DS18561882) with *in vivo* antitumor activity. *J. Med. Chem.* 62, 10204–10220. doi:10.1021/acs.jmedchem.9b01113

Kim, J., Yang, G., Kim, Y., Kim, J., and Ha, J. (2016). AMPK activators: mechanisms of action and physiological activities. *Exp. Mol. Med.* 48, e224. doi:10.1038/emm.2016.16

Lee, J., Chen, X., Wang, Y., Nishimura, T., Li, M., Ishikawa, S., et al. (2021). A novel oral inhibitor for one-carbon metabolism and checkpoint kinase 1 inhibitor as a rational combination treatment for breast cancer. *Biochem. Biophys. Res. Commun.* 584, 7–14. doi:10.1016/j.bbrc.2021.11.001

Nilsson, R., Jain, M., Madhusudhan, N., Sheppard, N. G., Strittmatter, L., Kampf, C., et al. (2014). Metabolic enzyme expression highlights a key role for MTHFD2 and the mitochondrial folate pathway in cancer. *Nat. Commun.* 5, 3128. doi:10.1038/ncomms4128

Nishimura, T., Nakata, A., Chen, X., Nishi, K., Meguro-Horike, M., Sasaki, S., et al. (2019). Cancer stem-like properties and gefitinib resistance are dependent on purine synthetic metabolism mediated by the mitochondrial enzyme MTHFD2. *Oncogene* 38, 2464–2481. doi:10.1038/s41388-018-0589-1

Scaletti, E. R., Gustafsson Westergren, R., Andersson, Y., Wiita, E., Henriksson, M., Homan, E. J., et al. (2022). The first structure of human MTHFD2L and its implications for the development of isoform-selective inhibitors. *ChemMedChem* 17, e202200274. doi:10.1002/cmdc.202200274

Shang, M., Yang, H., Yang, R., Chen, T., Fu, Y., Li, Y., et al. (2021). The folate cycle enzyme MTHFD2 induces cancer immune evasion through PD-L1 up-regulation. *Nat. Commun.* 12, 1940. doi:10.1038/s41467-021-22173-5

Su, C.-C., Hsieh, K.-L., Liu, P.-L., Yeh, H.-C., Huang, S.-P., Fang, S.-H., et al. (2019). AICAR induces apoptosis and inhibits migration and invasion in prostate cancer cells through an AMPK/mTOR-Dependent pathway. *Int. J. Mol. Sci.* 20, 1647. doi:10.3390/ijms20071647

Sugiura, A., Andrejeva, G., Voss, K., Heintzman, D. R., Xu, X., Madden, M. Z., et al. (2022). MTHFD2 is a metabolic checkpoint controlling effector and regulatory T cell fate and function. *Immunity* 55, 65–81.e9. doi:10.1016/j.immuni.2021.10.011

Yang, C., Zhang, J., Liao, M., Yang, Y., Wang, Y., Yuan, Y., et al. (2021). Folate-mediated one-carbon metabolism: a targeting strategy in cancer therapy. *Drug Discov. Today* 26, 817–825. doi:10.1016/j.drudis.2020.12.006

Yu, C., Yang, L., Cai, M., Zhou, F., Xiao, S., Li, Y., et al. (2020). Down-regulation of MTHFD2 inhibits NSCLC progression by suppressing cycle-related genes. *J. Cell. Mol. Med.* 24, 1568–1577. doi:10.1111/jcmm.14844

Zhao, L. N., Björklund, M., Caldez, M. J., Zheng, J., and Kaldis, P. (2021). Therapeutic targeting of the mitochondrial one-carbon pathway: perspectives, pitfalls, and potential. *Oncogene* 40, 2339–2354. doi:10.1038/s41388-021-01695-8

Zhao, R., Feng, T., Gao, L., Sun, F., Zhou, Q., Wang, X., et al. (2022). PPFIA4 promotes castration-resistant prostate cancer by enhancing mitochondrial metabolism through MTHFD2. *J. Exp. Clin. Cancer Res. CR* 41, 125. doi:10.1186/s13046-022-02331-3

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β-sitosterol alleviates dextran sulfate sodium-induced experimental colitis via inhibition of NLRP3/Caspase-1/GSDMD-mediated pyroptosis

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Background: Inflammation-related NLRP3/Caspase-1/GSDMD-mediated pyroptosis is involved in the progression of ulcerative colitis (UC). β-sitosterol (SIT) was reported to have anti-inflammatory effects on experimental colitis, while the regulation of SIT on pyroptosis is unclear. Therefore, the present study aimed to define the protective and healing effects of SIT on dextran sulfate sodium (DSS)-induced experimental UC rats and human epithelial colorectal adenocarcinoma cells (Caco-2) and explore the underlying mechanisms that are responsible for its effects on NLRP3/Caspase-1/GSDMD-mediated pyroptosis in UC.

Methods: UC model rats were established by oral 4% DSS. Following colitis injury, the animals received SIT (doses of 50, 100, and 200 mg/kg) treatment for 2 weeks. For *in vitro* study, we exposed Caco-2–50 mg/mL DSS with or without SIT (concentrations of 8 and 16 μg/mL). Disease activity index (DAI) and histopathological injury were assessed *in vivo*. Activation proteins of nuclear factor kappa B (NF-κB) signaling axis, and tight junction-related proteins of zonula occludens-1 (ZO-1) and occludin were detected in colon tissues. TNF-α, IL-1β, and IL-18 in serum and cell supernatant were measured by enzyme-linked immunosorbent assay (ELISA). Changes in NLRP3/Caspase-1/GSDMD-mediated pyroptosis signaling pathway activation were analyzed both in tissues and cells.

Results: Our findings suggested that SIT treatment attenuated the severity of 4% DSS-induced UC by protecting rats from weight and colon length loss, and macroscopic damage. SIT also reduced proinflammatory factors production (TNF-α, IL-1β, and IL-18) in serum and cell supernatant. Mechanistically, SIT

Abbreviations: ASC, apoptosis-associated speck-like protein; CON, control; DAI, disease activity index; DSS, dextran sulfate sodium; ELISA, enzyme-linked immunosorbent assay; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GSDMD, gasdermin D; GSDMD-N, gasdermin D-N-terminal; HD, high-dose; HRP, horse radish peroxidase; IHC, immunohistochemistry; IL, interleukin; IBD, inflammatory bowel disease; IL, interleukin; LD, low-dose; MD, middle-dose; NF-κB, nuclear transcription factor-kappa B; NLRP3, nucleotide-binding oligomerization domain (Nod)-like receptor thermal protein domain associated protein 3; PVDF, polyvinylidene fluoride; TNF, tumor necrosis factor; SD, standard deviation; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel; UC, ulcerative colitis; ZO, zonula occludens.

downregulated the expression levels of pyroptosis-related proteins including Caspase-1, cleaved-Caspase-1, NLRP3, GSDMD, and GSDMD-N in colon tissues and Caco-2 cells. Further analysis indicated that SIT maintained the colonic barrier integrity by enhancing the protein expression of ZO-1 and occludin.

Conclusion: We confirmed that SIT exerts protective and therapeutic effects on DSS-induced colitis injury by suppressing NLRP3/Caspase-1/GSDMD-mediated pyroptosis and inflammation response. These findings demonstrated that SIT could be a potential medication for UC treatment.

KEYWORDS

β-sitosterol, ulcerative colitis, inflammatory factors, Caspase-1, GSDMD, pyroptosis

1 Introduction

Ulcerative colitis (UC), a global public health problem, which is a chronic non-specific, non-infectious, and inflammatory intestinal disease. It is a continuous mucosal ulcer with unknown origin and usually begins in the rectum, involves the colonic mucosa and submucosa layer, and then spreads to the cecum for the longest, which is characterized by continuous and diffuse distribution. Clinical manifestations of this illness are chronic and recurrent with abdominal pain, diarrhea, fecal bleeding, weight loss, and intestinal mucosal (Ordás et al., 2012). In recent years, UC has had increasing prevalence and incidence in developing and developed countries worldwide due to the rapid economic development and westernized diet uptake (Ng et al., 2017). Additionally, the incidence among people with UC in China has nearly tripled in the past decades (Ye et al., 2013). Research shows that patients with long-term UC have a higher risk of developing colorectal cancer and colitis-associated cancer is a major cause of death in patients with UC (Rogler, 2014), thereby resulting in a high economic burden on individuals and society health systems. For the above reasons, effective intervention in the course of UC is urgent.

Though the etiology of UC remains undefined, the inflammatory response definitely exists in the pathogenesis of UC (Du and Ha, 2020). Studies have suggested the large aggregation of inflammatory molecules such as TNF-α, IL-1β, and IL-18 could lead to colonic tissue injury and epithelial integrity disruption in the gut while inhibiting cytokine-mediated inflammation could treat or cure UC (Elmaksoud et al., 2021). NF-κB is a key transcriptional regulator in the regulation of proinflammatory mediators and chemokines production and secretion, which leads to an inflammatory cascade (Cui et al., 2019). The abnormal activation of NF-κB signaling was founded in colonic tissues of UC patients and experimental models (Neurath et al., 1996). Pyroptosis is a newly found form of pro-inflammatory programmed cell death unlike traditional necrosis and apoptosis (Shi et al., 2015). Pyroptosis plays an essential role in host defense and inflammatory responses, in which Caspase-1 or Caspase-11/4/5 was activated during this process (Yu P. et al., 2021). Activation of inflammatory caspases can trigger pyroptosis and release pro-inflammatory cytokines IL-1β and IL-18, which are relevant to inflammatory diseases (Yuan et al., 2018). In addition, over-expression of pyroptosis-related proteins, including nucleotide-binding oligomerization segment-like receptor family 3 (NLRP3), apoptosis-associated speck-like protein (ASC), Caspase-1, and gasdermin D-N-terminal (GSDMD-N) in colonic tissues has been observed in experimental colitis (Chao et al., 2020; Wei et al., 2021) and suppression of the NLRP3/Caspase-1/GSDMD-mediated pyroptosis signaling pathway can attenuate the damage of UC (Jie et al., 2021), thus therapy targeting pyroptosis could be promising and merits further investigation.

While immunosuppressants, glucocorticoids, salicylic acid, and biological agents are the frontline therapy for clinical patients with UC (Nakamura et al., 2008; Berends et al., 2019), there are many side effects associated with these pharmaceutical medications (Moreau and Mas, 2015). Therefore, more researchers have paid attention to natural plant substances, which will be safe and beneficial with less deleterious effects (Cao et al., 2019). Phytosterols are a critical class of bioorganic compounds that are abundant in a range of organisms such as plants, animals, and fungi, and exert an important role in the physiological processes of eukaryotes (Zhang et al., 2022). β-sitosterol (SIT) (Figure 1), the major compound of phytosterol, is a plant-derived natural product widely present in many countries. Its pharmacological effects and biological activities were well documented in the literature including anti-inflammation (Loizou et al., 2010; Sun et al., 2020), anti-anxiety (Panayotis et al., 2021), antiviral properties (Chen et al., 2022), anti-oxidant stress, immunomodulation (Cheng et al., 2020), anti-diabetes (Babu et al., 2020), anti-tumor (Bae et al., 2021), anti-microbes (Pierre Luhata and Usuki, 2021), hepatoprotective effects (Kim et al., 2014), cardioprotective effects (Lin et al., 2020), anti-diabetes (Ponnulakshmi et al., 2019), as well as regulation of gut microbiota (Yu Y. et al., 2021). These studies showed that SIT could be a potential measure of various diseases. Furthermore, evidence from experimental studies on SIT indicates its anti-inflammatory ability and can be used as

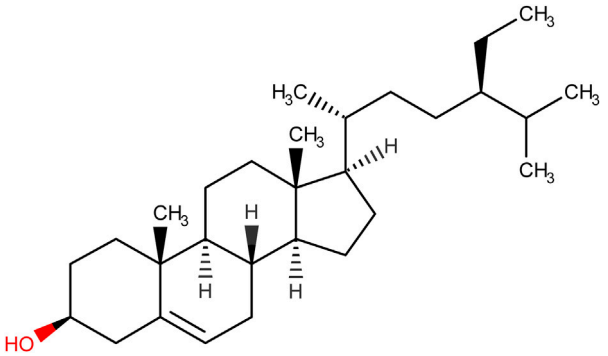

FIGURE 1
Chemical structure of β-sitosterol. (DrugBank Accession Number: DB14038).

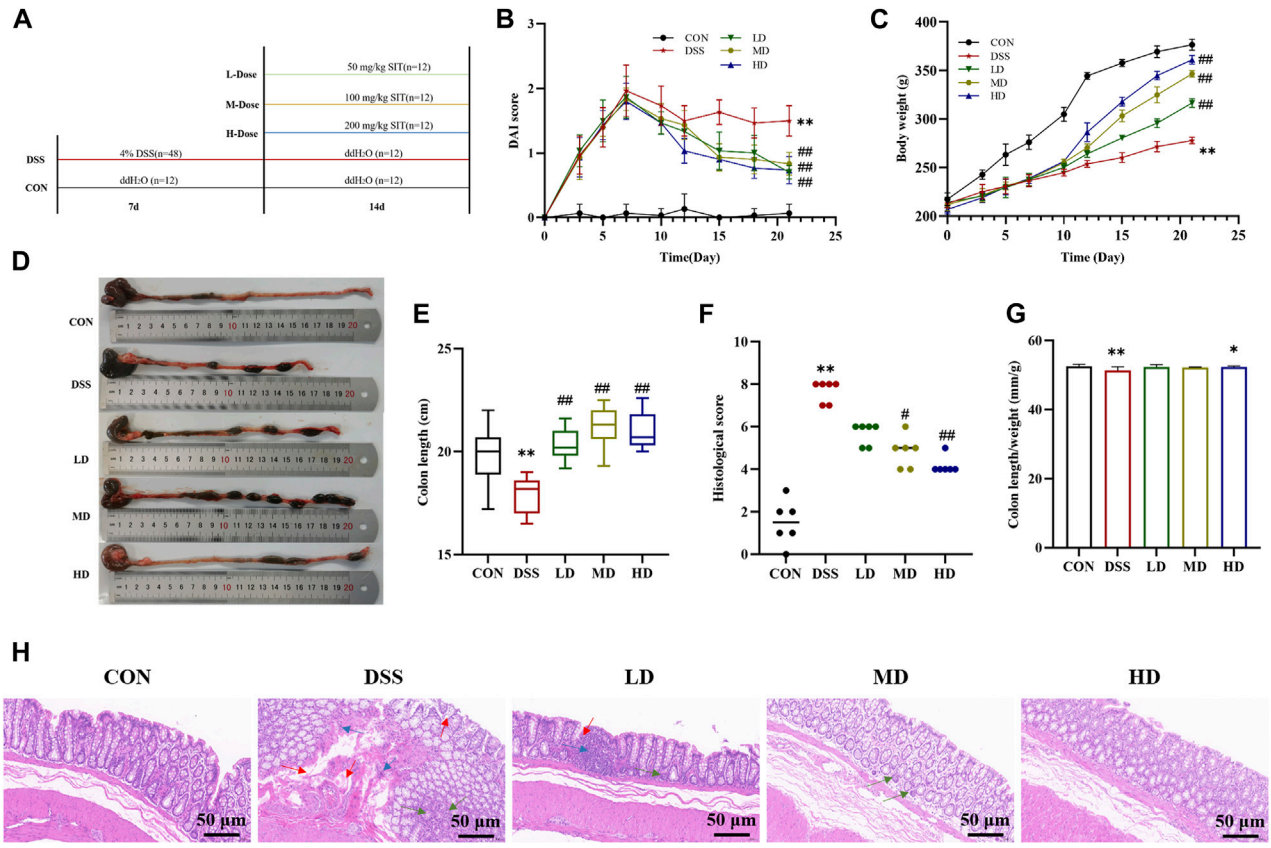

FIGURE 2
SIT protects rats from DSS-induced UC. **(A)** An experimental design *in vivo*. **(B)** Changes of body weight (N = 10 per group). **(C)** The disease activity index (DAI) scores (N = 10 per group). **(D)** Representative picture of the colon. **(E)** The colon lengths (N = 11 per group). **(F)** The colon length/weight ratio (N = 11 per group). **(G)** The colonic histopathological scores (N = 6 per group). **(H)** Representative histopathologic image of colon. H&E staining, 100 ×; scale bar, 50 μm. Red arrow: distortion or loss of crypts; Blue arrow: infiltration of inflammatory cells; Green arrow: depletion of goblet cells. The data are presented as mean ± SD. CON, control group; DSS, dextran sulfate sodium-induced group; SIT, β-sitosterol; LD, low-dose SIT group; MD, middle-dose SIT group; HD, high-dose SIT group; H&E, hematoxylin and eosin; SD, standard deviation. $^{**}p < 0.01$ compared with CON group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with DSS group.

a safe pharmaceutical complement in the treatment of experimental colitis (Lee et al., 2012; Aldini et al., 2014; Bin Sayeed et al., 2016; Feng et al., 2017; Ding et al., 2019). Of note, SIT has been proven experimentally that it does not produce cytotoxic impacts under long-term use (Malini and Vanithakumari, 1990; Paniagua-Pérez et al., 2005; Feng et al., 2020). Despite this, further experimental studies are required to uncover the role of SIT in the recovery of experimental UC and elucidate its possible anti-pyroptosis mechanism behind this.

Thus, in this study, we established the DSS-induced experimental colitis in rats and Caco-2 cells to assess the protective and therapeutic effects of SIT on UC and further explore its underlying mechanism.

2 Materials and methods

2.1 Drug, and reagents

Dextran sulfate sodium and β-sitosterol (SIT, purity >95% and HPLC ≥98%) were purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Carboxymethyl cellulose was purchased from Biotopped Technology Co. Ltd. (Beijing, China). Enzyme-linked immunosorbent assay (ELISA) kits of TNF-α, IL-1β, and IL-18 were obtained from Cloud-Clone Corp (Wuhan, China). Antibodies against NLRP3, occludin, β-Actin, goat-anti-rabbit IgG H&L (HRP), and goat-anti-mouse IgG H&L (HRP) were purchased from Abcam (Cambridge, United Kingdom). Antibodies against Caspase-1, GSDMD, IKB alpha, and ZO-1 were obtained from Proteintech (Wuhan, China). Antibodies against NF-kappaB p65, phospho-NF-kappaB p65, and phospho-IkB alpha were purchased from Invitrogen (Carlsbad, CA). Antibody against cleaved Caspase-1 was purchased from Cell Signalling Technology (Danvers, MA, United States). Antibody against GSDMD-N terminal was purchased from ABclonal (Wuhan, China). The Cell Counting Kit-8 (CCK-8) was purchased from Biorigin (Beijing, China).

The following reagents were obtained from GenePool Biotechnology (Beijing, China): Total RNA Extraction with DNase I Kit, mRNA cDNA Synthesis Kit, mRNA qPCR Kit, RNA Loading Buffer, BSA Blocking Buffer, SDS-PAGE Gel Ki, SDS-PAGE Loading Buffer, Tris-Glycine Running Buffer, WB Transfer Buffer, and TBST. The following reagents were

TABLE 1 Disease activity index (DAI) scoring system.

Score	Weight loss (%)	Stool consistency	Gross bleeding/rectal bleeding
0	0	Normal	None
1	1–5	Loose stool	Haemoccult positive
2	6–10	Loose stool	Haemoccult positive
3	11–20	Loose stool	Haemoccult positive
4	>20	Diarrhea	Severe bleeding

purchased from Beyotime Biotechnology (Shanghai, China): Cell lysis buffer for Western and IP, Protein Extraction Kit, BCA protein assay kit, enhanced chemiluminescence (ECL) kit, Citrate-EDTA Antigen Retrieval Solution, and Hematoxylin and Eosin Staining Kit. The following reagents were purchased from Gibco (Carlsbad, CA, United States): fetal bovine serum (FBS), minimum essential medium (MEM), Penicillin-Streptomycin antibiotics, non-essential amino acids (NEAA), and 0.25% trypsin solution with EDTA. All other chemicals were of reagent grade.

2.2 Animal experimental design and treatment

Sixty male Sprague-Dawley rats (6–8 weeks old, weighing 180–200 g) were purchased from Beijing Sibeifu Bioscience, Co., Ltd. (Beijing, China) (License NO. SCXK [Beijing] 2019-0010). All animals were raised under standard specific pathogen-free (SPF) conditions (temperature, 25°C ± 1°C; humidity, 60% ± 5%) with a 12 h light/dark cycle per day and allowed *ad libitum* eat and drink. Ethical approvals for the animal experiments were obtained from the animal ethics committee of Beijing University of Chinese Medicine (NO. BUCM-4-2022102901-4029).

This experimental design consists of two steps: a DSS-induced colitis stage and a drug treatment stage as described in Figure 2A. 60 rats were randomly assigned to five groups: 1) Control group (CON): received only double-drilled water; 2) DSS group (DSS): received 4% DSS (molecular weight: 40 000 Da; purity >98%) dissolved in double-drilled water in their daily drinking water; 3) Low-dose group (LD): received DSS and then gavaged with 50 mg/kg of SIT (purity >95%) suspended in 0.1% carboxymethyl cellulose; 4) Middle-dose group (MD): received DSS and then gavaged with 100 mg/kg SIT; 5) High-dose group (HD): received DSS and then gavaged with 200 mg/kg SIT. The intervention period of DSS was from day 1 to day 7 and the period of drug treatment was from day 8 to day 21.

Following the 14-day intervention, rats were all deeply anesthetized after 24 h fasting with 1% sodium pentobarbital (40 mg/kg). After recording the colon length and weight, blood samples were collected and centrifuged for serum. Then the colon specimens were immediately removed and then fixed in 4% paraformaldehyde for histopathological studies or stored at −80°C for molecular biology detection.

2.3 Observation of UC symptoms and signs in rats

During this experimental period, body weight, stool status, and rectal bleeding were recorded every day to calculate disease activity index (DAI) scores on a previously established scoring system (Kihara et al., 2003) and listed in Table 1. DAI score is the average of the three categories. All evaluations were performed while unaware of the conditions.

2.4 Histological examination

Hematoxylin and eosin (H&E) detection was conducted with a standard protocol. The colon tissues were fixed in 4% paraformaldehyde and embedded in paraffin. Then, the paraffin-blocked samples were cut into 5-μm sections on slides for staining with hematoxylin and eosin. Slides were scanned using a Panoramic MIDI Scan Whole Slide Scanner (3DHISTECH Ltd., Budapest, Hungary) and viewed with Panoramic Viewer 1.15.4 (3DHISTECH); in addition, histopathological scores for histopathological changes were calculated based on a previously developed scoring system (Dieleman et al., 1998) and listed in Table 2. The individual scoring was blinded to the identity of the slides.

2.5 Cell preparation and viability assay

Human epithelial colorectal adenocarcinoma (Caco-2) cell lines (originally obtained from ATCC) were purchased from IMMOCELL (Xiamen, China). The Caco-2 cells were maintained in minimum essential medium (MEM) containing 10% fetal bovine serum (FBS), 100 U/ml of penicillin, 100 μg/mL of streptomycin, and 1% non-essential amino acids supplements. Cells were maintained at 37°C with 5% CO_2 atmosphere.

Caco-2 cell viability was measured by CCK-8 assay followed by a standard protocol. After preparing single-cell suspensions, cells (5×10^4 cells/well) were added to 96-well plates and continued to culture till 80% confluency. Then, 10 μL/well of CCK-8 solution was added and incubated protected from light for 2 h at 37°C. The 450 nm absorbance values were detected with a multifunctional microplate reader (Thermo, Manassas, United States) to measure the cell growth inhibition rate.

TABLE 2 Histopathological scoring system.

Score	Inflammation	Mucosal damage	Regeneration	Crypt damage	Range of lesions (%)
0	None	None	Complete regeneration or normal tissue	None	0
1	Mild	Mucous layer	Alomost complete regeneration	Basal 1/3 damage	1%–25%
2	Moderate	Mucousa and submucosa	Regeneration with crypt depletion	Basal 2/3 damage	26%–50%
3	Severe	Transmural	Surface epithelium not intact	Crypt lost; surface epithelium present	51%–75%
4	-	-	No tissue repair	Crypt and surface epithelium lost	76%–100%

TABLE 3 Primers used in quantitative RT-PCR assay.

Gene		Primer sequences (5′-3′)
IL-1β	Forward	CCCAACTGGTACATCAGCACCTCTC
	Reverse	CTATGTCCCGACCATTGCTG
IL-18	Forward	CCGAACAGCCAACGAATCC
	Reverse	ACATCCTTCCATCCTTCACAGA
Caspase-1	Forward	CTGGTCTTGTGACTTGGAGGA
	Reverse	TCAGTGGTTGGCATCTGTAGT
NLRP3	Forward	AGACCTCCAAGACCACGACTG
	Reverse	CATCCGCAGCCAATGAACAGA
GSDMD	Forward	GCAGTGGTGAGCAGGTAGAG
	Reverse	CCAGAGCCTTAGTAGCCAGTAG
GAPDH	Forward	TGGAGTCTACTGGCGTCTT
	Reverse	TGTCATATTTCTCGTGGTTCA

2.6 Caco-2 inflammation model establishment and treatment

Cells were cultured overnight with or without SIT (HPLC ≥98%) dissolved in absolute ethanol (concentrations of 8 μg/mL as Low-dose group and 16 μg/mL as High-dose group). Supernatants of cells were discarded and washed with PBS, and 50 mg/mL DSS was added to establish the inflammation model.

2.7 Cytokine assays

The serum of rats and supernatant of cells were collected and then calculated for TNF-α, IL-1β, and IL-18 levels using ELISA kits according to the manufacturer's scheme.

2.8 Quantitative polymerase chain reaction

Relative levels of NLRP3, Caspase-1, GSDMD, IL-1β, and IL-18 mRNAs in colonic tissues were analyzed by a quantitative real-time (qRT)-PCR. Total RNA of colonic tissues was isolated using the Total RNA Extraction Kit and cDNA was synthesized by using the mRNA cDNA Synthesis Kit in accordance with the manufacturer's protocols. The specific primers for NLRP3, Caspase-1, GSDMD, IL-1β, and IL-18 were designed and synthesized by GenePool Biotechnology (Beijing, China) and listed in Table 3. The PCR reaction parameters were predetermined: 95°C for 5 min, followed by a 35 cycle-denaturation for 30 s at 95°C, 55°C for 30 s, and extension for 1 min at 72°C. Each sample underwent three biological replications for statistical analysis to determine significant differences. GAPDH was an internal control and relative gene expression levels were calculated using the $2^{-\Delta\Delta CT}$ method.

2.9 Western blot analysis

The total proteins of tissues and cells were extracted using Western and IP lysate buffer, BCA protein assay kit was applied to determine protein concentration. After homogenization, an equal quantity of proteins was separated with SDS-PAGE and transferred to PVDF membranes (Millipore corp., Massachusetts, United States), then blocked with 5% BSA. Primary antibodies of NLRP3 (dilution 1:1 000), Caspase-1 (dilution 1:2 000), cleaved-Caspase-1 (dilution 1:1 000), GSDMD (dilution 1:2 000), GSDMD-N (dilution 1:1 000), p65 (dilution 1:1 000), p-p65 (dilution 1:1 000), IκBα (dilution 1:1 000), p-IκBα (dilution 1:1 000), ZO-1 (dilution 1:5 000), occludin (dilution 1:1 000), and β-Actin (dilution 1:5 000) were incubated overnight at 4°C. After being washed with TBST three times, membranes were then incubated with secondary antibodies against Goat Anti-Rabbit IgG H&L (HRP) (dilution 1:5 000) or Goat Anti-Mouse IgG H&L (HRP) (dilution 1:5 000) for 1 h at 37°C, and bands were visualized with an ECL kit captured with Tanon 5200 Chemiluminescent Imaging System (Tanon, Shanghai, China). Finally, the relative grey values of the target proteins blots normalized to β-Actin were analyzed by the ImageJ software (National Institutes of Health, United States).

2.10 Statistical analysis

Statistical analysis was performed by SPSS 26.0 system. Quantitative results were expressed as arithmetic mean plus or minus the standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA) followed by least significant difference (LSD)'s multiple-comparison test, while Kruskal-Wallis test was performed for difference analysis of non-parametric data. p-value < 0.05 was considered statistically significant.

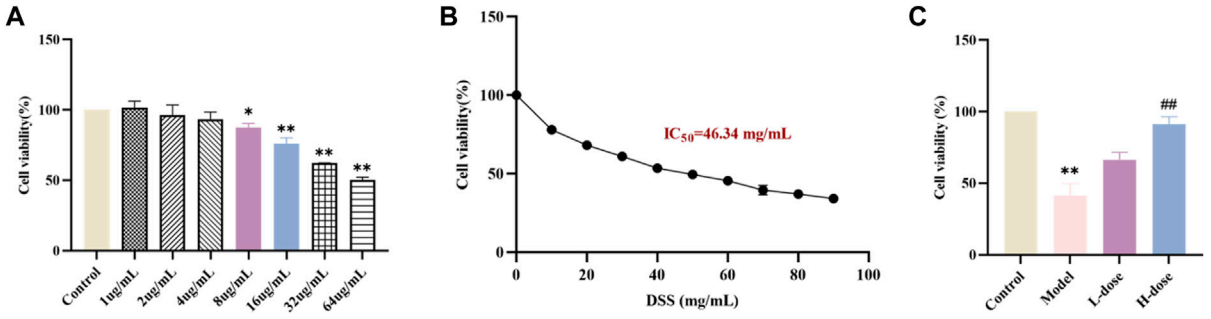

FIGURE 3
SIT protects Caco-2 cells from DSS-induced damage. **(A)** The viability of Caco-2 cells under the treatment with different concentrations SIT-containing medium for 24 h, and the viability was assayed by CCK-8. **(B)** Caco-2 cells were cultured with 10, 20, 30, 40, 50, 60, 70, 80, and 90 mg/mL DSS-containing medium for 24 h. **(C)** The cells were first cultured with SIT-containing medium for 24 h, incubated in 50 mg/mL DSS-containing medium for 6 h, and then replaced with fresh complete medium to culture for 18 h. N = 6 per group. The data are presented as mean ± SD. DSS, dextran sulfate sodium-induced group; SIT, β-sitosterol; SD, standard deviation. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with Control group; $^{\#\#}p < 0.01$ compared with Model group.

3 Results

3.1 SIT alleviates DSS-induced UC

In order to investigate the therapeutic effects of SIT *in vivo*, UC model rats were induced by 4% DSS for 7 consecutive days. During the experiment period, rats in the control group remained in a normal state, and body weight was rising steadily, while those in the model group represented a poor mental state with disheveled fur, decreased feeding, persistent fecal bleeding, and obvious body weight loss. Compared to the control group DAI score in the DSS-induced model group was significantly increased, which was consistent with the clinical characteristics. Notably, administration of SIT recovered the body weight and colon length gradually (Figures 2C–E) as well as decreased DAI score (Figure 2B) in DSS-induced rats, especially at the dose of 200 mg/kg.

Furthermore, colonic shortening caused by DSS was evidently mitigated after SIT intake. As can be seen from Figure 2F, DSS in the UC group led to a significant decrease in colon length/weight ratio compared with the control group. High dose of SIT agonist partly offset the decrease of colon length/weight ratio. Under microscopy, results by H&E staining showed that there were pathological lesions and superficial inflammation in the DSS-induced colonic tissues characterized by inflammatory cell infiltration, crypt architectural distortion or absence and mucosa defects or damage. Consistent with the remission of clinical signs, the pathological changes were improved to varying degrees following the administration of SIT (Figures 2F,G). Collectively, these results indicated that SIT is protective against DSS-induced colitis and the effects might be dose-dependent.

3.2 Effects of SIT on the viability of Caco-2 cells

Before the formal *in vitro* experiments, toxicity evaluation of different concentrations of SIT (1, 2, 4, 8, 16, 32, and 64 μg/mL) on Caco-2 was carried out using the CCK-8 assay. After the 24 h-intervention, we observed that there was a significant decline in cell viability of Caco-2 following the higher concentrations of SIT (Figure 3A). Based on this, we chose the relatively safe concentrations for low-dose SIT (LD, 8 μg/mL) and high-dose SIT (HD, 16 μg/mL), respectively. In addition, at the concentration of 46.34 mg/mL DSS, growth of 50% cells was inhibited (Figure 3B). For convenience, we used 50 mg/mL DSS for subsequent experimental Caco-2 modeling, and found that SIT serves protectively against DSS-induced Caco-2 damage, (Figure 3C).

