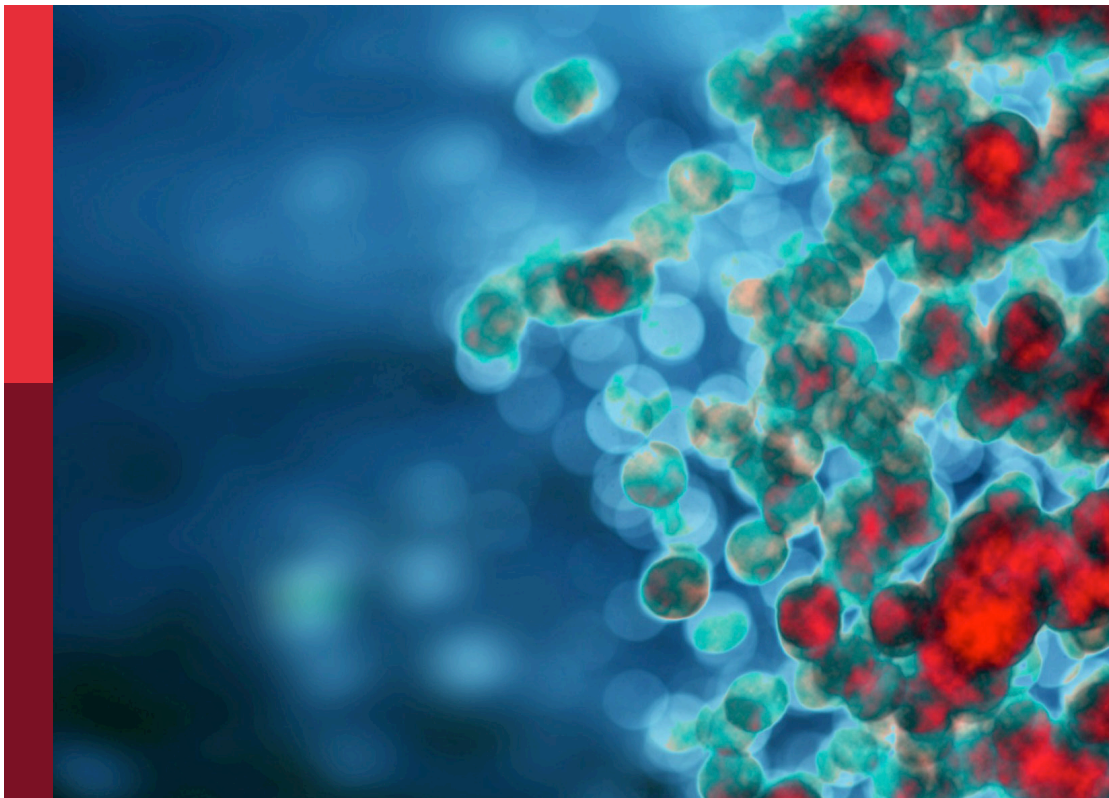
frontiers | Research Topics

The immunological regulation of extracellular vesicles on chronic diseases

Edited by
Zhiwen Luo, Chun Wai Mai, Jinhong Zhu and Shicheng Guo

Published in
Frontiers in Immunology



ISSN 1664-8714
ISBN 978-2-8325-5080-9
DOI 10.3389/978-2-8325-5080-9

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

The immunological regulation of extracellular vesicles on chronic diseases

Topic editors

Zhiwen Luo — Fudan University, China

Chun Wai Mai — UCSI University, Malaysia

Jinhong Zhu — Harbin Medical University Cancer Hospital, China

Shicheng Guo — Arrowhead Pharmaceuticals, United States

Citation

Luo, Z., Mai, C. W., Zhu, J., Guo, S., eds. (2024). *The immunological regulation of extracellular vesicles on chronic diseases*. Lausanne: Frontiers Media SA.

doi: 10.3389/978-2-8325-5080-9

Table of contents

frontiers | Frontiers in Immunology

TYPE Editorial
PUBLISHED 18 June 2024
DOI 10.3389/fimmu.2024.1442387

OPEN ACCESS

EDITED AND REVIEWED BY
Silvano Sozzani,
Sapienza University of Rome, Italy

*CORRESPONDENCE
Zhiwen Luo
zhiwen.luo_fudan@hotmail.com
Chun Wai Mai
maicw@ucsiuniversity.edu.my
Jie Mei
meijie1996@njmu.edu.cn
Jinhong Zhu
zhujinhong@hrbmu.edu.cn
Shicheng Guo
Shicheng.Guo@wisc.edu

†These authors have contributed equally to this work and share first authorship

RECEIVED 02 June 2024
ACCEPTED 10 June 2024
PUBLISHED 18 June 2024

CITATION
Wan R, Chen P, Guo S, Zhu J, Mei J, Mai CW and Luo Z (2024) Editorial: The immunological regulation of extracellular vesicles on chronic diseases.
Front. Immunol. 15:1442387.
doi: 10.3389/fimmu.2024.1442387

Editorial: The immunological regulation of extracellular vesicles on chronic diseases

Renwen Wan[1†], Peng Chen[2†], Shicheng Guo[3*], Jinhong Zhu[4*], Jie Mei[5*], Chun Wai Mai[6*] and Zhiwen Luo[1*]

[1]Department of Sports Medicine, Huashan Hospital, Fudan University, Shanghai, China, [2]Department of Sports Medicine, Peking University Shenzhen Hospital, Shenzhen, China, [3]Department of Medical Genetics, University of Wisconsin (UW)-Madison, Madison, WI, United States, [4]Department of Laboratory Medicine, Biobank Harbin Medical University Cancer Hospital, Harbin, Heilongjiang, China, [5]The First Clinical Medicine College, Nanjing Medical University, Nanjing, China, [6]Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, University College Sedaya International (UCSI), Kuala Lumpur, Malaysia

KEYWORDS

extracellular vesicles (EVs), chronic diseases, immunology, mechanisms, biomarker

Editorial on the Research Topic
The immunological regulation of extracellular vesicles on chronic diseases

Extracellular vesicles (EVs) are microscopic membrane structures that originate inside the cell and are then expelled into the extracellular matrix, especially exosomes (1). EVs are encapsulated by a lipid bilayer and harbor a variety of biomolecules, including proteins, lipids, and various forms of RNA and DNA. Primarily, EVs have been considered cellular waste. However, most researchers now found that EVs play a key role in mediating complex cellular communication (2–4). After over 30 years of exploration, the regulation of exosomes in intercellular transport mechanisms was further explored in depth. Scientists James E. Rothman, Randy W. Schekman, and Thomas C. Südho were jointly awarded the 2013 Nobel Prize for their outstanding contributions to this field. The cellular interactions responsible for exosomes are critical for a myriad of physiological processes and have implications for the pathogenesis of disease (5, 6). As our understanding of EVs continues to grow, the field has undergone a major shift and has begun to explore the potential of EVs for diagnostic and therapeutic applications (7–10). This collection of manuscripts on our topic - The Immunological Regulation of Extracellular Vesicles on Chronic Diseases, provides a comprehensive overview of the latest advances in EVs and immunology research. Experts have written 11 featuring articles in their respective fields. This Research Topic not only reveals innovative approaches to isolate and characterize EVs but also explores the functional roles of EVs in the regulation of chronic diseases. In addition, this Research Topic demonstrated EVs' emerging applications in the fields of targeted therapies and biomarker discovery shown in Figure 1.

An innovative study conducted by Lentilhas-Graca et al. investigated the impact of macrophage secretomes on recovery from spinal cord injuries. The research revealed that macrophage secretomes, exhibiting diverse polarization patterns, exert varied influences on neuronal growth and survival. Notably, secretomes activated by IL-10 and TGF-β1 were

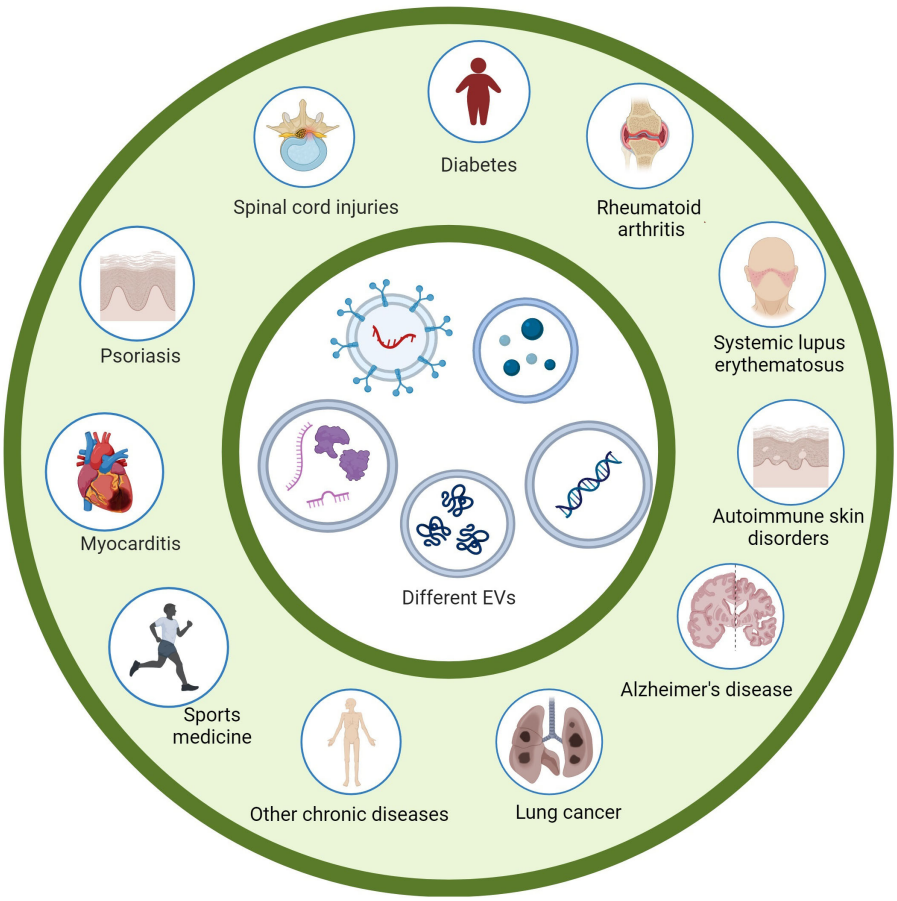


FIGURE 1
Various extracellular vesicles can play an immunomodulatory role in many chronic diseases.

found to significantly enhance axonal regeneration and contribute to functional recovery post-spinal cord injury. Proteomic analysis identified a suite of proteins within these secretomes that are pivotal in axon extension and the establishment of cell polarity, offering novel therapeutic avenues for spinal cord injury treatment.

In a comprehensive review, Iuliano et al. delineate the involvement of EVs in the pathogenesis of psoriasis and their potential therapeutic applications. The review articulates the critical function of EVs as conveyors of molecular signals across the psoriatic landscape, their utility as innovative biomarkers, and their capability as platforms for precision anti-inflammatory treatments. Furthermore, the discussion extends to the integration of EVs within the psoriasis microenvironment, their role in disease transmission, and the progression of related comorbidities, underscoring the potential of EV-based biotechnologies in both therapeutic and research settings.

Di Florio et al.'s review focuses on the role of mitochondrial EVs in autoimmune diseases, particularly myocarditis. The review highlights that viruses like Coxsackievirus B3 and SARS-CoV-2 can induce cells to release mitochondrial vesicles during infection, which subsequently trigger an immune response culminating in autoimmune reactions. Moreover, the presence of mitochondrial autoantibodies in myocarditis patients and the regulatory role of autoimmune regulatory factors (AIRE) in mitigating mitochondrial antigen-induced autoimmunity are explored. This study offers fresh perspectives on the mechanisms through which viral infections may precipitate autoimmune conditions.

A review conducted by Zhang et al. provides a comprehensive analysis of the roles of exosomes derived from various cellular origins in the context of rheumatoid arthritis (RA). The study elucidates that exosomes are intricately involved in the pathogenesis of RA and may serve pivotal roles as diagnostic markers and therapeutic agents. Notably, exosomes originating from mesenchymal stem cells demonstrate considerable potential in modulating immune responses, mitigating inflammation, and facilitating tissue repair, suggesting their viability as therapeutic modalities in RA management.

In a succinct mini-review, Zhang et al. highlight the critical functions of exosomal microRNAs (miRNAs) in autoimmune skin disorders. This review details how these miRNAs, abnormally expressed across various autoimmune skin conditions, influence disease progression by regulating the secretion of essential cytokines and directing immune cell differentiation. The potential of exosomal miRNAs as biomarkers for tracking disease activity,

recurrence, and therapeutic response is underscored, paving the way for novel targeted treatment approaches. The review calls for further investigation into the mechanisms of exosomal miRNAs to enhance clinical treatment strategies.

Ye et al.'s mini-review discusses the immunomodulatory properties of mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) in Alzheimer's disease (AD). The review proposes MSC-EVs as innovative agents for AD therapy by detailing their roles in suppressing glial cell activation, reducing inflammatory cytokine levels, and promoting both neuroprotection and amyloid β clearance. It also critically assesses the potential challenges and advantages of MSC-EVs in clinical applications, offering valuable insights for advancing extracellular vesicle-based therapies for neurodegenerative diseases.

Zhao et al.'s mini-review examines the involvement of exosomes in lung cancer progression, focusing on their roles in metastasis, diagnostic potential, and immunological interactions. Exosomes are described as crucial players in lung cancer dynamics, capable of enhancing metastatic processes and modulating immune responses. The review highlights the diagnostic potential of specific miRNAs within exosomes and discusses the innovative applications of engineered exosomes in lung cancer therapy. It also emphasizes the need for further studies to validate the safety and efficacy of exosomal applications in clinical settings.

A review authored by He et al. critically assesses the immunomodulatory functions and therapeutic potentials of natural killer cell-derived extracellular vesicles (NKEVs) in managing chronic diseases. These vesicles are enriched with a diverse array of cytotoxic proteins and nucleic acids, demonstrating promising therapeutic effects across various conditions, including malignant tumors, hepatic fibrosis, and pulmonary injuries. Despite certain challenges such as limited yield and suboptimal targeting capabilities, advancements in research concerning memory-like NK cells, their derived EVs, and engineered NKEVs are paving the way for enhanced treatment efficiency, specificity, and safety. Collectively, NKEVs are emerging as potent therapeutic agents in the realm of chronic disease management.

In the mini-review by Huang et al., the role of exosomes in sports medicine is explored, emphasizing their importance in managing chronic conditions and boosting athletic performance. The review elucidates the fundamental aspects of exosomes, including their biogenesis, release mechanisms, content profiles, and biological activities, and discusses their capabilities in facilitating muscle repair, arthritis treatment, and performance enhancement. The paper also addresses the ongoing challenges and future prospects of exosome application in sports medicine, underscoring their significant role in personalizing treatment and advancing clinical evaluations and technological innovations.

Wong et al. provide a comprehensive review on the use of mesenchymal stem cell (MSC)-derived extracellular vesicles (MSC-EVs) in treating systemic lupus erythematosus (SLE). The review highlights the vast potential of MSC-EVs as innovative, cell-free therapeutic options that leverage immunomodulation, MSC preconditioning techniques, and their diagnostic and therapeutic applications in SLE. It also points to existing gaps in understanding the precise mechanisms of MSC-EV actions and the hurdles in their clinical implementations, advocating for more research to optimize their therapeutic deployment in SLE.

Li et al.'s review offers an in-depth look at the emerging role of exosomes in the immunotherapy of diabetes. Serving as critical intercellular communicators, exosomes can reprogram immune responses associated with diabetes and its complications. This paper discusses how exosomes from immune cells like neutrophils, T lymphocytes, and macrophages, as well as from stem cells, exert immunomodulatory and anti-inflammatory effects in diabetes management. The review also considers engineered exosomes as novel therapeutic tools for diabetes, addressing current challenges in their clinical application and proposing new directions for future diabetes immunotherapy research.

Through these articles, we have not only expanded our understanding of the function of EVs but also opened new perspectives for future therapeutic strategies. This album is the result of our joint efforts and demonstrates how scientific research can reveal deeper mechanisms in biology and bring hope for the treatment of chronic diseases. We look forward to continuing this exciting journey of scientific discovery of EVs with researchers around the world.

Author contributions

RW: Writing – review & editing, Writing – original draft. PC: Writing – review & editing, Writing – original draft. SG: Writing – review & editing, Writing – original draft. JZ: Writing – review & editing, Writing – original draft. JM: Writing – review & editing, Writing – original draft. CM: Writing – review & editing, Writing – original draft. ZL: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Acknowledgments

We thank ChatGPT for the language modification. The work was supported by Medical Research Foundation of Guangdong Province (B2021198).

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.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

References

1. 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

2. Goulielmaki E, Ioannidou A, Tsekrekou M, Stratigi K, Poutakidou IK, Gkirtzimanaki K, et al. Tissue-infiltrating macrophages mediate an exosome-based metabolic reprogramming upon DNA damage. *Nat Commun*. (2020) 11(1):42. doi: 10.1038/s41467–019-13894–9

3. Dinh PUC, Paudel D, Brochu H, Popowski KD, Gracieux MC, Cores J, et al. Inhalation of lung spheroid cell secretome and exosomes promotes lung repair in pulmonary fibrosis. *Nat Commun*. (2020) 11(1):1064. doi: 10.1038/s41467–020-14344–7

4. Cheng M, Yang J, Zhao X, Zhang E, Zeng Q, Yu Y, et al. Circulating myocardial microRNAs from infarcted hearts are carried in exosomes and mobilise bone marrow progenitor cells. *Nat Commun*. (2019) 10(1):959. doi: 10.1038/s41467–019-08895–7

5. Safdar A, Saleem A, Tarnopolsky MA. The potential of endurance exercise-derived exosomes to treat metabolic diseases. *Nat Rev Endocrinol*. (2016) 12:504–17. doi: 10.1038/nrendo.2016.76

6. Chen Y, Sun Y, Luo Z, Lin J, Qi B, Kang X, et al. Potential mechanism underlying exercise upregulated circulating blood exosome miR-215–5p to prevent necroptosis of neuronal cells and a model for early diagnosis of alzheimer's disease. *Front Aging Neurosci*. (2022) 14:860364. doi: 10.3389/fnagi.2022.860364

7. Feng X, Peng Z, Yuan L, Jin M, Hu H, Peng X, et al. Research progress of exosomes in pathogenesis, diagnosis, and treatment of ocular diseases. *Front Bioeng Biotechnol*. (2023) 11:1100310. doi: 10.3389/fbioe.2023.1100310

8. Hu Y, Li X, Zhang Q, Gu Z, Luo Y, Guo J, et al. Exosome-guided bone targeted delivery of Antagomir-188 as an anabolic therapy for bone loss. *Bioact Mater*. (2021) 6 (9):2905–13. doi: 10.1016/j.bioactmat.2021.02.014

9. Yue J, Chen Z-S, Xu X-X, Li S. Functions and therapeutic potentials of exosomes in osteosarcoma. *Acta Materia Med*. (2022) 1:552–62. doi: 10.15212/amm-2022–0024

10. Dai S, Wen Y, Luo P, Ma L, Liu Y, Ai J, et al. Therapeutic implications of exosomes in the treatment of radiation injury. *Burns Trauma*. (2022) 10:tkab043. doi: 10.1093/burnst/tkab043

frontiers | Frontiers in Immunology

TYPE Review
PUBLISHED 27 July 2023
DOI 10.3389/fimmu.2023.1240747

OPEN ACCESS

EDITED BY
Zhiwen Luo,
Fudan University, China

REVIEWED BY
Zhen Peng,
Shanghai General Hospital, China
Xinting Feng,
Huashan Hospital,Fudan University, China
Beijie Qi,
Shanghai Pudong Hospital, China

*CORRESPONDENCE
Hailong Ma
mhlahpo@163.com

†These authors have contributed equally to this work

RECEIVED 15 June 2023
ACCEPTED 12 July 2023
PUBLISHED 27 July 2023

CITATION
Zhang S, Duan Z, Liu F, Wu Q, Sun X and Ma H (2023) The impact of exosomes derived from distinct sources on rheumatoid arthritis.
Front. Immunol. 14:1240747.
doi: 10.3389/fimmu.2023.1240747

The impact of exosomes derived from distinct sources on rheumatoid arthritis

Sicheng Zhang[1†], Zhen Duan[2†], Fang Liu[1], Qingjie Wu[1], Xiwei Sun[1] and Hailong Ma[1*]

[1]Department of Pediatric Orthopedics, Anhui Provincial Children's Hospital, Hefei, China, [2]The Function Examination Center, Anhui Chest Hospital, Hefei, China

Rheumatoid arthritis (RA) is an autoimmune disease that can induce joint deformities and functional impairment, significantly impacting the overall well-being of individuals. Exosomes, which are cellularly secreted vesicles, possess favorable biological traits such as biocompatibility, stability, and minimal toxicity. Additionally, they contain nucleic acids, lipids, proteins, amino acids, and metabolites, serving as mediators in cellular communication and information exchange. Recent studies have demonstrated the association between exosomes and the pathogenesis of RA. Exosomes derived from mesenchymal stem cells, dendritic cells, and neutrophils exert influence on the biological functions of immune cells and joint cells, however, the precise mechanism remains largely unclarified. This comprehensive review systematically analyzes and summarizes the biological characteristics and functionalities of exosomes derived from diverse cellular sources, thus establishing a scientific foundation for the utilization of exosomes as diagnostic targets and therapeutic modalities in the context of RA.