3.3 SIT inhibits proinflammatory mediators

To validate the suppressive effects of SIT on inflammation involved in UC, we detected the levels of inflammatory-related factors. The ELISA results showed that there was a significantly increase of TNF-α, IL-1β, and IL-18 levels after DSS treatment compared with the control group in rats and SIT effectively decreased the expression (Figure 4A). We further noticed the protective effects of SIT on Caco-2 from inflammation after being exposed to DSS (Figure 4B).

3.4 SIT regulates the NF-κB inflammatory pathway

NF-κB signaling participates in the regulation of inflammatory response. We wondered whether the anti-inflammatory role of SIT was associated with the signaling. The expression levels of the major proteins p-p65, p65, p-IκBα, and IκBα in the NF-kB signaling pathways were consequently measured by Western blot. The experimental results showed that the levels of p-p65 and p-IκBα in DSS-induced colonic tissues were abnormally higher compared to the control group. In comparison with the DSS group, the protein expression ratio of p-p65/p65 and p-IκBα/IκBα were attenuated after stimulation with SIT (Figures 4C,D) while none were statistically significant. These results indicate that NF-κB

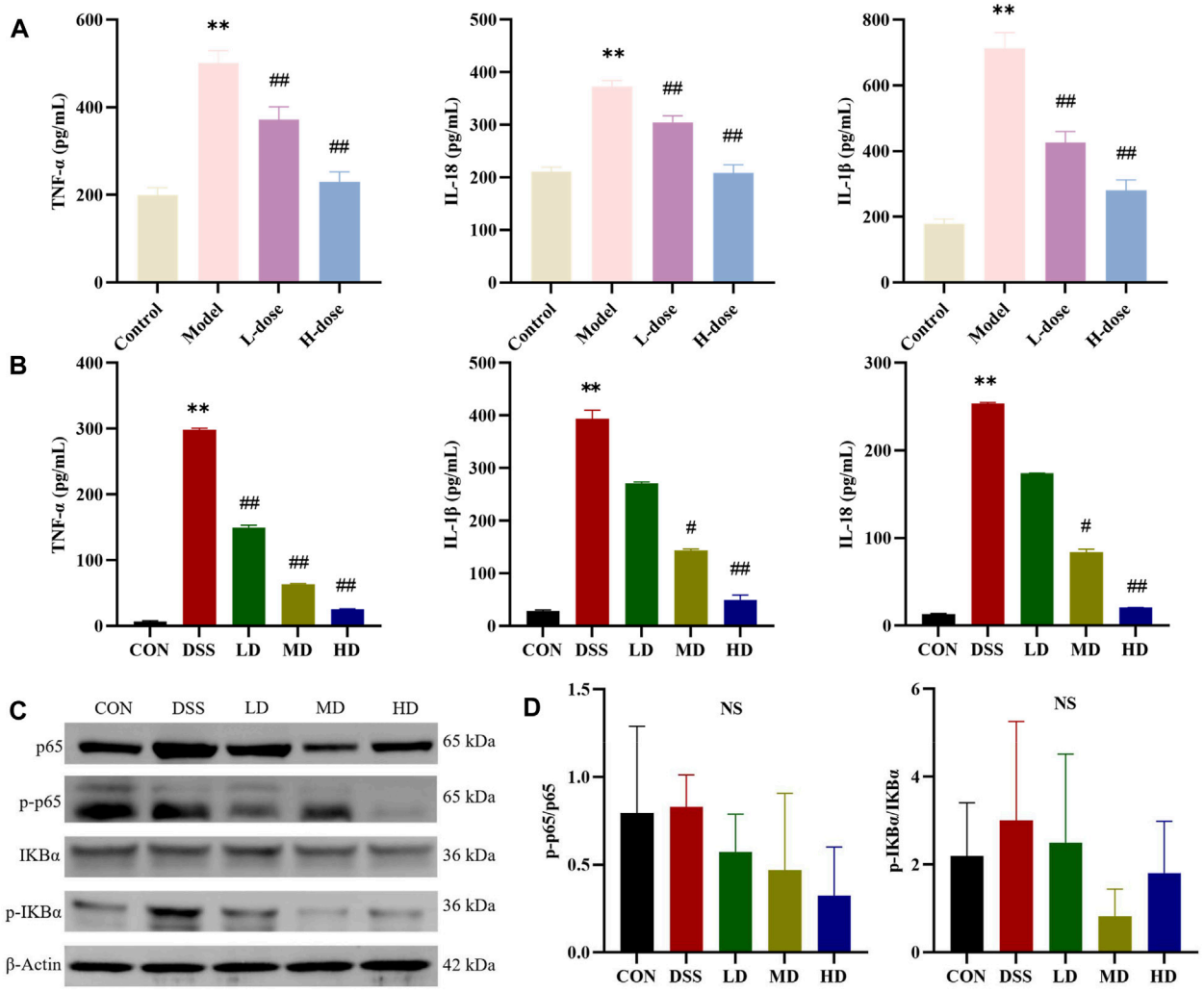

FIGURE 4
SIT regulates the pro-inflammatory cytokines and NF-κB pathway. The expression levels of TNF-α, IL-1β, and IL-18 in serum **(A)** and Caco-2 cells **(B)** were analyzed by ELISA (N = 6 per group). **(C)** Gel electrophoresis images of p-p65, p65, p-IκBα, and IκBα were analyzed by Western blot. **(D)** Analysis of protein expression levels (N = 3 per group). The data are presented as mean ± SD. NF-κB, nuclear transcription factor-kappa B; CON, control group; DSS, dextran sulfate sodium-induced group; SIT, β-sitosterol; LD, low-dose SIT group; MD, middle-dose SIT group; HD, high-dose SIT group; TNF, tumor necrosis factor; IL, interleukin; ELISA, enzyme-linked immunosorbent assay; SD, standard deviation. $^{**}p < 0.01$ compared with CON or Control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with DSS or Model group; NS, not statistically significant.

signaling might be involved in the reduction of DSS-induced UC inflammation by SIT administration.

3.5 SIT suppresses critical indicators activity of pyroptosis

Pyroptosis is a type of programmed cell death (PCD) and participates in inflammatory disease processes involved in UC. For this reason, we further measured the expression of critical indicators of NLRP3/Caspase-1/GSDMD-mediated pyroptosis to explicit the anti-inflammatory effects of SIT. RT-qPCR analysis was carried out to assess the gene expression of Caspase-1, NLRP3, GSDMD, IL-1β, and IL-18 in colonic tissues. When compared to the control group, the mRNA expression levels of the above genes were upregulated in the DSS group while significantly downregulated with SIT administration (Figure 5A).

As expected, Western blot analysis showed that protein expression levels of Caspase-1, Cleaved-Caspase-1, NLRP3, GSDMD, and GSDMD-N distinctly increased after DSS treatment in colonic tissues and Caco-2 cells and significantly counteracted following the intervention with SIT (Figures 5B–E). Meanwhile, ELISA indicated that SIT treatment significantly inhibited DSS-induced increased levels of IL-1β and IL-18 in serum and cell supernatant; it has been shown that secretion of cytokines IL-1β and IL-18 were associated with the NLRP3 inflammasome and induced an inflammatory cell death mode termed as pyroptosis (He et al., 2015). Comprehensively, the above results suggest that SIT plays a possible therapeutic role in UC via regulating Caspase-1-mediated pyroptosis.

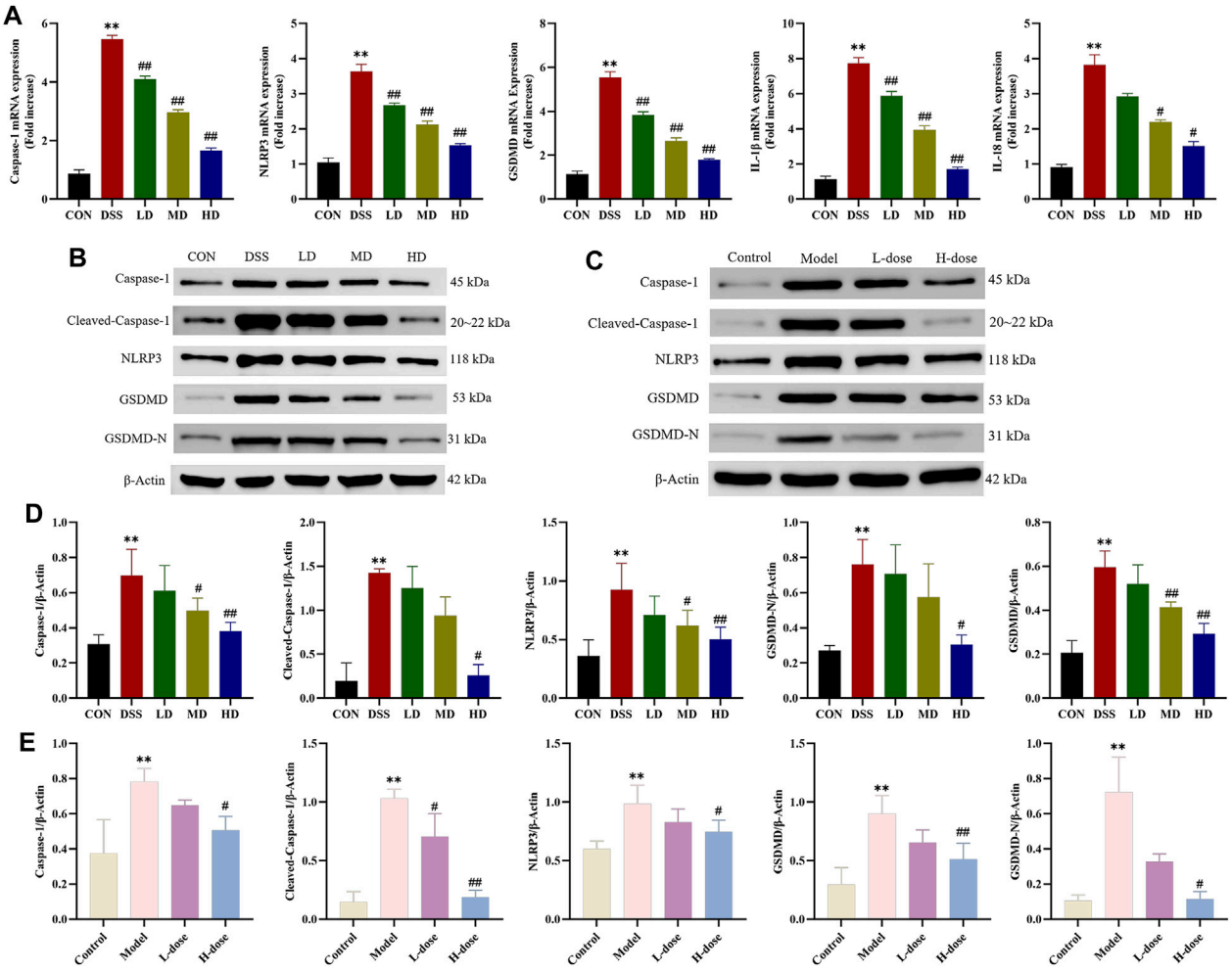

FIGURE 5
SIT inhibits DSS-induced Caspase-1 mediated pyroptosis pathway activation. **(A)** The mRNA levels of Caspase-1, NLRP3, GSDMD, IL-1β, and IL-18 in colon tissues. Gel electrophoresis images of Caspase-1, Cleaved-Caspase-1, NLRP3, GSDMD, and GSDMD-N in colon tissues **(B)** and Caco-2 cells **(D)** were analyzed by Western blot. Analysis of protein expression levels was analyzed and shown in **(C)** and **(E)**. N = 3 per group. The data are presented as mean ± SD. CON, control group; DSS, dextran sulfate sodium-induced group; SIT, β-sitosterol; LD, low-dose SIT group; MD, middle-dose SIT group; HD, high-dose SIT group; NLRP3, nucleotide-binding oligomerization domain (Nod)-like receptor thermal protein domain associated protein 3; GSDMD, gasdermin D; GSDMD-N, gasdermin D-N terminal; IL, interleukin; SD, standard deviation. $^{**}p < 0.01$ compared with CON or Control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with DSS or Model group.

3.6 SIT attenuates colonic mucosal barrier

Tight junction proteins are the essential parts for the maintenance of the gut mucosal barrier integrity, which could against the invasion of luminal detrimental substances and lower the risk of microbe-induced inflammation (Parikh et al., 2019). Therefore, we further verified whether the protective mechanism of SIT on the colon is related to the restoration of intestinal barrier function. ZO-1 and occludin, are important two tight junction-associated proteins (Ji et al., 2016), which were measured by Western blot. The expression levels of ZO-1 and occludin were decreased compared with the control group, which were significantly reversed by SIT as provided in Figure 6, suggesting that SIT could alleviate colitis through enhancing mucosal barrier integrity.

4 Discussion

UC is a main subtype of chronic relapsing inflammatory bowel disease and is highly prevalent worldwide. The nature of UC is complex and the pathogenesis of UC has not been well elucidated yet. Current therapeutic agents are not entirely desirable in terms of potency with many side effects, erratic efficacy, and recurrence or failure after reduction or termination of administration as well as a heavy financial burden (Bressler et al., 2015; Berends et al., 2019). Hence new alternative therapeutic medication from natural products is of high interest in research. SIT is a well-known bioactive phytosterol naturally plentiful in dietary and non-dietary plant cell membranes, accounting for about 65% of human herbal nutrition forming (Weihrauch and Gardner, 1978). They are not only highly found in lipid-rich plant foods such as nuts,

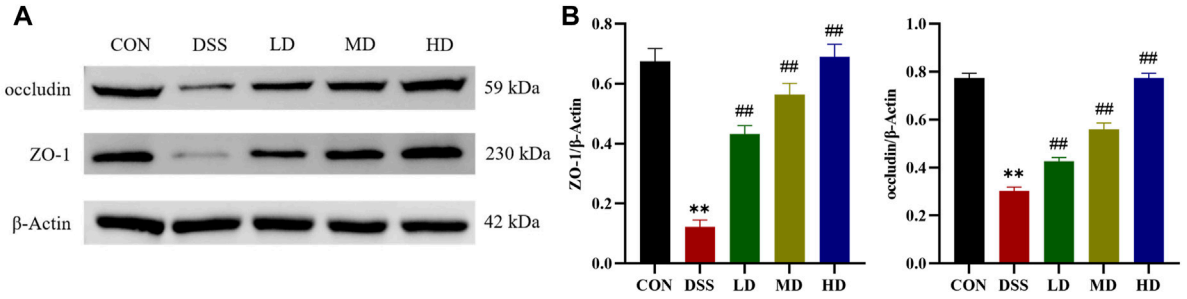

FIGURE 6
SIT alleviates colitis from barrier damage. **(A)** Gel electrophoresis images of occludin and ZO-1 in colon tissues were analyzed by Western blot. **(B)** Analysis of protein expression levels (N = 3 per group). The data are presented as mean ± SD. CON, control group; DSS, dextran sulfate sodium-induced group; SIT, β-sitosterol; LD, low-dose SIT group; MD, middle-dose SIT group; HD, high-dose SIT group; ZO, zonula occludens; SD, standard deviation. $^{**}p < 0.01$ compared with CON group; $^{\#\#}p < 0.01$ compared with DSS group.

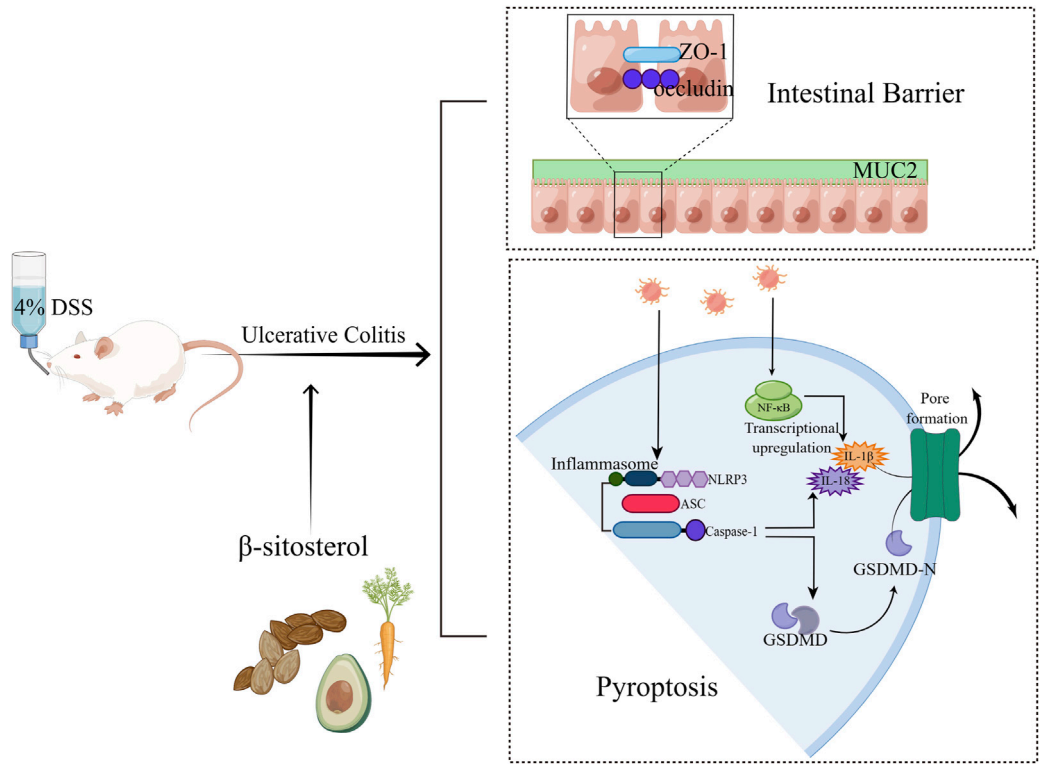

FIGURE 7
Overview of the effects of SIT on DSS-induced UC (By FigDraw, ID: SSITU8168e).

seeds, and legumes but also in vegetables and fruits (Khan et al., 2022). Over the past few decades, research on SIT was at an ever-accelerating pace and has suggested it may exert preventable and therapeutic effects on UC (Lee et al., 2012; Ding et al., 2019) with much less research on its role in pyroptosis.

DSS-induced experimental colitis is a reliable and mature animal model, which resembles clinically pathological symptoms and histological features in chronic UC (Tessner et al., 1998). In our current study, we demonstrated that SIT did significantly relieve the body weight loss in rats with 4% DSS-induced UC, with an increased DAI score, a main indicator in the assessment of the severity of UC. In addition, colonic shortening, another indirect indicator in the evaluation of the severity of UC, was attenuated by SIT treatment, indicating that SIT has therapeutic potential in UC rats. For colon pathological injury, we detected histological analysis and found that administration of SIT could improve cryptal glands and submucosa and reduce inflammatory cells infiltrating. Also, with the increase of SIT dosages, the therapeutic effects improve correspondingly, suggesting that SIT may be a potential drug of choice for UC treatment.

Self-limiting acute inflammation is crucial for the body to eliminate the danger and restore homeostasis, while unresolved

inflammation contributes to the pathogenesis of autoimmune diseases including UC (Afonina et al., 2017). An increasing body of evidence has shown that aberrant inflammatory responses are the key contributors to the progression and exacerbation of UC. Pyroptosis, a novel type of programmed cell death, is mainly elicited by either classical Caspase-1-mediated or non-classical Caspase-11/4/5/11-mediated pathways in inflammation (Fang et al., 2020). Research has indicated that both Caspase-1-mediated and Caspase-11-mediated pyroptosis are closely linked to the development of UC (Chao et al., 2020), in which NLRP3 inflammasome plays a key role (Chen et al., 2019; Zhen and Zhang, 2019). NLRP3 first interacts with ASC and combines with Caspase-1 to assemble the inflammasome complex (Lu et al., 2014). Cleaved Caspase-1 then cleaves GSDMD to the N-terminal fragment (GSDMD-N). After that, the activated N-terminal domain of GSDMD translocates and forms cell membrane pores, thus resulting in the occurrence of pyroptosis and triggering the damage of the epithelial cells in the gut, and releasing pro-inflammatory cytokines (Yuan et al., 2018). The secretion of IL-1β and IL-18 can further amplify and perpetuate the inflammatory reaction (He et al., 2015; Shi et al., 2015). Of note, as the co-substrate of multiple inflammasomes and two main pathways of pyroptosis, GSDMD performs the primary function of pyroptosis executioner (Kayagaki et al., 2015; Shi et al., 2015).

Inflammatory cytokines IL-1β and IL-18 are the main markers for pyroptosis and their overproduction was found in various regions of the colon in UC active patients (Thinwa et al., 2014). Recent findings suggested that both Caspase-1 and Caspase-11 activation possess the function to induce the release of IL-1β and IL-18, but only Caspase-1 could directly cleave them (Man and Kanneganti, 2015); in addition, epithelium IL-18 secretion was independent on NLRP3 but dependent on Caspase-1 (Song-Zhao et al., 2014). Numerous experimental studies on animals have demonstrated inhibiting Caspase-1-dependent pyroptosis could protect against DSS-induced colitis (Tian et al., 2020; Cui et al., 2022; Xue et al., 2022). In view of this, we observed the regulation effect of SIT on inflammatory mediators in our present study. The results showed that SIT alleviated DSS-induced inflammation both in UC rats and Caco-2 cells with downregulation of pro-inflammatory cytokines including TNF-α, IL-1β, and IL-18. Furthermore, we explored the canonical Caspase-1 dependent pathway which underlies UC and we found that SIT treatment downregulated the protein levels of Caspase-1, cleaved-Caspase-1, NLRP3, GSDMD, and GSDMD- N in tissues and cells as expected. Taken together, SIT did ameliorate experimental colitis by inhibiting the NLRP3/Caspase-1/GSDMD-dependent pyroptosis pathway.

Furthermore, NF-κB pathway played an important role in the inflammatory responses of UC (Tong et al., 2021). In mammals, NF-κB family can form homo- or heterodimers of which the most common form is a dimer of p50 and p65. Normally, NF-κB remains inactive with the combination of members of IKB family (Lawrence, 2009). Clinical studies also have demonstrated excessive inflammation activated by NF-κB exists in UC (Zhou et al., 2018). Activation of NF-κB signaling cascade response was reported to participate in the regulation of NLRP3 inflammasome transcription and Caspase-1 has been proven as an active activator of NF-κB (Danelishvili et al., 2011; Hu et al., 2019; Zhen and Zhang, 2019). Previous studies have demonstrated that SIT could reduce the secretion of inflammatory mediators such as TNF-α and IL-1β; in addition, SIT has the ability to suppress the initiation of NLRP3 and the activation of Caspase-1 as well as partial inhibition of NF-κB *in vitro* (Liao et al., 2018). In this study, we also assessed the major proteins of NF-κB pathway. We observed the over-expressed phosphorylation levels of p65 subunit and IκBα, the protein content in colon tissues was suppressed under the SIT treatment. However, it cannot be stated with certainty that SIT-mediated inflammation alleviation is associated with NF-κB pathway in UC in light of our present results.

The mechanical barrier is the most important barrier among the intestinal mucosal barrier, with the structural basis consisting of intact intestinal epithelial cells and tight junction (TJ) proteins between the cells (Odenwald and Turner, 2017). Once the intestinal barrier was disrupted, the bacteria and toxins from the gut would translocate to the mucosa and activate deleterious intestinal inflammation (Liao et al., 2018; Zhang et al., 2018). Previous literature has demonstrated that the dysfunction of intestinal barrier integrity is responsible for the exacerbation of inflammatory bowel disease including UC (Zhao et al., 2020). Therefore, restoring the intestinal barrier integrity can act in preventing or treating UC. To gain further insight into the actions of SIT on the colonic barrier function, we detected the expression levels of the TJ-associated proteins ZO-1 and occludin. Western blot results suggested that the protein expression of ZO-1 and occludin were markedly weakened by DSS treatment while increasing in response to SIT administration. These results suggest that SIT could protect the gut against the occurrence of intestinal inflammation by maintaining mucosal barrier integrity, and therefore halt UC progression.

5 Conclusion

In summary, SIT not only inhibits the production of pro-inflammatory mediators and suppresses pyroptosis via suppression the NLRP3/Caspase-1/GSDMD-dependent pathway, but also enhances the function of the intestinal mucosal barrier through regulation of epithelial tight junction proteins expression (Figure 7). These findings indicate that SIT is an effective drug candidate and may be potential in clinical applications of UC treatment in the future.

As far as we know, it is the first evidence to show that SIT is effective in regulating pyroptosis in experimental UC. Although many studies have shown that SIT has good safety profiles, its poor stability, low water solubility, and short half-life also confine its broader application, and await to be addressed.

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 authors.

Ethics statement

Ethical approval was not required for the studies on humans in accordance with the local legislation and institutional requirements because only commercially available established cell lines were used. The animal study was approved by the animal ethics committee of

Beijing University of Chinese Medicine. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

The contribution of the authors in this work is as follows, D-MZ and MC conceived and designed the research, and provided financial support. DZ performed the experiments, carried out the data processing, and drafted the original manuscript. JJ and FG assisted in the investigation and conducting experiments. Y-JL and F-RZ assisted in the manuscript revision. S-JZ and S-YW provided the test medicine and technical support. D-MZ and MC supervised the research and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1218477/full#supplementary-material

References

Afonina, I. S., Zhong, Z., Karin, M., and Beyaert, R. (2017). Limiting inflammation-the negative regulation of NF-κB and the NLRP3 inflammasome. *Nat. Immunol.* 18, 861–869. doi:10.1038/ni.3772

Aldini, R., Micucci, M., Cevenini, M., Fato, R., Bergamini, C., Nanni, C., et al. (2014). Antiinflammatory effect of phytosterols in experimental murine colitis model: prevention, induction, remission study. *PloS One* 9, e108112. doi:10.1371/journal.pone.0108112

Babu, S., Krishnan, M., Rajagopal, P., Periyasamy, V., Veeraraghavan, V., Govindan, R., et al. (2020). Beta-sitosterol attenuates insulin resistance in adipose tissue via IRS-1/Akt mediated insulin signaling in high fat diet and sucrose induced type-2 diabetic rats. *Eur. J. Pharmacol.* 873, 173004. doi:10.1016/j.ejphar.2020.173004

Bae, H., Park, S., Ham, J., Song, J., Hong, T., Choi, J.-H., et al. (2021). ER-mitochondria calcium flux by β-sitosterol promotes cell death in ovarian cancer. *Antioxidants (Basel, Switz.* 10, 1583. doi:10.3390/antiox10101583

Berends, S. E., Strik, A. S., Löwenberg, M., D'Haens, G. R., and Mathôt, R. A. A. (2019). Clinical pharmacokinetic and pharmacodynamic considerations in the treatment of ulcerative colitis. *Clin. Pharmacokinet.* 58, 15–37. doi:10.1007/s40262-018-0676-z

Bin Sayeed, M. S., Karim, S. M. R., Sharmin, T., and Morshed, M. M. (2016). Critical analysis on characterization, systemic effect, and therapeutic potential of beta-sitosterol: a plant-derived orphan phytosterol. *Med. (Basel, Switz.* 3, 29. doi:10.3390/medicines3040029

Bressler, B., Marshall, J. K., Bernstein, C. N., Bitton, A., Jones, J., Leontiadis, G. I., et al. (2015). Clinical practice guidelines for the medical management of nonhospitalized ulcerative colitis: the Toronto consensus. *Gastroenterology* 148, 1035–1058.e3. doi:10.1053/j.gastro.2015.03.001

Cao, F., Liu, J., Sha, B.-X., and Pan, H.-F. (2019). Natural products: experimental efficient agents for inflammatory bowel disease therapy. *Curr. Pharm. Des.* 25, 4893–4913. doi:10.2174/1381612825666191216154224

Chao, L., Li, Z., Zhou, J., Chen, W., Li, Y., Lv, W., et al. (2020). Shen-Ling-Bai-Zhu-San improves dextran sodium sulfate-induced colitis by inhibiting caspase-1/caspase-11-mediated pyroptosis. *Front. Pharmacol.* 11, 814. doi:10.3389/fphar.2020.00814

Chen, C., Shen, J.-L., Liang, C.-S., Sun, Z.-C., and Jiang, H.-F. (2022). First discovery of beta-sitosterol as a novel antiviral agent against white spot syndrome virus. *Int. J. Mol. Sci.* 23, 10448. doi:10.3390/ijms231810448

Chen, X., Liu, G., Yuan, Y., Wu, G., Wang, S., and Yuan, L. (2019). NEK7 interacts with NLRP3 to modulate the pyroptosis in inflammatory bowel disease via NF-κB signaling. *Cell Death Dis.* 10, 906. doi:10.1038/s41419-019-2157-1

Cheng, Y., Chen, Y., Li, J., Qu, H., Zhao, Y., Wen, C., et al. (2020). Dietary β-sitosterol regulates serum lipid level and improves immune function, antioxidant status, and intestinal morphology in broilers. *Poult. Sci.* 99, 1400–1408. doi:10.1016/j.psj.2019.10.025

Cui, L.-J., Yuan, W., Chen, F.-Y., Wang, Y.-X., Li, Q.-M., Lin, C., et al. (2022). Pectic polysaccharides ameliorate the pathology of ulcerative colitis in mice by reducing pyroptosis. *Ann. Transl. Med.* 10, 347. doi:10.21037/atm-22-877

Cui, L., Wang, W., Luo, Y., Ning, Q., Xia, Z., Chen, J., et al. (2019). Polysaccharide from Scutellaria baicalensis Georgi ameliorates colitis via suppressing NF-κB signaling and NLRP3 inflammasome activation. *Int. J. Biol. Macromol.* 132, 393–405. doi:10.1016/j.ijbiomac.2019.03.230

Danelishvili, L., Everman, J. L., McNamara, M. J., and Bermudez, L. E. (2011). Inhibition of the plasma-membrane-associated serine protease cathepsin G by *Mycobacterium tuberculosis* Rv3364c suppresses caspase-1 and pyroptosis in macrophages. *Front. Microbiol.* 2, 281. doi:10.3389/fmicb.2011.00281

Dieleman, L. A., Palmen, M. J., Akol, H., Bloemena, E., Peña, A. S., Meuwissen, S. G., et al. (1998). Chronic experimental colitis induced by dextran sulphate sodium (DSS) is characterized by Th1 and Th2 cytokines. *Clin. Exp. Immunol.* 114, 385–391. doi:10.1046/j.1365-2249.1998.00728.x

Ding, K., Tan, Y.-Y., Ding, Y., Fang, Y., Yang, X., Fang, J., et al. (2019). β-Sitosterol improves experimental colitis in mice with a target against pathogenic bacteria. *J. Cell. Biochem.* 120, 5687–5694. doi:10.1002/jcb.27853

Du, L., and Ha, C. (2020). Epidemiology and pathogenesis of ulcerative colitis. *Gastroenterology Clin. N. Am.* 49, 643–654. doi:10.1016/j.gtc.2020.07.005

Elmaksoud, H. A. A., Motawea, M. H., Desoky, A. A., Elharrif, M. G., and Ibrahimi, A. (2021). Hydroxytyrosol alleviate intestinal inflammation, oxidative stress and apoptosis resulted in ulcerative colitis. *Biomed. Pharmacother. = Biomedecine Pharmacother.* 142, 112073. doi:10.1016/j.biopha.2021.112073

Fang, Y., Tian, S., Pan, Y., Li, W., Wang, Q., Tang, Y., et al. (2020). Pyroptosis: a new frontier in cancer. *Biomed. Pharmacother. = Biomedecine Pharmacother.* 121, 109595. doi:10.1016/j.biopha.2019.109595

Feng, S., Belwal, T., Li, L., Limwachiranon, J., Liu, X., and Luo, Z. (2020). Phytosterols and their derivatives: potential health-promoting uses against lipid metabolism and associated diseases, mechanism, and safety issues. *Compr. Rev. Food Sci. Food Saf.* 19, 1243–1267. doi:10.1111/1541-4337.12560

Feng, S., Dai, Z., Liu, A., Wang, H., Chen, J., Luo, Z., et al. (2017). β-Sitosterol and stigmasterol ameliorate dextran sulfate sodium-induced colitis in mice fed a high fat Western-style diet. *Food and Funct.* 8, 4179–4186. doi:10.1039/c7fo00375g

He, W.-t., Wan, H., Hu, L., Chen, P., Wang, X., Huang, Z., et al. (2015). Gasdermin D is an executor of pyroptosis and required for interleukin-1β secretion. *Cell Res.* 25, 1285–1298. doi:10.1038/cr.2015.139

Hu, S., Xie, H., Luo, R., Feng, P., Liu, Q., Han, M., et al. (2019). Inhibition of IL-1β by aliskiren improved renal AQP2 expression and urinary concentration defect in ureteral obstruction and release. *Front. Physiology* 10, 1157. doi:10.3389/fphys.2019.01157

Ji, R., Wang, A., Shang, H., Chen, L., Bao, C., Wu, L., et al. (2016). Herb-partitioned moxibustion upregulated the expression of colonic epithelial tight junction-related proteins in Crohn's disease model rats. *Chin. Med.* 11, 20. doi:10.1186/s13020-016-0090-0

Jie, F., Xiao, S., Qiao, Y., You, Y., Feng, Y., Long, Y., et al. (2021). Kuijieling decoction suppresses NLRP3-Mediated pyroptosis to alleviate inflammation and experimental colitis *in vivo* and *in vitro*. *J. Ethnopharmacol.* 264, 113243. doi:10.1016/j.jep.2020.113243

Kayagaki, N., Stowe, I. B., Lee, B. L., O'Rourke, K., Anderson, K., Warming, S., et al. (2015). Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. *Nature* 526, 666–671. doi:10.1038/nature15541

Khan, Z., Nath, N., Rauf, A., Emran, T. B., Mitra, S., Islam, F., et al. (2022). Multifunctional roles and pharmacological potential of β-sitosterol: emerging evidence toward clinical applications. *Chemico-biological Interact.* 365, 110117. doi:10.1016/j.cbi.2022.110117

Kihara, N., de la Fuente, S. G., Fujino, K., Takahashi, T., Pappas, T. N., and Mantyh, C. R. (2003). Vanilloid receptor-1 containing primary sensory neurones mediate dextran sulphate sodium induced colitis in rats. *Gut* 52, 713–719. doi:10.1136/gut.52.5.713

Kim, K.-S., Yang, H. J., Lee, J.-Y., Na, Y.-C., Kwon, S.-Y., Kim, Y.-C., et al. (2014). Effects of β-sitosterol derived from Artemisia capillaris on the activated human hepatic stellate cells and dimethylnitrosamine-induced mouse liver fibrosis. *BMC Complementary Altern. Med.* 14, 363. doi:10.1186/1472-6882-14-363

Lawrence, T. (2009). The nuclear factor NF-kappaB pathway in inflammation. *Cold Spring Harb. Perspect. Biol.* 1, a001651. doi:10.1101/cshperspect.a001651

Lee, I.-A., Kim, E.-J., and Kim, D.-H. (2012). Inhibitory effect of β-sitosterol on TNBS-induced colitis in mice. *Planta Medica* 78, 896–898. doi:10.1055/s-0031-1298486

Liao, P.-C., Lai, M.-H., Hsu, K.-P., Kuo, Y.-H., Chen, J., Tsai, M.-C., et al. (2018). Identification of β-sitosterol as *in vitro* anti-inflammatory constituent in moringa oleifera. *J. Agric. Food Chem.* 66, 10748–10759. doi:10.1021/acs.jafc.8b04555

Lin, F., Xu, L., Huang, M., Deng, B., Zhang, W., Zeng, Z., et al. (2020). β-Sitosterol protects against myocardial ischemia/reperfusion injury via targeting PPARγ/NF-κB signalling. *Evidence-based Complementary Altern. Med.* 2020, 2679409. doi:10.1155/2020/2679409

Loizou, S., Lekakis, I., Chrousos, G. P., and Moutsatsou, P. (2010). Beta-sitosterol exhibits anti-inflammatory activity in human aortic endothelial cells. *Mol. Nutr. Food Res.* 54, 551–558. doi:10.1002/mnfr.200900012

Lu, A., Magupalli, V. G., Ruan, J., Yin, Q., Atianand, M. K., Vos, M. R., et al. (2014). Unified polymerization mechanism for the assembly of ASC-dependent inflammasomes. *Cell* 156, 1193–1206. doi:10.1016/j.cell.2014.02.008

Malini, T., and Vanithakumari, G. (1990). Rat toxicity studies with beta-sitosterol. *J. Ethnopharmacol.* 28, 221–234. doi:10.1016/0378-8741(90)90032-o

Man, S. M., and Kanneganti, T.-D. (2015). Regulation of inflammasome activation. *Immunol. Rev.* 265, 6–21. doi:10.1111/imr.12296

Moreau, J., and Mas, E. (2015). Drug resistance in inflammatory bowel diseases. *Curr. Opin. Pharmacol.* 25, 56–61. doi:10.1016/j.coph.2015.11.003

Nakamura, T., Nagahori, M., Kanai, T., and Watanabe, M. (2008). Current pharmacologic therapies and emerging alternatives in the treatment of ulcerative colitis. *Digestion* 77 (1), 36–41. doi:10.1159/000111486

Neurath, M. F., Pettersson, S., Meyer zum Büschenfelde, K. H., and Strober, W. (1996). Local administration of antisense phosphorothioate oligonucleotides to the p65 subunit of NF-kappa B abrogates established experimental colitis in mice. *Nat. Med.* 2, 998–1004. doi:10.1038/nm0996-998

Ng, S. C., Shi, H. Y., Hamidi, N., Underwood, F. E., Tang, W., Benchimol, E. I., et al. (2017). Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet (London, Engl.* 390, 2769–2778. doi:10.1016/S0140-6736(17)32448-0

Odenwald, M. A., and Turner, J. R. (2017). The intestinal epithelial barrier: a therapeutic target? *Nat. Rev. Gastroenterology Hepatology* 14, 9–21. doi:10.1038/nrgastro.2016.169

Ordás, I., Eckmann, L., Talamini, M., Baumgart, D. C., and Sandborn, W. J. (2012). Ulcerative colitis. *Lancet (London, Engl.* 380, 1606–1619. doi:10.1016/S0140-6736(12)60150-0

Panayotis, N., Freund, P. A., Marvaldi, L., Shalit, T., Brandis, A., Mehlman, T., et al. (2021). β-sitosterol reduces anxiety and synergizes with established anxiolytic drugs in mice. *Cell Rep. Med.* 2, 100281. doi:10.1016/j.xcrm.2021.100281

Paniagua-Pérez, R., Madrigal-Bujaidar, E., Reyes-Cadena, S., Molina-Jasso, D., Gallaga, J. P., Silva-Miranda, A., et al. (2005). Genotoxic and cytotoxic studies of beta-sitosterol and pteropodine in mouse. *J. Biomed. Biotechnol.* 2005, 242–247. doi:10.1155/JBB.2005.242

Parikh, K., Antanaviciute, A., Fawkner-Corbett, D., Jagielowicz, M., Aulicino, A., Lagerholm, C., et al. (2019). Colonic epithelial cell diversity in health and inflammatory bowel disease. *Nature* 567, 49–55. doi:10.1038/s41586-019-0992-y

Pierre Luhata, L., and Usuki, T. (2021). Antibacterial activity of β-sitosterol isolated from the leaves of Odontonema strictum (Acanthaceae). *Bioorg. Med. Chem. Lett.* 48, 128248. doi:10.1016/j.bmcl.2021.128248

Ponnulakshmi, R., Shyamaladevi, B., Vijayalakshmi, P., and Selvaraj, J. (2019). *In silico* and *in vivo* analysis to identify the antidiabetic activity of beta sitosterol in adipose tissue of high fat diet and sucrose induced type-2 diabetic experimental rats. *Toxicol. Mech. Methods* 29, 276–290. doi:10.1080/15376516.2018.1545815

Rogler, G. (2014). Chronic ulcerative colitis and colorectal cancer. *Cancer Lett.* 345, 235–241. doi:10.1016/j.canlet.2013.07.032

Shi, J., Zhao, Y., Wang, K., Shi, X., Wang, Y., Huang, H., et al. (2015). Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature* 526, 660–665. doi:10.1038/nature15514

Song-Zhao, G. X., Srinivasan, N., Pott, J., Baban, D., Frankel, G., and Maloy, K. J. (2014). Nlrp3 activation in the intestinal epithelium protects against a mucosal pathogen. *Mucosal Immunol.* 7, 763–774. doi:10.1038/mi.2013.94

Sun, Y., Gao, L., Hou, W., and Wu, J. (2020). β-Sitosterol alleviates inflammatory response via inhibiting the activation of ERK/p38 and NF-κB pathways in LPS-exposed BV2 cells. *BioMed Res. Int.* 2020, 7532306. doi:10.1155/2020/7532306

Tessner, T. G., Cohn, S. M., Schloemann, S., and Stenson, W. F. (1998). Prostaglandins prevent decreased epithelial cell proliferation associated with dextran sodium sulfate injury in mice. *Gastroenterology* 115, 874–882. doi:10.1016/s0016-5085(98)70259-8

Thinwa, J., Segovia, J. A., Bose, S., and Dube, P. H. (2014). Integrin-mediated first signal for inflammasome activation in intestinal epithelial cells. *J. Immunol.* 193, 1373–1382. doi:10.4049/jimmunol.1400145

Tian, M., Ma, P., Zhang, Y., Mi, Y., and Fan, D. (2020). Ginsenoside Rk3 alleviated DSS-induced ulcerative colitis by protecting colon barrier and inhibiting NLRP3 inflammasome pathway. *Int. Immunopharmacol.* 85, 106645. doi:10.1016/j.intimp.2020.106645

Tong, L., Hao, H., Zhang, Z., Lv, Y., Liang, X., Liu, Q., et al. (2021). Milk-derived extracellular vesicles alleviate ulcerative colitis by regulating the gut immunity and reshaping the gut microbiota. *Theranostics* 11, 8570–8586. doi:10.7150/thno.62046

Wei, Y.-Y., Fan, Y.-M., Ga, Y., Zhang, Y.-N., Han, J.-C., and Hao, Z.-H. (2021). Shaoyao decoction attenuates DSS-induced ulcerative colitis, macrophage and NLRP3 inflammasome activation through the MKP1/NF-κB pathway. *Phytomedicine* 92, 153743. doi:10.1016/j.phymed.2021.153743

Weihrauch, J. L., and Gardner, J. M. (1978). Sterol content of foods of plant origin. *J. Am. Dietetic Assoc.* 73, 39–47. doi:10.1016/s0002-8223(21)05668-6

Xue, S., Xue, Y., Dou, D., Wu, H., Zhang, P., Gao, Y., et al. (2022). Kui jie tong ameliorates ulcerative colitis by regulating gut microbiota and NLRP3/caspase-1 classical pyroptosis signaling pathway. *Dis. Markers* 2022, 2782112. doi:10.1155/2022/2782112

Ye, L., Cao, Q., and Cheng, J. (2013). Review of inflammatory bowel disease in China. *TheScientificWorldJournal* 2013, 296470. doi:10.1155/2013/296470

Yu, P., Zhang, X., Liu, N., Tang, L., Peng, C., and Chen, X. (2021a). Pyroptosis: mechanisms and diseases. *Signal Transduct. Target. Ther.* 6, 128. doi:10.1038/s41392-021-00507-5

Yu, Y., Cao, Y., Huang, W., Liu, Y., Lu, Y., and Zhao, J. (2021b). β-Sitosterol ameliorates endometrium receptivity in PCOS-like mice: the mediation of gut microbiota. *Front. Nutr.* 8, 667130. doi:10.3389/fnut.2021.667130

Yuan, Y.-Y., Xie, K.-X., Wang, S.-L., and Yuan, L.-W. (2018). Inflammatory caspase-related pyroptosis: mechanism, regulation and therapeutic potential for inflammatory bowel disease. *Gastroenterol. Rep.* 6, 167–176. doi:10.1093/gastro/goy011

Zhang, R., Han, Y., McClements, D. J., Xu, D., and Chen, S. (2022). Production, characterization, delivery, and cholesterol-lowering mechanism of phytosterols: a review. *J. Agric. Food Chem.* 70, 2483–2494. doi:10.1021/acs.jafc.1c07390

Zhang, Y., Zhao, X., Zhu, Y., Ma, J., Ma, H., and Zhang, H. (2018). Probiotic mixture protects dextran sulfate sodium-induced colitis by altering tight junction protein expressions and increasing tregs. *Mediat. Inflamm.* 2018, 9416391. doi:10.1155/2018/9416391

Zhao, B., Xia, B., Li, X., Zhang, L., Liu, X., Shi, R., et al. (2020). Sesamol supplementation attenuates DSS-induced colitis via mediating gut barrier integrity, inflammatory responses, and reshaping gut microbiome. *J. Agric. Food Chem.* 68, 10697–10708. doi:10.1021/acs.jafc.0c04370

Zhen, Y., and Zhang, H. (2019). NLRP3 inflammasome and inflammatory bowel disease. *Front. Immunol.* 10, 276. doi:10.3389/fimmu.2019.00276

Zhou, M., Xu, W., Wang, J., Yan, J., Shi, Y., Zhang, C., et al. (2018). Boosting mTOR-dependent autophagy via upstream TLR4-MyD88-MAPK signalling and downstream NF-κB pathway quenches intestinal inflammation and oxidative stress injury. *EBioMedicine* 35, 345–360. doi:10.1016/j.ebiom.2018.08.035

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Progress in studying the impact of hyperlipidemia and statins on rotator cuff injury and repair

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This review delves into the intersection of two prevalent conditions, hyperlipidemia and rotator cuff injuries, both of which bear substantial healthcare burdens. Our investigation begins with an exploration of rotator cuff injuries, common musculoskeletal disorders that severely impair shoulder functionality and quality of life. These injuries are notably pervasive among sports enthusiasts and the older adult, with an incidence rate estimated at 5–10% in the general population. Despite their widespread occurrence and the diverse, multifactorial etiological factors, effective treatment strategies remain elusive. We then examine hyperlipidemia, a metabolic disorder affecting approximately 40% of the global adult population. Characterized by elevated levels of cholesterol and triglycerides, hyperlipidemia can precipitate severe cardiovascular complications and presents a significant socioeconomic burden. Although current management strategies encompass lifestyle modifications and pharmacological interventions, the condition remains a formidable health challenge. Central to this review is the exploration of a potential association between hyperlipidemia and rotator cuff injuries. We aim to synthesize the current understanding of hyperlipidemia's role in the pathophysiology of rotator cuff injuries, thereby offering fresh insights into their common etiological underpinnings, potential therapeutic targets, and drugs, such as Statins. The influence of other lipid-lowering therapeutics on tendon health is also considered, and further research into the molecular pathways and potential therapeutic benefits of these drugs is required. This pursuit aligns with broader efforts to enhance patient outcomes, minimize healthcare burdens, and contribute to the global understanding of these prevalent conditions.

KEYWORDS

rotator cuff injury, hyperlipidemia, statin, mechanism, treatment, inflammation

Introduction

The rotator cuff, a sophisticated orchestration of muscles and tendons that envelope the shoulder joint, serves an indispensable role in bestowing stability and enabling a diverse array of shoulder movements (1–3). Afflictions to this vital assembly, which span the spectrum from benign inflammation or tendonitis to partial or even full-throttle tendon ruptures, predominantly impact the shoulder's soft tissues (4, 5). These injuries frequently culminate in acute pain, muscular weakness, and restricted mobility, thereby epitomizing a pervasive musculoskeletal disorder (6). Rotator cuff injuries manifest with particular frequency among sports aficionados and the geriatric population, often inducing substantial debilitation in shoulder functionality and a significant deterioration in the quality of life (3). The annual incidence rate of rotator cuff

injuries hovers around 5–10% within the general populace, with a conspicuous surge in prevalence commensurate with advancing age (asymptomatic tears were found in more than 20% of individuals over the age of 60) (7). A rough estimate of the economic burden for a single patient undergoing surgery for a rotator cuff injury can be in the range of $10,000–$30,000 or even more, including both direct and indirect costs. Thus, this condition represents a substantial quota of visits to orthopedic clinics, exerting a hefty strain on healthcare resources, and thereby emerging as a noteworthy public health quandary.

The precise etiology of rotator cuff injuries remains nebulous, likely being multifactorial in nature. The onslaught of years is a well-entrenched risk factor, with degenerative alterations in the rotator cuff tendons becoming increasingly commonplace as individuals age (8). Furthermore, lifestyle determinants such as smoking have been implicated, with empirical evidence indicating that smokers are at an escalated risk of succumbing to rotator cuff injuries in contrast to their non-smoking counterparts (9). Medical conditions such as diabetes and hypertension have also been correlated with an augmented risk of these injuries, thereby suggesting a role for systemic health in the integrity of the rotator cuff (10, 11). The stewardship of rotator cuff injuries is multifarious, with therapeutic options customized to the individual patient's needs, factoring in the severity of the injury, the patient's overarching health status, and their functional exigencies. Conservative management approaches, encompassing physical therapy, pain mitigation with non-steroidal anti-inflammatory drugs (NSAIDs), and corticosteroid injections, typically constitute the initial line of treatment (12). However, in cases of severe injuries or when conservative management proves ineffective, surgical intervention may be necessitated (8). Despite these treatment modalities, a significant number of patients persist in experiencing pain and functional limitations, thereby highlighting the need for further research into innovative therapeutic strategies (11, 13).

Hyperlipidemia, a pervasive metabolic aberration, is characterized by escalated levels of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C), coupled with a reduction in high-density lipoprotein cholesterol (HDL-C) (1, 14, 15). This condition often manifests covertly, with no symptoms in its incipient stages. However, unaddressed hyperlipidemia can precipitate atherosclerosis, engendering grave cardiovascular ramifications such as heart disease, cerebrovascular accident, and peripheral artery disease (10, 16, 17). As a substantial global health quandary, the World Health Organization posits that approximately 40% of the global adult populace grapples with elevated total cholesterol levels, a key indicator of hyperlipidemia. This widespread disorder imposes a significant socioeconomic encumbrance, accounting for a considerable proportion of healthcare expenditure. Therapeutic stratagems encompass lifestyle modifications, including dietary adjustments and consistent physical exertion, along with pharmacological interventions like statins, fibrates, and niacin (16, 18, 19). Despite these measures, hyperlipidemia persists as a formidable health challenge, underscoring the exigency for continued exploration into innovative therapeutic approaches and prophylactic strategies. The potential correlation between hyperlipidemia and rotator cuff injuries further accentuates the necessity for a comprehensive understanding and efficacious management of this disorder (16).

Given the substantial prevalence and socio-economic burden of hyperlipidemia (20, 21), and its potential association with rotator cuff injuries, a comprehensive understanding of the interplay between these two conditions is essential. Therefore, this review aims to amalgamate the current understanding of the role of hyperlipidemia in the pathophysiology of rotator cuff injuries. Our objective is to elucidate this connection, thereby providing novel insights for etiological research and clinical management. By probing into the underlying mechanisms, we expect to shed light on potential therapeutic targets and preventive strategies, thereby contributing to the global efforts in addressing these prevalent conditions.

Hyperlipidemia as a risk factor for rotator cuff injuries

The etiology of rotator cuff injuries is a complex interplay of multiple factors, inclusive of age-related degenerative changes, disease progression, and undue physical strain. In the scientific discourse of recent years, a considerable emphasis has been placed on the potential correlation between hyperlipidemia and rotator cuff injuries. This connection was evidenced in a prospectively designed study by Skovgaard et al. (16), wherein hypercholesterolemia surfaced as a factor escalating the risk of upper limb tendon injuries by a factor of 1.5. A similar correlation was delineated in Kara's research (10), where cardiovascular risk elements, including diabetes and hyperlipidemia, were identified as potential precipitators of rotator cuff injuries.

In a parallel vein, Djerbi et al. (22) uncovered a significant correlation between smoking, dyslipidemia, and the incidence rate of rotator cuff tears. An 11-year longitudinal follow-up study conducted by Lin et al. (15) further underscored hyperlipidemia as an independent risk factor for rotator cuff disorders, doubling the risk compared to non-hyperlipidemic cohorts. Recent studies echoed these findings, demonstrating a discernible link between hyperlipidemia and various manifestations of rotator cuff tendon diseases, with hyperlipidemic patients bearing a higher risk of complete tears.

However, it is imperative to note the existence of contrarian views, as several studies (23, 24) reported no significant correlation between dyslipidemia and rotator cuff tears. These incongruities might be attributed to variances in the demographic composition of the study populations, the parameters observed, and the criteria for grouping.

The impact of hyperlipidemia on the efficacy of rotator cuff injury repair

Hyperlipidemia, characterized by elevated lipid levels, has been increasingly identified as a critical factor that correlates not only with the onset of rotator cuff tears but also with the propensity for a re-tear following surgical intervention. This multifaceted influence of hyperlipidemia was substantiated in a large-scale, rigorous study conducted by Cancienne et al. (25). Their research, encompassing an extensive cohort of 30,638 patients subjected to rotator cuff repair, unveiled a significant correlation between perioperative total cholesterol and low-density lipoprotein cholesterol levels, and the rate of revision surgery following the initial repair. This finding underscores the potential for hyperlipidemia to complicate the post-operative trajectory and recovery in patients with rotator cuff tears.

Further amplifying this perspective, Garcia's research (14) revealed a distinct disparity in the risk of re-tear between hyperlipidemic and non-hyperlipidemic patients. The study found that, compared to their non-hyperlipidemic counterparts, patients with hyperlipidemia faced a staggering fourfold increase in the risk of re-tear following rotator cuff repair. This finding is indicative of the profound influence of lipid metabolic disorders on the integrity and healing capacity of rotator cuff tissues.

Complementing these findings, Kim's study (26) further reinforced the role of hyperlipidemia as a significant risk factor for re-tear subsequent to rotator cuff repair. The convergence of these findings across multiple studies underscores the necessity of considering hyperlipidemia in the strategic planning of both preventive and therapeutic interventions for rotator cuff injuries.

Taking this multifactorial approach a step further, Harada et al. (27) embarked on an investigation of the risk factors for re-tear following rotator cuff repair. The study's findings highlighted that the combination of hyperlipidemia, a tear size of ≥40 mm, and a critical shoulder angle of ≥37° collectively forecasted the highest rate of re-tear. This discovery underscores the importance of a comprehensive, multi-parametric assessment in predicting the risk of re-tear and devising more effective post-operative management strategies.

Molecular mechanism of rotator cuff injury induced by hyperlipidemia

The intricate role of hyperlipidemia in the pathogenesis of rotator cuff injury remains somewhat nebulous, but current research illuminates a series of molecular mechanisms that may underpin the deleterious effects of this metabolic disorder on tendon health (Figure 1).

Oxidative stress

A multitude of studies affirm the primacy of oxidative stress in triggering tendon degeneration, fibrosis, and adhesion (28, 29). Hyperlipidemia, particularly characterized by high cholesterol, can suppress the expression of tendon-related genes in tendon stem/progenitor cells via the reactive oxygen species (ROS)-activated nuclear factor kappa-B (NF-κB) signaling pathway (30, 31). Concurrently, it can instigate cell apoptosis and autophagy through the ROS-activated AKT/FOXO1 signaling pathway in these cells, culminating in dysfunction and ultimately precipitating tendon degeneration and tendinopathy (32, 33).

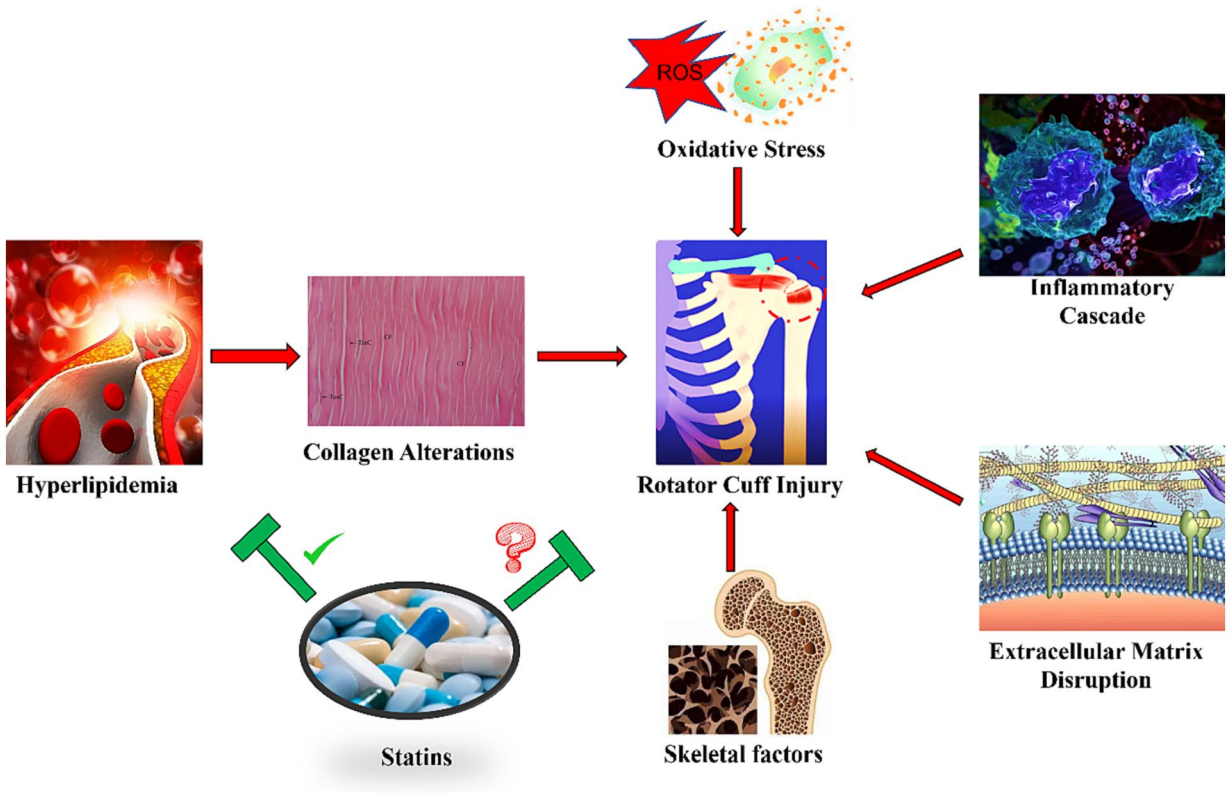

FIGURE 1
Mechanism of hyperlipidemia and statins on rotator cuff injury. The figure reflects several messages: 1. Hyperlipidemia may lead to a range of pathologic changes leading to a higher incidence of rotator cuff tears. 2. Collagen transformation, oxidative stress, inflammation, extracellular matrix disorders, and changes in the skeletal system underlie the co-morbidities of hyperlipidemia and rotator cuff injuries. 3. Statins have been clearly shown to intervene in hyperlipidemia, but the effect on rotator cuff injuries is controversial, and co-morbidities are not well understood. 4. The efficacy of statins in rotator cuff injury is controversial, and more research is needed to support the efficacy of statins in this co-morbid condition.

Collagen alterations

Normal tendons are predominantly constituted of type I collagen, interspersed with a minor proportion of type III collagen. Aberrant cholesterol deposition in tendon tissues can provoke alterations in collagen synthesis and organization, thereby inducing structural and biomechanical alterations within the tendon matrix (34).

Extracellular matrix disruption

The extracellular matrix (ECM) plays a pivotal role in maintaining tendon homeostasis (35). A hyperlipidemic milieu can induce changes in critical components of the ECM in tendon tissues and cells. The mechanism may involve low-density lipoprotein cholesterol (LDL-C) inducing dysfunction of tendon cells, contributing to alterations in the ECM components (36).

Inflammatory cascade

The inflammatory microenvironment is a key player in tendon degenerative damage (37–39), and hyperlipidemia has been closely linked to systemic inflammation (40). Cholesterol molecules can activate the NF-κB signaling pathway, augmenting the expression of pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α), interleukin 6 (IL-6) (41–43).

Skeletal factors

Hyperlipidemia can exert a dual detrimental impact on bone health by inhibiting bone formation and accelerating bone resorption, thereby promoting the onset of osteoporosis (44). Notably, osteoporosis has been identified as an independent risk factor for both the genesis of rotator cuff tears and re-tear following surgical repair (45, 46). This underscores the intersection of metabolic, inflammatory, and skeletal factors in the pathogenesis and prognosis of rotator cuff injuries.

Application of lipid-lowering drugs in rotator cuff injuries and repair

Statins, scientifically known as 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, represent the principal lipid-lowering agents in clinical practice (47). By inhibiting HMG-CoA reductase, the rate-limiting enzyme in cholesterol synthesis, statins effectively curtail cholesterol production. This, in turn, upregulates the expression of LDL receptors on the cell surface, thereby accelerating the metabolic clearance of serum LDL. However, the potential influence of statins on rotator cuff injuries and their recovery trajectory engenders a degree of controversy in the current literature.

Several studies have elucidated a beneficial role for statins in the context of rotator cuff injuries and their post-injury repair. Dolkart et al. (48) employed a rat model of rotator cuff tear to probe the impact of atorvastatin on tendon-bone healing following rotator cuff repair surgery. The experimental findings suggested that atorvastatin could activate the COX2/PGE2/EP4 pathways, thereby fostering tendon cell proliferation, migration, and differentiation, and ultimately bolstering the biomechanical integrity of the tendon-bone interface. Parallel findings were obtained in an animal study conducted by Hao et al. (49), demonstrating that silk protein fortified with simvastatin could stimulate osteogenic differentiation of bone marrow mesenchymal stem cells and collagen synthesis via the β-catenin signaling pathway, thereby enhancing tendon-bone interface healing. Lin et al. (15) conducted an 11-year longitudinal study corroborating that hyperlipidemia constitutes a risk factor for rotator cuff injuries, and statin therapy in hyperlipidemic patients could mitigate this risk. A clinical study by Cancienne et al. (25) revealed a positive correlation between perioperative lipid levels and the rate of revision surgery following rotator cuff repair, suggesting that statin therapy could potentially decrease this revision rate. Similarly, a recent case–control study by Lee et al. (50) incorporated 104 hyperlipidemic patients who underwent arthroscopic rotator cuff repair, among whom 66 individuals were in the statin group and 38 in the non-statin group. The follow-up findings indicated a lower re-tear rate in the statin group.

Conversely, several studies have reported potentially detrimental effects of statins on tendon health. Animal experiments conducted by Oliveira et al. (51, 52) demonstrated that statins could disrupt collagen fiber organization and alter tendon matrix composition, thereby diminishing the biomechanical resilience of the tendon and increasing the risk of rupture. Kaleağasıoğlu et al. (53) discerned that statins could precipitate tendon calcification, which could in turn compromise its biomechanical properties. Animal experiments and clinical cohort studies by Eliasson et al. (54, 55) showed that statins could suppress tendon cell proliferation and elicit deleterious effects on tendon collagen and matrix. Furthermore, some studies found that statins did not improve the prognosis following rotator cuff repair surgery. In a study conducted by Amit et al. (1), 77 hyperlipidemic patients underwent arthroscopic rotator cuff repair, among whom 38 individuals were on statins and 39 were not. The follow-up found no significant differences in shoulder joint function, postoperative fatty infiltration, and re-tear rates between the two groups. Similarly, a study by Zeng et al. (56) found no significant difference in postoperative shoulder function scores between hyperlipidemic patients undergoing arthroscopic rotator cuff repair who were or were not on statin therapy perioperatively.

Potential reasons for the varied outcomes observed across different studies may be due to different baseline population data, different administered doses, and different treatment times, etc. Finally, it should be mentioned that there are still some limitations in the literature cited in this current review. (1) Most of the clinical studies have a low grade of evidence. (2) Animal studies are not sufficiently mechanistically insightful. (3) There is no direct evidence for the effect of statins on co-morbidities, and more clinical and basic experimental evidence is needed to support it.

Summary and perspectives

The significance of hyperlipidemia in the context of rotator cuff injuries is progressively gaining momentum in medical research. The intricate interplay between hyperlipidemia and these injuries remains

somewhat enigmatic, necessitating large-scale, multicentric prospective studies to conclusively ascertain whether hyperlipidemia augments the susceptibility to such injuries. The molecular pathways through which hyperlipidemia instigates rotator cuff injuries are yet to be fully elucidated, thereby requiring a more comprehensive exploration through animal models and cellular experiments.

Moreover, the influence of statins on rotator cuff injuries and the subsequent healing of the tendon-bone interface post-surgery continues to be a subject of debate (57). The impact of other widely used lipid-lowering therapeutics, such as ezetimibe and probucol, as well as novel lipid-lowering agents, on tendon health remains nebulous and warrants a more thorough investigation.