KEYWORDS

exosome, stem cell, rheumatoid arthritis, inflammation, synovium

1 Introduction

RA is a long-lasting and widespread inflammatory autoimmune disease that is characterized by inflammation in the synovial membrane, gradual erosion of the joints, and involvement of other body parts outside the joints (1). The prevalence rate of RA in China ranges from 0.2% to 0.4%, while European and American countries experience a higher prevalence rate of up to 1%. The pathogenesis of RA remains incompletely understood, and this immune disease is associated with a high disability rate, poor prognosis, and susceptibility to recurrent attacks (2). Additionally, RA can impact the synovial joint lining, causing stiffness, pain, inflammation, limited mobility, and joint erosion (3). Furthermore, the articular cartilage primarily comprises an extracellular matrix (ECM) and a small population of cells. The ECM mainly consists of type II collagen,

proteoglycans, and aggrecans. In the context of RA, the synovium undergoes hyperplastic transformation into an invasive tissue that destructs cartilage and bone. Fibroblastoid synovial cells (FLSs), which line the joints, exhibit an aggressive phenotype in RA and play a crucial role in these pathological processes (4). FLSs, along with matrix metalloproteinases (MMPs) secreted by chondrocytes, constitute the key components contributing to cartilage tissue destruction (5). Abnormal proliferation of FLSs results in elevated levels of interleukin (IL)-6, IL-8 and other cytokines and chemokines, promoting the activation and migration of leukocytes from blood vessels to the synovium (6).

Exosomes are nanovesicles that originate from endosomes and possess a diameter ranging from 40 to 160 nm (with an average of 100 nm). These small vesicles are enclosed by lipid layers and can be released by various cells, and they are detectable in both tissues and biological fluids (7–10). Depending on their cellular origin, exosomes harbor diverse constituents, including DNA, RNA, lipids, metabolites, cytosolic proteins, and cell surface proteins (11, 12). Due to their ability to carry genetic information, exosomes serve as crucial mediators in intercellular communication and have been investigated as potential carriers for therapeutic molecules (13). Numerous studies have implicated exosomes in inflammatory processes, which play fundamental roles in the pathogenesis of numerous diseases such as cancer, type II diabetes, inflammatory bowel diseases, RA, and neurodegenerative diseases. Furthermore, exosomes have emerged as vital regulators of intercellular communication, exerting their influence locally and systemically by modulating a wide array of biological processes between cells. Notably, exosomes represent a cutting-edge treatment strategy for systemic immune diseases (14–16). Thus, aside from their involvement in the pathogenesis of RA, exosomes also exert significant influence in inflammation, cell signaling, immune regulation, and can potentially serve as biomarkers for diagnosing RA.

In this review, we conduct a comprehensive analysis and synthesis of the functions of various exosomes derived from cells in the pathogenesis of RA, as well as their potential preventive roles. Our findings offer valuable clinical insights into the potential diagnostic and therapeutic applications of exosomes as a means to identify future therapeutic targets for RA.

2 Exosomes derived from mesenchymal stem cells

Mesenchymal stem cells (MSCs) are a distinct population of cells characterized by their ability to undergo self-renewal and differentiate into multiple cell types. These cells possess the capacity to modulate inflammatory responses and play a crucial role in various pathological conditions associated with tissue repair and regeneration. MSCs can be obtained from diverse sources such as bone marrow, umbilical cord, adipose tissue, and other tissues (17). MSC therapies have been employed as cell-based therapeutic interventions for several decades due to their anti-inflammatory, immunomodulatory, and regenerative attributes (18). Several studies have suggested that exosomes released by MSCs (MSCs-Exo) not only demonstrate enhanced efficacy compared to the parent cells but also exhibit reduced toxicity and improved stability. These exosomes are capable of transferring various nucleic acids, proteins, and lipids from the donor cell to the recipient cell, thereby contributing to chronic inflammatory and immune processes (19, 20).

Exosomes derived from MSC can affect the occurrence and progression of RA through lncRNA, miRNA and circRNA. Su et al. discovered that exosomes derived from MSCs play a role in intercellular transfer of lncRNA HAND2-AS1, which leads to the suppression of RA-FLS activation through the miR-143-3p/TNFAIP3/NF-κB pathway. This finding provides a novel understanding of the pathogenesis and treatment of RA (21). Additionally, L. Chang et al. observed decreased levels of circFBXW7 and histone deacetylase 4 (HDAC4), along with elevated levels of miR-216a-3p in clinical RA samples compared to healthy samples. HDAC4 is involved in modulating immunity, inflammation, and osteoblast differentiation during the onset of RA, and it also contributes to the release of RA-related inflammatory cytokines by FLSs. L. Chang et al. demonstrated that treatment with exosomal circFBXW7 suppressed proliferation, migration, and inflammatory response of RA-FLSs, as well as attenuated damage in the RA model. The circFBXW7 directly acts as a sponge for miR-216a-3p, leading to the upregulation of HDAC4 expression. The therapeutic effects of exosomal circFBXW7 were diminished when HDAC4 was inhibited or miR-216a-3p was upregulated (22). Furthermore, H.Y. Meng et al. produced exosomes from human MSCs overexpressing miRNA-124a. They observed that co-incubation with HMSC-124a-EV effectively suppressed cell proliferation, migration, and promoted apoptosis in a fibroblast-like synoviocyte cell line. Their findings suggest that MSC-derived exosomes serve as efficient carriers for therapeutic miRNA, offering a promising avenue for developing new medicines and strategies to treat RA (23). Here, we summarize the role of different MSC derived exosomes in RA and the related mechanisms.

2.1 Exosomes derived from bone marrow mesenchymal stem cells

At present, the research on exosome in MSC s mainly focuses on bone marrow mesenchymal stem cells (BMSCs) (24–26). Fibrinogen-like protein 1 (FGL1) is a member of the fibrinogen family and can be recognized as an immune checkpoint target through an immune escape mechanism (27). FGL1 functions as an anti-inflammatory agent in collagen-induced RA (28). Subsequent investigations have demonstrated that FGL1 contained in MSCs-Exo exhibits therapeutic effects on RA without significant adverse reactions. Overexpression of FGL1 reduces the activity of the nuclear factor kappa B (NF-κB) pathway, thereby attenuating RA injury by inhibiting apoptosis of fibroblast-like synoviocytes (FLS) and promoting their proliferation (29). It is acknowledged that MMPs are involved in the degradation of the extracellular matrix (ECM). FLS can produce MMPs, among other matrix-degrading enzymes, which contribute to the destruction of cartilage in the affected joints of RA (30). Vascular endothelial growth factor

(VEGF) is a potent growth factor specific to endothelial cells and is upregulated by pro-inflammatory cytokines and hypoxia. Serum concentrations of VEGF are elevated in RA and associate with disease activity (31). In a study by Chen et al., MSCs were transfected with an miR-150-5p expression plasmid, and MSC-derived exosomes were harvested. MSC-derived exosomes containing miR-150-5p may play a beneficial role in ameliorating joint destruction in RA. *In vivo* experiments demonstrated that MSC-derived miR-150-5p exosomes inhibit synoviocyte hyperplasia and angiogenesis by reducing the migration and invasion of FLSs and downregulating tube formation in human umbilical vein endothelial cells (HUVECs) through the targeting of MMP14 and VEGF. Injection of MSC-derived miR-150-5p exosomes leads to a reduction in hind paw thickness and clinical arthritic scores in a mouse model of collagen-induced arthritis, thus facilitating the direct intracellular transfer of miRNAs between cells and representing a potential therapeutic strategy for RA (13). Moreover, BMSCs have emerged as a viable solution for treating inflammatory rheumatism, they also have the potential to promote inflammation. BMSCs exhibit low immunogenicity and possess immunomodulatory effects, enabling them to regulate various cell types through the transmission of exosomes. These exosomes derived from BMSCs carry specific regulatory molecules present in the parent cells, including programmed death (PD)-L1, galectin-1 (GAL-1), and transforming growth factor (TGF)-β1 (32). In a murine model of collagen-induced arthritis (CIA), which mimics human RA, PD-L1 demonstrates the capacity to modulate collagen type II (CII)-reactive T cells and subsequently mitigate joint destruction. Intraperitoneal administration of PD-L1-Ig in mice leads to a deceleration in the development rate of CIA and an improvement in associated clinical manifestations (33). GAL-1 exerts regulatory functions within the immune system, with a study verifying increased levels of GAL-1 serum (sGal1) in RA patients (34).

Additionally, Stella et al. conducted the initial investigation on the involvement of exosomes derived from bone marrow mesenchymal stem cells (BMSC-Exo) in models of RA. They demonstrated the effective therapeutic potential of BMSC-Exo in mitigating experimental RA. This was achieved by dose-dependent inhibition of T lymphocyte proliferation and reduction in the proportion of CD4+ and CD8+ T cell subsets. Notably, the administration of parental MSCs did not result in an increase in Treg cell population. In a study using delayed-type hypersensitivity (DTH) mice, a dose-dependent anti-inflammatory effect of BMSC-Exo was observed. Furthermore, BMSC-Exo effectively alleviated clinical symptoms of inflammation in a mouse model of collagen-induced arthritis (CIA). The beneficial impact of BMSC-Exo was correlated with a decrease in plasmablast numbers and an increase in Breg-like cell numbers in lymph nodes (26). Another study indicated that exosomes derived from BMSCs have the ability to hinder the release of IL-1β, tumor necrosis factor-α (TNF-α), and IL-18, as well as the activation of NLRP3 in macrophages and RA rats. Additionally, they confirmed that BMSC-derived exosomes containing miR-223 could ameliorate RA by inhibiting NLRP3 expression in macrophages (35). G.Q. Li et al. substantiated that BMSC-derived exosomes alleviate RA by delivering miR-21. The exosomal miR-21, in turn, alleviates RA by targeting the TET1/KLF4 regulatory axis. TET1, a member of the DNA demethylase family that governs the expression of numerous genes, has been associated with RA. It is noteworthy that KLF4, a key player in cell survival and proliferation, has been recently found to be upregulated in RA (36).

2.2 Exosomes from human umbilical cord mesenchymal stem cells

The easier obtainability of umbilical cord mesenchymal stem cells (UMSCs) compared to other cell types is complemented by their ability to maintain their biological properties unchanged even after cryopreservation. UMSCs have progressively emerged as the preferred cells for cell therapy, gradually replacing bone marrow-derived MSCs. Human UMSCs (HUMSCs) release exosomes that exhibit specific immunomodulatory functions in the context of RA. Notably, macrophages, B cells, T cells, particularly CD4+ T cells, assume pivotal roles in local inflammation development. The immune pathogenesis of RA is linked to the imbalanced response of memory Th17 and memory regulatory T cells. The progression of RA is regulated by T helper and regulatory T (Treg) cells, with synovial inflammation and pannus growth being attributed to Th17 cells. A particular study highlighted the significant role of HUMSC-Exo in regulating the balance between Th1/Th17 and Treg cells during immune and inflammatory responses, thus reducing the ratio of Th1/Th17 to Treg cells and inhibiting the development of RA. Additionally, HUMSC-Exo may exert a direct influence on macrophage and osteoclast differentiation (37). In light of these findings, it can be inferred that exosomes derived from HUMSCs have the potential to modulate the equilibrium between pro-inflammatory and anti-inflammatory cells, presenting a promising therapeutic avenue for RA.

Serum/glucocorticoid regulated kinase 1 (SGK1) serves as a crucial modulator in the process of osteo-/chondrogenic transdifferentiation and calcification in vascular smooth muscle cells. Exosomal miRNA-140-3p derived from HUMSCs effectively mitigates joint injury in rats with RA by downregulating the expression of SGK1. Overexpression of SGK1 reversed the inhibition of RASF growth caused by overexpression of miR-140-3p (38).

2.3 Exosomes derived from synovial mesenchymal stem cells

Zhang J. et al. discovered that the intracellular transfer of circEDIL3 through exosomes derived from synovial mesenchymal stem cells (SMSC-Exo) holds potential as a novel therapeutic approach for RA. The circEDIL3 molecule functions as a sponge that specifically targets miR-485-3p, which in turn regulates PIAS3. PIAS3, a member of the small Rho GTPase family, serves as a primary cellular inhibitor of STAT3. By inhibiting STAT3 activity, PIAS3 exosomes derived from SMSCs overexpressing circEDIL3 effectively downregulated the expression of the VEGF complex.

This effect was achieved by influencing the miR-485-3p target through PIAS3 and suppressing STAT3 activity, resulting in reduced downstream VEGF levels. These findings were observed in the supernatants of co-cultured RA-FLS (rheumatoid arthritis fibroblast-like synoviocytes) and human dermal microvascular endothelial cells (HDMECs), as well as in the cell lysate of co-cultured RA-FLSs (39). These studies have demonstrated that exosomes derived from MSCs effectively attenuate the invasion and migration of FLS to some extent.

The specific mechanisms of diverse MSC-derived exosomes on RA are summarized in Figure 1. Nevertheless, there is a dearth of research investigating the relationship between SMSC-Exo and RA, a lot of research needs to be carried out in the future.

3 Exosomes derived from dendritic cells

Dendritic cells (DCs) play a crucial role in initiating antigen-specific immune responses and promoting immune tolerance (40, 41). Recently, there has been growing interest in exploring and utilizing exosomes derived from DCs in the context of autoimmune diseases. A study demonstrated that exosomes derived from immature DCs exhibited therapeutic potential in treating mice with collagen-induced arthritis (CIA). These exosomes, when derived from immature DCs treated with IL-10, effectively suppressed inflammation and autoimmune responses by inhibiting pro-inflammatory cytokines such as IL-1 and TNF-α, as well as reducing Hsp70 levels. However, it was observed that DC-derived exosomes (DC-Exo) showed diminished responsiveness to regulatory T cells compared to DCs alone (42). Subsequent investigations revealed that exosomes obtained from DCs overexpressing indoleamine 2,3-dioxygenase (IDO) or CTLA-4Ig, an inducer of IDO, could reverse established CIA and alleviate inflammation in a model of delayed-type hypersensitivity (DTH) in mice. The DC-derived exosomes were found to suppress CD8+ effector T cells and interact with endogenous antigen-presenting cells through B7 costimulatory molecule-dependent mechanisms, thereby modulating their function (43). IDO, an immunomodulatory protein known for its role in inducing or maintaining peripheral tolerance and immunosuppression in autoimmune diseases, asthma, cancer, is upregulated by CTLA-4Ig and has shown promise in the treatment of RA (44, 45). Furthermore, exosomes secreted by genetically modified bone marrow-derived DCs were found to secrete IL-4 and exhibited therapeutic effects by reducing the severity and incidence of CIA, as well as inhibiting inflammation in DTH mice. These DC-derived exosomes exerted their inhibitory effects on the DTH response through MHC class II molecules, partially dependent on Fas ligands/Fas, thereby modulating the activity of collagen-reactive T cells in *in vivo* (46). Current research suggests that DC-derived exosomes not

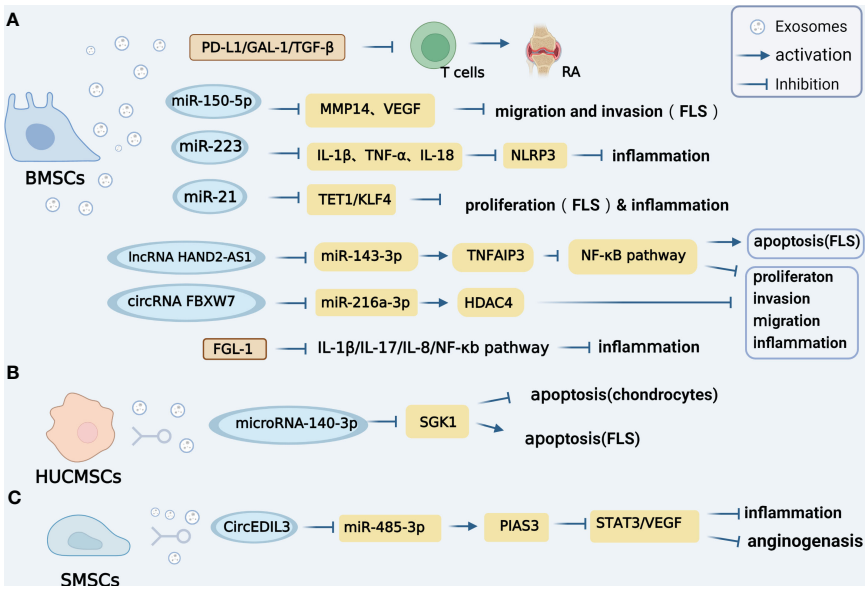


FIGURE 1
Mechanisms of diverse MSC-derived exosomes on RA. **(A)**. Exosomes derived from (BMSCs contain various regulatory molecules, such as PD-L1, GAL-1, and TGF-β1, which play a significant role in promoting the progression of RA. Additionally, BMSCs-derived exosomes carrying miR-150-5p have been found to effectively reduce the migration and invasion of RA-FLS cells by targeting VEGF and MMP14. Furthermore, the exosomal miR-223 derived from BMSCs exhibits inhibitory effects on the release of interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interleukin-18 (IL-18), as well as the activation of NLRP3 in rats, thereby alleviating inflammation. Notably, BMSCs-derived exosomes also participate in the intercellular transfer of lncRNA HAND2-AS1. The lncRNA HAND2-AS1 has been shown to have a positive impact on RA by inhibiting miR-143-3p and promoting the expression of TNFAIP3, resulting in the inhibition of the NF-κB pathway. In terms of circRNA, the BMSCS-derived exosome CircFBXW7 has been found to directly inhibit the activity of miR-216a-3p and up-regulate the expression of HDAC4, leading to the inhibition of proliferation, migration, and inflammatory response in RA-FLS. Furthermore, FGL1-MSC-Exo has the ability to reduce inflammatory cytokines such as IL1-β, IL-17, IL-8, and the NF-κB pathway, thereby attenuating RA injury. **(B)**. HUMSCs-derived exosomes miRNA-140-3p can attenuate joint injury of rats with rheumatoid arthritis by silencing SGK1. **(C)**. SMSCs-derived exosomes circEDIL3 can act as a sponge targeting miR-485-3p of PIAS3, inhibit STAT3 activity and reduce downstream VEGF.

only possess immunosuppressive properties but also hold potential as carriers for drug delivery in immune-related disorders. Triptolide, a compound known for its immunosuppressive effects, selectively targets DCs. *In vivo* experiments demonstrated that DC-derived exosomes can encapsulate triptolide, enabling targeted delivery of the compound and alleviating local inflammation and damage in mice with RA, while reducing toxicity. Additionally, triptolide-loaded DC-derived exosomes were found to reshape the immune microenvironment by reducing the levels of CD4+ T cells and increasing the levels of Treg cells in the body (47). Therefore, DC-derived exosomes can serve as carriers for anti-RA drugs and offer a novel non-cellular drug delivery system, presenting a promising approach for anti-RA therapy. Nevertheless, there is a paucity of research on the specific molecules present in exosomes derived from dendritic cells, which display therapeutic effects on RA. This deficiency requires prompt attention, and further investigations are imperative.