The unraveling of the mechanistic role of hyperlipidemia in rotator cuff diseases, coupled with a deeper understanding of the potential therapeutic benefits of lipid-lowering drugs, could potentially offer transformative insights into the etiological basis and clinical management of rotator cuff injuries. Such insights could pave the way for more personalized and effective therapeutic regimens, enhancing the prognosis for patients with these injuries.

Author contributions

YQ: Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing. HH: Conceptualization, Data curation, Investigation, Writing – original draft, Writing – review & editing. RW: Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. YZ: Conceptualization, Data curation, Formal analysis, Writing – original draft. XF: Data curation, Formal analysis, Writing – original draft. FX: Funding acquisition, Supervision, Validation, Writing – review & editing. ZL: Conceptualization, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. QW: Conceptualization, Funding acquisition, Resources, Supervision, Validation, Visualization, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Amit P, Kuiper JH, James S, Snow M. Does statin-treated hyperlipidemia affect rotator cuff healing or muscle fatty infiltration after rotator cuff repair? *J Shoulder Elb Surg*. (2021) 30:2465–74. doi: 10.1016/j.jse.2021.05.014

2. Gavriilidis I, Kircher J, Magosch P, Lichtenberg S, Habermeyer P. Pectoralis major transfer for the treatment of irreparable anterosuperior rotator cuff tears. *Int Orthop*. (2010) 34:689–94. doi: 10.1007/s00264-009-0799-9

3. Luo Z, Lin J, Sun Y, Zhu K, Wang C, Chen J. Outcome comparison of latissimus Dorsi transfer and pectoralis major transfer for irreparable subscapularis tendon tear: a systematic review. *Am J Sports Med*. (2022) 50:2032–41. doi: 10.1177/03635465211018216

4. Sanchez-Sotelo J, Athwal GS. How to optimize reverse shoulder arthroplasty for irreparable cuff tears. *Curr Rev Musculoskelet Med*. (2020) 13:553–60. doi: 10.1007/s12178-020-09655-7

5. Wang C, Song W, Chen B, Liu X, He Y. Exosomes isolated from adipose-derived stem cells: a new cell-free approach to prevent the muscle degeneration associated with torn rotator cuffs. *Am J Sports Med*. (2019) 47:3247–55. doi: 10.1177/0363546519876323

6. Sun Y, Lin J, Luo Z, Chen J. Preoperative lymphocyte to monocyte ratio can be a prognostic factor in arthroscopic repair of small to large rotator cuff tears. *Am J Sports Med*. (2020) 48:3042–50. doi: 10.1177/0363546520953427

7. Sun Y, Lin J, Luo Z, Zhang Y, Chen J. The serum from patients with secondary frozen shoulder following rotator cuff repair induces shoulder capsule fibrosis and promotes macrophage polarization and fibroblast activation. *J Inflamm Res*. (2021) 14:1055–68. doi: 10.2147/JIR.S304555

8. Sun Y, Luo Z, Chen Y, Lin J, Zhang Y, Qi B, et al. Si-Tgfbr1-loading liposomes inhibit shoulder capsule fibrosis via mimicking the protective function of exosomes from patients with adhesive capsulitis. *Biomater Res*. (2022) 26:39. doi: 10.1186/s40824-022-00286-2

9. Pietrzak M. Adhesive capsulitis: an age related symptom of metabolic syndrome and chronic low-grade inflammation? *Med Hypotheses*. (2016) 88:12–7. doi: 10.1016/j.mehy.2016.01.002

10. Applegate KA, Thiese MS, Merryweather AS, Kapellusch J, Drury DL, Wood E, et al. Association between cardiovascular disease risk factors and rotator cuff tendinopathy: a cross-sectional study. *J Occup Environ Med*. (2017) 59:154–60. doi: 10.1097/JOM.0000000000000929

11. Giri A, O'Hanlon D, Jain NB. Risk factors for rotator cuff disease: a systematic review and meta-analysis of diabetes, hypertension, and hyperlipidemia. *Ann Phys Rehabil Med*. (2023) 66:101631. doi: 10.1016/j.rehab.2022.101631

12. Luo Z, Sun Y, Qi B, Lin J, Chen Y, Xu Y, et al. Human bone marrow mesenchymal stem cell-derived extracellular vesicles inhibit shoulder stiffness via let-7a/Tgfbr1 axis. *Bioact Mater*. (2022) 17:344–59. doi: 10.1016/j.bioactmat.2022.01.016

13. Leong HT, Fu SC, He X, Oh JH, Yamamoto N, Hang S. Risk factors for rotator cuff tendinopathy: a systematic review and meta-analysis. *J Rehabil Med*. (2019) 51:627–37. doi: 10.2340/16501977-2598

14. Garcia GH, Liu JN, Wong A, Cordasco F, Dines DM, Dines JS, et al. Hyperlipidemia increases the risk of retear after arthroscopic rotator cuff repair. *J Shoulder Elb Surg*. (2017) 26:2086–90. doi: 10.1016/j.jse.2017.05.009

15. Lin TT-L, Lin C-H, Chang C-L, Chi C-H, Chang S-T, Sheu WH-H. The effect of diabetes, hyperlipidemia, and statins on the development of rotator cuff disease: a nationwide, 11-year, longitudinal, population-based follow-up study. *Am J Sports Med*. (2015) 43:2126–32. doi: 10.1177/0363546515588173

16. Skovgaard D, Siersma VD, Klausen SB, Visnes H, Haukenes I, Bang CW, et al. Chronic hyperglycemia, hypercholesterolemia, and metabolic syndrome are associated with risk of tendon injury. *Scand J Med Sci Sports*. (2021) 31:1822–31. doi: 10.1111/sms.13984

17. Yao Y. S., Li T.Di, Zeng Z. H. (2020). Mechanisms underlying direct actions of hyperlipidemia on myocardium: an updated review. *Lipids Health Dis* 19,:23. doi: 10.1186/s12944-019-1171-8

18. Karr S. Epidemiology and management of hyperlipidemia. *Am J Manag Care*. (2017) 23:S139–48.

19. Miao H, Chen H, Pei S, Bai X, Vaziri ND, Zhao Y-Y. Plasma lipidomics reveal profound perturbation of glycerophospholipids, fatty acids, and sphingolipids in diet-induced hyperlipidemia. *Chem Biol Interact*. (2015) 228:79–87. doi: 10.1016/j.cbi.2015.01.023

20. Lan Z, Tan L, Wang G, Tang M, Wang R, Wang S. Active compounds in RenShenJian decoction ameliorate insulin resistance *in vitro*. *Tradit Med Res*. (2022) 7:32. doi: 10.53388/tmr20221022002

21. Pan B, Zhang H, Li H, Yuan E, Luo J, Zhang T, et al. Advances in Chinese medicine treatment and research on endocrine diseases in 2021. *Tradit Med Res*. (2022) 7:49. doi: 10.53388/tmr20220208001

22. Djerbi I, Chammas M, Mirous M-P, Lazerges C, Coulet B. Impact of cardiovascular risk factor on the prevalence and severity of symptomatic full-thickness rotator cuff tears. *Orthop Traumatol Surg Res*. (2015) 101:S269–73. doi: 10.1016/j.otsr.2015.06.011

23. Longo UG, Franceschi F, Spiezia F, Forriol F, Maffulli N, Denaro V. Triglycerides and total serum cholesterol in rotator cuff tears: do they matter? *Br J Sports Med*. (2010) 44:948–51. doi: 10.1136/bjsm.2008.056440

24. Yamamoto N, Mineta M, Kawakami J, Sano H, Itoi E. Risk factors for tear progression in symptomatic rotator cuff tears: a prospective study of 174 shoulders. *Am J Sports Med*. (2017) 45:2524–31. doi: 10.1177/0363546517709780

25. Cancienne JM, Brockmeier SF, Rodeo SA, Werner BC. Perioperative serum lipid status and statin use affect the revision surgery rate after arthroscopic rotator cuff repair. *Am J Sports Med*. (2017) 45:2948–54. doi: 10.1177/0363546517717686

26. Kim Y-K, Jung K-H, Kim J-W, Kim U-S, Hwang D-H. Factors affecting rotator cuff integrity after arthroscopic repair for medium-sized or larger cuff tears: a retrospective cohort study. *J Shoulder Elb Surg*. (2018) 27:1012–20. doi: 10.1016/j.jse.2017.11.016

27. Harada N, Gotoh M, Ishitani E, Kakuma T, Yano Y, Tatara D, et al. Combination of risk factors affecting retear after arthroscopic rotator cuff repair: a decision tree analysis. *J Shoulder Elb Surg*. (2021) 30:9–15. doi: 10.1016/j.jse.2020.05.006

28. Miao Y, Chen R, Zhang Z, Liu Y, Yang F, Zhang J. Network pharmacology analysis of Xuanfei Baidu granule in the treatment of intestinal Flora disorder. *Adv Gut Microbiome Res*. (2022) 2022:1–13. doi: 10.1155/2022/7883756

29. Singh T, Kaur G, Kaur A. Dysbiosis—an etiological factor for cardiovascular diseases and the therapeutic benefits of gut microflora. *Adv Gut Microbiome Res*. (2023) 2023:1–8. doi: 10.1155/2023/7451554

30. Wang Z-H, Lin Z, Lai Y, Ding L, Wang H, Chen X, et al. The immunomodulatory effects of bone marrow-derived mesenchymal stem cells on lymphocyte in spleens of aging rats. *Biomed Eng Commun*. (2023) 2:13. doi: 10.53388/bmec2023013

31. Wu Y, Hu C, Wang Y-B. Recent advances in the application of biomimetic nanomedicines in disease treatment. *Biomed Eng Commun*. (2022) 1:4. doi: 10.53388/bmec2022004

32. Li K, Deng G, Deng Y, Chen S, Wu H, Cheng C, et al. High cholesterol inhibits tendon-related gene expressions in tendon-derived stem cells through reactive oxygen species-activated nuclear factor-κB signaling. *J Cell Physiol*. (2019) 234:18017–28. doi: 10.1002/jcp.28433

33. Li K, Deng Y, Deng G, Chen P, Wang Y, Wu H, et al. High cholesterol induces apoptosis and autophagy through the ROS-activated AKT/FOXO1 pathway in tendon-derived stem cells. *Stem Cell Res Ther*. (2020) 11:131. doi: 10.1186/s13287-020-01643-5

34. Steplewski A, Fertala J, Tomlinson R, Hoxha K, Han L, Thakar O, et al. The impact of cholesterol deposits on the fibrillar architecture of the Achilles tendon in a rabbit model of hypercholesterolemia. *J Orthop Surg Res*. (2019) 14:172. doi: 10.1186/s13018-019-1217-7

35. Figueiredo EA, Loyola LC, Belangero PS, Campos Ribeiro-dos-Santos ÂK, Emanuel Batista Santos S, Cohen C, et al. Rotator cuff tear susceptibility is associated with variants in genes involved in tendon extracellular matrix homeostasis. *J Orthop Res*. (2020) 38:192–201. doi: 10.1002/jor.24455

36. Fang W, Sekhon S, Teramoto D, Fung C, la V, Duong C, et al. Pathological alterations in the expression status of rotator cuff tendon matrix components in hyperlipidemia. *Mol Cell Biochem*. (2023) 478:1887–98. doi: 10.1007/s11010-022-04643-6

37. Chen H, Li H, Shi W, Qin H, Zheng L. The roles of m6A RNA methylation modification in cancer stem cells: new opportunities for cancer suppression. *Cancer Insight*. (2022) 1:1–18. doi: 10.58567/ci01020001

38. Liu C, Qin Q, Cong H. Research Progress on the relationship between mitochondrial Deoxyguanosine kinase and apoptosis and autophagy in lung adenocarcinoma cells. *Cancer Insight*. (2022) 1:53–62. doi: 10.58567/ci01010004

39. Shindle MK, Chen CCT, Robertson C, DiTullio AE, Paulus MC, Clinton CM, et al. Full-thickness supraspinatus tears are associated with more synovial inflammation and tissue degeneration than partial-thickness tears. *J Shoulder Elb Surg*. (2011) 20:917–27. doi: 10.1016/j.jse.2011.02.015

40. Luo Z-W, Sun Y-Y, Lin J-R, Qi B-J, Chen J-W. Exosomes derived from inflammatory myoblasts promote M1 polarization and break the balance of myoblast proliferation/differentiation. *World J Stem Cells*. (2021) 13:1762–82. doi: 10.4252/wjsc.v13.i11.1762

41. Bhatt BA, Dube JJ, Dedousis N, Reider JA, O'Doherty RM. Diet-induced obesity and acute hyperlipidemia reduce IkappaBalpha levels in rat skeletal muscle in a fiber-type dependent manner. *Am J Physiol Regul Integr Comp Physiol*. (2006) 290:R233–40. doi: 10.1152/ajpregu.00097.2005

42. Hu Y, Lin H, Dib B, Atik A, Bouzika P, Lin C, et al. Cholesterol crystals induce inflammatory cytokines expression in a human retinal pigment epithelium cell line by activating the NF-κB pathway. *Discov Med*. (2014) 18:7–14.

43. Luo Z, Sun Y-Y, Xia W, Xu J-Y, Xie D-J, Jiao C-M, et al. Physical exercise reverses immuno-cold tumor microenvironment via inhibiting SQLE in non-small cell lung cancer. *Mil Med Res*. (2023) 10:39. doi: 10.1186/s40779-023-00474-8

44. Mu Y, Gao W, Zhou Y, Xiao L, Xiao Y. Physiological and pathological/ectopic mineralization: from composition to microstructure. *Microstructures*. (2023) 3:2023030. doi: 10.20517/microstructures.2023.05

45. Chung SW, Oh JH, Gong HS, Kim JY, Kim SH. Factors affecting rotator cuff healing after arthroscopic repair: osteoporosis as one of the independent risk factors. *Am J Sports Med*. (2011) 39:2099–107. doi: 10.1177/0363546511415659

46. Hong J-P, Huang S-W, Lee C-H, Chen H-C, Charoenpong P, Lin H-W. Osteoporosis increases the risk of rotator cuff tears: a population-based cohort study. *J Bone Miner Metab*. (2022) 40:348–56. doi: 10.1007/s00774-021-01293-4

47. Mao W, Cai Y, Chen D, Jiang G, Xu Y, Chen R, et al. Statin shapes inflamed tumor microenvironment and enhances immune checkpoint blockade in non–small cell lung cancer. *JCI Insight*. (2022) 7:e161940. doi: 10.1172/jci.insight.161940

48. Dolkart O, Liron T, Chechik O, Somjen D, Brosh T, Maman E, et al. Statins enhance rotator cuff healing by stimulating the COX2/PGE2/EP4 pathway: an in vivo and *in vitro* study. *Am J Sports Med*. (2014) 42:2869–76. doi: 10.1177/0363546514545856

49. Hao L, Chen J, Shang X, Chen S. Surface modification of the simvastatin factor-loaded silk fibroin promotes the healing of rotator cuff injury through β-catenin signaling. *J Biomater Appl*. (2021) 36:210–8. doi: 10.1177/0885328221995926

50. Lee S, Lee N, Shin S-J. Relationship of missed statin therapy and 10-year atherosclerotic cardiovascular disease risk score to Retear rate after arthroscopic rotator cuff repair. *Am J Sports Med*. (2023) 51:1988–96. doi: 10.1177/03635465231175476

51. de Oliveira LP, Vieira CP, Guerra FD, Almeida MS, Pimentel ER. Structural and biomechanical changes in the Achilles tendon after chronic treatment with statins. *Food Chem Toxicol*. (2015) 77:50–7. doi: 10.1016/j.fct.2014.12.014

52. Oliveira LP, Vieira CP, Marques PP, Pimentel ER. Do different tendons exhibit the same response following chronic exposure to statins? *Can J Physiol Pharmacol*. (2017) 95:333–9. doi: 10.1139/cjpp-2016-0133

53. Kaleağasıoğlu F, Olcay E, Olgaç V. Statin-induced calcific Achilles tendinopathy in rats: comparison of biomechanical and histopathological effects of simvastatin, atorvastatin and rosuvastatin. *Knee Surg Sports Traumatol Arthrosc*. (2017) 25:1884–91. doi: 10.1007/s00167-015-3728-z

54. Eliasson P, Dietrich-Zagonel F, Lundin A-C, Aspenberg P, Wolk A, Michaëlsson K. Statin treatment increases the clinical risk of tendinopathy through matrix metalloproteinase release - a cohort study design combined with an experimental study. *Sci Rep*. (2019) 9:17958. doi: 10.1038/s41598-019-53238-7

55. Eliasson P, Svensson RB, Giannopoulos A, Eismark C, Kjær M, Schjerling P, et al. Simvastatin and atorvastatin reduce the mechanical properties of tendon constructs *in vitro* and introduce catabolic changes in the gene expression pattern. *PLoS One*. (2017) 12:e0172797. doi: 10.1371/journal.pone.0172797

56. Zeng GJS, Lee MJH, Chen JY, Ang BFH, Hao Y, Lie DTT. Dyslipidemia with perioperative statin usage is not associated with poorer 24-month functional outcomes after arthroscopic rotator cuff surgery. *Am J Sports Med*. (2020) 48:2518–24. doi: 10.1177/0363546520937266

57. Cai J, Xu J, Ye Z, Wang L, Zheng T, Zhang T, et al. Exosomes derived from Kartogenin-preconditioned mesenchymal stem cells promote cartilage formation and collagen maturation for enthesis regeneration in a rat model of chronic rotator cuff tear. *Am J Sports Med*. (2023) 51:1267–76. doi: 10.1177/03635465231155927

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Multifaceted oncostatin M: novel roles and therapeutic potential of the oncostatin M signaling in rheumatoid arthritis

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Rheumatoid arthritis (RA) is a self-immune inflammatory disease characterized by joint damage. A series of cytokines are involved in the development of RA. Oncostatin M (OSM) is a pleiotropic cytokine that primarily activates the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway, the mitogen-activated protein kinase (MAPK) signaling pathway, and other physiological processes such as cell proliferation, inflammatory response, immune response, and hematopoiesis through its receptor complex. In this review, we first describe the characteristics of OSM and its receptor, and the biological functions of OSM signaling. Subsequently, we discuss the possible roles of OSM in the development of RA from clinical and basic research perspectives. Finally, we summarize the progress of clinical studies targeting OSM for the treatment of RA. This review provides researchers with a systematic understanding of the role of OSM signaling in RA, which can guide the development of drugs targeting OSM for the treatment of RA.

KEYWORDS

oncostatin M (OSM), rheumatoid arthritis (RA), fibroblast-like synoviocytes (FLS), cytokines, cartilage and bone destruction, $CD4^+$ T cell, pannus formation

1 Introduction

Rheumatoid arthritis (RA) is an autoimmune disease of unknown etiology, which is believed to be associated with genetic, environmental, and immunological factors (1). The primary clinical manifestation of RA is symmetrical joint inflammation, which is based on synovitis, bone, and cartilage destruction (2). Cytokines play a crucial role in the pathogenesis of RA and are of paramount importance in RA pathology (3, 4). Cytokines

such as tumor necrosis α (TNF-α), interleukin 6 (IL-6), IL-1, IL-17, and IL-10 promote or inhibit the occurrence and development of RA. Fibroblast-like synoviocytes (FLS) and synovial macrophages in the joint cavity of RA patients secrete a large number of pro-inflammatory cytokines and chemokines, which are important mechanisms leading to joint inflammation, bone destruction, and angiogenesis in RA patients (5–7). Therefore, ongoing research is revealing the relationship between cytokines and RA, with the aim of further exploring the molecular mechanisms underlying RA, discovering new disease activity biomarkers, and even finding new targets for treating RA.

As a member of the IL-6 family, oncostatin M (OSM) has gradually received attention for its role and mechanism in the occurrence of rheumatoid arthritis (RA) (8). OSM is a pleiotropic cytokine that participates in the regulation of multiple signaling pathways and plays an important role in the pathogenesis of various autoimmune diseases (9). It has been observed that RA patients have high levels of OSM in their synovial fluid, and further research has found that OSM can promote the occurrence and development of RA through multiple pathways (9, 10). The present paper intends to introduce the research progress of OSM at the current stage from the aspects of OSM and its receptor complex, OSM signal transduction, OSM's biological functions, and the relationship between OSM and the pathogenesis of RA.

2 OSM and OSM receptor complex

The IL-6 cytokine family is one of the largest cytokine families, and the common feature of cytokines in this family is the presence of the signal receptor subunit gp130 in their receptor complexes. These cytokines include IL-6, IL-11, ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), OSM, IL-12, cardiotrophin-1 (CT-1), cardiotrophin-like cytokine factor 1 (CLCF1), and IL-27 (11). It is worth noting that the receptor complex of the cytokine IL-31 contains a gp130-like receptor (GPL), so some researchers consider IL-31 as part of the IL-6 cytokine family. OSM is an important member of the IL-6 family of cytokines, first discovered by Zarling et al. in the U-937 human lymphoma cell line (12). The human OSM gene is located in the chromosome 22q12.2 region, and the OSM polypeptide transcribed from the human OSM gene includes 252 amino acid residues, with N- and C-terminals consisting of 25 and 32 amino acid residues, respectively. After proteolytic processing, only 195 amino acid residues are retained. The mature human OSM protein has a molecular weight of 28 kDa, and its structure consists of four α-helices arranged in an "up-up-down-down" topology (13–15). OSM is widely expressed *in vivo*, and many immune cells such as T cells, monocytes/macrophages, and neutrophils can express OSM (16–19).

As mentioned earlier, the receptor complexes of the IL-6 family of receptors all contain the gp130 subunit (20). The receptor complexes of OSM are all heterodimers, and depending on the different second subunits in the receptor complexes, the receptor complexes can be divided into two types: type I and type II (21). The type I OSM receptor complex is composed of the α subunit gp130 and the β subunit LIFRβ (LIF receptor β subunit), while the type II OSM receptor complex is composed of the a subunit gp130 and the b subunit OSMRb (OSM receptor b subunit) (Figure 1) (22). To avoid confusion, in this article, OSMR specifically refers to the β subunit of the type II OSM receptor complex, while OSM receptor refers to both type I and type II OSM receptor complexes.

It is worth noting that the two subunits of the OSM receptor complex exist separately in the resting state. When both OSM and the two subunits of the OSM receptor complex are present, OSM first forms a low-affinity heterodimer with gp130, and then this heterodimer recruits OSMR or LIFR and binds to it (9). Further research through computational simulations has identified several key amino acid residues involved in the binding process between OSM and OSMR (23). Additionally, research using computational

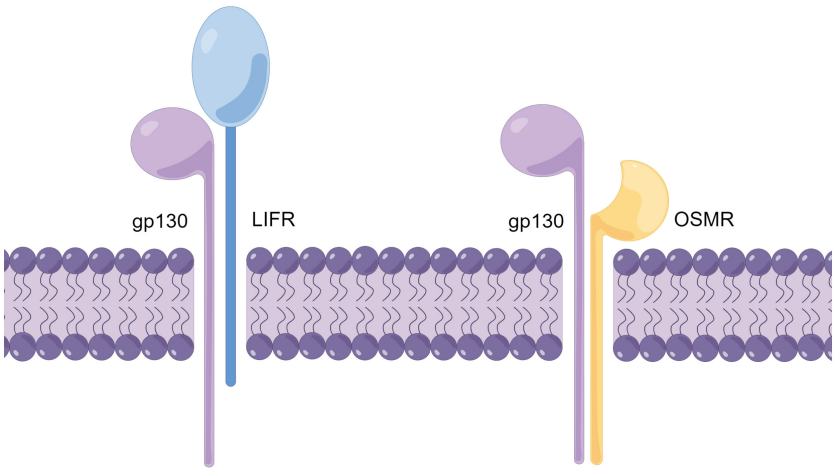

FIGURE 1
Schematic diagram of the OSM receptor complex structure (By Figdraw.). The OSM receptor complex is a heterodimer and can be classified into two types (Type I and Type II), with different subunits comprising each receptor complex. The Type I OSM receptor complex is composed of gp130 and LIFR, while the Type II OSM receptor complex is composed of gp130 and OSMR. The schematic diagram illustrates the structure of the OSM receptor complex.

simulations has revealed that the phenomenon of OSM and LIF sharing the LIFR receptor is related to the structural similarity between OSM and LIF (24). The binding of OSM and the OSM receptor complex is species-specific, and OSM from mice generally only binds to type II OSM receptor complexes. Only high concentrations of mouse-derived OSM can weakly activate LIFR, but there is also a single report that mouse-derived OSM can activate LIFR in mouse osteoblasts (Figure 2) (9, 25, 26). In contrast, OSM from rats or humans can bind to type I or type II OSM receptor complexes of the same species (27). Overall, OSMR as a unique subunit in the OSM receptor complex enables type II OSM receptor complexes to activate downstream pathways different from other IL-6 family signaling pathways, thereby exerting its unique biological functions.

3 OSM signaling transduction

The two subunits of the OSM receptor, like other members of the IL-6 family of receptors, have transmembrane structures. When the extracellular region binds to OSM, the intracellular region recruits and activates the Janus kinase (JAK) family. After binding to its receptor complex, the OSM receptor complex can activate JAK1 through gp130, and promote the phosphorylation and dimerization of the transcription factor signal transducer and activator of transcription (STAT), which is the downstream of JAK (20). The STAT protein then enters the nucleus and regulates the expression of target genes. In addition, OSM signaling can also trigger the RAS/mitogen-activated protein kinase (MAPK) signaling pathway, the c-Jun N-terminal kinase (JNK)/p38 MAPK signaling pathway, and the phosphatidylinositol 3-kinase/serine-threonine kinase (PI3K/AKT) signaling pathway (28, 29). It is worth noting that the OSM receptor complex of type II OSM receptor complex has the subunit OSMR, which is only present in the OSM receptor, unlike other cytokines in the IL-6 family. Therefore, OSM can exert its unique biological regulatory function through the type II OSM receptor complex. Like type I receptor complexes, type II receptor complexes of OSM can also bind and activate JAK, but the binding affinity of OSM's type II receptor complex with JAK1 or JAK2 is equivalent, while type I receptor complexes of OSM can bind JAK1 with high affinity (30).

4 The physiological functions of OSM signaling

The physiological functions of OSM were first identified in the human lymphoma cell line U-937, and it was subsequently found to have growth-inhibitory effects on melanoma cells, which led to its naming (12). OSM can regulate cell proliferation by controlling cell cycle checkpoints. The cell cycle refers to the entire process between two cell divisions, which can be divided into the mitotic phase and interphase, which is further divided into the G1 phase, S phase, and G2 phase. In eukaryotes, cell cycle checkpoints regulated by cyclins and cyclin-dependent kinases (CDKs) control whether cells can progress from G1 phase to S phase (31). In breast cancer cell lines treated with OSM, the number of cells before S phase significantly

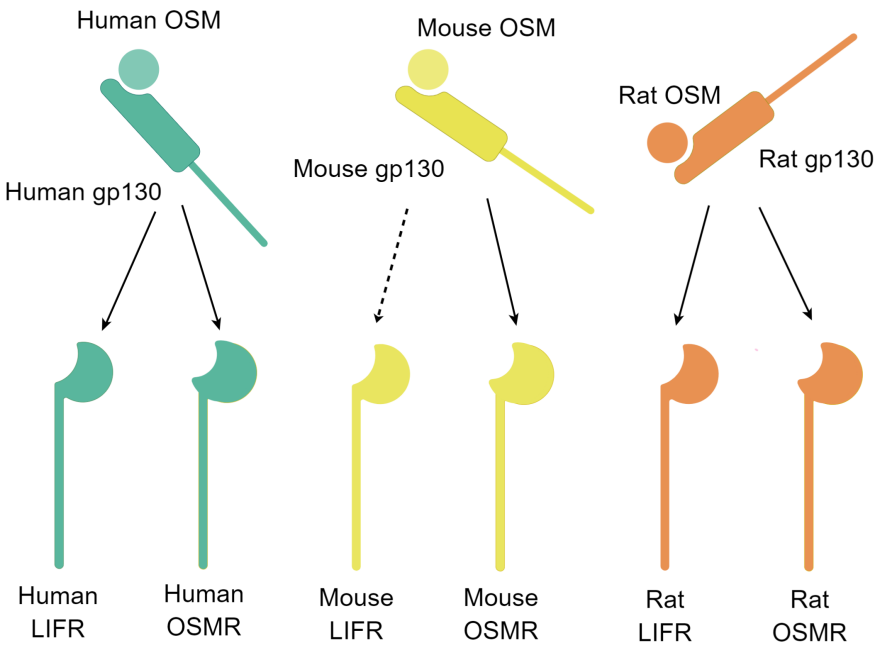

FIGURE 2
Schematic diagram of the OSM and receptor complex binding (By Figdraw.). Free OSM initially binds to gp130 and subsequently binds to another subunit, LIFR or OSMR. The binding ability of OSM and the two receptors differs among different species. The OSM-gp130 complex in humans and rats can bind to LIFR or OSMR of the same species, whereas in mice, the OSM-gp130 complex can bind to OSMR, and at high concentrations, it can also bind to LIFR (dashed line marked). The schematic diagram depicts the binding of the OSM and receptor complex.

increased, while the number of cells in S phase significantly decreased (32). Additionally, OSM treatment altered the levels of cyclins in breast cancer cell lines, indicating that OSM regulates the cell cycle by controlling cyclin levels, causing cells to stay at the cell cycle checkpoint and inhibiting the division of breast cancer cell lines. Furthermore, OSM can inhibit tumor cell division by regulating the expression levels of CDKs in melanoma cell line A375 and liver cancer cell line HepG2, hindering the tumors from progressing through the cell cycle checkpoint (33, 34). However, during liver regeneration, OSM promotes liver cell proliferation by promoting STAT3 phosphorylation (35). These studies suggest that the regulatory effects of OSM on cell proliferation are context-dependent and cannot be generalized.

In addition to its role in regulating cell proliferation, OSM also plays a regulatory role in many physiological processes, including inflammation, immune regulation, and hematopoiesis. As previously mentioned, OSM can activate inflammatory signaling pathways such as JAK/STAT and PI3K/AKT. In human vascular smooth muscle cells and mouse fibroblasts, OSM can also induce the expression of pro-inflammatory cytokine IL-6 (36, 37).

OSM also exhibits complex immunomodulatory effects. OSM has been shown to enhance innate immunity. Treatment of Huh7 liver cancer cells with OSM upregulates the expression of related innate immune molecules, intracellular adhesion molecule-1 (ICAM-1), and IL-15 receptor and enhances interferon-α-induced gene transcription, thereby enhancing interferon-α's ability to combat hepatitis A and B viruses (38). Additionally, OSM inhibits naive $CD4^+$ T cell differentiation into Th17 cells by activating suppressors of cytokine signaling 3 (SOCS3), STAT3, and STAT5 through cytokine signaling transduction (39). These findings demonstrate that OSM regulates both the innate and adaptive immune systems.

OSM is also closely related to hematopoiesis. $OSMR^{-/-}$ mice exhibit reduced numbers of peripheral circulating red blood cells and platelets, as well as a corresponding decrease in megakaryocyte-erythroid progenitor cell (MEP) numbers in the bone marrow (8, 40). During embryonic development in vertebrates, the fetal liver is a natural site for the expansion of hematopoietic stem cells, and in mice, approximately half of the hematopoietic cells reside in the liver during embryonic development. At the end of hematopoiesis, hepatic hematopoietic cells express OSM and act on OSM receptors on the fetal liver stromal cells, promoting the maturation of the fetal liver and the loss of hematopoietic support function (41).

5 OSM signaling and diseases

Abnormal levels of OSM are associated with various inflammatory diseases. Compared to healthy controls, OSM levels are elevated in the peripheral blood plasma of patients with coronavirus disease 2019 (COVID-19), and are associated with the severity of the disease (42). Colonic and serum OSM levels are not only elevated in patients with inflammatory bowel diseases (IBD) who experience postoperative recurrence, but also high levels of OSM in colonic tissue often indicate poor prognosis or insensitivity to biologic therapy in IBD patients (43).

Previously, it was mainly believed that OSM mainly affects the development of many diseases by promoting inflammation. For example, researchers have found that lipopolysaccharide (LPS) produced by respiratory microbiota imbalance can promote OSM secretion by macrophages in the respiratory tract, which not only promotes airway inflammation and mucus secretion in patients with severe asthma. However, blocking the OSM signal with OSM-specific antibodies can alleviate asthma-related pathological features without affecting important antibacterial immune responses in the body (44). In addition, OSM can also promote the expression of inflammatory factors CC chemokine ligand (CCL) 2, IL-6, and vascular endothelial growth factor (VEGF) by inducing human aortic adventitial fibroblasts and smooth muscle cells in synergy with LPS, ultimately promoting the development of atherosclerosis (45). However, some studies have shown that OSM can play an anti-inflammatory role in certain diseases. For example, OSM treatment inhibited the expression of TNF-α induced by lipopolysaccharide (LPS) in septic mice and reduced their mortality (46). OSM also exerts an anti-inflammatory effect by promoting macrophage polarization towards the M2 type in adipose tissue (47). Therefore, the regulatory effect of OSM on inflammation is quite complex, and it may have opposite effects in different diseases. It is a pleiotropic cytokine.

In addition, OSM can also affect the progression of diseases by regulating fibrosis through various pathways. Overexpression of OSM in mouse liver tissue leads to upregulation of the expression of the profibrotic cytokine transforming growth factor-β (TGF-β) in liver macrophages, which promotes liver tissue fibrosis (48). Tissue inhibitor of matrix metalloproteinase (TIMP) can inhibit the expression of matrix metallopeptidase (MMP) 1. In cardiac fibroblasts, OSM inhibits the expression of TIMP1, which promotes the deposition of local extracellular matrix, leading to tissue fibrosis (49). Some studies have also found that OSM directly binds to the extracellular matrix, which may increase the stability of the extracellular matrix and promote its deposition, indicating that OSM may directly promote fibrosis (50). The profibrotic effect of OSM may also be beneficial for disease recovery. *In vitro* studies have shown that OSM can accelerate wound healing by promoting collagen and glycosaminoglycan production in fibroblasts at the site of diabetic foot ulcers (51). Further animal experiments have found that local treatment with OSM promotes wound healing in diabetic mice (52).

Recent studies suggest that OSM may have a role in the development and progression of other diseases beyond regulating inflammation and fibrosis. For instance, OSM secreted by T cells and monocytes in the dermis may enhance the sensitivity of sensory neurons to pruritogens, leading to increased itchiness in inflammatory skin lesions (53).

It is evident that OSM, a pleiotropic cytokine, plays a crucial role in the pathogenesis of many inflammatory diseases. Numerous studies have demonstrated the significant involvement of OSM levels in rheumatic diseases. Single nucleotide polymorphisms (SNP) rs22922016 related to OSMR gene has been found to associated to systemic lupus erythematosus (SLE) (54). Moreover, OSM signaling pathway was identified as a potential pathway which related to SLE by analyzing transcriptomic and genome-wide

association studies data (55). Systemic sclerosis (SSc), a rheumatic disease characterized by skin thickening, hardening, and visceral fibrosis, is also associated with OSM signaling. Diffuse cutaneous systemic sclerosis (dcSSc) patients showed significantly elevated levels of OSM in their serum, and OSM and OSM-regulated genes were upregulated in dcSSc skin (56). Fibroblasts positive for OSM and pSTAT3 were increased in affected skin compared to unaffected skin. These findings suggest that OSM signaling activation in the skin of dcSSc patients may be one of the causes leading to downstream STAT3 signaling activation (56). In addition to SLE and SSc, there is also a close association between the OSM signaling pathway and RA. Subsequently, this article will focus on the relationship between OSM and the development of RA.

6 The clinical correlation of OSM signaling with RA

Hui et al. first investigated the levels of OSM in synovial fluid from RA patients and found that OSM was detected in 18 out of 20 RA samples, while it was not detected in 10 osteoarthritis (OA) samples used as controls. Additionally, they observed a positive correlation between OSM levels and synovial fluid white blood cell counts (57). Similarly, Cawston et al. detected elevated OSM levels only in RA patients but not in OA or healthy control samples (58). Manicourt et al. later reported a positive correlation between OSM levels in synovial fluid from RA patients and the levels of pro-inflammatory cytokines IL-6 and TNF-α, with a larger sample size (59). OSM in the joint cavity of RA patients could be derived from macrophages and neutrophils. Cawston et al. confirmed that $CD14^+$ macrophages were the primary source of OSM in synovial tissue from RA patients using immunohistochemistry (58). Cross et al. found that neutrophils from peripheral blood of RA patients rapidly expressed and released OSM upon stimulation with granulocyte-macrophage colony-stimulating factor (GM-CSF), while neutrophils from synovial fluid did not respond to GM-CSF stimulation (18). Considering that OSM is a secreted protein, neutrophils in synovial fluid from RA patients may have already released a significant amount of OSM in the joint cavity. Furthermore, genomic studies have shown that the T allele of the OSMR gene promoter region (rs22922016 locus) has a protective effect on RA patients (54). These findings suggest a close relationship between the OSM signaling pathway and the development of RA.

7 Potential effects of OSM signaling on the pathogenesis of RA

As previously mentioned, evidence from both tissue and blood samples of RA patients suggests that OSM may be involved in the pathogenesis of RA. In fact, OSM is not just a bystander in the pathogenesis of RA, but it also participates in regulating RA development through various mechanisms (Figure 3). The following section will focus on elucidating the molecular biological mechanisms through which OSM may impact RA.

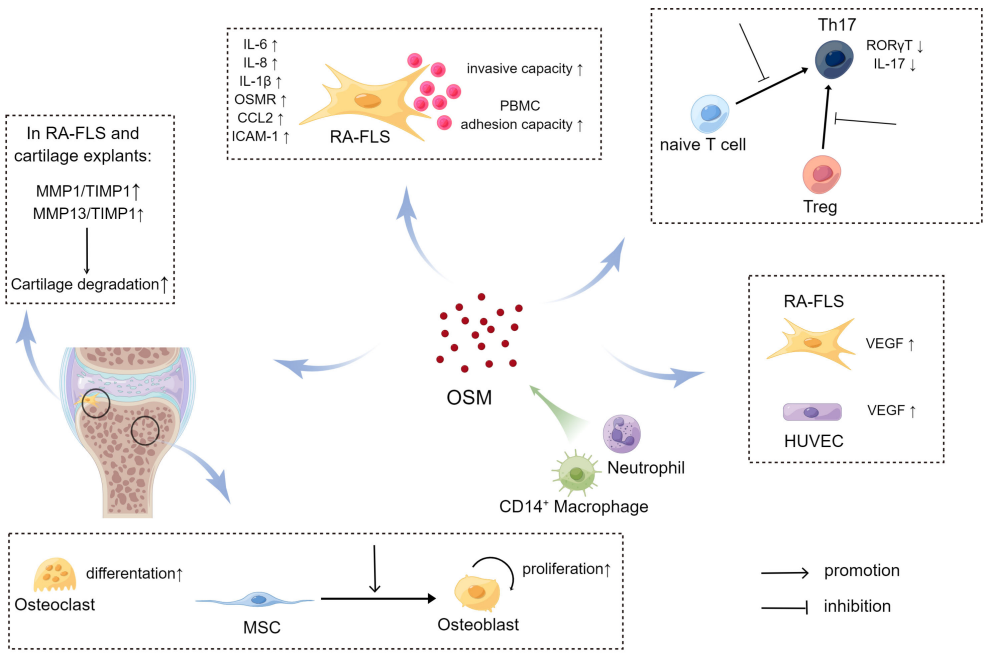

FIGURE 3
The impact of OSM on signal transduction (upper left) and metabolic profile (lower right) of RA-FLS (By Figdraw.). In RA-FLS, OSM activates JAK adjacent to the OSM receptor upon binding, followed by activation of downstream signaling pathways such as STAT and MAPK signaling, affecting the expression of pathogenic genes such as IL6 and IL8 in the cell nucleus. Tofacitinib inhibits downstream signaling pathways by blocking JAK activation. OSM signaling may activate transcription factor HIF-1α in RA-FLS and promote transcription of downstream glucose transporter (GLUT1) and glycolysis-related genes (HK2, PFKFB3), thereby increasing the ECAR/OCR of cells and promoting a shift in metabolic profile toward glycolysis in RA-FLS.

7.1 The impact of OSM signaling on RA-FLS

The synovium is a connective tissue that attaches to the surrounding cartilage of the joint and is an essential component of the joint. The synovium not only provides direct physical connections to the musculoskeletal system but also reduces friction between joints by secreting synovial fluid, provides nutrition to other tissues within the joint, and is critical for maintaining normal physiological function (60). Histologically, the human synovium can be divided into two layers: the synovial lining layer near the joint cavity and the sublining layer beneath the lining layer. Under normal conditions, the synovial lining layer consists of only 2-3 cell layers, mainly composed of synovial macrophages (SMs) and FLSs. Synovitis is one of the major pathological changes in RA, and FLSs play a key role in the pathogenesis of RA synovitis (61). After being stimulated by a series of pro-inflammatory cytokines, such as TNF-α, in the joints of RA patients, RA-FLS continue to produce inflammatory factors to maintain chronic inflammation in the synovium, while also proliferating, migrating, and invading to destroy joint cartilage tissue (62, 63).

Studies have shown that OSM can affect RA-FLS through multiple pathways. Migita et al. found *in vitro* that OSM treatment promoted JAK/STAT pathway activation by inducing JAK and STAT phosphorylation in RA-FLS, and also induced RA-FLS to secrete the pro-inflammatory cytokine IL-6 by activating the MAPK pathway (64). The JAK inhibitor tofacitinib was able to inhibit the aforementioned changes induced by OSM. A more in-depth study by Hanlon et al. found that OSM treatment not only promoted RA-FLS to secrete IL-6, but also promoted RA-FLS to secrete the pro-inflammatory cytokines IL-8, chemokine C-C Motif Chemokine Ligand 2 (CCL2), and adhesion molecule ICAM-1 (65, 66). Using mouse FLS, Goff et al. demonstrated that OSM can synergistically enhance the expression of IL-6 with other pro-inflammatory cytokines, such as TNF-α and IL-1β (67). Furthermore, OSM treatment increased the expression of OSMR and IL-1β receptors (IL-1βR) in human RA-FLS and wild-type mouse FLS, which further activated the inflammatory signaling pathway in FLS through positive feedback. Therefore, OSM stimulation of RA-FLS by inducing the secretion of a series of pro-inflammatory cytokines and chemokines aggravates synovitis and angiogenesis in RA (Figure 4). In addition to inducing RA-FLS to secrete cytokines and their receptors, OSM also increases the invasive ability of RA-FLS and the adhesion of peripheral blood mononuclear cells (PBMCs) to RA-FLS (65).

Interestingly, OSM also causes changes in the metabolic pathways of RA-FLS, known as metabolic reprogramming (Figure 4). Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) are metrics used to measure mitochondrial respiration and glycolysis, respectively. After OSM treatment, the ECAR/OCR ratio in RA-FLS

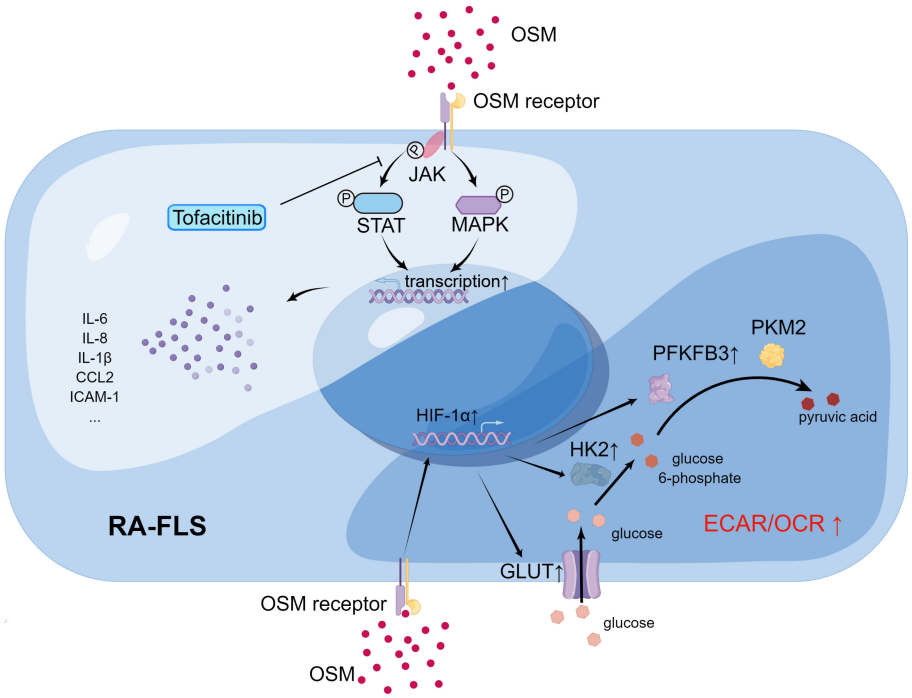

FIGURE 4
The source and pathogenic role of OSM in rheumatoid arthritis (RA) joints (By Figdraw.). In RA joints, $CD14^+$ macrophages and neutrophils are potential major sources of Oncostatin M (OSM), which plays a regulatory role in RA-FLS, bone and cartilage, pannus formation, and T cell differentiation. Through these pathways, OSM may impact the development of RA. In the case of RA-FLS, OSM promotes secretion of relevant cytokines, increases the invasive ability of RA-FLS, and enhances adhesion of RA-FLS and PBMC to promote joint damage. OSM also affects bone and cartilage metabolism. For instance, OSM enhances differentiation of MSC to osteoblasts, promotes the proliferation of osteoblasts, and stimulates the differentiation of osteoclasts. Additionally, OSM increases the secretion of MMPs, which further enhances cartilage degradation. OSM inhibits the differentiation of naive T cells to Th17 cells by suppressing the expression of RORγT. It also reduces the conversion of Treg to Th17 and decreases the secretion of IL17 from Th17 cells. Moreover, OSM promotes the secretion of VEGF, which facilitates formation of pannus.

cells increased, and OSM induced the expression of glucose transporters (GLUT)-1, hexokinase 2 (HK2), 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3), and hypoxia-inducible factor (HIF)-1α in RA-FLS, indicating that OSM promotes glycolytic metabolism in RA-FLS (65). It is worth noting that most tumor cells also rely on glycolysis to produce energy, and glycolysis-related metabolic intermediates promote the invasion and metastasis of tumor cells (68). As mentioned earlier, OSM treatment also enhances the invasive ability of RA-FLS, which is similar to tumor cells. Therefore, OSM is likely to enhance the intra-articular invasive ability of RA-FLS by promoting glycolytic metabolism in RA-FLS.

7.2 OSM signaling and bone homeostasis and cartilage metabolism

Under normal circumstances, a balance between osteoblast-mediated bone formation and osteoclast-mediated bone resorption maintains bone homeostasis. However, in patients with rheumatoid arthritis (RA), increased osteoclast generation and activity, as well as suppressed osteoblast-mediated bone formation, disrupts the normal bone homeostasis, leading to bone and cartilage destruction and bone loss (69). OSM has biological functions that regulate bone metabolism, which can be seen in its regulation of mesenchymal stem cell osteogenic differentiation and its modulation of osteoblast and osteoclast-mediated bone formation and resorption.

Studies have found that OSM is capable of promoting mesenchymal stem cell differentiation into osteoblasts. It has been shown that treatment with OSM can activate JAK, MAPK and other signaling molecules, promoting osteogenic differentiation of human or mouse adipose-derived mesenchymal stem cells (70, 71). Similarly, in mouse fibroblast cell line C3H10T1/2, OSM treatment can induce osteogenic differentiation and promote terminal calcium deposition and mineralized nodule formation (72). Synovial-derived mesenchymal stem cells (SMSCs) in synovial fluid also have the potential to differentiate into osteoblasts, and studies have attempted to induce SMSCs to differentiate in a directed manner for the treatment of joint bone defects (73). Therefore, it is reasonable to assume that higher levels of OSM in RA joints can promote osteogenic differentiation of SMSCs.

OSM also has the ability to promote the proliferation and osteogenic differentiation of osteoblasts. OSM can promote osteogenic differentiation of mouse embryo osteoblast precursor cells MC3T3-E1 by regulating CCL2 (74). Additionally, OSM can promote osteoclast differentiation. Studies have found that OSM, in combination with pro-inflammatory cytokines TNF-α or IL-1β, can activate the RANKL-RANK signaling pathway and significantly increase the number of TRAP-positive cells (i.e. osteoclasts) in mouse joints (75).

The aforementioned studies collectively indicate that OSM has a promoting effect on both bone formation and bone resorption. Therefore, *in vivo* studies exploring the comprehensive effects of OSM on bone formation and resorption are necessary. Previous studies have found that injection of OSM into mice with tibial injury can induce intra-membrane bone formation, suggesting that OSM promotes bone formation in mice (76). However, in a mouse model of arthritis, adenoviral overexpression of joint OSM promotes bone erosion, suggesting that excessive OSM can also contribute to pathological bone destruction (8, 75). Taken together, these two studies suggest that OSM may overall promote bone destruction in the inflammatory environment of arthritis.

Joint damage in RA patients is also closely related to cartilage destruction. High levels of MMPs in the joint cavity of RA patients can degrade the extracellular matrix and absorb cartilage, thereby promoting joint destruction (77). OSM can also regulate cartilage metabolism. Collagen fibers and proteoglycans are the main structural substances of joint cartilage. Clinical studies have found a positive correlation between OSM levels in the synovial fluid of RA patients and the levels of antigenic keratan sulfate (Ag KS) and D-pyridinoline (D-Pyr), suggesting that OSM may promote the degradation of proteoglycans and collagen in joint cartilage of RA patients (59). *In vitro* experiments have shown that OSM treatment of pig joint cartilage explants increases the release of proteoglycans while inhibiting synthesis (78). MMPs can degrade cartilage by cleaving collagen. Using co-cultures of RA-FLS and normal human joint cartilage explants, Fearon et al. found that OSM treatment increased the levels of MMP1 and the MMP inhibitor TIMP1, both of which can degrade the extracellular matrix (79). However, the ratio of MMP1 to TIMP1 increased after OSM treatment, and compared to the use of a single cytokine, the combined use of OSM and IL-1β significantly increased the ratio of MMP1 to TIMP1 and MMP13 to TIMP1. These results suggest that in an inflammatory environment, OSM can promote cartilage degradation.

7.3 OSM signaling and pannus formation

Pannus formation is another major pathological change in RA. As synovitis progresses in RA patients, a large number of immune cells infiltrate, the synovial lining layer proliferates and transforms into pannus, extending and adhering to cartilage, promoting the degradation of cartilage and bone tissue (80). Pannus consists of neovascularization, proliferating synovial cells, immune cells, and fibrinoid deposits, and angiogenesis mediated by cell factors such as VEGF is one of the important factors in angiogenesis formation (81, 82). Hanlon et al. found that OSM treatment not only promotes RA-FLS and human umbilical vein endothelial cells to secrete pro-inflammatory cytokines such as IL-6 and CCL2, but also promotes the expression of VEGF (65). Furthermore, OSM and IL-1β combined treatment significantly upregulated the levels of VEGF in the co-culture supernatant of RA-FLS and normal human joint cartilage explants (79). Therefore, it can be seen that OSM can enhance angiogenesis by promoting the expression of VEGF, thus exacerbating the formation of angiogenesis in synovial tissue of RA.

7.4 OSM signaling and $CD4^+$ T cells

The imbalance of $CD4^+$ T cell subsets also plays a significant role in the pathogenesis of RA. Among the various $CD4^+$ T cell

subsets, the imbalance between the pro-inflammatory Th17 subset and the immunosuppressive regulatory T (Treg) subset is closely related to the development and progression of RA. Currently, there are not many studies on the effects of OSM on $CD4^+$ T cell differentiation and function. One study found that OSM treatment inhibited the differentiation of $CD4^+$ T cells from mouse spleen into Th17 cells, suppressed Th17-related cytokine IL-17, and the Th17-specific transcription factor retinoic acid-related orphan receptor γt (RORγt) expression (39). Further research found that OSM inhibited $CD4^+$ T cell differentiation into Th17 cells by activating STAT3, STAT5, and suppressor of cytokine signaling (SOCS) 3 signaling in a dose-dependent manner. In addition, OSM also inhibited the proliferation of Th17 cells and the conversion of Treg cells into Th17 cells. Similarly, Shirshev et al. found that combined treatment with sex hormones and OSM increased the ratio of Treg cells in $CD4^+$ T cells derived from PBMCs, which helps to maintain pregnancy-related immune tolerance (83).

From the perspective of the effect of OSM on $CD4^+$ T cell subset differentiation, OSM can alleviate RA inflammatory responses. However, this contradicts many clinical studies and animal experiments. Considering that OSM is a pleiotropic cytokine, we speculate that although OSM inhibits Th17 cell differentiation, it also has the effect of promoting the secretion of inflammatory cytokines, promoting bone destruction and angiogenesis in RA. Overall, OSM still promotes the pathogenesis of RA.

8 The OSM signaling pathway in arthritis animal models

The aforementioned studies suggest that OSM is a pleiotropic cytokine, thus it is necessary to examine its overall regulatory effect in animal models of arthritis. Currently, most studies on animal models of arthritis suggest that OSM promotes the onset and progression of arthritis. In mice with collagen-induced arthritis (CIA), the mRNA levels of OSM in joint tissues are elevated, and the severity of arthritis and histopathological changes can be improved by neutralizing OSM with antibodies (84). In mice with pristane-induced arthritis (PIA), preemptive OSM neutralization with antibodies can prevent the onset of arthritis symptoms (84). Local overexpression of OSM in mouse joints using adenoviral vectors leads to synovial hyperplasia, mononuclear cell infiltration, and cartilage damage (85). In mice with antigen-induced arthritis (AIA), the expression levels of OSM and its type I and II receptors are elevated (67). In a rat model of peptidoglycan-polysaccharide (PGPS)-induced arthritis, OSM levels in the synovial membrane are higher than those in the control group, and the levels of OSM in the knee joints of rats are positively correlated with the Krenn score (used to evaluate synovitis histopathology) and the Mankin score (used to evaluate cartilage damage) (86). However, some studies have also shown that OSM can improve joint lesions in animal models of arthritis. Wahl et al. used four different monoclonal antibodies against collagen and LPS to construct a mouse model of arthritis and found that the mice in the arthritis group exhibited joint pathology changes such as angiogenesis, cartilage and bone erosion, while the joint pathology changes in the OSM-treated group were minimal (46). This result is obviously contradictory to other relevant studies. However, it is worth noting that Wahl et al. used human OSM in their study, which cannot bind to mouse OSMR and activate downstream signaling pathways, but can bind to receptors such as LIFR in mice and exert anti-inflammatory effects (9, 29).

9 Research on targeted OSM signaling therapy for patients with RA

There have been studies attempting to treat RA patients by antagonizing OSM signaling. Choy et al. attempted to construct a humanized monoclonal antibody against OSM (GSK315234) and conducted a Phase II clinical trial to test its efficacy for treating RA patients (87). The results showed that RA patients had good tolerance to GSK315234, and common adverse events included aggravation of RA, elevated alanine aminotransferase, fever, headache, hypertension, and diarrhea, but no one withdrew from the study due to adverse events. However, the therapeutic effect of GSK315234 on RA was not very satisfactory. In the multi-dose, intravenous infusion trial, GSK315234 improved the disease activity score using 28 joint counts (DAS28) in RA; in the single-dose, intravenous infusion control trial, there was no difference in DAS28 between the treatment group and the placebo group; in the single-dose, subcutaneous injection trial, there was a statistically significant difference in DAS28 between the treatment group and the placebo group. In all three clinical trials, there was no statistically significant difference in erythrocyte sedimentation rate or C-reactive protein between the treatment group and the placebo group. Pharmacokinetic and pharmacodynamic analyses revealed that the low affinity of GSK315234 for OSM may be the main reason for its unsatisfactory therapeutic effect on RA.

Besides GSK315234, there are other monoclonal antibodies targeting the OSM signaling pathway. GSK2330811, another anti-OSM monoclonal antibody, has demonstrated sufficient affinity and good tolerability in healthy individuals and has been explored for the treatment of SSc and Crohn's disease (88). In a completed multicenter, randomized, double-blind, placebo-controlled clinical trial, GSK2330811 was administered at doses of 100 mg or 300 mg weekly for 12 weeks in the treatment of SSc. However, the trial found that changes in inflammation or fibrosis biomarkers in SSc subjects following GSK2330811 treatment were not ideal. Additionally, GSK2330811 led to dose-dependent decreases in hemoglobin or platelet counts in 39% and 26% of the subjects, respectively. Overall, GSK2330811 does not appear to be an ideal choice for treating SSc. Another multicenter, randomized clinical trial (NCT04151225), which aimed to use GSK2330811 for the treatment of Crohn's disease, has been canceled by the sponsor due to a potentially narrow therapeutic window, and no relevant study data have been disclosed.

There are also studies attempting to target OSMR (OSM receptor β subunit) for the development of monoclonal antibodies to treat related diseases. Vixarelimab, also known as KPL-716, is a monoclonal antibody developed against OSMR. It has shown significant reduction in skin itchiness and improvement in quality of life caused by prurigo nodularis in clinical trials (89). Vixarelimab also demonstrated notable improvements in excessive skin keratinization and nodules in prurigo nodularis subjects. Furthermore, compared to the placebo group, the vixarelimab-treated group did not exhibit an increased incidence of overall infections, immune reactions, liver function abnormalities, hematological changes, malignancies, injection site reactions, or drug-related adverse events related to cardiac toxicity. However, the vixarelimab-treated group had a higher incidence of upper respiratory tract infections. Overall, vixarelimab, targeting OSMR, has shown significant efficacy and relatively sufficient drug safety for the treatment of prurigo nodularis, making it a potential emerging therapy for this condition. The relevant information of existing monoclonal antibodies targeting OSM has been summarized in Table 1.

It is worth mentioning that researchers are also developing small molecule drugs targeting OSM and OSMR. SMI-10B is a small molecule drug predicted through screening to target the OSM signaling pathway (90). The drug developers claim that further experiments have shown that this small molecule can significantly reduce OSM-induced STAT3 phosphorylation in cancer cells. Recently, additional unbiased molecular dynamics studies have revealed that SMI-10B can spontaneously bind to OSM (91). Compared to monoclonal antibodies, small molecule inhibitors, as clinical drugs, generally exhibit lower toxicity and antigenicity, as well as better patient compliance when administered orally. Therefore, it is promising to expect the use of small molecule inhibitors targeting the OSM signaling pathway in RA or other related diseases, although further clinical trials are needed to evaluate their therapeutic efficacy.

10 Conclusions

OSM, a secreted cytokine, can regulate JAK/STAT, RAS/MAPK, JNK/p38 MAPK, and PI3K/AKT signaling pathways. OSM can regulate physiological processes such as cell proliferation, inflammation, immune response, and hematopoiesis, and is associated with the pathogenesis of various diseases. In RA patients, the gene polymorphism of OSMR is related to disease activity, and OSM levels in synovial fluid and synovium are higher than those in healthy controls. Existing research indicates that OSM mainly affects the occurrence and development of RA by promoting the secretion of pro-inflammatory cytokines and chemokines by RA-FLS, enhancing the invasive ability of RA-FLS, promoting the adhesion of other immune cells to RA-FLS, regulating bone formation and bone destruction in RA joints, promoting angiogenesis, and regulating the differentiation of $CD4^+$ T cells. Although OSM has pleiotropic effects, considering clinical studies and animal models of arthritis, it is clear that OSM overall promotes the occurrence and development of RA.

However, it should be noted that the binding of OSM to its receptor has species specificity, which has affected some previous related studies. Currently, research on RA still cannot avoid animal models of arthritis, and the issue of OSM species specificity will be difficult to avoid in the future. Therefore, future researchers should provide detailed information on the species of the reagents used in experiments, and the interpretation of research results should also consider the species specificity of OSM.

Although the clinical study of the anti-OSM monoclonal antibody GSK315234 for treating RA patients did not achieve good results, this is related to the low affinity of GSK315234 for OSM. Future clinical trial research should learn from this lesson and develop anti-OSM antibodies and small molecule inhibitors with high affinity for use in clinical trials to better explore the potential of antagonizing OSM signaling for treating RA and provide new tools for clinical drug treatment of RA.

TABLE 1 Monoclonal antibody drugs targeting Oncostatin M signaling.

Name	Target	Diseases	National Clinical Trial number
GSK315234	OSM	rheumatoid arthritis	NCT00674635
GSK2330811		systemic sclerosis	NCT03041025
		Crohn's disease	NCT04151225
Vixarelimab/ KPL-716	OSMR	prurigo nodularis	NCT03816891

OSM, Oncostatin M; OSMR, Oncostatin M rreceptor β subunit.