4 Exosomes derived from neutrophils

Neutrophils are abundant in the synovial fluid of patients with RA. A study has identified an increased concentration of polymorphonuclear neutrophil-derived exosomes (PMN-Exo) in the synovial fluid of RA patients. PMN-Exo induces an elevation of Annexin A1 (AnxA1+) in the synovial fluid, which activates anabolic genes in chondrocytes and contributes to extracellular matrix (ECM) accumulation and cartilage protection by regulating IL-8 and prostaglandin E2 (PGE2). Correspondingly, *in vivo* experiments have demonstrated that intra-articular injection of AnxA1 (+)-containing exosomes attenuated cartilage degeneration in mice with inflammatory arthritis. Furthermore, direct co-culture of neutrophils with chondrocytes indicated that chondrocyte death was induced, whereas exposure to neutrophil exosomes exerted a protective effect. It was revealed that exosomes, rather than neutrophils themselves, possess the ability to penetrate cartilage (48). Another study suggested that exosomes secreted by neutrophils, due to nanase functionalization, exhibit excellent anti-inflammatory properties. Ultrasmall Prussian Blue nanoparticle exosomes (UPB-Exo) selectively accumulate in activated FLS, subsequently neutralizing pro-inflammatory factors, eliminating reactive oxygen species, and alleviating inflammatory stress. Moreover, UPB-Exo can effectively target inflammatory synovitis and penetrate the cartilage, enabling precise diagnosis of RA *in vivo* and triggering a series of anti-inflammatory events through regulation of Th17/Treg cell balance, thus significantly improving joint injury (49).

5 Exosomes derived from granulocytic myeloid-derived suppressor cells and macrophages

GMDSC-Exo, secreted by granulocytic myeloid-derived suppressor cells, exhibits regulatory effects on immune cells. In both *in vivo and in vitro* settings, GMDSC-Exo stimulates the secretion of IL-10 by regulatory B cells. Arthritis patients and mice demonstrate elevated levels of PGE2 in their serum and synovium. Upon injection of GMDSC-Exo into CIA mice, a decrease in arthritic index values and inflammatory cell infiltration is observed. Previous research has shown that PGE2 can elevate the levels of IL-10, an anti-inflammatory cytokine, and is a crucial factor in the production of IL-10 and Breg cells. Studies have revealed that GMDSC exosomes can generate PGE2, upregulate the phosphorylation of GSK-3β and CREB, and exert an anti-inflammatory role by promoting the secretion of IL-10 (50). Macrophages play a significant role in complex microenvironments and can be categorized into M1 and M2 subtypes. Imbalances between pro-inflammatory M1 and anti-inflammatory M2 macrophage activities induce synovial inflammation and autoimmunity, leading to joint injury. It has been reported that macrophage-derived exosomes not only enhance inflammation and cellular immune responses but also serve as nanocarriers for drug delivery in therapeutic applications. Plasmid DNA encoding the anti-inflammatory cytokine IL-10 (IL-10 pDNA) and the chemotherapy drug betamethasone sodium phosphate (BSP) can be encapsulated within exosomes (M2-Exo) derived from M2-type macrophages. This co-delivery system of M2 Exo/pDNA/BSP promotes the polarization of M1-to-M2 macrophages by reducing the secretion of pro-inflammatory cytokines (IL-1β and TNF-α) while increasing the expression of IL-10. *In vivo* experiments also demonstrate the potent anti-inflammatory activity of M2 Exo/pDNA/BSP. Furthermore, M. Chen et al. have discovered that high expression of miR-103a in exosomes derived from macrophages (RAW264.7) can exacerbate inflammation and angiogenesis in RA mice by downregulating hepatocyte nuclear factor 4 alpha (HNF-4α) and activating the JAK/STAT3 pathway, thereby aggravating RA in mice (51). These studies present empirical evidence indicating that exosomes originating from GMSCs and macrophages may mitigate the advancement of RA by means of their anti-inflammatory properties. In brief, Figure 2 illustrates the mechanisms of exosomes derived from DCs, neutrophils, GMDSCs, and macrophages within the framework of RA. Additional research is necessary to elucidate the exact molecular mechanisms that underlie exosome-mediated signaling pathways in various cell types in the future.

6 Exosomes derived from serum and plasma

In recent years, there has been a growing body of research focusing on the role of exosomes in arthritis serum. Several studies have been conducted by numerous researchers, who have extracted serum exosomes from both clinical RA patients and collagen-induced arthritis (CIA) mouse models. These investigations aimed to analyze the influence of serum exosomes on RA morbidity, clinical score, and bone degradation. The results of these studies suggest a noteworthy association between serum exosomes and the occurrence of RA. As a result, the timely identification and prevention of RA are of utmost importance. However, it remains unclear whether serum exosomes

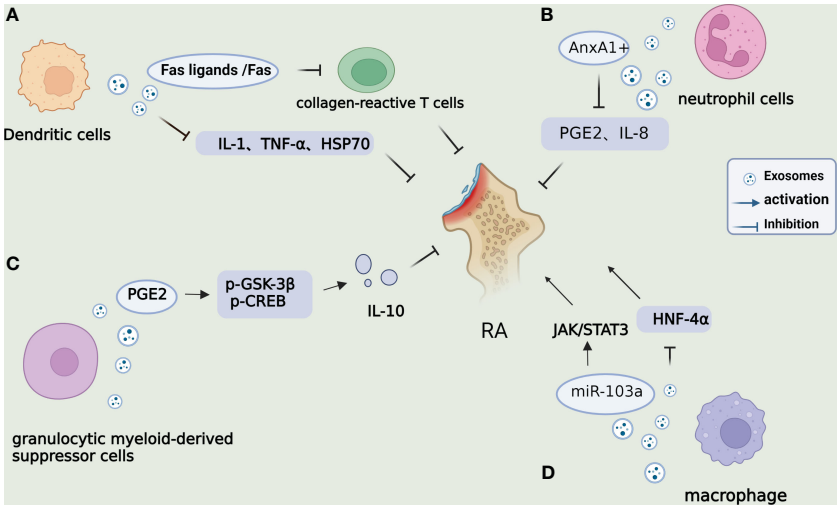


FIGURE 2
Mechanisms of other cell-derived exosomes on RA. **(A)**. DC-derived exosomes can inhibit the DTH mice response through MHC class II, partially dependent on Fas ligands/Fas, thereby regulating the activity of collagen-reactive T cells. DCs treated with IL-10 can inhibit inflammation and autoimmune responses by inhibiting pro-inflammatory cytokines IL-1 and TNF-α and reduce Hsp70 levels. **(B)**. Annexin A1 (AnxA1^{+}) is overexpressed by PMN-derived exosomes in synovium fluid, which can activate anabolic genes in chondrocytes and play a role in ECM accumulation and cartilage protection by regulating IL-8 and PGE2. **(C)**. Exosomes derived from G-MDSC can produce PGE2 and upregulate phosphorylation of GSK-3β and CREB, promoting the secretion of IL-10 from regulatory B cells and ameliorating arthritis in mice. **(D)**. miR-103a in RAW264.7-derived exosomes from macrophages can promote inflammation and angiogenesis in RA mice by targeting the downregulation of HNF-4α and activation of the JAK/STAT3 pathway, thus, aggravating RA in mice.

accurately reflect the content of exosomes in synovial fluid. Exosomes were isolated from the serum and synovial fluid of osteoarthritis patients, and miRNA expression was compared through miRNA sequencing. The comparison revealed 31 upregulated miRNAs and 33 downregulated miRNAs in the synovial fluid compared to the serum. Further transcription analysis demonstrated that these differentially expressed miRNAs primarily relate to intercellular processes and metabolic pathways. Hence, the results suggest that serum-derived exosomes do not fully represent synovial exosomes (52). In another study utilizing global miRNA screening in plasma exosomes, performed with a custom microarray on both RA patients and healthy controls, researchers identified four abnormally expressed exosomal miRNAs in RA patients. The downregulation of exosomal miR-204-5p was confirmed in both the replication group (30 RA patients vs. 30 healthy controls) and the validation group (56 RA patients vs. 60 healthy controls). Furthermore, Spearman correlation analysis indicated an inverse correlation between the expression of plasma exosomal miR-204-5p and disease parameters of RA patients, including rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) levels (53). Another study demonstrated that overexpression of serum-derived exosomes containing nuclear-enriched abundant transcript 1 (NEAT1) or ROCK2 promotes the proliferation of immune cells (CD4+ T cells), differentiation of Th17 cells, and cell migration in response to stimulation. NEAT1 binds to and inhibits the expression of miR-144-3p, and knockout of the NEAT1 gene induces the expression of miR-144-3p in CD4+ T cells. MiR-144-3p is associated with the activation of ROCK2 in RA, thereby activating the Wnt/β-catenin pathway (54). Moreover, a novel group of serum biomarkers consisting of exomiR-451a, exomiR-25-3p, and serum sTWEAK levels may be utilized for early clinical diagnosis of RA. Additionally, a newly identified predictive RNA target gene, YHWAB, may play a significant role in the development of RA (55). Osteoclasts directly transform into osteoblasts, and elevated levels of miR-214-3p in osteoclasts are associated with increased serum exosome miR-214-3p and reduced bone formation in older women with fractures and ovariectomized (OVX) mice. Targeted inhibition of miR-214-3p promotes bone formation in aging OVX mice (56). Furthermore, exosome-encapsulated miR-6089 exhibits a significant reduction in serum exosomes in 76 RA patients compared to 20 controls. MiR-6089 regulates the production of IL-6, IL-29, and TNF-α by targeting TLR4 signal transduction, thereby inhibiting cell proliferation induced by lipopolysaccharide (LPS) and macrophage-like activation of THP-1 cells. Therefore, exosome-encapsulated miR-6089 holds promise as a new biomarker for RA (57). Moreover, the expression of miR-3a-76p in serum exosomes and peripheral blood mononuclear cells (PBMCs) of RA patients (n = 20) was significantly downregulated compared to healthy controls (n = 548). Serum exosome miR-548a-3p exhibited a negative correlation with serum CRP, RF, and ESR levels in RA patients.

Toll-like receptors (TLRs) are transmembrane proteins that recognize diverse pathogen-associated molecular patterns, triggering an inflammatory immune response. All TLRs engage the myD88-dependent pathway, which activates NF-κB, a classical inflammatory pathway, and ultimately elicits the release of inflammatory mediators and cytokines. Wang et al. previously identified the distinct exosome-encapsulated miRNA profile in serum samples of RA patients using miRNA microarray analysis. This profile encompasses 20 differentially expressed serum exosomal miRNAs. Notably, miR-548a-3p stands out as one of the significantly

down-regulated exosome-delivered miRNAs in the serum of RA patients when compared to healthy controls. Subsequently, they determined that miR-548a-3p exerts inhibitory effects on the proliferation and activation of pTHP-4 cells by modulating the TLR1/NF-κB signaling pathway (58). Furthermore, the expression level of HOX antisense intergenic RNA (Hotair) in serum exosomes of patients with RA exhibited a substantial increase, leading to the migration of activated macrophages. Conversely, inhibition of Hotair resulted in the reduction of MMP-2 and MMP-13 levels. These findings indicate that, in addition to miRNAs, lncRNAs transcribed in reverse orientation may also contribute to the pathogenesis of RA through serum-derived exosomes (59).

7 Discussion

In conclusion, RA is a systemic and progressive inflammatory disorder that results in joint and periarticular structural damage as well as systemic inflammation-related consequences. Exosomes, which are small nanovesicles released by nearly all cells, contain genetic information and have emerged as crucial mediators of intercellular communication in various biological processes. Through the investigation and analysis of the presence of miRNAs and lncRNAs in exosomes derived from different cell types and serum sources, it has been observed that exosomes predominantly contribute to immune regulation, control of inflammatory response, and reduction of inflammatory cytokine release. Exosomes actively participate in the pathogenesis of RA, offering potential therapeutic prospects in inflammation management, cellular signaling, and immune regulation. Furthermore, exosomes exhibit great potential as biomarkers for RA diagnosis and as a diagnostic tool for precise identification and targeted treatment. Among these, MSC-Exo demonstrates significant potential in the treatment of RA. Neutrophils, granulocytic myeloid-derived suppressor cells, and macrophage-secreted exosomes also exert immunological regulatory and anti-inflammatory roles in RA. The miRNAs and lncRNAs enclosed within exosomes derived from serum could serve as novel groups of serum biomarkers for early clinical detection of RA. Additionally, certain exosomes derived from stem cells act as therapeutic agents in RA by functioning as drug carriers. Consequently, scrutinizing the roles of exosomes derived from different sources in the pathogenesis of RA and their preventive functions holds promise for the identification of future therapeutic targets.

Author contributions

SZ designed and wrote this manuscript, ZD wrote and checked this manuscript, FL wrote thismanuscript, QW checked the manuscript, XS checked themanuscript, HM designed and checked the manuscript. Allauthors contributed to the article and submitted and approvedthe submitted section.

Funding

This study is supported by the Health research project of Anhui Province (No. AHWJ2022b008); The Natural Science Foundation project of Anhui Province (No. 2208085QHQ241). The Natural Science Foundation project of Anhui Medical University (No. 2022xkj112); The Research project of Anhui Children's Hospital (No. 21etyy002).

Conflict of interest

The author declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Publisher's note

References

1. Li K, Wang M, Zhao L, Liu Y, Zhang X. ACPA-negative rheumatoid arthritis: From immune mechanisms to clinical translation. *EBioMedicine* (2022) 83:104233. doi: 10.1016/j.ebiom.2022.104233

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

3. Lopez-Santalla M, Fernandez-Perez R, Garin MI. Mesenchymal stem/stromal cells for rheumatoid arthritis treatment: an update on clinical applications. *Cells* (2020) 9(8):1852. doi: 10.3390/cells9081852

4. Nygaard G, Firestein GS. Restoring synovial homeostasis in rheumatoid arthritis by targeting fibroblast-like synoviocytes. *Nat Rev Rheumatol* (2020) 16(6):316–33. doi: 10.1038/s41584-020-0413-5

5. Kumar LD, Karthik R, Gayathri N, Sivasudha T. Advancement in contemporary diagnostic and therapeutic approaches for rheumatoid arthritis. *BioMed Pharmacother* (2016) 79:52–61. doi: 10.1016/j.biopha.2016.02.001

6. Miao C, Wang X, Zhou W, Huang J. The emerging roles of exosomes in autoimmune diseases, with special emphasis on microRNAs in exosomes. *Pharmacol Res* (2021) 169:105680. doi: 10.1016/j.phrs.2021.105680

7. Mirzaei R, Zamani F, Hajibaba M, Rasouli-Saravani A, Noroozbeygi M, Gorgani M, et al. The pathogenic, therapeutic and diagnostic role of exosomal microRNA in the autoimmune diseases. *J Neuroimmunol* (2021) 358:577640. doi: 10.1016/j.jneuroim.2021.577640

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(1):39. doi: 10.1186/s40824-022-00286-2

9. Chen Y, Sun Y, Luo Z, Lin J, Qi B, Kang X, et al. Potential mechanism underlying exercise upregulated circulating blood exosome miR-215-5p to prevent necroptosis of neuronal cells and a model for early diagnosis of Alzheimer's disease. *Front Aging Neurosci* (2022) 14:860364. doi: 10.3389/fnagi.2022.860364

10. Tomiyama E, Matsuzaki K, Fujita K, Shiromizu T, Narumi R, Jingushi K, et al. Proteomic analysis of urinary and tissue-exudative extracellular vesicles to discover

novel bladder cancer biomarkers. *Cancer Sci* (2021) 112(5):2033–45. doi: 10.1111/cas.14881

11. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. *Science* (2020) 367(6478). doi: 10.1126/science.aau6977

12. Feng X, Peng Z, Yuan L, Jin M, Hu H, Peng X, et al. Research progress of exosomes in pathogenesis, diagnosis, and treatment of ocular diseases. *Front Bioeng Biotechnol* (2023) 11:1100310. doi: 10.3389/fbioe.2023.1100310

13. Chen Z, Wang H, Xia Y, Yan F, Lu Y. Therapeutic potential of mesenchymal cell-derived miRNA-150-5p-expressing exosomes in rheumatoid arthritis mediated by the modulation of MMP14 and VEGF. *J Immunol* (2018) 201(8):2472–82. doi: 10.4049/jimmunol.1800304

14. Console L, Scalise M, Indiveri C. Exosomes in inflammation and role as biomarkers. *Clin Chim Acta* (2019) 488:165–71. doi: 10.1016/j.cca.2018.11.009

15. Wang Y, Liu J, Ma J, Sun T, Zhou Q, Wang W, et al. Exosomal circRNAs: biogenesis, effect and application in human diseases. *Mol Cancer* (2019) 18(1):116. doi: 10.1186/s12943-019-1041-z

16. Luo Z, Qi B, Sun Y, Chen Y, Lin J, Qin H, et al. Engineering bioactive M2 macrophage-polarized, anti-inflammatory, miRNA-based liposomes for functional muscle repair: from exosomal mechanisms to biomaterials. *Small* (2022) 18(34): e2201957. doi: 10.1002/smll.202201957

17. Yang J, Chen X, Yuan T, Yang X, Fan Y, Zhang X. Regulation of the secretion of immunoregulatory factors of mesenchymal stem cells (MSCs) by collagen-based scaffolds during chondrogenesis. *Mater Sci Eng C Mater Biol Appl* (2017) 70(Pt 2):983–91. doi: 10.1016/j.msec.2016.04.096

18. Hwang JJ, Rim YA, Nam Y, Ju JH. Recent developments in clinical applications of mesenchymal stem cells in the treatment of rheumatoid arthritis and osteoarthritis. *Front Immunol* (2021) 12:631291. doi: 10.3389/fimmu.2021.631291

19. Liu H, Li R, Liu T, Yang L, Yin G, Xie Q. Immunomodulatory effects of mesenchymal stem cells and mesenchymal stem cell-derived extracellular vesicles in rheumatoid arthritis. *Front Immunol* (2020) 11:1912. doi: 10.3389/fimmu.2020.01912

20. Wang S, Lei B, Zhang E, Gong P, Gu J, He L, et al. Targeted therapy for inflammatory diseases with mesenchymal stem cells and their derived exosomes: from basic to clinics. *Int J Nanomed* (2022) 17:1757–81. doi: 10.2147/IJN.S355366

21. Su Y, Liu Y, Ma C, Guan C, Ma X, Meng S. Mesenchymal stem cell-originated exosomal lncRNA HAND2-AS1 impairs rheumatoid arthritis fibroblast-like synoviocyte activation through miR-143-3p/TNFAIP3/NF-κB pathway. *J Orthop Surg Res* (2021) 16(1):116. doi: 10.1186/s13018-021-02248-1

22. Chang L, Kan L. Mesenchymal Stem Cell-Originated Exosomal Circular RNA circFBXW7 Attenuates Cell Proliferation, Migration and Inflammation of Fibroblast-Like Synoviocytes by Targeting miR-216a-3p/HDAC4 in Rheumatoid Arthritis. *J Inflammation Res* (2021) 14:6157–71. doi: 10.2147/JIR.S336099

23. Meng HY, Chen LQ, Chen LH. The inhibition by human MSCs-derived miRNA-124a overexpression exosomes in the proliferation and migration of rheumatoid arthritis-related fibroblast-like synoviocyte cell. *BMC Musculoskelet Disord* (2020) 21(1):150. doi: 10.1186/s12891-020-3159-y