Author contributions

LH: Data curation, Investigation, Writing – original draft. JY: Investigation, Visualization, Writing – review & editing. TL: Investigation, Visualization, Writing – review & editing. WL: Investigation, Writing – review & editing. YaH: Writing – review & editing, Funding acquisition. PS: Writing – review & editing, Funding acquisition. XB: Writing – review & editing. YiH: Writing – review & editing, Funding acquisition. KQ: Writing – review & editing, Funding acquisition. YG: Visualization, Writing – review & editing. HW: Visualization, Writing – review & editing. KZ: Visualization, Writing – review & editing. YL: Writing – review & editing. YW: Writing – review & editing. ZC: Writing – review & editing, Funding acquisition. ST: Supervision, Writing – review & editing, Funding acquisition.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Scherer HU, Häupl T, Burmester GR. The etiology of rheumatoid arthritis. *J Autoimmun* (2020) 110:102400. doi: 10.1016/j.jaut.2019.102400

2. Aletaha D, Smolen JS. Diagnosis and management of rheumatoid arthritis: A review. *Jama* (2018) 320:1360–72. doi: 10.1001/jama.2018.13103

3. Chen Z, Bozec A, Ramming A, Schett G. Anti-inflammatory and immune-regulatory cytokines in rheumatoid arthritis. *Nat Rev Rheumatol* (2019) 15:9–17. doi: 10.1038/s41584-018-0109-2

4. Ridgley LA, Anderson AE, Pratt AG. What are the dominant cytokines in early rheumatoid arthritis? *Curr Opin Rheumatol* (2018) 30:207–14. doi: 10.1097/bor.0000000000000470

5. Leech MT, Morand EF. Fibroblasts and synovial immunity. *Curr Opin Pharmacol* (2013) 13:565–9. doi: 10.1016/j.coph.2013.04.001

6. Boutet MA, Courties G, Nerviani A, Le Goff B, Apparailly F, Pitzalis C, et al. Novel insights into macrophage diversity in rheumatoid arthritis synovium. *Autoimmun Rev* (2021) 20:102758. doi: 10.1016/j.autrev.2021.102758

7. Nygaard G, Firestein GS. Restoring synovial homeostasis in rheumatoid arthritis by targeting fibroblast-like synoviocytes. *Nat Rev Rheumatol* (2020) 16:316–33. doi: 10.1038/s41584-020-0413-5

8. West NR, Owens BMJ, Hegazy AN. The oncostatin M-stromal cell axis in health and disease. *Scand J Immunol* (2018) 88:e12694. doi: 10.1111/sji.12694

9. Richards CD. The enigmatic cytokine oncostatin m and roles in disease. *ISRN Inflammation* (2013) 2013:512103. doi: 10.1155/2013/512103

10. Stawski L, Trojanowska M. Oncostatin M and its role in fibrosis. *Connect Tissue Res* (2019) 60:40–9. doi: 10.1080/03008207.2018.1500558

11. Kim JW, Marquez CP, Sperberg RAP, Wu J, Bae WG, Huang PS, et al. Engineering a potent receptor superagonist or antagonist from a novel IL-6 family cytokine ligand. *Proc Natl Acad Sci USA* (2020) 117:14110–8. doi: 10.1073/pnas.1922729117

12. Zarling JM, Shoyab M, Marquardt H, Hanson MB, Lioubin MN, Todaro GJ. Oncostatin M: a growth regulator produced by differentiated histiocytic lymphoma cells. *Proc Natl Acad Sci USA* (1986) 83:9739–43. doi: 10.1073/pnas.83.24.9739

13. Bravo J, Heath JK. Receptor recognition by gp130 cytokines. *EMBO J* (2000) 19:2399–411. doi: 10.1093/emboj/19.11.2399

14. Deller MC, Hudson KR, Ikemizu S, Bravo J, Jones EY, Heath JK. Crystal structure and functional dissection of the cytostatic cytokine oncostatin M. *Structure* (2000) 8:863–74. doi: 10.1016/s0969-2126(00)00176-3

15. Plun-Favreau H, Perret D, Diveu C, Froger J, Chevalier S, Lelièvre E, et al. Leukemia inhibitory factor (LIF), cardiotrophin-1, and oncostatin M share structural binding determinants in the immunoglobulin-like domain of LIF receptor. *J Biol Chem* (2003) 278:27169–79. doi: 10.1074/jbc.M303168200

16. Pothoven KL, Norton JE, Suh LA, Carter RG, Harris KE, Biyasheva A, et al. Neutrophils are a major source of the epithelial barrier disrupting cytokine oncostatin M in patients with mucosal airways disease. *J Allergy Clin Immunol* (2017) 139:1966–78.e9. doi: 10.1016/j.jaci.2016.10.039

17. Hergovits S, Mais C, Haan C, Costa-Pereira AP, Hermanns HM. Oncostatin M induces RIG-I and MDA5 expression and enhances the double-stranded RNA response in fibroblasts. *J Cell Mol Med* (2017) 21:3087–99. doi: 10.1111/jcmm.13221

18. Cross A, Edwards SW, Bucknall RC, Moots RJ. Secretion of oncostatin M by neutrophils in rheumatoid arthritis. *Arthritis Rheum* (2004) 50:1430–6. doi: 10.1002/art.20166

19. Torossian F, Guerton B, Anginot A, Alexander KA, Desterke C, Soave S, et al. Macrophage-derived oncostatin M contributes to human and mouse neurogenic heterotopic ossifications. *JCI Insight* (2017) 2:e96034. doi: 10.1172/jci.insight.96034

20. Rose-John S. Interleukin-6 family cytokines. *Cold Spring Harb Perspect Biol* (2018) 10:a028415. doi: 10.1101/cshperspect.a028415

21. Auguste P, Guillet C, Fourcin M, Olivier C, Veziers J, Pouplard-Barthelaix A, et al. Signaling of type II oncostatin M receptor. *J Biol Chem* (1997) 272:15760–4. doi: 10.1074/jbc.272.25.15760

22. Tanaka M, Hara T, Copeland NG, Gilbert DJ, Jenkins NA, Miyajima A. Reconstitution of the functional mouse oncostatin M (OSM) receptor: molecular cloning of the mouse OSM receptor beta subunit. *Blood* (1999) 93:804–15. doi: 10.1182/blood.V93.3.804

23. Du Q, Qian Y, Xue W. Molecular simulation of oncostatin M and receptor (OSM-OSMR) interaction as a potential therapeutic target for inflammatory bowel disease. *Front Mol Biosci* (2020) 7:29. doi: 10.3389/fmolb.2020.00029

24. Du Q, Qian Y, Xue W. Cross-reactivity of two human IL-6 family cytokines OSM and LIF explored by protein-protein docking and molecular dynamics simulation. *Biochim Biophys Acta Gen Subj* (2021) 1865:129907. doi: 10.1016/j.bbagen.2021.129907

25. Adrian-Segarra JM, Schindler N, Gajawada P, Lörchner H, Braun T, Pöling J. The AB loop and D-helix in binding site III of human Oncostatin M (OSM) are required for OSM receptor activation. *J Biol Chem* (2018) 293:7017–29. doi: 10.1074/jbc.RA118.001920

26. Walker EC, McGregor NE, Poulton IJ, Solano M, Pompolo S, Fernandes TJ, et al. Oncostatin M promotes bone formation independently of resorption when signaling through leukemia inhibitory factor receptor in mice. *J Clin Invest* (2010) 120:582–92. doi: 10.1172/jci40568

27. Drechsler J, Grötzinger J, Hermanns HM. Characterization of the rat oncostatin M receptor complex which resembles the human, but differs from the murine cytokine receptor. *PloS One* (2012) 7:e43155. doi: 10.1371/journal.pone.0043155

28. Kurosawa T, Yamada A, Takami M, Suzuki D, Saito Y, Hiranuma K, et al. Expression of nephronectin is inhibited by oncostatin M *via* both JAK/STAT and MAPK pathways. *FEBS Open Bio* (2015) 5:303–7. doi: 10.1016/j.fob.2015.04.001

29. Houben E, Hellings N, Broux B, Oncostatin M. an underestimated player in the central nervous system. *Front Immunol* (2019) 10:1165. doi: 10.3389/fimmu.2019.01165

30. Hermanns HM. Oncostatin M and interleukin-31: Cytokines, receptors, signal transduction and physiology. *Cytokine Growth Factor Rev* (2015) 26:545–58. doi: 10.1016/j.cytogfr.2015.07.006

31. Swaffer MP, Jones AW, Flynn HR, Snijders AP, Nurse P. CDK substrate phosphorylation and ordering the cell cycle. *Cell* (2016) 167:1750–61.e16. doi: 10.1016/j.cell.2016.11.034

32. Douglas AM, Grant SL, Goss GA, Clouston DR, Sutherland RL, Begley CG. Oncostatin M induces the differentiation of breast cancer cells. *Int J Cancer* (1998) 75:64–73. doi: 10.1002/(sici)1097-0215(19980105)75:1<64::aid-ijc11>3.0.co;2-d

33. Kortylewski M, Heinrich PC, Mackiewicz A, Schniertshauer U, Klingmüller U, Nakajima K, et al. Interleukin-6 and oncostatin M-induced growth inhibition of human A375 melanoma cells is STAT-dependent and involves upregulation of the cyclin-dependent kinase inhibitor p27/Kip1. *Oncogene* (1999) 18:3742–53. doi: 10.1038/sj.onc.1202708

34. Klausen P, Pedersen L, Jurlander J, Baumann H. Oncostatin M and interleukin 6 inhibit cell cycle progression by prevention of p27kip1 degradation in HepG2 cells. *Oncogene* (2000) 19:3675–83. doi: 10.1038/sj.onc.1203707

35. Nakamura K, Nonaka H, Saito H, Tanaka M, Miyajima A. Hepatocyte proliferation and tissue remodeling is impaired after liver injury in oncostatin M receptor knockout mice. *Hepatology* (2004) 39:635–44. doi: 10.1002/hep.20086

36. Bernard C, Merval R, Lebret M, Delerive P, Dusanter-Fourt I, Lehoux S, et al. Oncostatin M induces interleukin-6 and cyclooxygenase-2 expression in human vascular smooth muscle cells : synergy with interleukin-1beta. *Circ Res* (1999) 85:1124–31. doi: 10.1161/01.res.85.12.1124

37. Smyth DC, Kerr C, Richards CD. Oncostatin M-induced IL-6 expression in murine fibroblasts requires the activation of protein kinase Cdelta. *J Immunol* (2006) 177:8740–7. doi: 10.4049/jimmunol.177.12.8740

38. Larrea E, Aldabe R, Gonzalez I, Segura V, Sarobe P, Echeverria I, et al. Oncostatin M enhances the antiviral effects of type I interferon and activates immunostimulatory functions in liver epithelial cells. *J Virol* (2009) 83:3298–311. doi: 10.1128/jvi.02167-08

39. Son HJ, Lee SH, Lee SY, Kim EK, Yang EJ, Kim JK, et al. Oncostatin M suppresses activation of IL-17/Th17 *via* SOCS3 regulation in CD4+ T cells. *J Immunol* (2017) 198:1484–91. doi: 10.4049/jimmunol.1502314

40. Tanaka M, Hirabayashi Y, Sekiguchi T, Inoue T, Katsuki M, Miyajima A. Targeted disruption of oncostatin M receptor results in altered hematopoiesis. *Blood* (2003) 102:3154–62. doi: 10.1182/blood-2003-02-0367

41. Miyajima A, Kinoshita T, Tanaka M, Kamiya A, Mukouyama Y, Hara T. Role of Oncostatin M in hematopoiesis and liver development. *Cytokine Growth Factor Rev* (2000) 11:177–83. doi: 10.1016/s1359-6101(00)00003-4

42. Arunachalam PS, Wimmers F, Mok CKP, Perera R, Scott M, Hagan T, et al. Systems biological assessment of immunity to mild versus severe COVID-19 infection in humans. *Science* (2020) 369:1210–20. doi: 10.1126/science.abc6261

43. Verstockt S, Verstockt B, Machiels K, Vancamelbeke M, Ferrante M, Cleynen I, et al. Oncostatin M is a biomarker of diagnosis, worse disease prognosis, and therapeutic nonresponse in inflammatory bowel disease. *Inflammation Bowel Dis* (2021) 27:1564–75. doi: 10.1093/ibd/izab032

44. Headland SE, Dengler HS, Xu D, Teng G, Everett C, Ratsimandresey RA, et al. Oncostatin M expression induced by bacterial triggers drives airway inflammatory and mucus secretion in severe asthma. *Sci Transl Med* (2022) 14:eabf8188. doi: 10.1126/scitranslmed.abf8188

45. Schnittker D, Kwofie K, Ashkar A, Trigatti B, Richards CD. Oncostatin M and TLR-4 ligand synergize to induce MCP-1, IL-6, and VEGF in human aortic adventitial fibroblasts and smooth muscle cells. *Mediators Inflammation* (2013) 2013:317503. doi: 10.1155/2013/317503

46. Wahl AF, Wallace PM. Oncostatin M in the anti-inflammatory response. *Ann Rheum Dis* (2001) 60 Suppl 3:iii75–80. doi: 10.1136/ard.60.90003.iii75

47. Komori T, Morikawa Y. Oncostatin M in the development of metabolic syndrome and its potential as a novel therapeutic target. *Anat Sci Int* (2018) 93:169–76. doi: 10.1007/s12565-017-0421-y

48. Matsuda M, Tsurusaki S, Miyata N, Saijou E, Okochi H, Miyajima A, et al. Oncostatin M causes liver fibrosis by regulating cooperation between hepatic stellate cells and macrophages in mice. *Hepatology* (2018) 67:296–312. doi: 10.1002/hep.29421

49. Weiss TW, Kvakan H, Kaun C, Zorn G, Speidl WS, Pfaffenberger S, et al. The gp130 ligand oncostatin M regulates tissue inhibitor of metalloproteinases-1 through ERK1/2 and p38 in human adult cardiac myocytes and in human adult cardiac fibroblasts: a possible role for the gp130/gp130 ligand system in the modulation of extracellular matrix degradation in the human hea rt. *J Mol Cell Cardiol* (2005) 39:545–51. doi: 10.1016/j.yjmcc.2005.03.015

50. Ryan RE, Martin B, Mellor L, Jacob RB, Tawara K, McDougal OM, et al. Oncostatin M binds to extracellular matrix in a bioactive conformation: implications for inflammation and metastasis. *Cytokine* (2015) 72:71–85. doi: 10.1016/j.cyto.2014.11.007

51. Lim HW, Chun KW, Han SK, Kim WK. Effect of oncostatin M on wound healing activity of diabetic fibroblasts. *vitro. J Korean Soc Plast Reconstr Surg* (2008) 35:355–9.

52. Shin SH, Han SK, Jeong SH, Kim WK. Potential of oncostatin M to accelerate diabetic wound healing. *Int Wound J* (2014) 11:398–403. doi: 10.1111/j.1742-481X.2012.01107.x

53. Tseng PY, Hoon MA. Oncostatin M can sensitize sensory neurons in inflammatory pruritus. *Sci Transl Med* (2021) 13:eabe3037. doi: 10.1126/scitranslmed.abe3037

54. Lin YZ, Li RN, Lin CH, Ou TT, Wu CC, Tsai WC, et al. Association of OSMR gene polymorphisms with rheumatoid arthritis and systemic lupus erythematosus patients. *Autoimmunity* (2014) 47:23–6. doi: 10.3109/08916934.2013.849701

55. Gorji AE, Roudbari Z, Alizadeh A, Sadeghi B. Investigation of systemic lupus erythematosus (SLE) with integrating transcriptomics and genome wide association information. *Gene* (2019) 706:181–7. doi: 10.1016/j.gene.2019.05.004

56. Feeney M, Syed F, Khan K, Shiwen X, Sully K, Trinder S, et al. Oncostatin M as a potential molecular target in systemic sclerosis [abstract]. *Arthritis Rheumatol* (2015) 67(suppl 10).

57. Hui W, Bell M, Carroll G. Detection of oncostatin M in synovial fluid from patients with rheumatoid arthritis. *Ann Rheum Dis* (1997) 56:184–7. doi: 10.1136/ard.56.3.184

58. Cawston TE, Curry VA, Summers CA, Clark IM, Riley GP, Life PF, et al. The role of oncostatin M in animal and human connective tissue collagen turnover and its localization within the rheumatoid joint. *Arthritis Rheum* (1998) 41:1760–71. doi: 10.1002/1529-0131(199810)41:10<1760::Aid-art8>3.0.Co;2-m

59. Manicourt DH, Poilvache P, Van Egeren A, Devogelaer JP, Lenz ME, Thonar EJ. Synovial fluid levels of tumor necrosis factor alpha and oncostatin M correlate with levels of markers of the degradation of crosslinked collagen and cartilage aggrecan in rheumatoid arthritis but not in osteoarthritis. *Arthritis Rheum* (2000) 43:281–8. doi: 10.1002/1529-0131(200002)43:2<281::Aid-anr7>3.0.Co;2-7

60. Scanzello CR, Goldring SR. The role of synovitis in osteoarthritis pathogenesis. *Bone* (2012) 51:249–57. doi: 10.1016/j.bone.2012.02.012

61. Huang YZ, Xie HQ, Silini A, Parolini O, Zhang Y, Deng L, et al. Mesenchymal stem/progenitor cells derived from articular cartilage, synovial membrane and synovial fluid for cartilage regeneration: current status and future perspectives. *Stem Cell Rev Rep* (2017) 13:575–86. doi: 10.1007/s12015-017-9753-1

62. Loh C, Park SH, Lee A, Yuan R, Ivashkiv LB, Kalliolias GD. TNF-induced inflammatory genes escape repression in fibroblast-like synoviocytes: transcriptomic and epigenomic analysis. *Ann Rheum Dis* (2019) 78:1205–14. doi: 10.1136/annrheumdis-2018-214783

63. Bustamante MF, Garcia-Carbonell R, Whisenant KD, Guma M. Fibroblast-like synoviocyte metabolism in the pathogenesis of rheumatoid arthritis. *Arthritis Res Ther* (2017) 19:110. doi: 10.1186/s13075-017-1303-3

64. Migita K, Komori A, Torigoshi T, Maeda Y, Izumi Y, Jiuchi Y, et al. CP690,550 inhibits oncostatin M-induced JAK/STAT signaling pathway in rheumatoid synoviocytes. *Arthritis Res Ther* (2011) 13:R72. doi: 10.1186/ar3333

65. Hanlon MM, Rakovich T, Cunningham CC, Ansboro S, Veale DJ, Fearon U, et al. STAT3 mediates the differential effects of oncostatin M and TNFα on RA synovial fibroblast and endothelial cell function. *Front Immunol* (2019) 10:2056. doi: 10.3389/fimmu.2019.02056

66. Matsushima K, Yang D, Oppenheim JJ. Interleukin-8: An evolving chemokine. *Cytokine* (2022) 153:155828. doi: 10.1016/j.cyto.2022.155828

67. Le Goff B, Singbrant S, Tonkin BA, Martin TJ, Romas E, Sims NA, et al. Oncostatin M acting via OSMR, augments the actions of IL-1 and TNF in synovial fibroblasts. *Cytokine* (2014) 68:101–9. doi: 10.1016/j.cyto.2014.04.001

68. Abdel-Wahab AF, Mahmoud W, Al-Harizy RM. Targeting glucose metabolism to suppress cancer progression: prospective of anti-glycolytic cancer therapy. *Pharmacol Res* (2019) 150:104511. doi: 10.1016/j.phrs.2019.104511

69. Steffen U, Schett G, Bozec A. How autoantibodies regulate osteoclast induced bone loss in rheumatoid arthritis. *Front Immunol* (2019) 10:1483. doi: 10.3389/fimmu.2019.01483

70. Song HY, Jeon ES, Kim JI, Jung JS, Kim JH. Oncostatin M promotes osteogenesis and suppresses adipogenic differentiation of human adipose tissue-derived mesenchymal stem cells. *J Cell Biochem* (2007) 101:1238–51. doi: 10.1002/jcb.21245

71. Smyth DC, Takenaka S, Yeung C, Richards CD. Oncostatin M regulates osteogenic differentiation of murine adipose-derived mesenchymal progenitor cells through a PKCdelta-dependent mechanism. *Cell Tissue Res* (2015) 360:309–19. doi: 10.1007/s00441-014-2099-y

72. Zou F, Xu JC, Wu GH, Zhou LL, Wa QD, Peng JQ, et al. Effects of oncostatin M on cell proliferation and osteogenic differentiation in C3H10T1/2. *J Musculoskelet Neuronal Interact* (2016) 16:377–85.

73. Diaz-Rodriguez P, Erndt-Marino JD, Gharat T, Munoz Pinto DJ, Samavedi S, Bearden R, et al. Toward zonally tailored scaffolds for osteochondral differentiation of synovial mesenchymal stem cells. *J BioMed Mater Res B Appl Biomater* (2019) 107:2019–29. doi: 10.1002/jbm.b.34293

74. Zheng W, Guan J. Oncostatin M promotes the osteogenic differentiation of mouse MC3T3−E1osteoblasts through the regulation of monocyte chemotactic protein −1. *Mol Med Rep* (2018) 18:2523–30. doi: 10.3892/mmr.2018.9261

75. Hui W, Cawston TE, Richards CD, Rowan AD. A model of inflammatory arthritis highlights a role for oncostatin M in pro-inflammatory cytokine-induced bone destruction via RANK/RANKL. *Arthritis Res Ther* (2005) 7:R57–64. doi: 10.1186/ar1460

76. Guihard P, Boutet MA, Brounais-Le Royer B, Gamblin AL, Amiaud J, Renaud A, et al. Oncostatin m, an inflammatory cytokine produced by macrophages, supports intramembranous bone healing in a mouse model of tibia injury. *Am J Pathol* (2015) 185:765–75. doi: 10.1016/j.ajpath.2014.11.008

77. Ribbens C, Andre B, Kaye O, Kaiser MJ, Bonnet V, Jaspar JM, et al. Synovial fluid matrix metalloproteinase-3 levels are increased in inflammatory arthritides whether erosive or not. *Rheumatol (Oxford)* (2000) 39:1357–65. doi: 10.1093/rheumatology/39.12.1357

78. Hui W, Bell M, Carroll G, Oncostatin M. (OSM) stimulates resorption and inhibits synthesis of proteoglycan in porcine articular cartilage explants. *Cytokine* (1996) 8:495–500. doi: 10.1006/cyto.1996.0067

79. Fearon U, Mullan R, Markham T, Connolly M, Sullivan S, Poole AR, et al. Oncostatin M induces angiogenesis and cartilage degradation in rheumatoid arthritis synovial tissue and human cartilage cocultures. *Arthritis Rheum* (2006) 54:3152–62. doi: 10.1002/art.22161

80. Xiao C, Lv C, Sun S, Zhao H, Ling H, Li M, et al. TSP1 is the essential domain of SEMA5A involved in pannus formation in rheumatoid arthritis. *Rheumatol (Oxford)* (2021) 60:5833–42. doi: 10.1093/rheumatology/keab133

81. Tsai CY, Shiau AL, Chen SY, Chen YH, Cheng PC, Chang MY, et al. Amelioration of collagen-induced arthritis in rats by nanogold. *Arthritis Rheum* (2007) 56:544–54. doi: 10.1002/art.22401

82. MacDonald IJ, Liu SC, Su CM, Wang YH, Tsai CH, Tang CH. Implications of angiogenesis involvement in arthritis. *Int J Mol Sci* (2018) 19:2012. doi: 10.3390/ijms19072012

83. Shirshev SV, Nekrasova IV, Gorbunova OL, Orlova EG. Regulation of recombinase rag-1 expression by female sex steroids in treg and th17 lymphocytes: role of oncostatin M. *Dokl Biochem Biophys* (2019) 484:73–7. doi: 10.1134/s1607672919010198

84. Plater-Zyberk C, Buckton J, Thompson S, Spaull J, Zanders E, Papworth J, et al. Amelioration of arthritis in two murine models using antibodies to oncostatin M. *Arthritis Rheum* (2001) 44:2697–702. doi: 10.1002/1529-0131(200111)44:11<2697::aid-art450>3.0.co;2-#

85. Langdon C, Kerr C, Hassen M, Hara T, Arsenault AL, Richards CD. Murine oncostatin M stimulates mouse synovial fibroblasts in *vitro* and induces inflammation and destruction in mouse joints in *vivo*. *Am J Pathol* (2000) 157:1187–96. doi: 10.1016/s0002-9440(10)64634-2

86. Garcia JP, Utomo L, Rudnik-Jansen I, Du J, Zuithoff NPA, Krouwels A, et al. Association between oncostatin M expression and inflammatory phenotype in experimental arthritis models and osteoarthritis patients. *Cells* (2021) 10:508. doi: 10.3390/cells10030508

87. Choy EH, Bendit M, McAleer D, Liu F, Feeney M, Brett S, et al. Safety, tolerability, pharmacokinetics and pharmacodynamics of an anti- oncostatin M monoclonal antibody in rheumatoid arthritis: results from phase II randomized, placebo-controlled trials. *Arthritis Res Ther* (2013) 15:R132. doi: 10.1186/ar4312

88. Reid J, Zamuner S, Edwards K, Rumley SA, Nevin K, Feeney M, et al. *In vivo* affinity and target engagement in skin and blood in a first-time-in-human study of an anti-oncostatin M monoclonal antibody. *Br J Clin Pharmacol* (2018) 84:2280–91. doi: 10.1111/bcp.13669

89. Sofen H, Bissonnette R, Yosipovitch G, Silverberg JI, Tyring S, Loo WJ, et al. Efficacy and safety of vixarelimab, a human monoclonal oncostatin M receptor β antibody, in moderate-to-severe prurigo nodularis: a randomised, double-blind, placebo-controlled, phase 2a study. *EClinicalMedicine* (2023) 57:101826. doi: 10.1016/j.eclinm.2023.101826

90. Mass OA, Tuccinardi J, Woodbury L, Wolf CL, Grantham B, Holdaway K, et al. Bioactive recombinant human oncostatin M for NMR-based screening in drug discovery. *Sci Rep* (2021) 11:16174. doi: 10.1038/s41598-021-95424-6

91. Du Q, Tu G, Qian Y, Yang J, Yao X, Xue W. Unbiased molecular dynamics simulation of a first-in-class small molecule inhibitor binds to oncostatin M. *Comput Biol Med* (2023) 155:106709. doi: 10.1016/j.compbiomed.2023.106709

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GPR35 acts a dual role and therapeutic target in inflammation

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GPR35 is a G protein-coupled receptor with notable involvement in modulating inflammatory responses. Although the precise role of GPR35 in inflammation is not yet fully understood, studies have suggested that it may have both pro- and anti-inflammatory effects depending on the specific cellular environment. Some studies have shown that GPR35 activation can stimulate the production of pro-inflammatory cytokines and facilitate the movement of immune cells towards inflammatory tissues or infected areas. Conversely, other investigations have suggested that GPR35 may possess anti-inflammatory properties in the gastrointestinal tract, liver and certain other tissues by curbing the generation of inflammatory mediators and endorsing the differentiation of regulatory T cells. The intricate role of GPR35 in inflammation underscores the requirement for more in-depth research to thoroughly comprehend its functional mechanisms and its potential significance as a therapeutic target for inflammatory diseases. The purpose of this review is to concurrently investigate the pro-inflammatory and anti-inflammatory roles of GPR35, thus illuminating both facets of this complex issue.

KEYWORDS

GPR35, pro-inflammatory, anti-inflammatory, inflammatory diseases, therapeutic target

Introduction

Inflammation is a multifaceted physiological reaction that serves a critical role in the immune response against infection, tissue injury, and other provocations. It orchestrates a series of cellular and molecular activities with the ultimate goals of eradicating pathogens, repairing damaged tissues, and reinstating homeostasis (1). However, a dysregulated or persistent inflammatory response can pave the way for numerous diseases, encompassing autoimmune disorders where the body mistakenly attacks its cells, chronic inflammatory conditions that endure over extended periods, and even cancer where abnormal cells

proliferate uncontrollably (2–4). The link between inflammation and such diverse diseases underscores the importance of understanding and effectively managing inflammation.

G protein-coupled receptors (GPCRs) constitute a vast family of cell surface receptors that fundamentally mediate cellular responses to a myriad of stimuli, including inflammatory (5, 6). Among these receptors, GPR35 has recently drawn considerable interest due to its suspected role in inflammatory processes. Discovered in 1998, GPR35 is highly expressed in the intestine and various immune cells. Owing to its wide expression and the significance of its function, it is perceived as a prospective therapeutic target for various diseases (7–9).

The involvement of GPR35 in inflammation is complex and context-dependent, demonstrating both pro-inflammatory and anti-inflammatory effects in disparate cellular and tissue settings. GPR35 has been implicated in modulating an array of inflammatory responses, including the activation of immune cells, production of cytokines, and chemotactic movements (10–12). It interacts with several signaling pathways, such as MAPK, NF-κB and others, thereby modulating inflammatory signaling dynamics. Furthermore, the functionality of GPR35 can be influenced by various ligands, ranging from endogenous metabolites to synthetic compounds, thus impacting its downstream effects in the inflammation process (13). Through these complex interactions and functions, GPR35 illustrates the multifactorial nature of inflammation and the intricate molecular dance that enables the body to respond appropriately to internal and external stressors. Yet, it also underscores the delicate balance that must be maintained, as dysregulated signaling via pathways involving GPR35 could potentially lead to pathological inflammation, underscoring the need for further research to elucidate its precise role and regulatory mechanisms.

In this comprehensive review, we explored the current understanding of GPR35, with a particular focus on its role in inflammation. We began by presenting a detailed overview of GPR35, discussing aspects such as its expression pattern, signaling mechanisms, and the variety of ligands it interacts with. From there, we investigated the pro-inflammatory role of GPR35, and its activation influences the immune response, including the impact on immune cell activation, cytokine production, and chemotactic activity within various immune cells such as neutrophils, macrophages, and invariant natural killer T (iNKT) cells. Subsequently, we discussed an in-depth examination of the anti-inflammatory role of GPR35 and covered aspects such as its impact on immune cell signaling, cytokine generation, and the resolution of inflammation. In addition, we explored the intricate signaling pathways and mechanisms underpinning the pro-inflammatory and anti-inflammatory effects that are triggered following GPR35 activation. This comprehensive exploration will serve as a map guiding us through the complicated roles of GPR35 in inflammation, shedding light on its significance and potential therapeutic value.

Expression pattern of GPR35

GPR35 is classified as a class A orphan GPCR compromising nearly 85% of all GPCRs. GPCRs play a pivotal role in metabolite sensing within the intestine, acting as a significant connector linking the microbiota, immune system, and intestinal epithelium (14). Most GPCRs are predicted to be responsible for encoding olfactory receptors, while the rest are divided between receptors that bind with recognized endogenous compounds and those termed as orphan receptors because their specific endogenous activators remain unidentified (15).

Despite various endogenous ligands of GPR35, a definitive endogenous activator has not yet been firmly established, which maintains its status as an orphan receptor (13). Human GPR35 gene is transcribed and translated into three distinct variants (16). Both variants 2 and 3 encode the longer isoform GPR35b, which diverges from GPR35a by possessing an extended N-terminal sequence of 31 additional amino acids, thereby lengthening its extracellular domain. Despite this distinction, the subsequent sequences of the two isoforms are identical. mRNA expression of GPR35a and GPR35b have been identified in human tissues. Notably, GPR35b, which is highly expressed in gastric and colon cancer tissues, may have associations with carcinogenesis (17). Intriguingly, GPR35b demonstrates a lower agonist response efficacy than GPR35a (18). It was reported that the elongated N-terminus of the longer isoform may constrain G protein activation while boosting the interaction with β-arrestin (19).

GPR35 expression pattern suggests its pivotal role in immune responses and inflammation. GPR35 expression is particularly prominent in the small intestine and colon, with moderate but noticeable expression in the stomach, liver, spleen, kidney, and sympathetic neurons (20, 21). This widespread presence across various organ systems indicates its broad functionality within the body. Moreover, GPR35 expression is not limited to these organ systems. It also appears in various immune cells, including monocytes ($CD14^+$), T-cells ($CD3^+$), neutrophils, assorted dendritic cells, and invariant natural killer T cells (22–24), highlighting its integral role in immune responses. Among these, dendritic cells ($CD103^+/CD11b^-$) and macrophage clusters from the lamina propria and Peyer's patch cells in mouse small intestine are noted for their high expression of GPR35 (25).

Furthermore, there is a significant upregulation of GPR35 in neutrophils and intestinal tissues during the invasion of pathogenic microbes, as well as in mast cells during the stimulation of IgE antibodies, indicating a pronounced inflammatory response (21, 26, 27). Additionally, GPR35 presence in epithelial and endothelial cells, which are key participants in the inflammatory response (28), further underscores broad impact across numerous cell types and physiological processes. This ubiquitous distribution and varied presence underscore GPR35 potential as a major player in immune responses and inflammation. GPR35 presents the intriguing features with tissue specificity and different isoforms, taking into consideration when examining the potential of GPR35 as a therapeutic target for inflammation-related conditions.

GPR35 involved inflammation-related pathways

G proteins serve as the primary effector proteins for GPCRs. Typically, G proteins exist as a trimeric complex composed of α, β, and γ subunits. Upon activation of the GPCR, it binds to the Gα

subunit and facilitates the exchange of GDP for GTP on Gα. This process leads to the disassociation of Gα from the Gβγ dimer. Following this separation, both Gα and Gβγ can independently initiate their respective signaling pathways (29).

Four principal Gα protein families exist, and GPR35 has been found to couple predominantly with Gα12/13 and Gαi/o proteins (7, 24, 30) (Figure 1A). Gαi/o protein interacts with adenylate cyclase (AC), inhibiting its activity and consequently reducing intracellular cAMP levels, resulting in the dampening of the MAPK/ERK pathway (31). Interestingly, ERK may serve as an anti-inflammatory signal, suppressing the production of NF-κB dependent inflammatory factors through the inhibition of IKK activity (32). Gβγ dimer can activate phospholipase C beta (PLCβ), which results in phosphoinositide generation and activation of the PI3K/AKT pathway. This leads to the activation of a host of downstream transcription factors, notably NF-κB, a major player in the expression of genes encoding inflammatory factors (33). In the case of Gα12/13, activation leads to Rho-mediated cytoskeleton reorganization, collaborating with Gβγ to regulate immune cell chemotaxis to inflammation sites - these actions primarily result in pro-inflammatory effects (34, 35). Gαq coupled with GPR35 directly inhibits the PI3K catalytic subunit and then suppresses Akt activation. Additionally, Gαq plays a role in the activation of ERK through the PLCβ/Ca^{2+}/Src signaling cascade (19, 36).

GPR35 has been demonstrated to directly engage with β-arrestin upon activation by agonists, leading to its internalization and desensitization (37). Beyond that, β-arrestins also function as signaling scaffolds, interacting with various pathways including the c-Jun N-terminal kinase, protein kinase B, and the extracellular signal-regulated kinase (ERK1/2) pathway in a G protein independent manner, leading to anti-inflammatory effects (38–40). In addition, β-arrestins interact with IκBα, leading to the suppression of NF-κB activation (41). It is worth noting that there are certain ligands capable of activating GPR35 without facilitating the interaction between GPR35 and β-arrestin, such as kynurenic acid (19, 42), 5-HIAA (42) and DHICA analogues (43). Moreover, GPR35 interacts with the sodium-potassium pump (Na/K-ATPase), a vital regulator of cellular electrochemical gradients and Src family kinase signaling (44). This interaction enhances Na/K-ATPase pump function, aiding in the regulation of Ca^{2+} homeostasis. Also, Na/K-ATPase directly activates the kinase Src, leading to ERK activation in macrophages and enterocytes (45). Given that elevated cytoplasmic Ca^{2+} levels promote ERK and NF-κB activation via PKC in inflammation, alterations in Ca^{2+} homeostasis have context-dependent impacts on inflammation (46, 47) (Figure 1B).

Furthermore, GPR35 has also been reported to modulate the production of reactive oxygen species (ROS), key mediators of inflammation. Under mechanical stress, GPR35 activation has been shown to enhance ROS production, and excessive ROS triggers further GPR35 expression (48). Conversely, GPR35 activation with Kynurenic acid in macrophages suppresses NLRP3 inflammasome activation and related inflammation by reducing mitochondrial damage and mitochondrial ROS production (49). These studies, despite showing divergent outcomes, reveal the significant role of GPR35 in modulating inflammation via ROS-mediated pathways.