24. 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

25. Qin F, Tang H, Zhang Y, Zhang Z, Huang P, Zhu J. Bone marrow-derived mesenchymal stem cell-derived exosomal microRNA-208a promotes osteosarcoma cell proliferation, migration, and invasion. *J Cell Physiol* (2020) 235(5):4734–45. doi: 10.1002/jcp.29351

26. Cosenza S, Toupet K, Maumus M, Luz-Crawford P, Blanc-Brude O, Jorgensen C, et al. Mesenchymal stem cells-derived exosomes are more immunosuppressive than microparticles in inflammatory arthritis. *Theranostics* (2018) 8(5):1399–410. doi: 10.7150/thno.21072

27. Qian W, Zhao M, Wang R, Li H. Fibrinogen-like protein 1 (FGL1): the next immune checkpoint target. *J Hematol Oncol* (2021) 14(1):147. doi: 10.1186/s13045-021-01161-8

28. Lin WW, Ho KW, Su HH, Fang TF, Tzou SC, Chen IJ, et al. Fibrinogen-like protein 1 serves as an anti-inflammatory agent for collagen-induced arthritis therapy in mice. *Front Immunol* (2021) 12:767868. doi: 10.3389/fimmu.2021.767868

29. Xu W, Liu X, Qu W, Wang X, Su H, Li W, et al. Exosomes derived from fibrinogen-like protein 1-overexpressing bone marrow-derived mesenchymal stem cells ameliorates rheumatoid arthritis. *Bioengineered* (2022) 13(6):14545–61. doi: 10.1080/21655979.2022.2090379

30. Araki Y, Mimura T. Matrix metalloproteinase gene activation resulting from disordred epigenetic mechanisms in rheumatoid arthritis. *Int J Mol Sci* (2017) 18 (5):905. doi: 10.3390/ijms18050905

31. Taylor PC. Serum vascular markers and vascular imaging in assessment of rheumatoid arthritis disease activity and response to therapy. *Rheumatol (Oxford)* (2005) 44(6):721–8. doi: 10.1093/rheumatology/keh524

32. Shen Z, Huang W, Liu J, Tian J, Wang S, Rui K. Effects of mesenchymal stem cell-derived exosomes on autoimmune diseases. *Front Immunol* (2021) 12:749192. doi: 10.3389/fimmu.2021.749192

33. Wang G, Hu P, Yang J, Shen G, Wu X. The effects of PDL-Ig on collagen-induced arthritis. *Rheumatol Int* (2011) 31(4):513–9. doi: 10.1007/s00296-009-1249-0

34. Triguero-Martínez A, Roy-Vallejo E, Tomero EG, Montes N, Fuente H, Ortiz AM, et al. Galectin-1: A potential biomarker differentiating between early rheumatoid arthritis and spondyloarthritis. *J Clin Med* (2022) 11(21):6313. doi: 10.3390/jcm11216313

35. Huang Y, Lu D, Ma W, Liu J, Ning Q, Tang F, et al. miR-223 in exosomes from bone marrow mesenchymal stem cells ameliorates rheumatoid arthritis *via* downregulation of NLRP3 expression in macrophages. *Mol Immunol* (2022) 143:68–76. doi: 10.1016/j.molimm.2022.01.002

36. Li GQ, Fang YX, Liu Y, Meng FR, Wu X, Zhang CW, et al. MicroRNA-21 from bone marrow mesenchymal stem cell-derived extracellular vesicles targets TET1 to suppress KLF4 and alleviate rheumatoid arthritis. *Ther Adv Chronic Dis* (2021) 12:20406223211007369. doi: 10.1177/20406223211007369

37. Fu Y, Li J, Zhang Z, Ren F, Wang Y, Jia H, et al. Umbilical cord mesenchymal stem cell-derived exosomes alleviate collagen-induced arthritis by balancing the population of Th17 and regulatory T cells. *FEBS Lett* (2022) 596(20):2668–77. doi: 10.1002/1873-3468.14460

38. Huang Y, Chen L, Chen D, Fan P, Yu H. Exosomal microRNA-140-3p from human umbilical cord mesenchymal stem cells attenuates joint injury of rats with rheumatoid arthritis by silencing SGK1. *Mol Med* (2022) 28(1):36. doi: 10.1186/s10020-022-00451-2

39. Zhang J, Zhang Y, Ma Y, Luo L, Chu M, Zhang Z. Therapeutic Potential of Exosomal circRNA Derived from Synovial Mesenchymal Cells *via* Targeting circEDIL3/miR-485-3p/PIAS3/STAT3/VEGF Functional Module in Rheumatoid Arthritis. *Int J Nanomed* (2021) 16:7977–94. doi: 10.2147/IJN.S333465

40. Li M, Li S, Du C, Zhang Y, Li Y, Chu L, et al. Exosomes from different cells: Characteristics, modifications, and therapeutic applications. *Eur J Med Chem* (2020) 207:112784. doi: 10.1016/j.ejmech.2020.112784

41. Karami Fath M, Azami J, Jaafari N, Akbari Oryani M, Jafari N, Karim Poor A, et al. Exosome application in treatment and diagnosis of B-cell disorders: leukemias, multiple sclerosis, and arthritis rheumatoid. *Cell Mol Biol Lett* (2022) 27(1):74. doi: 10.1186/s11658-022-00377-x

42. Kim SH, Lechman ER, Bianco N, Menon R, Keravala A, Nash J, et al. Exosomes derived from IL-10-treated dendritic cells can suppress inflammation and collagen-induced arthritis. *J Immunol* (2005) 174(10):6440–8. doi: 10.4049/jimmunol.174.10.6440

43. Bianco NR, Kim SH, Ruffner MA, Robbins PD. Therapeutic effect of exosomes from indoleamine 2,3-dioxygenase-positive dendritic cells in collagen-induced arthritis and delayed-type hypersensitivity disease models. *Arthritis Rheum* (2009) 60(2):380–9. doi: 10.1002/art.24229

44. Baban B, Hansen AM, Chandler PR, Manlapat A, Bingaman A, Kahler DJ, et al. A minor population of splenic dendritic cells expressing CD19 mediates IDO-dependent T cell suppression *via* type I IFN signaling following B7 ligation. *Int Immunol* (2005) 17(7):909–19. doi: 10.1093/intimm/dxh271

45. Szántó S, Koreny T, Mikecz K, Glant TT, Szekanecz Z, Varga J. Inhibition of indoleamine 2,3-dioxygenase-mediated tryptophan catabolism accelerates collagen-induced arthritis in mice. *Arthritis Res Ther* (2007) 9(3):R50. doi: 10.1186/ar2205

46. Kim SH, Bianco N, Menon R, Lechman ER, Shufesky WJ, Morelli AE, et al. Exosomes derived from genetically modified DC expressing FasL are anti-inflammatory and immunosuppressive. *Mol Ther* (2006) 13(2):289–300. doi: 10.1016/j.ymthe.2005.09.015

47. Rao Q, Ma G, Li M, Wu H, Zhang Y, Zhang C, et al. Targeted delivery of triptolide by dendritic cell-derived exosomes for colitis and rheumatoid arthritis therapy in murine models. *Br J Pharmacol* (2023) 180(3):330–46. doi: 10.1111/bph.15958

48. Headland SE, Jones HR, Norling LV, Kim A, Souza PR, Corsiero E, et al. Neutrophil-derived microvesicles enter cartilage and protect the joint in inflammatory arthritis. *Sci Transl Med* (2015) 7(315):315ra190. doi: 10.1126/scitranslmed.aac5608

49. Zhang L, Qin Z, Sun H, Chen X, Dong J, Shen S, et al. Nanoenzyme engineered neutrophil-derived exosomes attenuate joint injury in advanced rheumatoid arthritis *via* regulating inflammatory environment. *Bioact Mater* (2022) 18:1–14. doi: 10.1016/j.bioactmat.2022.02.017

50. Wu X, Zhu D, Tian J, Tang X, Guo H, Ma J, et al. Granulocytic myeloid-derived suppressor cell exosomal prostaglandin E2 ameliorates collagen-induced arthritis by enhancing IL-10(+) B cells. *Front Immunol* (2020) 11:588500. doi: 10.3389/fimmu.2020.588500

51. Chen M, Li MH, Zhang N, Sun WW, Wang H, Wang YA, et al. Pro-angiogenic effect of exosomal microRNA-103a in mice with rheumatoid arthritis *via* the downregulation of hepatocyte nuclear factor 4 alpha and activation of the JAK/STAT3 signaling pathway. *J Biol Regul Homeost Agents* (2021) 35(2):629–40. doi: 10.23812/20-537-A

52. Xie Y, Chen W, Zhao M, Xu Y, Yu H, Qin J, et al. Exploration of exosomal miRNAs from serum and synovial fluid in arthritis patients. *Diagn (Basel)* (2022) 12 (2):239. doi: 10.3390/diagnostics12020239

53. Wu LF, Zhang Q, Mo XB, Lin J, Wu YL, Lu X, et al. Identification of novel rheumatoid arthritis-associated MiRNA-204-5p from plasma exosomes. *Exp Mol Med* (2022) 54(3):334–45. doi: 10.1038/s12276-022-00751-x

54. Liu R, Jiang C, Li J, Li X, Zhao L, Yun H, et al. Serum-derived exosomes containing NEAT1 promote the occurrence of rheumatoid arthritis through regulation

of miR-144-3p/ROCK2 axis. *Ther Adv Chronic Dis* (2021) 12:2040622321991705. doi: 10.1177/2040622321991705

55. Rodríguez-Muguruza S, Altuna-Coy A, Castro-Oreiro S, Poveda-Elices MJ, Fontova-Garrofé R, Chacón MR. A Serum Biomarker Panel of exomiR-451a, exomiR-25-3p and Soluble TWEAK for Early Diagnosis of Rheumatoid Arthritis. *Front Immunol* (2021) 12:790880. doi: 10.3389/fimmu.2021.790880

56. Li D, Liu J, Guo B, Liang C, Dang L, Lu C, et al. Osteoclast-derived exosomal miR-214-3p inhibits osteoblastic bone formation. *Nat Commun* (2016) 7:10872. doi: 10.1038/ncomms10872

57. Xu D, Song M, Chai C, Wang J, Jin C, Wang X, et al. Exosome-encapsulated miR-6089 regulates inflammatory response *via* targeting TLR4. *J Cell Physiol* (2019) 234(2):1502–11. doi: 10.1002/jcp.27014

58. Wang Y, Zheng F, Gao G, Yan S, Zhang L, Wang L, et al. MiR-548a-3p regulates inflammatory response *via* TLR4/NF-κB signaling pathway in rheumatoid arthritis. *J Cell Biochem* (2019) 120(2):1133–40. doi: 10.1002/jcb.26659

59. Song J, Kim D, Han J, Kim Y, Lee M, Jin EJ. PBMC and exosome-derived Hotair is a critical regulator and potent marker for rheumatoid arthritis. *Clin Exp Med* (2015) 15(1):121–6. doi: 10.1007/s10238-013-0271-4

frontiers | Frontiers in Immunology

TYPE Mini Review
PUBLISHED 01 December 2023
DOI 10.3389/fimmu.2023.1307455

OPEN ACCESS

EDITED BY
Zhiwen Luo,
Fudan University, China

REVIEWED BY
Giorgio Mangino,
Sapienza University of Rome, Italy

*CORRESPONDENCE
Dong Li
lidong_2022@hust.edu.cn

RECEIVED 04 October 2023
ACCEPTED 16 November 2023
PUBLISHED 01 December 2023

CITATION
Zhang R, Wei Y, Wang T, Nie X, Shi Z, Deng Y and Li D (2023) Exosomal miRNAs in autoimmune skin diseases. *Front. Immunol.* 14:1307455. doi: 10.3389/fimmu.2023.1307455

Exosomal miRNAs in autoimmune skin diseases

Ri Zhang, Yujia Wei, Tingmei Wang, Xiaoqi Nie, Zeqi Shi, Yunhua Deng and Dong Li*

Department of Dermatology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Exosomes, bilaterally phospholipid-coated small vesicles, are produced and released by nearly all cells, which comprise diverse biological macromolecules, including proteins, DNA, RNA, and others, that participate in the regulation of their biological functions. An increasing number of studies have revealed that the contents of exosomes, particularly microRNA(miRNA), play a significant role in the pathogenesis of various diseases, including autoimmune skin diseases. MiRNA is a class of single-stranded non-coding RNA molecules that possess approximately 22 nucleotides in length with the capability of binding to the untranslated as well as coding regions of target mRNA to regulate gene expression precisely at the post-transcriptional level. Various exosomal miRNAs have been found to be significantly expressed in some autoimmune skin diseases and involved in the pathogenesis of conditions via regulating the secretion of crucial pathogenic cytokines and the direction of immune cell differentiation. Thus, exosomal miRNAs might be promising biomarkers for monitoring disease progression, relapse and reflection to treatment based on their functions and changes. This review summarized the current studies on exosomal miRNAs in several common autoimmune skin diseases, aiming to dissect the underlying mechanism from a new perspective, seek novel biomarkers for disease monitoring and lay the foundation for developing innovative target therapy in the future.

KEYWORDS

exosome, miRNA, autoimmune skin disease, immunology, skin

1 Introduction

MicroRNA (miRNA), first discovered within nematodes by Ambro et al. (1) in 1993, is a class of single-stranded non-coding RNA molecules typically consisting of approximately 22 nucleotides in length. MiRNA binds to the 3' untranslated region (UTR) and coding region of target mRNA through base pairing, leading to either degradation or inhibition of translation of the target mRNA (2), enabling precise control of gene expression at the post-transcriptional level (3). MiRNA is involved in various cellular activities, including proliferation, differentiation, apoptosis, and metabolism (4). It has emerged as a

potential biomarker and therapeutic target for numerous diseases, making it a prominent focus of research in the medical field in recent years (5).

Exosomes, vesicular structures with a diameter ranging from 40 to 160nm enclosed within a bilayer phospholipid membrane, contain diverse biological molecules, including nucleic acids, lipids and proteins. They are widely distributed and can be generated by almost all cells and detected in various body fluids (6–9).

Unlike freely circulating miRNA in body fluids, miRNA within exosomes resists RNA degradation by ribonucleases, making it more stable (10). Furthermore, exosomal miRNA exhibits potential homing properties (11) and selective enrichment, enabling disease occurrence, progression and relapse monitoring (12), which is critical for clinical management of autoimmune diseases.

Autoimmune skin diseases are defined as autoimmune diseases characterized by excessive activation of the immune system leading to abnormal immune reactions and responses against self-antigens that are normally tolerated, including but not limited to skin involvement, such as vitiligo, psoriasis, systemic lupus erythematosus and dermatomyositis. In recent years, there has been a steady increase in the incidence and prevalence of autoimmune skin diseases, accounting for an essential part of the global disease mortality and economic burden. The uncertainty of etiology, the complexity of pathogenesis, and the unpredictable nature of disease progression have posed significant challenges to patients treating and managing autoimmune skin diseases, significantly impacting patients' quality of life. Therefore, searching for new therapeutic targets and identifying biomarkers that can predict disease progression and treatment effectiveness is crucial. As mentioned above, exosomal miRNA can be a promising choice for its function and nature. A comprehensive map illustrating how exosomal miRNAs are involved in autoimmune skin diseases by regulating transcripts, pathways, immune system differentiation, and their interactions with terminal cells such as keratinocytes, fibroblasts, and immune cells is no doubt important yet still lacking. Here, we reviewed recent evidence on the role of exosomal miRNAs within autoimmune skin diseases and discussed their impact on these diseases (Table 1), aiming to facilitate a better understanding of the pathogenetic mechanisms of autoimmune skin diseases and clinical management.

2 Exosomal miRNAs in several autoimmune skin diseases

2.1 Exosomal miRNAs in vitiligo

Vitiligo is an acquired pigmentary skin disorder that involves the participation of various innate and adaptive immune cells, leading to damage to melanocytes in the skin and hair follicles (41), resulting in depigmented patches.

Several researches have yielded positive results that circulating exosomal miRNAs contribute to vitiligo's pathogenesis. Zhang et al. (13) co-cultured circulating exosomes from vitiligo patients with the human melanocyte cell line PIG1. They observed the inhibition of melanogenesis, decreased tyrosinase activity, and altered expression of genes related to melanogenesis in melanocytes. Furthermore, they detected significantly higher expression of miR-21-5p in exosomes from vitiligo patients and confirmed that miR-21-5p inhibits melanogenesis, as evidenced by changes in the levels of tyrosinase and tyrosinase-related protein 1 (*TYP1*) and tyrosinase-related protein 2 (*TYP2*). Luo et al. (14) found that circulating exosomal miR-487b-3p in advanced-stage vitiligo patients was significantly downregulated before glucocorticoid treatment but recovered to normal levels after intervention. Enrichment analysis suggested that this miRNA primarily affects metabolic pathways.

Additionally, skin keratinocytes (42) and fibroblasts (43) are involved in this disease's abnormal immune environment that promotes local T-cell infiltration by secreting *CXCL9, CXCL10*, and various cytokines. Moreover, melanocytes appear to be not solely victims of the abnormal immune response but also participants in initiating the immune dysfunction (44). Zhao et al. (15) found that melanocytes exhibited a significant decrease in melanin content and tyrosinase activity after being cultured with exosomes from vitiligo patients' skin lesional keratinocytes. Furthermore, they discovered that miR-200C, downregulated in these exosomes, could promote melanogenesis, potentially mediated by the inhibition of *SOX1* to activate the *Wnt* pathway. Li et al. (16) performed high-throughput sequencing and correlation analysis of circulating exosomal miRNAs from segmental vitiligo patients and healthy controls with disease progression and staging, screened and expanded the specimens to verify that miR-493-3p, which was highly expressed in circulating exosomes of the patients as well as keratinocytes of the lesions. They subsequently demonstrated *in vitro* the miR-493-3p-*hnRNPU-COMT-DA* axis on the initial damage of melanocytes. However, there are no current studies on exosomal miRNAs referring to the role of fibroblasts or melanocytes initiating abnormal immunity, which might be worth investigating in the future to uncover the mechanism of vitiligo from multidimensional perspectives.

2.2 Exosomal miRNAs in psoriasis

Psoriasis is a chronic inflammatory skin disease characterized by abnormal activation and infiltration of T-cells and excessive proliferation of keratinocytes, clinically manifesting red plaques and papules covered with thick silver-white scales. Th17 cells and *IL-17A*/*IL-23* play a crucial role in the immune dysfunction in psoriasis (45). Jiang et al. (17) discovered that exosomes derived from keratinocytes treated with psoriasis cytokines (*IL-17A*, *IL-22*, *IFN-γ*, *TNF-α*) can induce the differentiation of CD4-positive T-cells into Th1 and Th17 cells and upregulate the expression of various cytokines including *IL-17A*, *IL-17F*, *IL-22*, *IL-23*, *IL-36*, *IFN-γ*, and *TNF-α*. MiR-381-3p was found to upregulate the expression of the indicated cytokines in CD4-positive T-cells. *UBR5* and *FOXO1* were identified as critical downstream target genes, playing essential roles in the immune response in psoriasis.

Chen et al. (18) sequenced circulating exosomal miRNAs in 15 patients with psoriasis vulgaris and 15 healthy controls and identified 246 differentially expressed miRNAs. Subsequently,

10.3389/fimmu.2023.1307455

TABLE 1 Summary of exosomal miRNAs profiles of indicated autoimmune skin diseases.

Disease	Exo-miRNA	State in disease group/ specific cells	Origin of exosome	Target cell	Target gene	Significance	References
vitiligo	miR-21-5p	up	plasma	melanocyte	*STAB1*	inhibit melanogenesis	(13)
	miR-487b-3p	down	plasma	-	-	biomarker for monitoring disease progression	(14)
	miR-200C	down	primary keratinocyte	melanocyte	*SOX1*	promote melanogenesis, potentially mediated by the *Wnt* pathway	(15)
	miR-493-3p	up	plasma	keratinocyte	*hnRNPU*	miR-493-3p/*hnRNPU*/ *COMT*/ *DA* axis is involved in the initial damage of melanocytes	(16)
psoriasis	miR-381-3p	up	keratinocyte	CD4-positive T-cell	*UBR5* and *FOXO1*	induce Th1 and Th17 polarization and promote psoriasis development	(17)
	246 miRNAs	up/down	plasma	-	-	provide abundant circulating exosomal miRNAs, target genes and signaling pathways for further research	(18)
	let-7b-5p and miR-30e-5p	down	plasma	-	-	biomarkers for arthritis in psoriasis patients	(19)
	miR-151a-3p, miR-199a-5p, miR-370-3p, miR-589-5p, and miR-769-5p	up	plasma	-	-	participate in the common pathogenesis of psoriasis vulgaris, psoriatic arthritis, rheumatoid arthritis and gouty arthritis	(20)
atopic dermatitis	miR-147	down	plasma	HaCaT cell	*TLSP*	exert protective effects by inhibiting *TLSP* expression	(21)
	25 miRNAs	up/down	plasma	-	-	biomarkers for psychological stress	(22)
severe drug eruption	miR-375-3p	up	plasma	keratinocyte	*XIAP*	promote apoptosis in keratinocytes by downregulating *XIAP*	(23)
	miR-18a	up	plasma	keratinocyte	*BCL2L10*	induce keratinocyte apoptosis by downregulating *BCL2L10*	(24)
	miR-4488	up	peripheral blood mononuclear cell	HaCaT cell	-	promote HaCaT cell apoptosis	(25)
	miR-96-5p	down					
dermatomyositis	miR-125a-3p, miR-1246, and miR-3614-5p	up	plasma	human skeletal muscle myocyte		clinical biomarkers and therapeutic targets	(26)
	miR-4488	up/down	plasma	-	*DDX39B*	biomarkers for DM-ILD-*MDA5* Ab (+)	(27)
	miR-1228-5p				*ZBTB22* and *MDM 2*		
	10 miRNAs	up/down	neutrophil	-	-	modulate *PI3K-Akt*, *MAPK*, *AMPK*, and *FoxO* pathways	(28)
	10 miRNAs	up/down	plasma	human aortic endothelial cell	59 genes	involved in vessel-related inflammation in juvenile dermatomyositis	(29)

(Continued)

TABLE 1 Continued

Disease	Exo-miRNA	State in disease group/ specific cells	Origin of exosome	Target cell	Target gene	Significance	References
scleroderma	17 miRNAs	up/down	plasma	normal human dermal fibroblast	-	identify 17 fibrosis-related miRNAs in systemic sclerosis	(30)
	22 miRNAs	up/down	neutrophil	human dermal fibroblast and human dermal microvascular endothelial cell	-	uncover the role of neutrophil in systemic sclerosis	(31)
	miR-214	down	bone marrow mesenchymal stem cell	fibroblast	*IL-33*	inhibit *IL-33*/*ST2* signaling	(32)
	miR-196b-5p	up	mesenchymal stem cell	fibroblast	*COL1A2*	alleviate skin fibrosis by inhibiting *COL1A2* expression	(33)
	miR-151-5p	up	mesenchymal stem cell	bone marrow mesenchymal stem cell	*IL-4Ra*	improve bone loss by targeting *IL-4Ra*	(34)
systemic lupus erythematosus	miR-574	up	plasma	plasmacytoid dendritic cell	*IFN-a*	structures resembling *IFN*-inducing motifs promote pDCs maturation and secretion	(35)
	miR-155	up	peripheral blood B-cell	B-cell	*SHIP-1*	regulate cell proliferation and activation of peripheral blood B-cells through targeting SHIP-1	(36)
	miR-19b	down	peripheral blood mononuclear cell	CD4-positive T-cell	*KLF13*	result in the differentiation of CD4-positive T-cells into Tregs and reduce the production of inflammatory cytokines	(37)
	miR-146a	down	plasma	bone marrow mesenchymal stem cell	*TRAF6*	rescue the senescence of BM-MSCs via inhibiting TRAF6/NF-κB pathway	(38)
	miR-146-5p	up	umbilical cord blood mesenchymal stem cell	macrophage	*NOTCH1*	suppress excessive inflammatory responses and protect against alveolar injury by inhibiting *NOTCH1* expression	(39)
	miR-195-5p	down	urine	-	*CXCL10*	biomarker for lupus nephritis	(40)

they discovered that enrichment analysis could enrich some target genes in inflammatory metabolic pathways. This study facilitated other researchers to select circulating exosomal miRNAs and target genes of interest at the subsequent step of investigating in psoriasis.

In addition to skin involvement, joints can also be affected, known as psoriatic arthritis (PsA). Pasquali et al. (19) identified 15 circulating exosomal miRNAs from 14 patients with psoriasis vulgaris (PsV) and 15 PsA patients. After expanding the sample size for validation along with regression analysis, they found that the expression levels of let-7b-5p and miR-30e-5p were negatively correlated with PsA group, which might be possible biomarkers for PsA. Chen et al. (20) extracted plasma exosomes from 15 PsV patients, 30 PsA patients, 15 patients with rheumatoid arthritis, 15 patients with gouty arthritis, and 15 healthy controls and identified five miRNAs (hsa-miR-151a-3p, hsa-miR-199a-5p, hsa-miR-370-3p, hsa-miR-589-5p, and hsa-miR-769-5p) that mainly participate in the common pathogenesis of these four diseases by affecting inflammation and bone metabolism.

2.3 Exosomal miRNAs in atopic dermatitis

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by intense itching, alternating acute episodes, and remissions, significantly affecting patients' quality of life. Crosstalk between keratinocytes and various immune cells results in skin barrier impairment via secreting diversified cytokines, such as *TNF-α* and *IFN-γ* (46). Shi et al. (21) detected significantly downregulated levels of miR-147 in the plasma, lesional tissues of a mouse model of AD and HaCaT cell models compared to the negative control group. The expression of miR-147 was negatively correlated with *TLSP* and *VEGFA*, two vascular growth-related factors considered important in the pathogenesis of AD (47).

Treatment with *TNF-α/IFN-γ* decreased the viability of HaCaT cells, upregulated *TLSP* and *VEGFA* expression, and promoted cell apoptosis. However, overexpression of miR-147 reversed these damaging processes in HaCaT cells. Furthermore, they found that exosomes from adipose-derived stem cells overexpressing miR-147 exerted similar protective effects and inhibited *TLSP* expression (21). These results indicate exosomal miR-147 as a potential target for AD therapy.

Besides, some studies have indicated a correlation between the onset of atopic dermatitis and psychological stress. Moreover, it has been demonstrated that psychotherapy can alleviate some patients' symptoms (48). Sung et al. (22) performed sequencing and differential analysis of plasma-derived neuronal exosomes from AD mouse models, identifying 9 significantly upregulated and 16 considerably downregulated miRNAs, which can be utilized to unveil mechanism of AD regarding psychological factors.

2.4 Exosomes miRNAs in severe drug eruption

Drug eruption, one of the most common adverse drug reactions, manifests as inflammatory skin and mucosal lesions with possible systemic involvement. Stevens-Johnson Syndrome (SJS), Toxic Epidermal Necrolysis (TEN), Acute Generalized Exanthemata's Pustulosis (AGEP) and Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS) are collectively known as severe drug eruptions characterized by large-scale death of keratinocytes (49). High mortality rates of severe drug eruptions urge dermatologists to excavate disease mechanisms and develop improved therapies. exosomes miRNA helps better understand the pathogenesis of severe drug eruptions that remain unclear.

Zhang et al. (23) identified upregulation of miR-375-3p in exosomes from patients with SJS and TEN. Overexpression of miR-375-3p in primary human keratinocytes reduced cell viability and promoted apoptosis via downregulating *XIAP*. Furthermore, they observed a positive correlation between the expression levels of miR-375-3p and the affected body surface area and epidermal necrosis score in patients with severe drug eruptions. Salinas et al. (24) investigated circulating exosomal miRNAs of patients with severe drug eruptions (9 cases of DRESS, 8 cases of SJS/TEN) and identified 24 significantly upregulated miRNAs, which were predominantly involved in T-cell activation, cell apoptosis, and inflammation processes. From these findings, they verified differential overexpression of miR-18a, consistent with previous research (50) that showed significant upregulation of miR-18a in the skin lesions and plasma of TEN patients, and it was found to induce keratinocyte apoptosis by downregulating *BCL2L10*. Suthumchai et al. (25) observed cytotoxic effects on HaCaT cells by exosomes secreted by peripheral blood mononuclear cells from 12 patients with SJS/TEN and identified upregulated miR-4488 and downregulated miR-486-5p, miR-96-5p, and miR-132-3p. Furthermore, both overexpression of miR-4488 and reduced expression of miR-96-5p promoted HaCaT cells apoptosis.

2.5 Exosomal miRNAs in dermatomyositis

Dermatomyositis (DM) is an autoimmune disease characterized by edematous purplish-red patches on the upper eyelids, as well as flat brownish-red papules on exposed areas.

Weakness and myalgia are the main manifestations of muscle involvement. Li et al. (26) confirmed the upregulation of has-miR-125a-3p, has-miR-1246, and has-miR-3614-5p in circulating exosomes from untreated DM patients and in human skeletal muscle myoblasts cells stimulated by these exosomes, which returned to normal levels after antirheumatic treatment. Furthermore, these three miRNAs were related to the cellular autophagy pathway and positively correlated with specific indicators in DM patients, such as serum creatine kinase levels and myositis antibody titers, suggesting them as potential biomarkers for DM.

The existence of anti-MDA5 antibodies was related to interstitial lung disease and a worse prognosis in DM patients (51). Zhong et al. (27) sequenced circulating exosomal miRNAs from 5 patients with DM accompanied by interstitial lung disease and positive anti-*MDA5* antibody, 5 patients with DM without myositis antibodies, and 5 healthy controls and found significant differences in has-miR-4488 and hsa-miR-1228-5p in all three comparisons. *H2AFX* and *MDM2* were identified as two essential hub genes that may be involved in the pathogenesis of DM. Nonetheless, this study didn't focus on the mechanism underlying the distinction between MDA5 positive and MDA5 negative DM patients.

Neutrophil/lymphocyte ratio in peripheral blood was found associated with disease activity, lung involvement, and overall survival in DM patients (52). Additionally, proteases within neutrophil cytoplasm seem to participate in muscle inflammation (53). Li et al. (28) validated 10 differentially expressed miRNAs in neutrophils-derived exosomes from DM patients' peripheral blood and determined *PI3K-Akt*, *MAPK*, *AMPK*, and *FoxO* as the main downstream signaling pathways, demonstrated and partially explained the role of neutrophil in DM.

In addition, evidences indicate a close relationship between the pathogenesis of DM and vascular changes (54). Jiang et al. (29) identified 10 differentially expressed circulating exosomal miRNAs in adolescent DM patients and 59 differentially expressed genes after co-culturing exosomes with human aortic endothelial cells. Through reciprocal prediction, they found specific downregulated genes in the patient group corresponding to upregulated miRNAs in the exosomal sequencing data, providing some miRNA-target gene axes for future research.

2.6 Exosomal miRNAs in scleroderma

Scleroderma, a chronic autoimmune skin disease characterized by abnormal activation of fibroblasts leading to progressive skin and visceral fibrosis, can be classified into two types: localized cutaneous scleroderma and systemic scleroderma, also known as systemic sclerosis (SSc) (55).

Wermuth et al. (30) validated the upregulation of 9 pro-fibrotic miRNAs and the downregulation of 8 anti-fibrotic miRNAs in exosomes derived from plasma of SSc patients and detected an upregulation of type I collagen fibers and fibronectin in normal human dermal fibroblasts stimulated by these exosomes, indicating the pro-fibrotic function of circulating exosomal miRNAs in SSc.

It has been found that neutrophil-derived exosomes from the peripheral blood of patients can inhibit endothelial cell proliferation and migration (56). Li et al. (31) discovered 22 differentially expressed miRNAs in neutrophil-derived exosomes from the peripheral blood of SSc patients and identified the involvement of the *Wnt*, *IL-23*, *NOTCH* and *AMPK* pathways. Further co-culturing the exosomes above with primary human dermal fibroblasts and human dermal microvascular endothelial cells from healthy individuals validated the presence of negative correlations between specific miRNAs and target genes associated with these molecular pathways, suggesting neutrophil as one of culprits in SSc.

Mesenchymal stem cells (MSCs) are a type of pluripotent stem cell derived from various tissues such as bone marrow, adipose tissue, placenta, and umbilical cord (57). These cells have demonstrated beneficial effects in improving the manifestations of scleroderma through their anti-inflammatory and anti-fibrotic properties (58), and it is believed that these effects are primarily mediated through the production of extracellular vesicles including exosomes (59). Xie et al. (32) identified downregulation of miR-214 in the peripheral blood of the SSc patients and discovered that bone marrow- MSC-derived exosomes could transport miR-214 to fibroblasts and inhibit their proliferation, migration, and expression of fibrosis-related genes via inhibiting *IL-33*/*ST2* signaling. Baral et al. (33) found that injection of exosomes derived from MSCs could alleviate skin fibrosis in bleomycin-induced SSc mice. They further identified upregulated miR-196b-5p and overexpression of this miRNA in mouse fibroblasts downregulated *COL1A2* expression, speculating that miR-196b-5p may play a role in the anti-fibrotic effects of MSC-derived exosomes. Chen et al. (34) discovered that MSCs transplantation could improve bone loss in systemic sclerosis mice by modulating the differentiation of recipient bone marrow-MSCs probably attributed to miR-151-5p derived from MSC-derived exosomes by targeting IL-4Ra, consistent with a previous study identifying IL-4 as a suspicious pathway (60).

These findings exhibited therapeutic potential of MSC-derived exosomal miRNAs for SSc.

2.7 Exosomal miRNAs in systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a complex autoimmune skin disease involving multiple systems. The characteristic skin lesions of SLE include edematous butterfly rash on the face, discoid rash, vasculitis-like lesions in the distal limbs, oral ulcers, and easily breakable hair at the frontal hairline.

Type I interferon plays a crucial role in the pathogenesis of SLE (61). Salvi et al. (35) demonstrated that circulating exosomal miRNAs with structures resembling interferon-inducing motifs from SLE patients can promote the maturation of human peripheral blood plasmacytoid dendritic cells(pDCs) and their secretion of type I interferon and other pro-inflammatory factors.

In addition to SSc, MSCs were also found therapeutic in SLE by modulating adaptive immunity. Patients exist various autoantibodies in their bodies, exemplified by anti-double-stranded DNA and anti-Sm antibodies (62), demonstrating the dominance of humoral immunity in SLE. Zhao et al. (36) found significantly higher numbers of peripheral blood B-cells in untreated SLE patients compared to healthy individuals, validated the upregulation of miR-155 and confirmed the binding relation between miR-155 and *SHIP-1*. Additionally, exosomes derived from umbilical cord blood-derived MSCs could upregulate *SHIP-1* expression in B-cells and inhibit cell proliferation, activation, and promotion of apoptosis.

However, cellular immunity shouldn't be easily neglected in SLE. Tu et al. (37) determined that miR-19b was downregulated while *KLF13* was upregulated in peripheral blood mononuclear cells of patients with SLE and verified the binding and negative correlation between miR-19b and *KLF13*. They further discovered that umbilical cord blood-derived MSCs could enrich miR-19b in exosomes, resulting in the differentiation of CD4-positive T-cells into Tregs and reducing the production of inflammatory cytokines.

On the contrary, MSCs might be victims surrounded by abnormal immune microenvironment in SLE. Dong et al. (38) discovered that plasma and plasma exosomes derived from SLE patients could promote the senescence of bone marrow MSCs, accelerate the degradation of *IκBα*, phosphorylation and translocation of *p65*, and exosomal miR-146a was downregulated. Based on previous studies and prediction websites, miR-146a is predicted to target and inhibit the expression of *TRAF6*, indicating that plasma-derived exosomal miR-146a may regulate the senescence of bone marrow MSCs through negative regulation of the *TRAF6*/*NF-κB* pathway.

Studies of exosomal miRNAs also focus on lupus nephritis and lung damage in SLE. Chen et al. (39) found that exosomes derived from umbilical cord blood-derived MSCs could alleviate diffuse alveolar hemorrhage in SLE mice and demonstrated that exosomes from umbilical cord blood-derived MSCs could transport miR-146-5p to target and inhibit the expression of *NOTCH1*, thus promoting the polarization of M2 macrophages, leading to the suppression of excessive inflammatory responses and protection against alveolar injury. Cheng et al. (40) demonstrated downregulation of miR-195-5p in the urine of lupus nephritis patients and its negative modulation of *CXCL10*, consistent with their initial result based on online available data. Furthermore, this miRNA was negatively correlated with urinary protein, renal damage, serum complement levels, and disease severity.

3 Discussion

Autoimmune skin diseases impose significant burdens on patients, families, and society. Despite the availability of various treatment options, the lack of a clear understanding of the underlying mechanisms of the diseases, coupled with their tendency for progression and relapse, often leads to unsatisfactory outcomes in terms of treatment and management. Exosomes, extracellular vesicles

that transport important molecules such as miRNA to recipient cells, play a crucial role in modulating gene expression and altering cellular functions. Studies of exosomal miRNA can provide insights into the intrinsic mechanisms of diseases, facilitate the identification of potential biomarkers and molecular targets, and lay the foundation for disease monitoring and the development of novel therapies and drugs from a new perspective. However, there is limited foundational research on exosomal miRNA in autoimmune skin diseases, with a scarcity of clinical studies and low levels of evidence. Therefore, more standardized research designs and larger-scale studies to explore the role of exosomal miRNA in autoimmune skin diseases are warranted in the future.