In sum, a growing body of evidence proposes a sophisticated and intricate role of GPR35 in inflammation. It appears to exert both pro-inflammatory and anti-inflammatory influences, which depend on the type of cell, the specific signaling pathways, and the

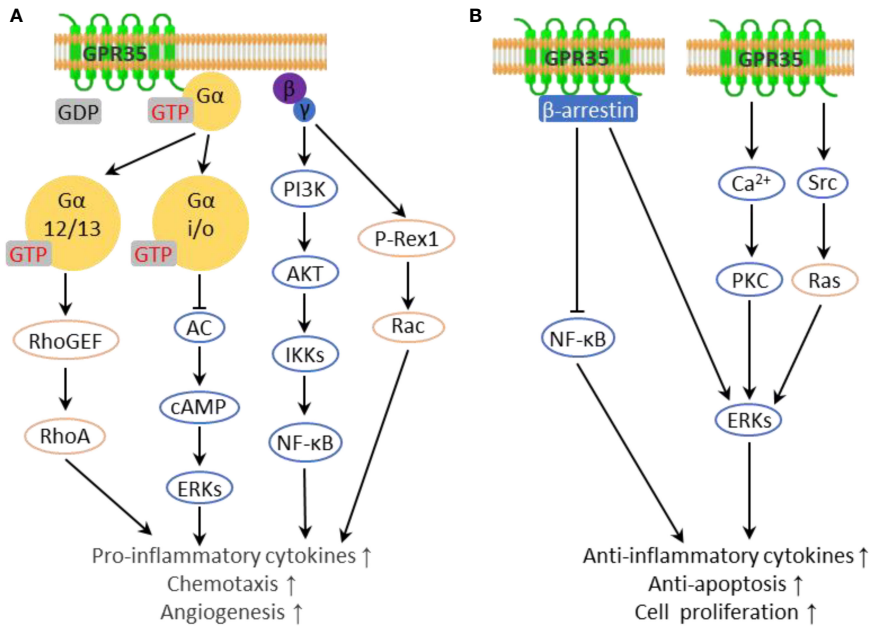

FIGURE 1
Inflammation-related downstream pathways of GPR35. **(A)** G proteins interacting with GPR35 transmit pro-inflammatory downstream pathways. **(B)** GPR35 transmits anti-inflammatory downstream pathways.

availability of ligands. The precise mechanisms through which GPR35 impacts inflammation-related pathways, however, remain somewhat elusive. Therefore, it necessitates additional research to comprehensively understand the subtleties of its signaling.

Primary ligands with activity at GPR35

A pivotal element influencing the activity of GPR35 are its ligands, otherwise referred to as agonists and antagonists. Agonists are molecules that initiate signaling by binding to the receptor, thereby triggering downstream cellular responses. Conversely, antagonists exert opposing effects. Furthermore, the activation of receptors is not always fully "on or off" scenario while some agonists can activate the corresponding receptors in a biased manner. For instance, in the case of the kynurenic acid/GPR35 axis, while kynurenic acid triggers the activation of G proteins downstream of GPR35, it scarcely facilitates the interaction between GPR35 and β-arrestin (42, 43).

There are multiple single nucleotide polymorphisms (SNPs) located within the GPR35 gene linked with various immune and inflammation-related diseases, such as inflammatory bowel diseases, ankylosing spondylitis, and primary sclerosing cholangitis (9). One of the most prevalent variants, rs3749171, induces the replacement of a threonine with methionine in transmembrane domain III (T108M), demonstrating a significant correlation with inflammatory bowel disease (50). Although most SNP-induced variations do not significantly affect the potency of agonist ligands, the V76M variant (rs13387859) does exhibit a reduction in agonist potency (51). While this variant is present at a 2% allele frequency, it has not been associated with any disease.

In recent years, a multitude of both endogenous and synthetic GPR35 ligands have been discovered, enriching our understanding of this involvement in inflammation and a host of other biological phenomena (Table 1).

Endogenous agonists

Kynurenic acid, a metabolite derived from tryptophan, is noted for its roles in the central nervous system, as well as anti-inflammatory property (73, 74). Its ability to activate GPR35 is significantly more potent in mice and rats compared to humans, where it requires 40- to 100-fold higher concentrations (20), occasionally failing to activate human GPR35 at high doses (27, 54). This species-selective aspect of the kynurenic acid/GPR35 axis has stirred discussion on whether this interaction is truly physiological, particularly in humans, leaving kynurenic acid as a potential endogenous ligand for GPR35 (7). Recently, it was demonstrated that pre-treatment with kynurenic acid mitigated the injuries sustained by both human iPS-cardiomyocytes and mouse cardiomyocytes following simulated ischemia/reperfusion (I/R) *ex vivo* (8). In mice, kynurenic acid stimulates the migration of $CX3CR1^{+}$/$GPR35^{+}$ macrophages in the small intestine, but not $GPR35^{-}$ macrophages (75).

Lysophosphatidic acid (LPA), an active phospholipid derivative, is present in cell membranes and can be produced extracellularly to activate six known GPCRs including LPAR1-6 (76). It was displayed that the Ca^{2+} response triggered by 2-acyl LPA was significantly stronger in GPR35-expressed HEK293 cells compared to the response in the control cells, which pronounced difference was not observed when 1-acyl LPA was applied (54). GPR35 deficiency was found to inhibit LPA-induced Ca^{2+} signaling in bone marrow-derived macrophages (BMDMs). However, it was posited that GPR35 deficiency might compromise LPA signaling via other LPA receptors (45). Recently, GPR35 was affirmed as a potential LPA receptor linked to an inhibitory G protein (Gi) (27). However, there LPA failed to activate GPR35 in other experimental settings, which leaves the question open on whether LPA acts as an endogenous agonist for GPR35 (26, 77).

CXCL17, a homeostatic chemokine in mucosa, attracts dendritic cells and macrophages but can be expressed elsewhere during inflammation (78). It was reported that CXCL17 influenced GPR35 at nanomolar levels within a physiological range, unlike kynurenic acid (56). However, subsequent studies failed to show that CXCL17 induced migration or signaling responses in GPR35-expressing cells (79, 80). Further, the actions of CXCL17 in a neuropathic pain model in mice were decreased by kynurenic acid and zaprinast, suggesting the presence of a CXCL17 receptor other than GPR35 (81).

Recent findings indicate that 5-Hydroxyindoleacetic acid (5-HIAA), a metabolite of 5-hydroxytryptamine generated by activated platelets and extravascular mast cells, can act as an agonist for GPR35, promoting the recruitment of neutrophils (26) and the migration of eosinophils (82). Interestingly, this perspective offers fresh insight into the role GPR35 regulating pain sensations due to the association of 5-HIAA with pain and sensory neurons (83). Additionally, GPR35 plays a role in modulating cAMP production and inhibiting N-type Ca^{2+} channels in neurons and astrocytes, showcasing its potential involvement in pain management treatments (84). Moreover, other endogenous molecules such as 5,6-dihydroxyindole-2-carboxylic acid (DHICA), reverse T3 (3,3,5- triiodothyronine), and cyclic guanosine 3′-5′ monophosphate (cGMP) have demonstrated a degree of activity towards GPR35, albeit with modest potency. However, these observations require further validation (40, 58).

Synthetic agonists

Zaprinast (2-(2-propyloxyphenyl) -8-azapurin-6-one), originally identified as a cGMP phosphodiesterase inhibitor, is one of the earliest discovered GPR35 ligands. Notably, the effects of Zaprinast on GPR35 can be separated from its cGMP phosphodiesterase inhibition properties with the intracellular calcium mobilization (59). With its moderate-to-high potency across human, mouse, and rat orthologs, Zaprinast has established its status as a go-to reference agonist for GPR35 research (30, 72). Its efficacy and wide applicability across different species have aided in comparative studies and offered

TABLE 1 A schematic representation of GPR35 ligands.

Name	Type	Potency (EC50)	Role in inflammation	References
Kynurenic acid	Metabolite of L-tryptophan	Human: 217 μM Rat: 66 μM	Anti-inflammatory	(52, 53)
LPA	Phospholipid derivative	Not reported	Pro-inflammatory	(54, 55)
5-HIAA	Serotonin metabolite	Not reported	Pro-inflammatory in Neutrophils	(26)
CXCL17	Endogenous peptide	Not reported	Anti-inflammatory	(56, 57)
cGMP	Cyclic nucleotide derived from GTP	Human: 131 μM	No conclusion	(58)
DHICA	Intermediate in the biosynthesis of elanin	Human: 22 μM	No conclusion	(40)
Reverse T3	Hormone produced in the thyroid gland	Human: 100 μM	No conclusion	(40)
Zaprinast	Synthetic agonist	Human: 2-8 μM Mouse: 1 μM Rat: 100 nM	No conclusion	(59)
Pamoic acid	Synthetic agonist	Human: 30-50 nM Mouse: Inactive Rat: >100 μM	Anti-inflammatory	(60, 61)
YE120	Synthetic agonist	Human: 30-35 nM	Anti-inflammatory	(62, 63)
Lodoxamide	Synthetic agonist	Human: 4 nM Rat: 13 nM	Allergic inhibition	(51)
Bufrolin	Synthetic agonist	Human: 13 nM Rat: 10 nM	Allergic inhibition	(51)
Compound 1	Synthetic agonist	Human: 26 nM Mouse: 17 μM Rat: 8 μM	No conclusion	(64)
PSB-13253	Synthetic agonist	Human: 12 nM Mouse: Inactive Rat: 1.4 μM	No conclusion	(65)
Amoxanox	Synthetic agonist	Human: 4 μM Rat: 23 nM	Mast cell stabilizer,anti-asthma and anti-allergy medication	(51)
Cromolyn disodium	Synthetic agonist	Not reported	Mast cell stabilizer, mitigate asthma	(21)
Nedocromil	Synthetic agonist	Human: 0.13 μM Mouse: 7.3 μM Rat: 2.7 μM	Mast cell stabilizer, mitigate asthma	(21)
Doxantrazole	Synthetic agonist	Human: 3.4 μM Rat: 300 nM	Mast cell stabilizer	(51)
Pemirolast	Synthetic agonist	Human: Inactive Rat: 95 nM	Mast cell stabilizer	(51)
Furosemide	Synthetic agonist	Human: 8.3 μM	Anti-inflammatory	(66)
Tyrphostin-51	Synthetic agonist	Human: 8 μM (DMR 120 nM)	No conclusion	(67)
2,3,5-THB	Synthetic agonist	Human: 8.4 μM (DMR 250 nM)	No conclusion	(68)
Gallic acid	Synthetic agonist	Human: 11.4 μM (DMR 1.16 μM)	Anti-inflammatory	(69)
wedelolactone	Synthetic agonist	Human: 1.39 μM (DMR 2.73 μM)	Anti-inflammatory	(69)
Ellagic acid	Synthetic agonist	Human: 2.96 μM (DMR 110 nM)	Anti-inflammatory	(69)

(Continued)

TABLE 1 Continued

Name	Type	Potency (EC50)	Role in inflammation	References
Aminosalicylate	Synthetic agonist	Not reported	Anti-inflammatory	(70)
Compound 50	Synthetic agonist	Human: 5.8 nM	No conclusion	(71)
CID2745687	Synthetic antagonist	Human (Ki): 10-20 nM	No conclusion	(60)
ML-145	Synthetic antagonist	Human (Ki): 25 nM	No conclusion	(72)

insights into the roles GPR35 plays in a myriad of biological processes.

Pamoic acid (5-nitro-2-(3-phenylproplyamino) benzoic acid) emerged as a potential GPR35 ligand following screening exercises within the Prestwick Chemical Library. However, its activity at rat and mouse orthologs of GPR35 is noticeably less potent compared to its interaction with the human version. This lower efficacy significantly impedes its usage in these preclinical studies (30, 60). Despite this limitation, the discovery of pamoic acid interaction with GPR35 has contributed to the expanding catalog of ligands and may potentially prompt further development of more effective analogs, thereby expanding our understanding of GPR35 physiological role.

YE120 (2-(3-cyano-5-(3,4-dichlorophenyl)-4,5-dimethylfuran-2(5H)-ylidene) malononitrile) is another compound that was identified as a GPR35 agonist through dynamic mass redistribution (DMR) assays performed in the native cell line HT-29. Remarkably, it has demonstrated superior potency compared to zaprinast (62). This discovery indicates the continuing progress in our understanding of GPR35, providing a new promising candidate for the functionality and potentially enabling the development of more effective therapeutic strategies targeting GPR35.

Lodoxamide, a widely used anti-inflammatory mast cell stabilizer, is another synthetic agonist that targets GPR35. Its application in the treatment of allergic keratoconjunctivitis attests to its significant role in modulating inflammatory responses. However, despite its high potency towards human and rat GPR35, the effectiveness of lodoxamide is notably diminished to for the mouse orthologue, with its potency being a 100-fold lower (51, 85). This highlights the importance of species-specific investigations in understanding the precise role of GPR35 and its ligands in mediating inflammatory responses. Recently, there was a significant development in understanding the structural attributes of GPR35 through its structure when bound to a GPR35 agonist lodoxamide, which shows a novel site for divalent cation coordination and a distinctive ionic regulatory mechanism. This helps understanding the affinity of GPR35 for other anti-asthma and anti-allergy agents, especially those featuring symmetric diacid structures like lodoxamide. providing a clear pathway for the binding process (86)

Aminosalicylates, a first-line treatment for inflammatory bowel diseases (IBDs), have shown activity on both human and mouse GPR35, although their exact target remains undefined. Of these, the pro-drug olsalazine exhibits the greatest potency in terms of GPR35 agonism, promoting ERK phosphorylation and the translocation of β-arrestin2. Notably, in a model of dextran sodium sulfate (DSS)-induced colitis, the protective effects of olsalazine on disease progression and its inhibitory effect on TNFα mRNA expression, as well as the NF-κB and JAK-STAT3 pathways, are significantly reduced in mice with GPR35 knockout, thus suggesting a critical role of GPR35 in these anti-inflammatory actions (70).

Recently, a group of 2H-chromen-2-one derivatives has been identified as agonists for GPR35 using dynamic mass redistribution assays in HT-29 cells. The compound 6-Bromo-7-hydroxy-8-nitro-3-(1H-tetrazol-5-yl)-2H-chromen-2-one (Compound 50) emerged as the most potent GPR35 agonist with an EC50 of 5.8 nM (71). In another development, GPR35 fluorescent probes were designed based on known GPR35 agonists. These serve as valuable tools for GPR35 research and for the discovery of new synthetic GPR35 agonists. The most promising compound from this series exhibited the highest binding potency, along with a minimal nonspecific Bioluminescence Resonance Energy Transfer (BRET) binding signal, with a Kd value of 3.9 nM (87).

Antagonists

CID2745687, known as methyl-5- [(tert-butylcarbamothioylhydrazinylidene)methyl] -1-(2,4- difluorophenyl)pyrazole-4-carboxylate, is a well-known antagonist of GPR35. It demonstrates the ability to obstruct the effects of the agonists pamoic acid and zaprinast in cells expressing either variant of human GPR35a or GPR35b. It is estimated that the inhibitor constant Ki is in the range of 10-20 nM, suggesting potential competitive function in a competitive manner (60). CID2745687 has a higher affinity for human GPR35 compared to its mouse and rat counterparts, limiting its application primarily to *in vitro* studies rather than rodent models (88). Another antagonist of GPR35 is ML-145, or 2-hydroxy-4- [4-(5Z)-5-[(E)-2-methyl-3- phenylprop-2-enylidene] -4-oxo-2-sulfanylidene-1,3- thiazolidin-3-yl] butanoylamino] benzoic acid. This compound also demonstrates high affinity for human GPR35 but shows appreciable affinity for the mouse and rat orthologues (72).

These antagonists and their specificity for the human GPR35 over rodent versions provide valuable insights into the diverse functionality and pharmacology of GPR35. They underline the challenges of studying GPR35 across different species but also offer opportunities for the development of novel therapeutic agents targeting the human GPR35 receptor.

Pro-inflammatory roles of GPR35

GPR35 activation has been demonstrated to induce a pro-inflammatory state within cells, leading to increased production

of pro-inflammatory cytokines and chemokines, which act as signal carriers, promoting the recruitment and activation of specific types of immune cells to the inflammation site (Figure 2). This process, in essence, highlights a crucial role of GPR35 in orchestrating immune responses, contributing to the exacerbation of inflammation, and potentially influencing the progression of various inflammatory conditions and diseases. There have been two diseases that are connected to the pro-inflammatory effects of GPR35. GPR35 plays a vital role in detecting bacteroides fragilis toxin and triggering an immune response in bacteroides fragilis toxin-induced colitis (89), and GPR35 loss in nucleus pulposus cells significantly reduces intervertebral disc degeneration typically triggered by inflammation induced by ROS or mechanical stress (49). In addition, encephalomyelitis (EAE) is worsened through the accumulation of $GPR35^{+}/Ly6C^{+}$ macrophages in the small intestine, while the blockage of KYNA-GPR35 signaling can alleviate EAE (75).

GPR35 plays a pro-inflammatory role in neutrophils

Neutrophils, the most populous immune cells in human blood, act as first responders in the face of tissue damage and pathogenic infections. The journey of neutrophils from blood vessels to target tissues is complex, traditionally classified into four stages: 1. The egress of neutrophils from bone marrow into the bloodstream. 2. A multi-step adhesion cascade resulting in the congregation of adherent neutrophils in microvessels. 3. Diapedesis of neutrophils across the vascular wall, granting access to the extravascular space. 4. Interstitial migration of neutrophils to their ultimate target sites (90). Various small molecules and cytokines orchestrate these stages, resulting in a highly redundant regulatory system, which complicates the task of identifying dominant chemokines (91).

Interestingly, GPR35 expression is observed to be relatively low in inactive neutrophils, while it increases substantially in activated neutrophils, which aligns with the role of GPR35 in promoting neutrophil recruitment to inflammatory tissues (26). While GPR35 deficiency was found to somewhat dampen neutrophil adherence to blood vessel walls, this effect was not significantly observed in animal studies. Nevertheless, the role of GPR35 in neutrophil diapedesis has been identified as non-redundant (92). Moreover, 5-HIAA produced by activated platelets and extravascular mast cells, can activate GPR35 as an agonist, thus encouraging the recruitment of neutrophils (93).

In summary, through the complex interactions of these various mechanisms, GPR35 plays a significant role in promoting an inflammatory response in neutrophils by facilitating neutrophil recruitment, reinforcing its central role in the body response to inflammation.

GPR35 plays a pro-inflammatory role in macrophages

Macrophages are specialized cells derived from monocytes in the bloodstream, and they play crucial roles in identifying, engulfing, and destroying bacteria, thereby initiating inflammation. These activated macrophages are classified into two types based on their involvement in inflammation: pro-inflammatory (M1) and anti-inflammatory (M2). M1 macrophages secrete pro-inflammatory cytokines such as IL-1, IL-

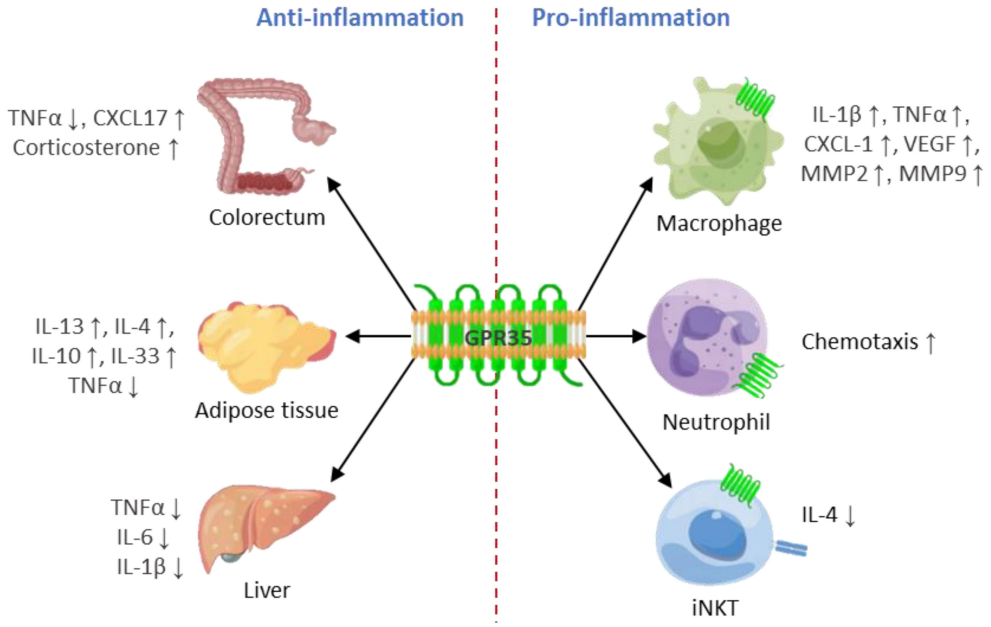

FIGURE 2
GPR35 plays a double-edged sword role in inflammation. GPR35 plays an anti-inflammatory role in colorectum, adipose tissue and liver, while plays a pro-inflammatory role in macrophages, neutrophils and iNKT.

6, TNF-α, as well as metalloproteinases MMP-2 and MMP-9, which help to reshape the extracellular matrix at inflammation sites. However, M2 macrophages produce IL-10, PDGF, IGF-1, TGF-β, and other cytokines that suppress inflammation and foster tissue repair (94–96).

In vitro experiments reveal that the absence of GPR35 does not influence the differentiation of macrophages into M1 and M2 subtypes. However, the level of secreted substances such as CXCL-1, VEGF, MMP2, and MMP9 is reduced in GPR35-deficient macrophages derived from mice with GPR35 deletion, specifically the $LysM^+$ cells (44, 45). When observing macrophages in the colon, a notable difference in cytokine expression was seen between $GPR35^{+/+}$ and $GPR35^{-/-}$ macrophages. Proinflammatory cytokines like IL-1 and TNF were highly expressed in $GPR35^{+/+}$ macrophages. Moreover, GPR35 was predominantly expressed in monocyte subgroups exhibiting high to medium levels of Ly6C in the intestine, with the level of intestinal TNF significantly dropping in $GPR35^{-/-}$ macrophages (27). $Ly6C^+$ macrophages, differentiated from monocytes expressing high to medium levels of Ly6C, are known to be pro-inflammatory (97).

Additionally, GPR35 deficiency hinders the adhesion of human peripheral monocytes induced by kynurenic acid (20). In contrast, the activation of GPR35 elevates the infiltration level of macrophages in gastric tissues (11). Taken together, it becomes evident that GPR35 contributes to the pro-inflammatory phenotype of macrophages.

GPR35 has pro-inflammatory function in iNKT cells

Human iNKT cells constitute a unique subset of T cells that feature an invariant alpha-beta T-cell receptor (TCR) along with numerous surface molecules characteristic of natural killer (NK) cells. These iNKT cells can become activated either directly through the engagement of the invariant TCR with glycolipid antigens and CD1d, or indirectly through activated antigen-presenting cell (98). Notably, iNKT cells are critical immunoregulatory components capable of rapid, abundant cytokine production, thus influencing the behavior of other immune cells (99). iNKT cells have been found to express GPR35 at high levels. Following receptor activation, GPR35 undergoes internalization within these iNKT cells. It has been observed that specific GPR35 agonists significantly decrease the release of Interleukin-4 (IL-4), but not Interferon gamma (IFN-γ) (22). IL-4 secretion by iNKT cells plays a crucial role in anti-inflammatory processes, such as M2 polarization of macrophages, the transition of monocytes (from $Ly6C^{hi}$ to $Ly6C^{lo}$), and tissue repair, particularly in the liver (100–102). Thus, the activation of GPR35 in iNKT cells, which in turn reduces the release of IL-4, has the potential to stimulate inflammation.

Overall, it is increasingly apparent that GPR35 can play a pro-inflammatory role under certain circumstances. This is evidenced by the role of GPR35 in the stimulation of pro-inflammatory cytokines and chemokines production, the activation of inflammatory signaling pathways, and the amplification of pro-inflammatory responses in particular cell types. However, it is crucial to emphasize that the pro-inflammatory effects of GPR35 may be modulated by various factors. These include the microenvironment in which the receptor operates, the existence of other receptor types and signaling pathways, as well as the specific cellular type expressing GPR35. Further in-depth research is required to unravel the underlying mechanisms and conclusively establish the exact role of GPR35 within the context of inflammation. The complexity of these dynamics underscores the intricate interplay of processes within our immune responses and the need for a comprehensive understanding of GPR35.

Anti-inflammatory role of GPR35

Although GPR35 is predominantly linked with pro-inflammatory actions across a range of immune cells, recent studies reveal its potential anti-inflammatory capabilities under certain circumstances. This perspective brings to light the anti-inflammatory roles of GPR35 (Figure 2), enriching our understanding of its part in maintaining immune homeostasis and potentially revealing novel therapeutic targets for treating various inflammatory conditions.

GPR35 displays an anti-inflammatory role, particularly in colonic inflammation. It is prominently expressed in the gastrointestinal tract, playing a pivotal role in regulating the healing process of gastrointestinal injuries. This means GPR35 can inhibit inflammation by maintaining gastrointestinal homeostasis. In fact, GPR35 demonstrates protective qualities in scenarios such as DSS-induced colitis (27, 63, 103). GPR35 agonists such as YE120, zaprinast, and pamoic acid have been shown to expedite wound repair in mouse colon epithelial cells by boosting cell migration through the augmentation of fibronectin expression and ERK phosphorylation (63). Interestingly, the recruitment of β-arrestin 2 and the activation of ERK1/2, mediated by pamoic acid, can be obstructed by the GPR35 antagonist CID2745687. The endogenous ligand of GPR35, 5-HIAA, has been recognized to mitigate the symptoms of ulcerative colitis, while the precise role of GPR35 was not delineated further (104). This insight suggests a potential therapeutic strategy, hinting that manipulating the activity of GPR35 could control the inflammatory response in colonic tissues (60).

GPR35 deficiency in intestinal epithelial cells leads to the reduction in both the number of goblet cells and the expression of Muc2, which occurs due to an increase in the pyroptosis of goblet cells (77). Pyroptosis is a form of cell death that contributes to inflammation, thus exacerbating inflammatory conditions. In consequence of this, the epithelial barrier is weakened, leading to increased susceptibility to infections such as those caused by Citrobacter rodentium (105, 106). In DSS-induced colitis mouse models, the specific deletion of GPR35 in macrophages resulted in elevated inflammation associated with a decrease in TNF production in macrophages (27). Although TNF is generally recognized for its pro-inflammatory role and is routinely targeted in treatments for IBD (107), it also serves an anti-inflammatory function. Specifically, it can induce the production of corticosterone in intestinal epithelial cells, a hormone that plays a key role in

mitigating inflammation. The interplay between GPR35 and TNF provides new insights into the mechanisms through which inflammation can be regulated and controlled in gastrointestinal disorders (108).

Recently, it was discovered that when GPR35 was eliminated in the liver of non-alcoholic fatty liver disease (NAFLD) mice, there was an increase in inflammatory cytokines such as TNFα, IL6, and IL1β in the liver. This led to a worsening of hepatitis. However, the overexpression of GPR35 in the liver reversed these effects, hinting at its potential anti-inflammatory role in liver disease (109). Additionally, Kynurenic acid was found to improve energy metabolism and reduce inflammation in mice fed with a high-fat diet. However, TNF in adipose tissue increased in the absence of GPR35, and the anti-inflammatory effects of kynurenic acid were abolished via reducing anti-inflammatory cytokines such as IL-13, IL-4, IL-10, and IL-33 (74). This finding suggests that GPR35 might also exhibit anti-inflammatory effects in adipose tissue. Therefore, understanding the precise function of GPR35 could provide novel insights into inflammation in diseases like NAFLD and obesity. Interestingly, it is worth noting that kynurenic acid is recognized for its anti-inflammatory effects (74, 110, 111). However, kynurenic acid is known to interact with multiple receptors, not just GPR35, including α7 nicotinic acetylcholine receptor (a7nAChR), the aryl hydrocarbon receptor (AhR), and ionotropic glutamate receptors (112). Therefore, it is challenging to discern the specific influence of GPR35 in the observed anti-inflammatory effects of kynurenic acid.

In various tissues, different types of cells contribute to the anti-inflammatory effects orchestrated by GPR35. In the colorectum, it mainly operates through epithelial cells and goblet cells to exert anti-inflammatory effects. While it is known that GPR35 also facilitates anti-inflammatory in both the adipose tissue and the liver, the specific cell types involved in these processes have yet to be identified. When looking at immune cells, it was highlighted that the activation of GPR35 with kynurenic acid in macrophages leads to the suppression of NLRP3 inflammasome activation, consequently reducing related inflammation (49). Moreover, it was revealed that GPR35, along with its platelet- and mast-cell-derived ligand 5-HIAA, facilitates eosinophils recruitment to the lungs infected with cryptococcus neoformans and exacerbation of disease (82).

Overall, activation of GPR35 has been demonstrated to suppress the production of pro-inflammatory cytokines, stimulate the output of anti-inflammatory cytokines in a variety of tissues and organs, and fortify the integrity of the gut barrier. These findings highlight the multifaceted and context-dependent nature of GPR35 in inflammation. Nonetheless, a full understanding of the intricacies involved in GPR35 in counteracting anti-inflammatory demands further investigation.

Conclusion and discussion

GPR35 is a distinctive G protein-coupled receptor with a significant role in the regulation of inflammation, demonstrating both pro-inflammatory and anti-inflammatory properties. Its pro-inflammatory effects have been witnessed in neutrophils, macrophages, and iNKT cells, while its anti-inflammatory attributes have been noted in varying contexts, including those in the colorectum, adipose tissue, and liver. Furthermore, GPR35 is increasingly recognized for its role in cancer and the related immune response. It facilitates angiogenesis within the tumor microenvironment, and GPR35 elimination in macrophages results in reduced immune cell infiltration in colon tumors (44). It was found that activating GPR35 in group 2 innate lymphoid cells fosters an immunosuppressive environment in lung cancer, thereby advancing the progression of lung cancer (113, 114). Consequently, GPR35 exhibits promising potential as a therapeutic target in cancer immunotherapy.

The question arises, "Could GPR35 be a potential target for treating inflammation-associated diseases?" The dual role of GPR35 in inflammation could have considerable consequences for devising therapeutic strategies centered on this receptor. For instance, the use of GPR35 agonists or antagonists could serve to regulate inflammatory responses in diverse diseases. In conditions characterized by excessive inflammation, such as IBD, inhibiting the pro-inflammatory actions of GPR35 could be advantageous. Conversely, in conditions requiring inflammation, such as wound healing or tissue repair, augmenting the anti-inflammatory effects of GPR35 activation may be beneficial. However, considering the intricate nature of GPR35 in inflammation, more comprehensive research is required to determine its exact impact and potential as a therapeutic target.

Currently, only two drugs targeting GPR35 have progressed to clinical trials. The first, Lodoxamide, has been employed for the treatment of allergic keratocon junctivitis (51), while the other, sodium cromoglycate (also known as RVT-1601 or PA101), is being used for treating idiopathic pulmonary fibrosis and chronic cough (115). Despite this, the potential for GPR35-focused treatment remains promising. GPR35 offers several advantages as a therapeutic target. Firstly, as a receptor situated on the cell surface, it is at the top of the signal transduction pathway. This is particularly significant given the numerous redundancies and compensatory mechanisms that exist within inflammation-related signal pathways, which can minimize these effects at the starting point.

Furthermore, GPR35 exhibits a tissue-specific expression pattern with high expression levels observed in immune cells and the gastrointestinal tract. The robust expression of GPR35 in immune cells aligns with its role in the regulation of inflammation. The substantial presence of GPR35 in the gastrointestinal tract, along with the strong correlation between GPR35 mutation and IBD, has led to the consideration of GPR35 as a potential target for IBD treatment. However, it is important to acknowledge the dual role GPR35 plays in IBD. While inhibiting GPR35 activity can diminish the proinflammatory response of macrophages and neutrophils, it may concurrently hinder the repair of gastrointestinal damage. Consequently, the risk-benefit balance of targeting GPR35 for IBD treatment remains somewhat elusive and requires further investigation.