Author contributions

RZ: Writing - original draft, Data curation. YW: Writing - original draft, Writing - review & editing. TW: Writing - review & editing. XN: Writing - review & editing. ZS: Writing - review & editing. YD: Writing - review & editing. DL: Conceptualization, Writing - review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

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. Lee RC, Feinbaum RL, Ambros V. The C. Elegans heterochronic gene lin-4 encodes small rnas with antisense complementarity to lin-14. *Cell* (1993) 75(5):843–54. doi: 10.1016/0092-8674(93)90529-y

2. Iwakawa HO, Tomari Y. Life of risc: formation, action, and degradation of rna-induced silencing complex. *Mol Cell* (2022) 82(1):30–43. doi: 10.1016/j.molcel.2021.11.026

3. Wu L, Fan J, Belasco JG. Micrornas direct rapid deadenylation of mrna. *Proc Natl Acad Sci U.S.A.* (2006) 103(11):4034–9. doi: 10.1073/pnas.0510928103

4. Cai Y, Yu X, Hu S, Yu J. A brief review on the mechanisms of mirna regulation. *Genomics Proteomics Bioinf* (2009) 7(4):147–54. doi: 10.1016/s1672-0229(08)60044-3

5. He B, Zhao Z, Cai Q, Zhang Y, Zhang P, Shi S, et al. Mirna-based biomarkers, therapies, and resistance in cancer. *Int J Biol Sci* (2020) 16(14):2628–47. doi: 10.7150/ijbs.47203

6. Dixon CL, Sheller-Miller S, Saade GR, Fortunato SJ, Lai A, Palma C, et al. Amniotic fluid exosome proteomic profile exhibits unique pathways of term and preterm labor. *Endocrinology* (2018) 159(5):2229–40. doi: 10.1210/en.2018-00073

7. Vojtech L, Woo S, Hughes S, Levy C, Ballweber L, Sauteraud RP, et al. Exosomes in human semen carry a distinctive repertoire of small non-coding rnas with potential regulatory functions. *Nucleic Acids Res* (2014) 42(11):7290–304. doi: 10.1093/nar/gku347

8. Pisitkun T, Shen RF, Knepper MA. Identification and proteomic profiling of exosomes in human urine. *Proc Natl Acad Sci U.S.A.* (2004) 101(36):13368–73. doi: 10.1073/pnas.0403453101

9. Akers JC, Ramakrishnan V, Kim R, Skog J, Nakano I, Pingle S, et al. Mir-21 in the extracellular vesicles (Evs) of cerebrospinal fluid (Csf): A platform for glioblastoma biomarker development. *PloS One* (2013) 8(10):e78115. doi: 10.1371/journal.pone.0078115

10. Siles-Lucas M, Morchon R, Simon F, Manzano-Roman R. Exosome-transported micrornas of helminth origin: new tools for allergic and autoimmune diseases therapy? *Parasite Immunol* (2015) 37(4):208–14. doi: 10.1111/pim.12182

11. Pegtel DM, Gould SJ. Exosomes. *Annu Rev Biochem* (2019) 88:487–514. doi: 10.1146/annurev-biochem-013118-111902

12. Turpin D, Truchetet ME, Faustin B, Augusto JF, Contin-Bordes C, Brisson A, et al. Role of extracellular vesicles in autoimmune diseases. *Autoimmun Rev* (2016) 15(2):174–83. doi: 10.1016/j.autrev.2015.11.004

13. Zhang C, Guo W, Wang S, Di Y, Wu D. Peripheral blood of vitiligo patients-derived exosomal mir-21-5p inhibits melanocytes melanogenesis via targeting satb1. *Iranian J Public Health* (2022) 51(12):2706–16. doi: 10.18502/ijph.v51i12.11461

14. Luo H, Xie B, Xu J, Zhu Y, Sun J, Shen Y, et al. Differential Expression of Serum Exosomal Hsa-Mir-487b-3p in Progressive Vitiligo before and after Systemic Corticosteroid Treatment. *Clinical cosmetic investigational Dermatol* (2022) 15:1377–86. doi: 10.2147/ccid.S372112

15. Zhao C, Wang D, Wang X, Mao Y, Xu Z, Sun Y, et al. Down-regulation of exosomal mir-200c derived from keratinocytes in vitiligo lesions suppresses melanogenesis. *J Cell Mol Med* (2020) 24(20):12164–75. doi: 10.1111/jcmm.15864

16. Li D, Zhou T, She Q, Nie X, Liu Z, Pan R, et al. Circulating exosomal mir-493-3p affects melanocyte survival and function by regulating epidermal dopamine concentration in segmental vitiligo. *J Invest Dermatol* (2022) 142(12):3262–73.e11. doi: 10.1016/j.jid.2022.05.1086

17. Jiang M, Fang H, Dang E, Zhang J, Qiao P, Yu C, et al. Small extracellular vesicles containing mir-381-3p from keratinocytes promote T helper type 1 and T helper type 17 polarization in psoriasis. *J Invest Dermatol* (2021) 141(3):563–74. doi: 10.1016/j.jid.2020.07.009

18. Chen XM, Yao DN, Wang MJ, Wu XD, Deng JW, Deng H, et al. Deep sequencing of serum exosomal microrna level in psoriasis vulgaris patients. *Front Med* (2022) 9:895564. doi: 10.3389/fmed.2022.895564

19. Pasquali L, Svedbom A, Srivastava A, Rosén E, Lindqvist U, Ståhle M, et al. Circulating micrornas in extracellular vesicles as potential biomarkers for psoriatic arthritis in patients with psoriasis. *J Eur Acad Dermatol Venereology JEADV* (2020) 34(6):1248–56. doi: 10.1111/jdv.16203

20. Chen XM, Zhao Y, Wu XD, Wang MJ, Yu H, Lu JJ, et al. Novel findings from determination of common expressed plasma exosomal micrornas in patients with psoriatic arthritis, psoriasis vulgaris, rheumatoid arthritis, and gouty arthritis. *Discovery Med* (2019) 28(151):47–68.

21. Shi C, Pei S, Ding Y, Tao C, Zhu Y, Peng Y, et al. Exosomes with overexpressed mir 147a suppress angiogenesis and infammatory injury in an experimental model of atopic dermatitis. *Sci Rep* (2023) 13(1):8904. doi: 10.1038/s41598-023-34418-y

22. Sung M, Sung SE, Kang KK, Choi JH, Lee S, Kim K, et al. Serum-derived neuronal exosomal micrornas as stress-related biomarkers in an atopic dermatitis model. *Biomedicines* (2021) 9(12):1764. doi: 10.3390/biomedicines9121764

23. Zhang C, Zhu Z, Gao J, Yang L, Dang E, Fang H, et al. Plasma exosomal mir-375-3p regulates mitochondria-dependent keratinocyte apoptosis by targeting xiap in severe drug-induced skin reactions. *Sci Trans Med* (2020) 12(574):eaaw6142. doi: 10.1126/scitranslmed.aaw6142

24. Salinas-Jaramillo O, Monroy-Arreola A, Herrera-Noreña S, Guzmán-Ortiz AL, Hernández-Hernández A, Méndez-Flores S, et al. Extracellular vesicles from human plasma show a distinctive proteome and mirnome profile in patients with severe cutaneous adverse reactions. *Chem Res Toxicol* (2021) 34(7):1738–48. doi: 10.1021/acs.chemrestox.1c00047

25. Suthumchai N, Buranapraditkun S, Thantiworasit P, Rerknimitr P, Wongpiyabovorn J, Khanaraksombat S, et al. Exosomal micrornas from pbmcs

stimulated with culprit drugs enhanced keratinocyte cell death in stevens-johnson syndrome/toxic epidermal necrolysis. *J Eur Acad Dermatol Venereology JEADV* (2023) 37(7):1375–84. doi: 10.1111/jdv.19000

26. Li L, Zuo X, Liu D, Luo H, Zhang H, Peng Q, et al. Plasma exosomal rnas have potential as both clinical biomarkers and therapeutic targets of dermatomyositis. *Rheumatol (Oxford England)* (2022) 61(6):2672–81. doi: 10.1093/rheumatology/keab753

27. Zhong D, Wu C, Xu D, Bai J, Wang Q, Zeng X. Plasma-derived exosomal hsa-mir-4488 and hsa-mir-1228-5p: novel biomarkers for dermatomyositis-associated interstitial lung disease with anti-melanoma differentiation-associated protein 5 antibody-positive subset. *BioMed Res Int* (2021) 2021:6676107. doi: 10.1155/2021/6676107

28. Li L, Zuo X, Liu D, Luo H, Zhu H. The functional roles of rnas cargoes released by neutrophil-derived exosomes in dermatomyositis. *Front Pharmacol* (2021) 12:727901. doi: 10.3389/fphar.2021.727901

29. Jiang K, Karasawa R, Hu Z, Chen Y, Holmes L, O'Neil KM, et al. Plasma exosomes from children with juvenile dermatomyositis are taken up by human aortic endothelial cells and are associated with altered gene expression in those cells. *Pediatr Rheumatol Online J* (2019) 17(1):41. doi: 10.1186/s12969-019-0347-0

30. Wermuth PJ, Piera-Velazquez S, Jimenez SA. Exosomes isolated from serum of systemic sclerosis patients display alterations in their content of profibrotic and antifibrotic microrna and induce a profibrotic phenotype in cultured normal dermal fibroblasts. *Clin Exp Rheumatol* (2017) 35 Suppl 106(4):21–30.

31. Li L, Zuo X, Liu D, Luo H, Zhu H. The profiles of mirnas and lncrnas in peripheral blood neutrophils exosomes of diffuse cutaneous systemic sclerosis. *J Dermatol Sci* (2020) 98(2):88–97. doi: 10.1016/j.jdermsci.2020.02.009

32. Xie L, Long X, Mo M, Jiang J, Zhang Q, Long M, et al. Bone marrow mesenchymal stem cell-derived exosomes alleviate skin fibrosis in systemic sclerosis by inhibiting the il-33/st2 axis via the delivery of microrna-214. *Mol Immunol* (2023) 157:146–57. doi: 10.1016/j.molimm.2023.03.017

33. Baral H, Uchiyama A, Yokoyama Y, Sekiguchi A, Yamazaki S, Amalia SN, et al. Antifibrotic effects and mechanisms of mesenchymal stem cell-derived exosomes in a systemic sclerosis mouse model: possible contribution of mir-196b-5p. *J Dermatol Sci* (2021) 104(1):39–47. doi: 10.1016/j.jdermsci.2021.08.006

34. Chen C, Wang D, Moshaverinia A, Liu D, Kou X, Yu W, et al. Mesenchymal stem cell transplantation in tight-skin mice identifies mir-151-5p as a therapeutic target for systemic sclerosis. *Cell Res* (2017) 27(4):559–77. doi: 10.1038/cr.2017.11

35. Salvi V, Gianello V, Busatto S, Bergese P, Andreoli L, D'Oro U, et al. Exosome-delivered micrornas promote ifn-A Secretion by human plasmacytoid dcs via tlr7. *JCI Insight* (2018) 3(10):e98204. doi: 10.1172/jci.insight.98204

36. Zhao Y, Song W, Yuan Z, Li M, Wang G, Wang L, et al. Exosome derived from human umbilical cord mesenchymal cell exerts immunomodulatory effects on B cells from sle patients. *J Immunol Res* (2023) 2023:3177584. doi: 10.1155/2023/3177584

37. Tu J, Zheng N, Mao C, Liu S, Zhang H, Sun L. Uc-bscs exosomes regulate th17/treg balance in patients with systemic lupus erythematosus via mir-19b/klf13. *Cells* (2022) 11(24):4123. doi: 10.3390/cells11244123

38. Dong C, Zhou Q, Fu T, Zhao R, Yang J, Kong X, et al. Circulating exosomes derived-mir-146a from systemic lupus erythematosus patients regulates senescence of mesenchymal stem cells. *BioMed Res Int* (2019) 2019:6071308. doi: 10.1155/2019/6071308

39. Chen X, Su C, Wei Q, Sun H, Xie J, Nong G. Exosomes derived from human umbilical cord mesenchymal stem cells alleviate diffuse alveolar hemorrhage associated with systemic lupus erythematosus in mice by promoting M2 macrophage polarization via the microrna-146a-5p/notch1 axis. *Immunol investigations* (2022) 51(7):1975–93. doi: 10.1080/08820139.2022.2090261

40. Cheng C, Guo F, Yang H, Ma J, Li H, Yin L, et al. Identification and analysis of the predictive urinary exosomal mir-195-5p in lupus nephritis based on renal mirna-mrna co-expression network. *Lupus* (2022) 31(14):1786–99. doi: 10.1177/09612033221133684

41. Le Poole IC, Das PK, van den Wijngaard RM, Bos JD, Westerhof W. Review of the etiopathomechanism of vitiligo: A convergence theory. *Exp Dermatol* (1993) 2 (4):145–53. doi: 10.1111/j.1600-0625.1993.tb00023.x

42. Hlača N, Kaštelan M, Brajac I, Prpić-Massari L. Current concepts of vitiligo immunopathogenesis. *Biomedicines* (2022) 10(7):1639. doi: 10.3390/biomedicines10071639

43. Peng L, Lu Y, Gu Y, Liang B, Li Y, Li H, et al. Mechanisms of Action of Lycium Barbarum Polysaccharide in Protecting against Vitiligo Mice through Modulation of the Stat3-Hsp70-Cxcl9/Cxcl10 Pathway. *Pharm Biol* (2023) 61(1):281–7. doi: 10.1080/13880209.2022.2163406

44. Richmond JM, Frisoli ML, Harris JE. Innate immune mechanisms in vitiligo: danger from within. *Curr Opin Immunol* (2013) 25(6):676–82. doi: 10.1016/j.coi.2013.10.010

45. Blauvelt A, Chiricozzi A. The immunologic role of il-17 in psoriasis and psoriatic arthritis pathogenesis. *Clin Rev Allergy Immunol* (2018) 55(3):379–90. doi: 10.1007/s12016-018-8702-3

46. Beck LA, Cork MJ, Amagai M, De Benedetto A, Kabashima K, Hamilton JD, et al. Type 2 inflammation contributes to skin barrier dysfunction in atopic dermatitis. *JID Innov skin Sci molecules to population Health* (2022) 2(5):100131. doi: 10.1016/j.xjidi.2022.100131

47. Lee HJ, Hong YJ, Kim M. Angiogenesis in chronic inflammatory skin disorders. *Int J Mol Sci* (2021) 22(21):12035. doi: 10.3390/ijms222112035

48. Suárez AL, Feramisco JD, Koo J, Steinhoff M. Psychoneuroimmunology of psychological stress and atopic dermatitis: pathophysiologic and therapeutic updates. *Acta dermato-venereologica* (2012) 92(1):7–15. doi: 10.2340/00015555-1188

49. Zhang J, Lei Z, Xu C, Zhao J, Kang X. Current perspectives on severe drug eruption. *Clin Rev Allergy Immunol* (2021) 61(3):282–98. doi: 10.1007/s12016-021-08859-0

50. Ichihara A, Wang Z, Jinnin M, Izuno Y, Shimozono N, Yamane K, et al. Upregulation of mir-18a-5p contributes to epidermal necrolysis in severe drug eruptions. *J Allergy Clin Immunol* (2014) 133(4):1065–74. doi: 10.1016/j.jaci.2013.09.019

51. Wu W, Guo L, Fu Y, Wang K, Zhang D, Xu W, et al. Interstitial lung disease in anti-mda5 positive dermatomyositis. *Clin Rev Allergy Immunol* (2021) 60(2):293–304. doi: 10.1007/s12016-020-08822-5

52. Yang W, Wang X, Zhang W, Ying H, Xu Y, Zhang J, et al. Neutrophil-lymphocyte ratio and platelet-lymphocyte ratio are 2 new inflammatory markers associated with pulmonary involvement and disease activity in patients with dermatomyositis. *Clinica chimica acta; Int J Clin Chem* (2017) 465:11–6. doi: 10.1016/j.cca.2016.12.007

53. Gao S, Zuo X, Liu D, Xiao Y, Zhu H, Zhang H, et al. The roles of neutrophil serine proteinases in idiopathic inflammatory myopathies. *Arthritis Res Ther* (2018) 20 (1):134. doi: 10.1186/s13075-018-1632-x

54. Lundberg IE, Fujimoto M, Vencovsky J, Aggarwal R, Holmqvist M, Christopher-Stine L, et al. Idiopathic inflammatory myopathies. *Nat Rev Dis Primers* (2021) 7(1):86. doi: 10.1038/s41572-021-00321-x

55. Denton CP, Khanna D. Systemic sclerosis. *Lancet (London England)* (2017) 390 (10103):1685–99. doi: 10.1016/s0140-6736(17)30933-9

56. Li L, Zuo X, Xiao Y, Liu D, Luo H, Zhu H. Neutrophil-derived exosome from systemic sclerosis inhibits the proliferation and migration of endothelial cells. *Biochem Biophys Res Commun* (2020) 526(2):334–40. doi: 10.1016/j.bbrc.2020.03.088

57. da Silva Meirelles L, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. *J Cell Sci* (2006) 119(Pt 11):2204–13. doi: 10.1242/jcs.02932

58. Cras A, Farge D, Carmoi T, Lataillade JJ, Wang DD, Sun L. Update on mesenchymal stem cell-based therapy in lupus and scleroderma. *Arthritis Res Ther* (2015) 17:301. doi: 10.1186/s13075-015-0819-7

59. Nooshabadi VT, Mardpour S, Yousefi-Ahmadipour A, Allahverdi A, Izadpanah M, Daneshimehr F, et al. The extracellular vesicles-derived from mesenchymal stromal cells: A new therapeutic option in regenerative medicine. *J Cell Biochem* (2018) 119 (10):8048–73. doi: 10.1002/jcb.26726

60. Wynn TA. Fibrotic disease and the T(H)1/T(H)2 paradigm. *Nat Rev Immunol* (2004) 4(8):583–94. doi: 10.1038/nri1412

61. Rönnblom L. The importance of the type I interferon system in autoimmunity. *Clin Exp Rheumatol* (2016) 34(4 Suppl 98):21–4.

62. Kiriakidou M, Ching CL. Systemic lupus erythematosus. *Ann Internal Med* (2020) 172(11):Itc81–itc96. doi: 10.7326/aitc202006020

frontiers | Frontiers in Immunology

TYPE Mini Review
PUBLISHED 14 December 2023
DOI 10.3389/fimmu.2023.1326667

OPEN ACCESS

EDITED BY
Jinhong Zhu,
Harbin Medical University Cancer Hospital, China

REVIEWED BY
Yang Du,
Peking Union Medical College Hospital (CAMS), China
Reza Rahbarghazi,
Tabriz University of Medical Sciences, Iran
Huashan Shi,
Sichuan University, China

*CORRESPONDENCE
Chunyan He
chunyanhe530@163.com
Zhen Zhu
zzesky@hotmail.com

†These authors have contributed equally to this work and share first authorship

RECEIVED 23 October 2023
ACCEPTED 04 December 2023
PUBLISHED 14 December 2023

CITATION
Zhao J, Li X, Liu L, Zhu Z and He C (2023) Exosomes in lung cancer metastasis, diagnosis, and immunologically relevant advances.
Front. Immunol. 14:1326667.
doi: 10.3389/fimmu.2023.1326667

Exosomes in lung cancer metastasis, diagnosis, and immunologically relevant advances

Jianhua Zhao[1†], Xiwen Li[2†], Lele Liu[3†], Zhen Zhu[1*] and Chunyan He[3*]

[1]Department of Thoracic Surgery, Kunshan Hospital of Chinese Medicine, Affiliated Hospital of Yangzhou University, Kunshan, China, [2]Department of Central Laboratory, Kunshan Hospital of Chinese Medicine, Affiliated Hospital of Yangzhou University, Kunshan, China, [3]Department of Clinical Laboratory, Kunshan Hospital of Chinese Medicine, Affiliated Hospital of Yangzhou University, Kunshan, China

Lung cancer is a chronic wasting disease with insidious onset and long treatment cycle. Exosomes are specialized extracellular vesicles, at first exosomes were considered as a transporter of cellular metabolic wastes, but recently many studies have identified exosomes which contain a variety of biologically active substances that play a role in the regulation of cellular communication and physiological functions. Exosomes play an important role in the development of lung cancer and can promote metastasis through a variety of mechanisms. However, at the same time, researchers have also discovered that immune cells can also inhibit lung cancer through exosomes. In addition, researchers have discovered that some specific miRNAs in exosomes can be used as markers for early diagnosis of lung cancer. Engineering exosomes may be one of the strategies to enhance the clinical translational application of exosomes in the future, for example, strategies such as modifying exosomes to enhance targeting or utilizing exosomes as carriers for drug delivery have been explored. but more studies are needed to verify the safety and efficacy. This article reviews the latest research on exosomes in the field of lung cancer, from the mechanism of lung cancer development, the functions of immune cell-derived exosomes and tumor-derived exosomes, to the early diagnosis of lung cancer.

KEYWORDS

exosome, lung cancer, immunotherapy, miRNA, EMT, neovascularization

1 Introduction

Cancer is essentially a disease of uncontrolled growth, unlimited proliferation and metastasis to distant sites, with an insidious onset and a long treatment period (1, 2). The American Cancer Society reported in 2020 that lung cancer currently ranks second in new cancer cases and first in cancer-related deaths worldwide (3, 4). Lung cancer is mainly categorized into two types: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC is a predominant type of lung cancer, accounting for approximately 85% to 90% of lung cancer cases. In contrast to SCLC, NSCLC typically exhibits slower growth with better prognosis compared to SCLC (5).

Almost all cell types can secrete extracellular vesicles (EVs). Currently, there is no gold standard for classifying extracellular vesicles (EVs). They can be categorized into microvesicles, exosomes, apoptotic bodies, and more. Different types of EVs exhibit distinct biological characteristics and origins. For instance, microvesicles, ranging in size from 100-1000nm, are directly secreted from the cellular membrane. Apoptotic bodies, generated during cell apoptosis, typically have a size of 50-500nm (6). Various EVs carry different surface markers, allowing for isolation and purification. The biogenesis of exosomes involves the inward budding of the plasma membrane and the formation of intraluminal vesicles (ILVs) within multivesicular bodies (MVBs). Through MVB fusion with the plasma membrane and exocytosis, ILVs are eventually released as exosomes (7).

Exosomes are specialized extracellular vesicles produced by the endocytosis pathway with a lipid bilayer closed structure, with diameters ranging from 30-150 nm, and are widely distributed in body fluids including urine, saliva, plasma, cerebrospinal fluid, and bile (7, 8). They contain a variety of biologically active substances, including proteins, DNA, microRNA, lipids, etc (Figure 1). Researchers have conducted extensive research to verify their biological functions (9). Cell-to-cell communication can be realized through exosomes, which have also been found to be involved in various physiopathological processes and intracellular mechanism regulation (10).

Exosomes were first discovered and named in 1983. But for a long time afterward, exosomes were not thought to be valuable components until 2007 that it was found to have the capability of intercellular communication, and more studies have subsequently found that exosomes have a variety of physiological functions (11). In other areas such as endocrine, cardiovascular, and ophthalmologic diseases, exosomes can serve as biomarkers for clinical diagnosis (12, 13). The function and contents within the exosomes are heterogeneous due to different origins, and even exosomes secreted by the same cell at different time can exhibit different morphologies and functions. In recent years, exosomes have been found to play an important role in tumor metastasis, immune microenvironment formation, and non-programmed tumor cell death (14).

There are various strategies for exosome isolation, including ultracentrifugation, ultrafiltration, size-exclusion chromatography, precipitation, immunoaffinity-based capture, and microfluidic separation (8, 15, 16). All these techniques allows researchers to unveil the mystery of exosomes. Currently, several researches on exosomes in lung cancer have made good progress, revealing the mechanism in lung cancer progression, drug resistance, and metastasis, and emphasizing the significance of exosomes in the early diagnosis (17). With the increasing knowledge of exosomes, researchers have used innovative techniques to generate engineered exosomes as a novel therapeutic strategy for lung cancer (18).

In this paper, we will briefly introduce the relevant mechanism of exosomes in lung cancer development in recent years. Then based on the previous understanding, we analyze the potential of exosomes as diagnostic markers as well as drug delivery carriers. Finally, we discuss the challenges and potential directions for future applications of exosomes in lung cancer research.

2 Exosomes promote the development of lung cancer

2.1 Exosomes in tumor drug resistance

Drug resistance refers to the development of tolerance in tumor cells to antitumor therapy, which can greatly reduce the effectiveness of antitumor therapy. Tumor drug resistance can be achieved through a variety of mechanisms, such as altered drug kinetics and enhanced drug efflux and metabolism, affecting tumor cell cycle and promoting proliferation and inhibiting apoptosis (19). Existing studies have shown that exosomes derived from different sources of cells in the tumor microenvironment(TME) can affect tumor drug resistance by influencing tumor proliferation and immunity (20), which include immune cells, tumor cells, and tumor-associated fibroblasts.

Exosomes can act as intermediate carriers to deliver drug resistance information from drug-resistant tumor cells to tumor cells that have not acquired resistance For example, researchers found that cisplatin-resistant NSCLS cells induced by hypoxic environment could secrete PKM2 exosomes to transfer resistance capability to cisplatin-sensitive NSCLS (21). As a result, exosomes can also serve as a drug-carrying tool to inhibit tumor drug resistance in lung (22).

2.2 Exosomes in tumor epithelial mesenchymal transition

Tumor metastasis is a major cause of poor prognosis and death, and epithelial mesenchymal transition (EMT) is one of the important pathways for tumor progression and metastasis (23). Normally, EMT helps cells to migrate in the embryo, and tumor cells that undergo EMT show changes of weakened intercellular adhesion and enhanced cell motility, which increases the likelihood of metastasis of tumor cells (24).

Exosomes from tumor-associated fibroblasts (CAFs) enhance cell stemness and EMT in colorectal cancer, thereby promoting tumor metastasis (25). KRT6B in tumor-derived exosomes was also found to promote cancer metastasis by inducing EMT in bladder

10.3389/fimmu.2023.1326667

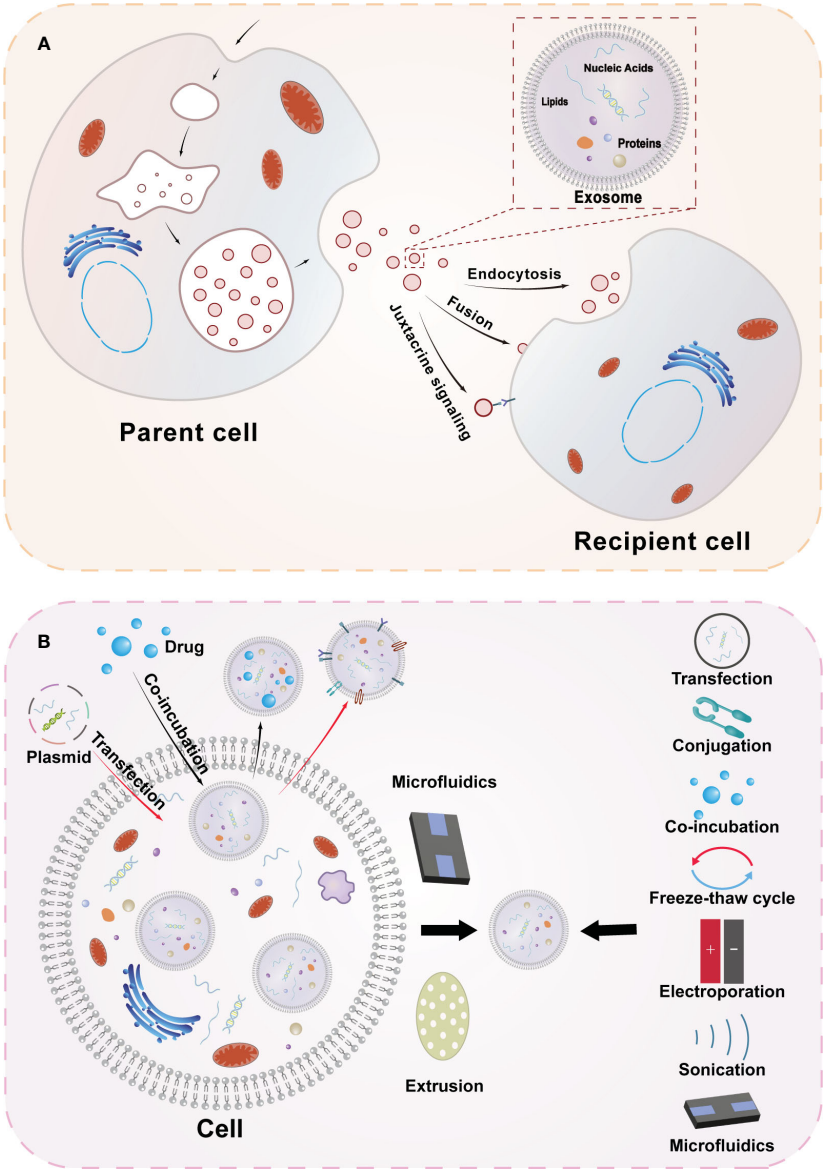


FIGURE 1
Overview diagram of exosome biogenesis and engineered exosomes. **(A)** Secretion and uptake of exosomes and the composition of exosomes. The cell membrane of the parent cell invaginates to form early endosomes, which subsequently mature into late endosomes and multivesicular vesicles, and the vesicles in the lumen of the multivesicular vesicles are exosomes secreted by the cell after membrane fusion. After exosomes are released to the outside of the cell, they can transmit information to the receptor cells in three ways(Endocytosis, Fusion, Juxtacrine signaling) to achieve the corresponding functions. **(B)** Extraction methods of exosomes and strategies for engineering exosomes. Engineered exosomes can be obtained by modifying exosomes at both cellular and exosome levels. At the cellular level, engineered exosomes can be obtained by processing cells through transfection and co-incubation, etc. At the exosome level, after extracting cellular exosomes through microfluidics and other techniques, information is loaded into exosomes using transfection, cojugation, freeze-thaw cycling, electroporation, sonication and other techniques.

cancer (26). Similar phenomena were found in kidney cancer (27), liver cancer (28), and breast cancer (29). In lung cancer, exosomes constituting the TME can also mediate EMT in lung cancer, thus promoting metastasis. Researchers found that miRNAs contained in exosomes produced by mysenchymal stem cells under hypoxic environments could elevate the expression of markers associated with EMT in lung cancer by regulating the STAT3 signaling pathway (30). Exosomes from hypoxic LUAD cells could significantly increase the migration of normoxic lung adenocarcinoma cells via SATB2to activate MEK/ERK pathway-mediated EMT (31).

Apart from a direct effect on EMT, lung adenocarcinoma cells may indirectly regulate EMT in lung adenocarcinoma by interfering with exosome secretion from CAFs as well (32). Additionally, different states of tumor cells and other cells in the TME can exert an influence on the EMT process. Exosomes, as one of the constitutive components of the TME, thereby intervene in the progression of the tumor process greatly.

2.3 Tumor exosomes and neovascularization

Neovascularization is required for lung cancer growth and metastasis, and is also a factor that affects the prognosis of lung cancer (33–35). The significance of anti-vascularization in lung cancer treatment has been noted early on, and anti-vascular endothelial growth factor (VEGF) drugs have been approved for use in the clinic (36). Studies on the mechanism of neovascularization in lung cancer are more limited, but some specific miRNAs, sirtuin1, and notch pathway have been found to play a role in neovascularization (37, 38). The Tumor Microenvironment (TME) refers to the collective assembly of cells, molecules, and physical factors surrounding a tumor, interacting with tumor cells and influencing the growth, spread, and therapeutic responses of the tumor. Hypoxic conditions are common in the TME. One study indicated that miR-23a in exosomes of lung cancer cells under hypoxia targets ZO-1 protein and prolyl hydroxylase, thereby enhancing angiogenesis and vascular permeability (39), while another study found that miR-197-3p in exosomes of lung adenocarcinoma origin targets TIMP2, TIMP3 to promote neoangiogenesis (40). MiR-3157-3p in exosomes from NSCLS was also found to down-regulate the expression of TIMP2, KLF2, ZO-1, and Occludin, and up-regulate the expression of VEGF, MMP2, and MMP9, ultimately leading to increased angiogenesis (41). Currently, anti-angiogenic drugs, such as bevacizumab, apatinib, abciximab, have been applied in the clinical treatment of lung cancer patients, with good efficacy. Since lung cancer exosomes is believed to be one of the mechanisms of neovascularization, and existing research found that the miRNAs therein seem to be the key, one may opens up the idea that if the application of the inhibitors of these miRNAs, or the inhibition of secretion of tumor exosomes, is possible to achieve good results? Of course, this requires more in-depth research to verify the safety and efficacy.

2.4 Tumor exosomes in immune cells regulation and immune efficacy of related research

The human immune system recognizes and removes tumor cells from the body, and tumor cells can evade immune recognition and removal by inhibiting immune cell proliferation or activation. For example, tumor-derived exosomes have been found to alter mitochondrial function to inhibit the proliferation of cytotoxic T cells (42). Natural killer cells(NKC) are sentinel cells of the immune system (43) that recognize tumor cells and remove them without additional activation. During immune evasion of tumor cells, a large number of bioactive molecules contained in tumor cell-derived exosomes, such as transforming growth factor-β (TGF-β), programmed death ligand (PD-L1), ligand for natural killer cell activated receptor NKG2D (MICA/B), apoptosis-associated protein Fas-L, etc., will recognize the cognate receptor on NK cells and abolish the anti-tumor activity. Regarding macrophages, tumor-derived exosomes have also been found to inhibit polarization, achieving immunosuppression and allowing tumor progression (44, 45).

The lymphatic system is responsible for immune cell activation, and the researchers found that exosomes secreted by tumor cells containing immunosuppressive protein PD-L1 can inactivate immune cells to protect themselves from being killed (46). This observation gives clue to researchers that if one can inhibit some tumor cell-derived exosomes, it will also be possible to address the dilemma of insensitivity to immune checkpoint inhibitors in many patients. However, this also raises new challenges in developing medications that can act on tumor exosomes.

Besides the effect of tumor-derived exosomesaffect on immune cells, exosomes secreted by immune cells also exert impact on the tumor immune response. In addition to recognizing and killing tumor cells, NK cells exosomes also modulate the immune response of T cells (47) and are cytotoxic to cancer cells (48). Exosomes secreted by M1-polarized macrophages were found to promote M1 polarization of macrophages, thereby enhancing anti-tumor immunity and inhibiting tumor growth (49).

2.5 Research progress of exosomes in the early diagnosis of lung cancer

Large number of researches have validated the function of exosomes in the progression of lung cancer (50), and people are also thinking about the role of exosomes in the clinical context. Early diagnosis of lung cancer can significantly improve the survival rate of patients (51, 52). Although low-dose computed tomography (LDCT) has improved the diagnosis rate of lung nodules, LDCT has poor specificity and is prone to unnecessary surgery, so non-invasive means to diagnose benign and malignant lung nodules is a very promising research direction.

Based on the changes of miRNA profiles in plasma exosomes, researchers identified miR-500a-3p, miR-501-3p, and miR-502-3p, up-regulated within lung cancer, which revealed the possibility of early diagnosis of lung cancer by plasma exosomes (53). Subsequently, another researcher team constructed a diagnostic model to distinguish benign and malignant lung nodules based on plasma exosomal miRNAs, which was also reported encouraging outcomes for lung nodules with a diameter of less than 1 cm (54). Combined with the current deep learning algorithm Shin et al. validated the feasibility of human plasma exosomes as potential tumor-associated biomarkers (55). Subsequently, a set of miRNAs, i.e., miR-200b-3p, miR-3124-5p and miR-92b-5p, in serum exosomes could be used as diagnostic and prognostic markers for SCLC (56) (Figure 2). Another study showed that exosomes of SCLC cells with molecules such as Hippo, Rap1, and Wnt could also be used as indicators to determine prognosis (57). At present, a series of studies have verified the feasibility of exosomes in the early diagnosis of lung cancer. Combining the advantages of non-invasiveness of exosomal examination with existing tests is expected to enhance the early diagnosis and prognosis of lung cancer.

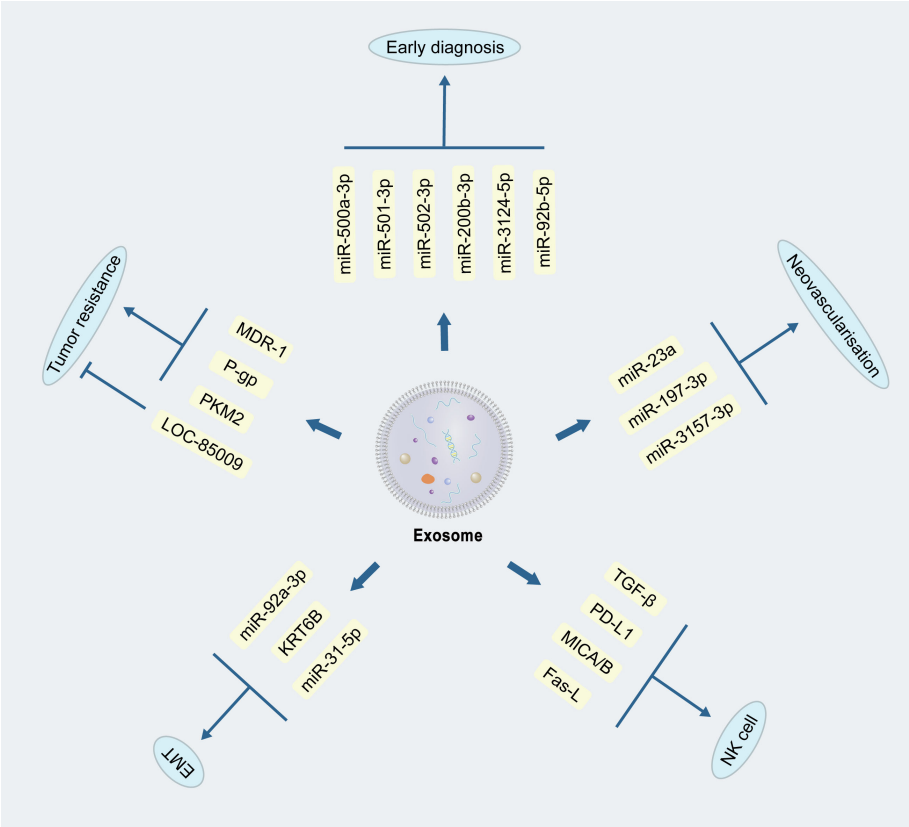


FIGURE 2
Mechanisms of exosomes in the development of lung cancer. Exosomes can promote or inhibit lung cancer development through different bioactive substances, as reflected in tumour drug resistance, epithelial-mesenchymal cell transformation, neovascularization, immunomodulation, and early diagnosis.

3 Discussion

Exosomes act as a carrier of information passed from cell to cell, altering the responsiveness of the immune system and the microenvironment. Understanding how the immune system reads the information in exosomes and how exosomes affect immune cells is important for the development of new strategies that will allow immune cells to behave effectively. For example, exosomes secreted by lung cancer cells contain miR-21 and miR-29a, which can be taken up by surrounding tumor-associated macrophages (TAMs) to influence cytokine secretion (58), which regulate tumor growth and metastasis. Exosome secretion has been found to be pH-sensitive, with the pH of the tumor microenvironment affecting both exosome secretion and the contents (59). The acidic environment in the tumor microenvironment tends to lead to the death of immune cells. This is why some researches have proposed that neutralizing the acidic environment of the TME using proton pump inhibitors or other buffering therapies can reduce the immune escape of tumor (60), and these may be the directions in the future to produce an anti-tumor therapy by restricting the function of tumor exosomes. Exosomes may be a potential treatment for tumors as well. While drug therapy can lead to drug resistance and cell therapy can lead to the risk of so-called "cytokine storm" in the body, exosomes may be able to circumvent these side effects. In addition, exosomes have been shown to cross the blood-brain and blood-testis barriers, a vital property in the context of cancer cells, since these cells can evade elimination by immune cells through various immune escape mechanisms. Nevertheless, exosomes have the potential to bypass such mechanisms and still target and kill cancer cells effectively.

One of the problems in the development of exosome-related tools is that the content is variable and complex. On the other hand, the development of engineered exosomes allows researchers to use exosomes as carriers, in which specific content is loaded. This technology may be one of the future drug delivery strategies for targeting tumor cells, and good results have been detected in lab. Still, more research is needed.

Apart from the deepened understanding of exosomes in lung cancer in recent years, there is still much limitation in the clinical application of exosome-related knowledge and techniques. First, the difference in the delivery mechanisms of exosomes from various cancer cells as compared to normal cells are not clearly investigated. Unveiling the factors influencing category and amount of cargo within the exosomes will remarkably help us gain a better picture of cancer biology. Secondly, one should keep in mind that the distribution and concentration of exosomes is not even across different systems. For example, blood-brain barrier has a significant impact on exosome biology and induces a divergence between those in circulation and in cerebrospinal fluids (61, 62). Does alveolar-capillary barrier has

similar effect should be depicted in the future, as this will provide valuable clue to the development of better laboratory methods with more accuracy. Third, a dynamic observation of exosomes from sputum, blood, and within the tumor in response to therapies is still poorly conducted in detail. A comparison and integration of these information can shed light on cancer biology and therapeutic targets.