The precise mechanisms driving the dual roles of GPR35 in promoting and inhibiting inflammation across various cells and

tissues remain unclear and necessitate more in-depth research. The effects of GPR35 activation on inflammatory responses are likely shaped by numerous factors. These include cell types, tissue microenvironments, and the co-existence of other signaling pathways. Additionally, the expression levels and functional activity of GPR35 agonists may also be instrumental in steering the direction of the inflammatory response. In conclusion, GPR35 is a versatile receptor that can adopt either pro-inflammatory or anti-inflammatory stances within distinct immune cells and tissues. This warrants additional comprehensive research into GPR35 intricacies. Recently, the unveiling of the first protein structure of GPR35 marked a significant advancement in our understanding of GPR35 (86). As our knowledge of GPR35 deepens, we can anticipate the development and application of a broader range of GPR35-targeted drugs in the future.

Author contributions

YW: Writing – original draft. PZ: Writing – original draft, Writing – review & editing. HF: Writing – review & editing. CZ: Writing – review & editing. PY: Writing – review & editing. XL: Conceptualization, Supervision. YC: Writing – review & editing, Conceptualization, Funding acquisition, Supervision.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Weavers H, Martin P. The cell biology of inflammation: From common traits to remarkable immunological adaptations. *J Cell Biol* (2020) 219(7):e202004003. doi: 10.1083/jcb.202004003

2. Pagan AJ, Ramakrishnan L. The formation and function of granulomas. *Annu Rev Immunol* (2018) 36:639–65. doi: 10.1146/annurev-immunol-032712-100022

3. Gurevich DB, French KE, Collin JD, Cross SJ, Martin P. Live imaging the foreign body response in zebrafish reveals how dampening inflammation reduces fibrosis. *J Cell Sci* (2019) 133(5). doi: 10.1242/jcs.236075

4. Sofias AM, De Lorenzi F, Pena Q, Azadkhah Shalmani A, Vucur M, Wang JW, et al. Therapeutic and diagnostic targeting of fibrosis in metabolic, proliferative and viral disorders. *Adv Drug Delivery Rev* (2021) 175:113831. doi: 10.1016/j.addr.2021.113831

5. Seyedabadi M, Ghahremani MH, Albert PR. Biased signaling of G protein coupled receptors (GPCRs): Molecular determinants of GPCR/transducer selectivity and therapeutic potential. *Pharmacol Ther* (2019) 200:148–78. doi: 10.1016/j.pharmthera.2019.05.006

6. Insel PA, Snead A, Murray F, Zhang L, Yokouchi H, Katakia T, et al. GPCR expression in tissues and cells: are the optimal receptors being used as drug targets? *Br J Pharmacol* (2012) 165(6):1613–6. doi: 10.1111/j.1476-5381.2011.01434.x

7. Milligan G. Orthologue selectivity and ligand bias: translating the pharmacology of GPR35. *Trends Pharmacol Sci* (2011) 32(5):317–25. doi: 10.1016/j.tips.2011.02.002

8. Wyant GA, Yu W, Doulamis IP, Nomoto RS, Saeed MY, Duignan T, et al. Mitochondrial remodeling and ischemic protection by G protein-coupled receptor 35 agonists. *Science* (2022) 377(6606):621–9. doi: 10.1126/science.abm1638

9. Quon T, Lin LC, Ganguly A, Tobin AB, Milligan G. Therapeutic opportunities and challenges in targeting the orphan G protein-coupled receptor GPR35. *ACS Pharmacol Transl Sci* (2020) 3(5):801–12. doi: 10.1021/acsptsci.0c00079

10. Im DS. Recent advances in GPR35 pharmacology; 5-HIAA serotonin metabolite becomes a ligand. *Arch Pharm Res* (2023) 46(6):550–63. doi: 10.1007/s12272-023-01449-y

11. Shu C, Wang C, Chen S, Huang X, Cui J, Li W, et al. ERR-activated GPR35 promotes immune infiltration level of macrophages in gastric cancer tissues. *Cell Death Discovery* (2022) 8(1):444. doi: 10.1038/s41420-022-01238-4

12. Kaya B, Melhem H, Niess JH. GPR35 in intestinal diseases: from risk gene to function. *Front Immunol* (2021) 12:717392. doi: 10.3389/fimmu.2021.717392

13. Milligan G. GPR35: from enigma to therapeutic target. *Trends Pharmacol Sci* (2023) 44(5):263–73. doi: 10.1016/j.tips.2023.03.001

14. Feng Z, Sun R, Cong Y, Liu Z. Critical roles of G protein-coupled receptors in regulating intestinal homeostasis and inflammatory bowel disease. *Mucosal Immunol* (2022) 15(5):819–28. doi: 10.1038/s41385-022-00538-3

15. Zhou Q, Yang D, Wu M, Guo Y, Guo W, Zhong L, et al. Common activation mechanism of class A GPCRs. *Elife* (2019) 8:e50279. doi: 10.7554/eLife.50279

16. Okumura S, Baba H, Kumada T, Nanmoku K, Nakajima H, Nakane Y, et al. Cloning of a G-protein-coupled receptor that shows an activity to transform NIH3T3 cells and is expressed in gastric cancer cells. *Cancer Sci* (2004) 95(2):131–5. doi: 10.1111/j.1349-7006.2004.tb03193.x

17. Ali H, AbdelMageed M, Olsson L, Israelsson A, Lindmark G, Hammarstrom ML, et al. Utility of G protein-coupled receptor 35 expression for predicting outcome in colon cancer. *Tumour Biol* (2019) 41(6):1010428319858885. doi: 10.1177/1010428319858885

18. Marti-Solano M, Crilly SE, Malinverni D, Munk C, Harris M, Pearce A, et al. Combinatorial expression of GPCR isoforms affects signalling and drug responses. *Nature* (2020) 587(7835):650–6. doi: 10.1038/s41586-020-2888-2

19. Schihada H, Klompstra TM, Humphrys LJ, Cervenka I, Dadvar S, Kolb P, et al. Isoforms of GPR35 have distinct extracellular N-termini that allosterically modify receptor-transducer coupling and mediate intracellular pathway bias. *J Biol Chem* (2022) 298(9):102328. doi: 10.1016/j.jbc.2022.102328

20. Barth MC, Ahluwalia N, Anderson TJ, Hardy GJ, Sinha S, Alvarez-Cardona JA, et al. Kynurenic acid triggers firm arrest of leukocytes to vascular endothelium under flow conditions. *J Biol Chem* (2009) 284(29):19189–95. doi: 10.1074/jbc.M109.024042

21. Yang Y, Lu JY, Wu X, Summer S, Whoriskey J, Saris C, et al. G-protein-coupled receptor 35 is a target of the asthma drugs cromolyn disodium and nedocromil sodium. *Pharmacology* (2010) 86(1):1–5. doi: 10.1159/000314164

22. Fallarini S, Magliulo L, Paoletti T, de Lalla C, Lombardi G. Expression of functional GPR35 in human iNKT cells. *Biochem Biophys Res Commun* (2010) 398 (3):420–5. doi: 10.1016/j.bbrc.2010.06.091

23. O'Dowd BF, Nguyen T, Marchese A, Cheng R, Lynch KR, Heng HH, et al. Discovery of three novel G-protein-coupled receptor genes. *Genomics* (1998) 47 (2):310–3. doi: 10.1006/geno.1998.5095

24. Guo J, Williams DJ, Puhl HL3rd, Ikeda SR. Inhibition of N-type calcium channels by activation of GPR35, an orphan receptor, heterologously expressed in rat sympathetic neurons. *J Pharmacol Exp Ther* (2008) 324(1):342–51. doi: 10.1124/jpet.107.127266

25. Xu H, Ding J, Porter CBM, Wallrapp A, Tabaka M, Ma S, et al. Transcriptional atlas of intestinal immune cells reveals that neuropeptide alpha-CGRP modulates

group 2 innate lymphoid cell responses. *Immunity* (2019) 51(4):696–708 e9. doi: 10.1016/j.immuni.2019.09.004

26. De Giovanni M, Tam H, Valet C, Xu Y, Looney MR, Cyster JG. GPR35 promotes neutrophil recruitment in response to serotonin metabolite 5-HIAA. *Cell* (2022) 185 (5):815–30 e19. doi: 10.1016/j.cell.2022.01.010

27. Kaya B, Donas C, Wuggenig P, Diaz OE, Morales RA, Melhem H, et al. Lysophosphatidic acid-mediated GPR35 signaling in CX3CR1(+) macrophages regulates intestinal homeostasis. *Cell Rep* (2020) 32(5):107979. doi: 10.1016/j.celrep.2020.107979

28. Divorty N, Mackenzie AE, Nicklin SA, Milligan G. G protein-coupled receptor 35: an emerging target in inflammatory and cardiovascular disease. *Front Pharmacol* (2015) 6:41. doi: 10.3389/fphar.2015.00041

29. Sriram K, Insel PA. G protein-coupled receptors as targets for approved drugs: how many targets and how many drugs? *Mol Pharmacol* (2018) 93(4):251–8. doi: 10.1124/mol.117.111062

30. Jenkins L, Brea J, Smith NJ, Hudson BD, Reilly G, Bryant NJ, et al. Identification of novel species-selective agonists of the G-protein-coupled receptor GPR35 that promote recruitment of beta-arrestin-2 and activate Galpha13. *Biochem J* (2010) 432 (3):451–9. doi: 10.1042/BJ20101287

31. Belcheva MM, Coscia CJ. Diversity of G protein-coupled receptor signaling pathways to ERK/MAP kinase. *Neurosignals* (2002) 11(1):34–44. doi: 10.1159/000057320

32. Maeng YS, Min JK, Kim JH, Yamagishi A, Mochizuki N, Kwon JY, et al. ERK is an anti-inflammatory signal that suppresses expression of NF-kappaB-dependent inflammatory genes by inhibiting IKK activity in endothelial cells. *Cell Signal* (2006) 18(7):994–1005. doi: 10.1016/j.cellsig.2005.08.007

33. Vuong B, Hogan-Cann AD, Alano CC, Stevenson M, Chan WY, Anderson CM, et al. NF-kappaB transcriptional activation by TNFalpha requires phospholipase C, extracellular signal-regulated kinase 2 and poly(ADP-ribose) polymerase-1. *J Neuroinflammation.* (2015) 12:229. doi: 10.1186/s12974-015-0448-8

34. Sun L, Ye RD. Role of G protein-coupled receptors in inflammation. *Acta Pharmacol Sin* (2012) 33(3):342–50. doi: 10.1038/aps.2011.200

35. Guo P, Tai Y, Wang M, Sun H, Zhang L, Wei W, et al. Galpha(12) and galpha (13): versatility in physiology and pathology. *Front Cell Dev Biol* (2022) 10:809425. doi: 10.3389/fcell.2022.809425

36. Zhang L, Shi G. Gq-coupled receptors in autoimmunity. *J Immunol Res* (2016) 2016:3969023. doi: 10.1155/2016/3969023

37. Jenkins L, Alvarez-Curto E, Campbell K, de Munnik S, Canals M, Schlyer S, et al. Agonist activation of the G protein-coupled receptor GPR35 involves transmembrane domain III and is transduced via Galpha(1)(3) and beta-arrestin-2. *Br J Pharmacol* (2011) 162(3):733–48. doi: 10.1111/j.1476-5381.2010.01082.x

38. Jean-Charles PY, Kaur S, Shenoy SK. G protein-coupled receptor signaling through beta-arrestin-dependent mechanisms. *J Cardiovasc Pharmacol* (2017) 70 (3):142–58. doi: 10.1097/FJC.0000000000000482

39. Eishingdrelo H, Sun W, Li H, Wang L, Eishingdrelo A, Dai S, et al. ERK and beta-arrestin interaction: a converging point of signaling pathways for multiple types of cell surface receptors. *J Biomol Screen.* (2015) 20(3):341–9. doi: 10.1177/1087057114557233

40. Deng H, Hu H, Fang Y. Multiple tyrosine metabolites are GPR35 agonists. *Sci Rep* (2012) 2:373. doi: 10.1038/srep00373

41. Gu YJ, Sun WY, Zhang S, Wu JJ, Wei W. The emerging roles of beta-arrestins in fibrotic diseases. *Acta Pharmacol Sin* (2015) 36(11):1277–87. doi: 10.1038/aps.2015.74

42. De Giovanni M, Chen H, Li X, Cyster JG. GPR35 and mediators from platelets and mast cells in neutrophil migration and inflammation. *Immunol Rev* (2023) 317 (1):187–202. doi: 10.1111/imr.13194

43. Deng H, Fang Y. Synthesis and agonistic activity at the GPR35 of 5,6-dihydroxyindole-2-carboxylic acid analogues. *ACS Med Chem Lett* (2012) 3(7):550–4. doi: 10.1021/ml300076u

44. Pagano E, Elias JE, Schneditz G, Saveljeva S, Holland LM, Borrelli F, et al. Activation of the GPR35 pathway drives angiogenesis in the tumour microenvironment. *Gut* (2022) 71(3):509–20. doi: 10.1136/gutjnl-2020-323363

45. Schneditz G, Elias JE, Pagano E, Zaeem Cader M, Saveljeva S, Long K, et al. GPR35 promotes glycolysis, proliferation, and oncogenic signaling by engaging with the sodium potassium pump. *Sci Signal* (2019) 12(562):eaau9048. doi: 10.1126/scisignal.aau9048

46. Zophel D, Hof C, Lis A. Altered ca(2+) homeostasis in immune cells during aging: role of ion channels. *Int J Mol Sci* (2020) 22(1):110. doi: 10.3390/ijms22010110

47. Iamartino L, Brandi ML. The calcium-sensing receptor in inflammation: Recent updates. *Front Physiol* (2022) 13:1059369. doi: 10.3389/fphys.2022.1059369

48. Sun T, Xie R, He H, Xie Q, Zhao X, Kang G, et al. Kynurenic acid ameliorates NLRP3 inflammasome activation by blocking calcium mobilization via GPR35. *Front Immunol* (2022) 13:1019365. doi: 10.3389/fimmu.2022.1019365

49. Chen Z, Jiao Y, Zhang Y, Wang Q, Wu W, Zheng J, et al. G-protein coupled receptor 35 induces intervertebral disc degeneration by mediating the influx of calcium ions and upregulating reactive oxygen species. *Oxid Med Cell Longev* (2022) 2022:5469220. doi: 10.1155/2022/5469220

50. Venkateswaran S, Prince J, Cutler DJ, Marigorta UM, Okou DT, Prahalad S, et al. Enhanced contribution of HLA in pediatric onset ulcerative colitis. *Inflammation Bowel Dis* (2018) 24(4):829–38. doi: 10.1093/ibd/izx084

51. MacKenzie AE, Caltabiano G, Kent TC, Jenkins L, McCallum JE, Hudson BD, et al. The antiallergic mast cell stabilizers lodoxamide and bufrolin as the first high and equipotent agonists of human and rat GPR35. *Mol Pharmacol* (2014) 85(1):91–104. doi: 10.1124/mol.113.089482

52. Dantzer R. Role of the kynurenine metabolism pathway in inflammation-induced depression: preclinical approaches. *Curr Top Behav Neurosci* (2017) 31:117–38. doi: 10.1007/7854_2016_6

53. Wang J, Simonavicius N, Wu X, Swaminath G, Reagan J, Tian H, et al. Kynurenic acid as a ligand for orphan G protein-coupled receptor GPR35. *J Biol Chem* (2006) 281(31):22021–8. doi: 10.1074/jbc.M603503200

54. Oka S, Ota R, Shima M, Yamashita A, Sugiura T. GPR35 is a novel lysophosphatidic acid receptor. *Biochem Biophys Res Commun* (2010) 395(2):232–7. doi: 10.1016/j.bbrc.2010.03.169

55. Mathew D, Torres RM. Lysophosphatidic acid is an inflammatory lipid exploited by cancers for immune evasion via mechanisms similar and distinct from CTLA-4 and PD-1. *Front Immunol* (2020) 11:531910. doi: 10.3389/fimmu.2020.531910

56. Maravillas-Montero JL, Burkhardt AM, Hevezi PA, Carnevale CD, Smit MJ, Zlotnik A. Cutting edge: GPR35/CXCR8 is the receptor of the mucosal chemokine CXCL17. *J Immunol* (2015) 194(1):29–33. doi: 10.4049/jimmunol.1401704

57. Xiao S, Xie W, Zhou L. Mucosal chemokine CXCL17: What is known and not known. *Scand J Immunol* (2021) 93(2):e12965. doi: 10.1111/sji.12965

58. Southern C, Cook JM, Neetoo-Isseljee Z, Taylor DL, Kettleborough CA, Merritt A, et al. Screening beta-arrestin recruitment for the identification of natural ligands for orphan G-protein-coupled receptors. *J Biomol Screen.* (2013) 18(5):599–609. doi: 10.1177/1087057113475480

59. Taniguchi Y, Tonai-Kachi H, Shinjo K. Zaprinast, a well-known cyclic guanosine monophosphate-specific phosphodiesterase inhibitor, is an agonist for GPR35. *FEBS Lett* (2006) 580(21):5003–8. doi: 10.1016/j.febslet.2006.08.015

60. Zhao P, Sharir H, Kapur A, Cowan A, Geller EB, Adler MW, et al. Targeting of the orphan receptor GPR35 by pamoic acid: a potent activator of extracellular signal-regulated kinase and beta-arrestin2 with antinociceptive activity. *Mol Pharmacol* (2010) 78(4):560–8. doi: 10.1124/mol.110.066746

61. De Leo F, Rossi A, De Marchis F, Cigana C, Melessike M, Quilici G, et al. Pamoic acid is an inhibitor of HMGB1.CXCL12 elicited chemotaxis and reduces inflammation in murine models of Pseudomonas aeruginosa pneumonia. *Mol Med* (2022) 28(1):108. doi: 10.1186/s10020-022-00535-z

62. Deng H, Hu H, He M, Hu J, Niu W, Ferrie AM, et al. Discovery of 2-(4-methylfuran-2(5H)-ylidene)malononitrile and thieno[3,2-b]thiophene-2-carboxylic acid derivatives as G protein-coupled receptor 35 (GPR35) agonists. *J Med Chem* (2011) 54(20):7385–96. doi: 10.1021/jm200999f

63. Tsukahara T, Hamouda N, Utsumi D, Matsumoto K, Amagase K, Kato S. G protein-coupled receptor 35 contributes to mucosal repair in mice via migration of colonic epithelial cells. *Pharmacol Res* (2017) 123:27–39. doi: 10.1016/j.phrs.2017.06.009

64. Neetoo-Isseljee Z, MacKenzie AE, Southern C, Jerman J, McIver EG, Harries N, et al. High-throughput identification and characterization of novel, species-selective GPR35 agonists. *J Pharmacol Exp Ther* (2013) 344(3):568–78. doi: 10.1124/jpet.112.201798

65. Funke M, Thimm D, Schiedel AC, Muller CE. 8-Benzamidochromen-4-one-2-carboxylic acids: potent and selective agonists for the orphan G protein-coupled receptor GPR35. *J Med Chem* (2013) 56(12):5182–97. doi: 10.1021/jm400587g

66. Yang Y, Fu A, Wu X, Reagan JD. GPR35 is a target of the loop diuretic drugs bumetanide and furosemide. *Pharmacology* (2012) 89(1-2):13–7. doi: 10.1159/000335127

67. Deng H, Hu H, Fang Y. Tyrphostin analogs are GPR35 agonists. *FEBS Lett* (2011) 585(12):1957–62. doi: 10.1016/j.febslet.2011.05.026

68. Deng H, Hu J, Hu H, He M, Fang Y. Thieno[3,2-b]thiophene-2-carboxylic acid derivatives as GPR35 agonists. *Bioorg Med Chem Lett* (2012) 22(12):4148–52. doi: 10.1016/j.bmcl.2012.04.057

69. Deng H, Fang Y. Anti-inflammatory gallic Acid and wedelolactone are G protein-coupled receptor-35 agonists. *Pharmacology* (2012) 89(3-4):211–9. doi: 10.1159/000337184

70. Otkur W, Wang J, Hou T, Liu F, Yang R, Li Y, et al. Aminosalicylates target GPR35, partly contributing to the prevention of DSS-induced colitis. *Eur J Pharmacol* (2023) 949:175719. doi: 10.1016/j.ejphar.2023.175719

71. Wei L, Wang J, Zhang X, Wang P, Zhao Y, Li J, et al. Discovery of 2H-chromen-2-one derivatives as G protein-coupled receptor-35 agonists. *J Med Chem* (2017) 60 (1):362–72. doi: 10.1021/acs.jmedchem.6b01431

72. Jenkins L, Harries N, Lappin JE, MacKenzie AE, Neetoo-Isseljee Z, Southern C, et al. Antagonists of GPR35 display high species ortholog selectivity and varying modes of action. *J Pharmacol Exp Ther* (2012) 343(3):683–95. doi: 10.1124/jpet.112.198945

73. Vecsei L, Szalardy L, Fulop F, Toldi J. Kynurenines in the CNS: recent advances and new questions. *Nat Rev Drug Discovery* (2013) 12(1):64–82. doi: 10.1038/nrd3793

74. Agudelo LZ, Ferreira DMS, Cervenka I, Bryzgalova G, Dadvar S, Jannig PR, et al. Kynurenic acid and gpr35 regulate adipose tissue energy homeostasis and inflammation. *Cell Metab* (2018) 27(2):378–92 e5. doi: 10.1016/j.cmet.2018.01.004

75. Miyamoto K, Sujino T, Harada Y, Ashida H, Yoshimatsu Y, Yonemoto Y, et al. The gut microbiota-induced kynurenic acid recruits GPR35-positive macrophages to

promote experimental encephalitis. *Cell Rep* (2023) 42(8):113005. doi: 10.1016/j.celrep.2023.113005

76. Ye X, Chun J. Lysophosphatidic acid (LPA) signaling in vertebrate reproduction. *Trends Endocrinol Metab* (2010) 21(1):17–24. doi: 10.1016/j.tem.2009.08.003

77. Melhem H, Kaya B, Kaymak T, Wuggenig P, Flint E, Roux J, et al. Epithelial GPR35 protects from Citrobacter rodentium infection by preserving goblet cells and mucosal barrier integrity. *Mucosal Immunol* (2022) 15(3):443–58. doi: 10.1038/s41385-022-00494-y

78. Burkhardt AM, Tai KP, Flores-Guiterrez JP, Vilches-Cisneros N, Kamdar K, Barbosa-Quintana O, et al. CXCL17 is a mucosal chemokine elevated in idiopathic pulmonary fibrosis that exhibits broad antimicrobial activity. *J Immunol* (2012) 188 (12):6399–406. doi: 10.4049/jimmunol.1102903

79. Binti Mohd Amir NAS, Mackenzie AE, Jenkins L, Boustani K, Hillier MC, Tsuchiya T, et al. Evidence for the existence of a CXCL17 receptor distinct from GPR35. *J Immunol* (2018) 201(2):714–24. doi: 10.4049/jimmunol.1700884

80. Park SJ, Lee SJ, Nam SY, Im DS. GPR35 mediates lodoxamide-induced migration inhibitory response but not CXCL17-induced migration stimulatory response in THP-1 cells; is GPR35 a receptor for CXCL17? *Br J Pharmacol* (2018) 175(1):154–61. doi: 10.1111/bph.14082

81. Rojewska E, Ciapala K, Mika J. Kynurenic acid and zaprinast diminished CXCL17-evoked pain-related behaviour and enhanced morphine analgesia in a mouse neuropathic pain model. *Pharmacol Rep* (2019) 71(1):139–48. doi: 10.1016/j.pharep.2018.10.002

82. De Giovanni M, Dang EV, Chen KY, An J, Madhani HD, Cyster JG. Platelets and mast cells promote pathogenic eosinophil recruitment during invasive fungal infection via the 5-HIAA-GPR35 ligand-receptor system. *Immunity* (2023) 56(7):1548–60 e5. doi: 10.1016/j.immuni.2023.05.006

83. Chen Y, Palm F, Lesch KP, Gerlach M, Moessner R, Sommer C. 5-hydroxyindolacetic acid (5-HIAA), a main metabolite of serotonin, is responsible for complete Freund's adjuvant-induced thermal hyperalgesia in mice. *Mol Pain.* (2011) 7:21. doi: 10.1186/1744-8069-7-21

84. Ciapala K, Mika J, Rojewska E. The kynurenine pathway as a potential target for neuropathic pain therapy design: from basic research to clinical perspectives. *Int J Mol Sci* (2021) 22(20):11055. doi: 10.3390/ijms222011055

85. Kim MJ, Park SJ, Nam SY, Im DS. Lodoxamide attenuates hepatic fibrosis in mice: involvement of GPR35. *Biomol Ther (Seoul).* (2020) 28(1):92–7. doi: 10.4062/biomolther.2018.227

86. Duan J, Liu Q, Yuan Q, Ji Y, Zhu S, Tan Y, et al. Insights into divalent cation regulation and G(13)-coupling of orphan receptor GPR35. *Cell Discovery* (2022) 8 (1):135. doi: 10.1038/s41421-022-00499-8

87. Wei L, Xiang K, Kang H, Yu Y, Fan H, Zhou H, et al. Development and characterization of fluorescent probes for the G protein-coupled receptor 35. *ACS Med Chem Lett* (2023) 14(4):411–6. doi: 10.1021/acsmedchemlett.2c00461

88. Mackenzie AE, Quon T, Lin LC, Hauser AS, Jenkins L, Inoue A, et al. Receptor selectivity between the G proteins Galpha(12) and Galpha(13) is defined by a single leucine-to-isoleucine variation. *FASEB J* (2019) 33(4):5005–17. doi: 10.1096/fj.201801956R

89. Boleij A, Fathi P, Dalton W, Park B, Wu X, Huso D, et al. G-protein coupled receptor 35 (GPR35) regulates the colonic epithelial cell response to enterotoxigenic Bacteroides fragilis. *Commun Biol* (2021) 4(1):585. doi: 10.1038/s42003-021-02014-3

90. Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nat Rev Immunol* (2007) 7 (9):678–89. doi: 10.1038/nri2156

91. Petri B, Sanz MJ. Neutrophil chemotaxis. *Cell Tissue Res* (2018) 371(3):425–36. doi: 10.1007/s00441-017-2776-8

92. Gonzalez RJ, von Andrian UH. Quo vadis, neutrophil? *Cell* (2022) 185(5):759–61. doi: 10.1016/j.cell.2022.02.009

93. Bordon Y. Platelet-derived 5-HIAA helps neutrophils enter tissue. *Nat Rev Immunol* (2022) 22(4):206–7. doi: 10.1038/s41577-022-00699-z

94. Martinez FO, Gordon S. The M1 and M2 paradigm of macrophage activation: time for reassessment. *F1000Prime Rep* (2014) 6:13. doi: 10.12703/P6-13

95. Ploeger DT, Hosper NA, Schipper M, Koerts JA, de Rond S, Bank RA. Cell plasticity in wound healing: paracrine factors of M1/ M2 polarized macrophages influence the phenotypical state of dermal fibroblasts. *Cell Commun Signal* (2013) 11 (1):29. doi: 10.1186/1478-811X-11-29

96. Nguyen-Chi M, Laplace-Builhe B, Travnickova J, Luz-Crawford P, Tejedor G, Phan QT, et al. Identification of polarized macrophage subsets in zebrafish. *Elife* (2015) 4:e07288. doi: 10.7554/eLife.07288

97. Kratofil RM, Kubes P, Deniset JF. Monocyte conversion during inflammation and injury. *Arterioscler Thromb Vasc Biol* (2017) 37(1):35–42. doi: 10.1161/ATVBAHA.116.308198

98. Godfrey DI, MacDonald HR, Kronenberg M, Smyth MJ, Van Kaer L. NKT cells: what's in a name? *Nat Rev Immunol* (2004) 4(3):231–7. doi: 10.1038/nri1309

99. Van Kaer L, Parekh VV, Wu L. Invariant natural killer T cells: bridging innate and adaptive immunity. *Cell Tissue Res* (2011) 343(1):43–55. doi: 10.1007/s00441-010-1023-3

100. Liew PX, Lee WY, Kubes P. iNKT cells orchestrate a switch from inflammation to resolution of sterile liver injury. *Immunity* (2017) 47(4):752–65 e5. doi: 10.1016/j.immuni.2017.09.016

101. Ahn S, Jeong D, Oh SJ, Ahn J, Lee SH, Chung DH. GM-CSF and IL-4 produced by NKT cells inversely regulate IL-1beta production by macrophages. *Immunol Lett* (2017) 182:50–6. doi: 10.1016/j.imlet.2017.01.003

102. Yoshimoto T. The hunt for the source of primary interleukin-4: how we discovered that natural killer T cells and basophils determine T helper type 2 cell differentiation in vivo. *Front Immunol* (2018) 9:716. doi: 10.3389/fimmu.2018.00716

103. Farooq SM, Hou Y, Li H, O'Meara M, Wang Y, Li C, et al. Disruption of GPR35 exacerbates dextran sulfate sodium-induced colitis in mice. *Dig Dis Sci* (2018) 63 (11):2910–22. doi: 10.1007/s10620-018-5216-z

104. Zhang Q, Wang Q, Li X, Wu M, Wu X, Zhao Q, et al. *Gut microbiota-derived 5-hydroxyindoleacetic acid from pumpkin polysaccharides supplementation alleviates colitis through epac/rap1 signaling activation*. (2023). doi: 10.21203/rs.3.rs-3123790/v1.

105. Lei-Leston AC, Murphy AG, Maloy KJ. Epithelial cell inflammasomes in intestinal immunity and inflammation. *Front Immunol* (2017) 8:1168. doi: 10.3389/fimmu.2017.01168

106. Churchill MJ, Mitchell PS, Rauch I. Epithelial pyroptosis in host defense. *J Mol Biol* (2022) 434(4):167278. doi: 10.1016/j.jmb.2021.167278

107. Hanauer SB, Feagan BG, Lichtenstein GR, Mayer LF, Schreiber S, Colombel JF, et al. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. *Lancet* (2002) 359(9317):1541–9. doi: 10.1016/S0140-6736(02)08512-4

108. Noti M, Corazza N, Mueller C, Berger B, Brunner T. TNF suppresses acute intestinal inflammation by inducing local glucocorticoid synthesis. *J Exp Med* (2010) 207(5):1057–66. doi: 10.1084/jem.20090849

109. Wei X, Yin F, Wu M, Xie Q, Zhao X, Zhu C, et al. G protein-coupled receptor 35 attenuates nonalcoholic steatohepatitis by reprogramming cholesterol homeostasis in hepatocytes. *Acta Pharm Sin B* (2023) 13(3):1128–44. doi: 10.1016/j.apsb.2022.10.011

110. Zheng X, Hu M, Zang X, Fan Q, Liu Y, Che Y, et al. Kynurenic acid/GPR35 axis restricts NLRP3 inflammasome activation and exacerbates colitis in mice with social stress. *Brain Behav Immun* (2019) 79:244–55. doi: 10.1016/j.bbi.2019.02.009

111. Wang D, Li D, Zhang Y, Chen J, Zhang Y, Liao C, et al. Functional metabolomics reveal the role of AHR/GPR35 mediated kynurenic acid gradient sensing in chemotherapy-induced intestinal damage. *Acta Pharm Sin B* (2021) 11 (3):763–80. doi: 10.1016/j.apsb.2020.07.017

112. Cervenka I, Agudelo LZ, Ruas JL. Kynurenines: Tryptophan's metabolites in exercise, inflammation, and mental health. *Science* (2017) 357(6349):eaaf9794. doi: 10.1126/science.aaf9794

113. Mackiewicz T, Wlodarczyk J, Zielinska M, Wlodarczyk M, Durczynski A, Hogendorf P, et al. Increased GPR35 expression in human colorectal and pancreatic cancer samples: A preliminary clinical validation of a new biomarker. *Adv Clin Exp Med* (2023) 32(7):783–9. doi: 10.17219/acem/157291

114. Yue J, Guo H, Xu P, Ma J, Wu Y. Activation of the GPR35 on ILC2 drives immunosuppression to promote lung cancer progression. *Am J Cancer Res* (2023) 13 (6):2426–38.

115. Birring SS, Wijsenbeek MS, Agrawal S, van den Berg JWK, Stone H, Maher TM, et al. A novel formulation of inhaled sodium cromoglicate (PA101) in idiopathic pulmonary fibrosis and chronic cough: a randomised, double-blind, proof-of-concept, phase 2 trial. *Lancet Respir Med* (2017) 5(10):806–15. doi: 10.1016/S2213-2600(17)30310-7

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