People first discovered exosomes in the culture fluid of reticulocytes, which was then thought to be nothing more than a garbage truck for cells to remove waste. Decades later, exosomes once again attract much attention with a new face. With the deepening of research, it is found that exosomes have rich biological functions, which can regulate the growth of the cells, signaling. Then, researchers found that exosomes have specificity, and some of their special components can be used as markers for identifying exosomes. (fig4, Progress in the understanding of exosome research, three stages). Researches also found that the contents in exosomes are related to the cell state and cell origin, which also laid the foundation for exosome-based therapeutics and diagnostics. In recent years, the proposal of engineered exosomes has expanded the applications of exosomes, allowing researchers to modify the surface of exosomes to make them targeted or to evade recognition by other cells. The contents of exosomes can also be customized to carry specific drugs or miRNAs. In short, with the deepening of research, the functional development of exosomes will be more complete.

4 Limitation

Apart from the deepened understanding of exosomes in lung cancer in recent years, there is still much limitation in the clinical application of exosome-related knowledge and techniques. First, the difference in the delivery mechanisms of exosomes from various cancer cells as compared to normal cells are not clearly investigated. Unveiling the factors influencing category and amount of cargo within the exosomes will remarkably help us gain a better picture of cancer biology. Secondly, one should keep in mind that the distribution and concentration of exosomes is not even across different systems. For example, blood-brain barrier has a significant impact on exosome biology and induces a divergence between those in circulation and in cerebrospinal fluids (61, 62). Does alveolar-capillary barrier has similar effect should be depicted in the future, as this will provide valuable clue to the development of better laboratory methods with more accuracy. Third, a dynamic observation of exosomes from sputum, blood, and within the tumor in response to therapies is still poorly conducted in detail. A comparison and integration of these information can shed light on cancer biology and therapeutic targets.

5 Conclusion

Exosomes of lung cancer origin can influence the metastasis and development of lung cancer through multiple mechanisms, while exosomes secreted by immune cells can also influence the progression of lung cancer. Exosomes have the potential to be a complementary means of early diagnostic tool for lung cancer, but more exploration is still needed.

Author contributions

JZ: Writing – original draft, Conceptualization, Methodology. XL: Writing – original draft, Writing – review & editing, Data curation. LL: Writing – original draft, Data curation. ZZ: Writing – review & editing, Methodology. CH: Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

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. Gupta SC, Kunnumakkara AB, Aggarwal S, Aggarwal BB. Inflammation, a double-edge sword for cancer and other age-related diseases. *Front Immunol* (2018) 9:2160. doi: 10.3389/fimmu.2018.02160

2. Pituskin E, Joy AA, Fairchild A. Advanced cancer as a chronic disease: introduction. *Semin Oncol Nurs* (2021) 37:151176. doi: 10.1016/j.soncn.2021.151176

3. Nasim F, Sabath BF, Eapen GA. Lung cancer. *Med Clin North Am* (2019) 103:463–73. doi: 10.1016/j.mcna.2018.12.006

4. Siegel RL, Miller KD, Jemal A. Cancer statistics 2020. *CA Cancer J Clin* (2020) 70:7–30. doi: 10.3322/caac.21590

5. Jeon DS, Kim HC, Kim SH, Kim TJ, Kim HK, Moon MH, et al. Five-year overall survival and prognostic factors in patients with lung cancer: results from the Korean association of lung cancer registry (KALC-R) 2015. *Cancer Res Treat* (2023) 55:103–11. doi: 10.4143/crt.2022.264

6. Sahoo S, Adamiak M, Mathiyalagan P, Kenneweg F, Kafert-Kasting S, Thum T. Therapeutic and diagnostic translation of extracellular vesicles in cardiovascular diseases: roadmap to the clinic. *Circulation* (2021) 143:1426–49. doi: 10.1161/CIRCULATIONAHA.120.049254

7. Kalluri R, Lebleu VS. The biology, function, and biomedical applications of exosomes. *Science* (2020) 367(6478). doi: 10.1126/science.aau6977

8. Feng X, Peng Z, Yuan L, Jin M, Hu H, Peng X, et al. Research progress of exosomes in pathogenesis, diagnosis, and treatment of ocular diseases. *Front Bioeng Biotechnol* (2023) 11:1100310. doi: 10.3389/fbioe.2023.1100310

9. Yang D, Zhang W, Zhang H, Zhang F, Chen L, Ma L, et al. Progress, opportunity, and perspective on exosome isolation - efforts for efficient exosome-based theranostics. *Theranostics* (2020) 10:3684–707. doi: 10.7150/thno.41580

10. Mathieu M, Martin-Jaular L, Lavieu G, Thery C. Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell-to-cell communication. *Nat Cell Biol* (2019) 21:9–17. doi: 10.1038/s41556-018-0250-9

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

12. Couch Y, Buzas EI, Di Vizio D, Gho YS, Harrison P, Hill AF, et al. A brief history of nearly EV-erything - The rise and rise of extracellular vesicles. *J Extracell Vesicles* (2021) 10:e12144. doi: 10.1002/jev2.12144

13. Yu W, Hurley J, Roberts D, Chakrabortty SK, Enderle D, Noerholm M, et al. Exosome-based liquid biopsies in cancer: opportunities and challenges. *Ann Oncol* (2021) 32:466–77. doi: 10.1016/j.annonc.2021.01.074

14. Weng Z, Zhang B, Wu C, Yu F, Han B, Li B, et al. Therapeutic roles of mesenchymal stem cell-derived extracellular vesicles in cancer. *J Hematol Oncol* (2021) 14:136. doi: 10.1186/s13045-021-01141-y

15. Romano E, Netti PA, Torino E. Exosomes in gliomas: biogenesis, isolation, and preliminary applications in nanomedicine. *Pharm (Basel)* (2020) 13(10):319. doi: 10.3390/ph13100319

16. Zhu L, Sun HT, Wang S, Huang SL, Zheng Y, Wang CQ, et al. Isolation and characterization of exosomes for cancer research. *J Hematol Oncol* (2020) 13:152. doi: 10.1186/s13045-020-00987-y

17. Li MY, Liu LZ, Dong M. Progress on pivotal role and application of exosome in lung cancer carcinogenesis, diagnosis, therapy and prognosis. *Mol Cancer* (2021) 20:22. doi: 10.1186/s12943-021-01312-y

18. Zhang J, JI C, Zhang H, Shi H, Mao F, Qian H, et al. Engineered neutrophil-derived exosome-like vesicles for targeted cancer therapy. *Sci Adv* (2022) 8:eabj8207. doi: 10.1126/sciadv.abj8207

19. Vasan N, Baselga J, Hyman DM. A view on drug resistance in cancer. *Nature* (2019) 575:299–309. doi: 10.1038/s41586-019-1730-1

20. Li I, Nabet BY. Exosomes in the tumor microenvironment as mediators of cancer therapy resistance. *Mol Cancer* (2019) 18:32. doi: 10.1186/s12943-019-0975-5

21. Wang D, Zhao C, Xu F, Zhang A, Jin M, Zhang K, et al. Cisplatin-resistant NSCLC cells induced by hypoxia transmit resistance to sensitive cells through exosomal PKM2. *Theranostics* (2021) 11:2860–75. doi: 10.7150/thno.51797

22. Yu Z, Tang H, Chen S, Xie Y, Shi L, Xia S, et al. Exosomal LOC85009 inhibits docetaxel resistance in lung adenocarcinoma through regulating ATG5-induced autophagy. *Drug Resist Update* (2023) 67:100915. doi: 10.1016/j.drup.2022.100915

23. Wei C, Yang C, Wang S, Shi D, Zhang C, Lin X, et al. Crosstalk between cancer cells and tumor associated macrophages is required for mesenchymal circulating tumor cell-mediated colorectal cancer metastasis. *Mol Cancer* (2019) 18:64. doi: 10.1186/s12943-019-0976-4

24. Pastushenko I, Blanpain C. EMT transition states during tumor progression and metastasis. *Trends Cell Biol* (2019) 29:212–26. doi: 10.1016/j.tcb.2018.12.001

25. Hu JL, Wang W, Lan XL, Zeng ZC, Liang YS, Yan YR, et al. CAFs secreted exosomes promote metastasis and chemotherapy resistance by enhancing cell stemness and epithelial-mesenchymal transition in colorectal cancer. *Mol Cancer* (2019) 18:91. doi: 10.1186/s12943-019-1019-x

26. Song Q, Yu H, Cheng Y, Han J, Li K, Zhuang J, et al. Bladder cancer-derived exosomal KRT6B promotes invasion and metastasis by inducing EMT and regulating the immune microenvironment. *J Transl Med* (2022) 20:308. doi: 10.1186/s12967-022-03508-2

27. Wang L, Yang G, Zhao D, Wang J, Bai Y, Peng Q, et al. CD103-positive CSC exosome promotes EMT of clear cell renal cell carcinoma: role of remote MiR-19b-3p. *Mol Cancer* (2019) 18:86. doi: 10.1186/s12943-019-0997-z

28. Lang Z, Li Y, Lin L, Li X, Tao Q, Hu Y, et al. Hepatocyte-derived exosomal miR-146a-5p inhibits hepatic stellate cell EMT process: a crosstalk between hepatocytes and hepatic stellate cells. *Cell Death Discov* (2023) 9:304. doi: 10.1038/s41420-023-01602-y

29. Jafari N, Kolla M, Meshulam T, Shafran JS, Qiu Y, Casey AN, et al. Adipocyte-derived exosomes may promote breast cancer progression in type 2 diabetes. *Sci Signal* (2021) 14:eabj2807. doi: 10.1126/scisignal.abj2807

30. Zhang X, Sai B, Wang F, Wang L, Wang Y, Zheng L, et al. Hypoxic BMSC-derived exosomal miRNAs promote metastasis of lung cancer cells via STAT3-induced EMT. *Mol Cancer* (2019) 18:40. doi: 10.1186/s12943-019-0959-5

31. Yu F, Liang M, Huang Y, Wu W, Zheng B, Chen C. Hypoxic tumor-derived exosomal miR-31-5p promotes lung adenocarcinoma metastasis by negatively regulating SATB2-reversed EMT and activating MEK/ERK signaling. *J Exp Clin Cancer Res* (2021) 40:179. doi: 10.1186/s13046-021-01979-7

32. Bai X, Shao J, Duan T, Liu X, Wang M, Li X, et al. Exo-miR-1290-induced by COX-2 overexpression promotes cancer-associated fibroblasts activation and tumor progression by CUL3-Nrf2 pathway in lung adenocarcinoma. *Cell Commun Signal* (2023) 21:242. doi: 10.1186/s12964-023-01268-0

33. Altorki NK, Markowitz GJ, Gao D, Port JL, Saxena A, Stiles B, et al. The lung microenvironment: an important regulator of tumour growth and metastasis. *Nat Rev Cancer* (2019) 19:9–31. doi: 10.1038/s41568-018-0081-9

34. Tan HW, Xu YM, Qin SH, Chen GF, Lau ATY. Epigenetic regulation of angiogenesis in lung cancer. *J Cell Physiol* (2021) 236:3194–206. doi: 10.1002/jcp.30104

35. Fang H, Sun Q, Zhou J, Zhang H, Song Q, Zhang H, et al. m(6)A methylation reader IGF2BP2 activates endothelial cells to promote angiogenesis and metastasis of lung adenocarcinoma. *Mol Cancer* (2023) 22:99. doi: 10.1186/s12943-023-01791-1

36. Herbst RS, Morgensztern D, Boshoff C. The biology and management of non-small cell lung cancer. *Nature* (2018) 553:446–54. doi: 10.1038/nature25183

37. Xie M, Liu M, He CS. SIRT1 regulates endothelial Notch signaling in lung cancer. *PloS One* (2012) 7:e45331. doi: 10.1371/journal.pone.0045331

38. Dimitrova N, Gocheva V, Bhutkar A, Resnick R, Jong RM, Miller KM, et al. Stromal expression of miR-143/145 promotes neoangiogenesis in lung cancer development. *Cancer Discov* (2016) 6:188–201. doi: 10.1158/2159-8290.CD-15-0854

39. Hsu YL, Hung JY, Chang WA, Lin YS, Pan YC, Tsai PH, et al. Hypoxic lung cancer-secreted exosomal miR-23a increased angiogenesis and vascular permeability by targeting prolyl hydroxylase and tight junction protein ZO-1. *Oncogene* (2017) 36:4929–42. doi: 10.1038/onc.2017.105

40. Chang RM, Fu Y, Zeng J, Zhu XY, Gao Y. Cancer-derived exosomal miR-197-3p confers angiogenesis via targeting TIMP2/3 in lung adenocarcinoma metastasis. *Cell Death Dis* (2022) 13:1032. doi: 10.1038/s41419-022-05420-5

41. Ma Z, Wei K, Yang F, Guo Z, Pan C, He Y, et al. Tumor-derived exosomal miR-3157-3p promotes angiogenesis, vascular permeability and metastasis by targeting TIMP/KLF2 in non-small cell lung cancer. *Cell Death Dis* (2021) 12:840. doi: 10.1038/s41419-021-04037-4

42. Bland CL, Byrne-Hoffman CN, Fernandez A, Rellick SL, Deng W, Klinke D. J., 2ND. Exosomes derived from B16F0 melanoma cells alter the transcriptome of cytotoxic T cells that impacts mitochondrial respiration. *FEBS J* (2018) 285:1033–50. doi: 10.1111/febs.14396

43. Terren I, Orrantia A, Vitalle J, Zenarruzabeitia O, Borrego F. NK cell metabolism and tumor microenvironment. *Front Immunol* (2019) 10:2278. doi: 10.3389/fimmu.2019.02278

44. Liu J, Fan L, Yu H, Zhang J, He Y, Feng D, et al. Endoplasmic reticulum stress causes liver cancer cells to release exosomal miR-23a-3p and up-regulate programmed death ligand 1 expression in macrophages. *Hepatology* (2019) 70:241–58. doi: 10.1002/hep.30607

45. Luo C, Xin H, Zhou Z, Hu Z, Sun R, Yao N, et al. Tumor-derived exosomes induce immunosuppressive macrophages to foster intrahepatic cholangiocarcinoma progression. *Hepatology* (2022) 76:982–99. doi: 10.1002/hep.32387

46. Poggio M, Hu T, Pai CC, Chu B, Belair CD, Chang A, et al. Suppression of exosomal PD-L1 induces systemic anti-tumor immunity and memory. *Cell* (2019) 177:414–427.e13. doi: 10.1016/j.cell.2019.02.016

47. Dosil SG, Lopez-Cobo S, Rodriguez-Galan A, Fernandez-Delgado I, ramirez-huesca M, Milan-Rois P, et al. Natural killer (NK) cell-derived extracellular-vesicle shuttled microRNAs control T cell responses. *Elife* (2022) 11:e76319. doi: 10.7554/eLife.76319.sa2

48. Lugini L, Cecchetti S, Huber V, Luciani F, Macchia G, Spadaro F, et al. Immune surveillance properties of human NK cell-derived exosomes. *J Immunol* (2012) 189:2833–42. doi: 10.4049/jimmunol.1101988

49. Gunassekaran GR, Poongkavithai Vadevoo SM, Baek MC, Lee B. M1 macrophage exosomes engineered to foster M1 polarization and target the IL-4 receptor inhibit tumor growth by reprogramming tumor-associated macrophages into M1-like macrophages. *Biomaterials* (2021) 278:121137. doi: 10.1016/j.biomaterials.2021.121137

50. Khan FH, Reza MJ, Shao YF, Perwez A, Zahra H, Dowlati A, et al. Role of exosomes in lung cancer: A comprehensive insight from immunomodulation to theragnostic applications. *Biochim Biophys Acta Rev Cancer* (2022) 1877:188776. doi: 10.1016/j.bbcan.2022.188776

51. Liu J, Ren L, Li S, Li W, Zheng X, Yang Y, et al. The biology, function, and applications of exosomes in cancer. *Acta Pharm Sin B* (2021) 11:2783–97. doi: 10.1016/j.apsb.2021.01.001

52. Chang L, Li J, Zhang R. Liquid biopsy for early diagnosis of non-small cell lung carcinoma: recent research and detection technologies. *Biochim Biophys Acta Rev Cancer* (2022) 1877:188729. doi: 10.1016/j.bbcan.2022.188729

53. Zhang JT, Qin H, Man Cheung FK, Su J, Zhang DD, Liu SY, et al. Plasma extracellular vesicle microRNAs for pulmonary ground-glass nodules. *J Extracell Vesicles* (2019) 8:1663666. doi: 10.1080/20013078.2019.1663666

54. Zheng D, Zhu Y, Zhang J, Zhang W, Wang H, Chen H, et al. Identification and evaluation of circulating small extracellular vesicle microRNAs as diagnostic biomarkers for patients with indeterminate pulmonary nodules. *J Nanobiotechnol* (2022) 20:172. doi: 10.1186/s12951-022-01366-0

55. Shin H, Oh S, Hong S, Kang M, Kang D, Ji YG, et al. Early-stage lung cancer diagnosis by deep learning-based spectroscopic analysis of circulating exosomes. *ACS Nano* (2020) 14:5435–44. doi: 10.1021/acsnano.9b09119

56. Kim DH, Park H, Choi YJ, Im K, Lee CW, Kim DS, et al. Identification of exosomal microRNA panel as diagnostic and prognostic biomarker for small cell lung cancer. *biomark Res* (2023) 11:80. doi: 10.1186/s40364-023-00517-1

57. Cao B, Wang P, Gu L, Liu J. Use of four genes in exosomes as biomarkers for the identification of lung adenocarcinoma and lung squamous cell carcinoma. *Oncol Lett* (2021) 21:249. doi: 10.3892/ol.2021.12510

58. Fabbri M, Paone A, Calore F, Galli R, Gaudio E, Santhanam R, et al. MicroRNAs bind to Toll-like receptors to induce prometastatic inflammatory response. *Proc Natl Acad Sci USA* (2012) 109:E2110–6. doi: 10.1073/pnas.1209414109

59. Logozzi M, Mizzoni D, Angelini DF, Di Raimo R, Falchi M, Battistini L, et al. Microenvironmental pH and exosome levels interplay in human cancer cell lines of different histotypes. *Cancers (Basel)* (2018) 10(10):370. doi: 10.3390/cancers10100370

60. Gillies RJ, Pilot C, Marunaka Y, Fais S. Targeting acidity in cancer and diabetes. *Biochim Biophys Acta Rev Cancer* (2019) 1871:273–80. doi: 10.1016/j.bbcan.2019.01.003

61. Ramos-Zaldivar HM, Polakovicova I, Salas-Huenuleo E, Corvalan AH, Kogan MJ, Yefi CP, et al. Extracellular vesicles through the blood-brain barrier: a review. *Fluids Barriers CNS* (2022) 19:60. doi: 10.1186/s12987-022-00359-3

62. Mardi N, Salahpour-Anarjan F, Nemati M, Shahsavari Baher N, Rahbarghazi R, Zarebkohan A. Exosomes; multifaceted nanoplatform for targeting brain cancers. *Cancer Lett* (2023) 557:216077. doi: 10.1016/j.canlet.2023.216077

frontiers | Frontiers in Immunology

TYPE Review
PUBLISHED 03 January 2024
DOI 10.3389/fimmu.2023.1328094

OPEN ACCESS

EDITED BY
Zhiwen Luo,
Fudan University, China

REVIEWED BY
Yuxuan Song,
Peking University People's Hospital, China
Yong Zhang,
Fujian Medical University, China
Yudong Cao,
Central South University, China

*CORRESPONDENCE
Hao Chi
Chihao7511@gmail.com
Qibiao Wu
qbwu@must.edu.mo
Guanhu Yang
guanhuyang@gmail.com

†These authors have contributed equally to this work

RECEIVED 26 October 2023
ACCEPTED 11 December 2023
PUBLISHED 03 January 2024

CITATION
He S, Su L, Hu H, Liu H, Xiong J, Gong X, Chi H, Wu Q and Yang G (2024) Immunoregulatory functions and therapeutic potential of natural killer cell-derived extracellular vesicles in chronic diseases. *Front. Immunol.* 14:1328094. doi: 10.3389/fimmu.2023.1328094

Immunoregulatory functions and therapeutic potential of natural killer cell-derived extracellular vesicles in chronic diseases

Shuang He[1,2†], Lanqian Su[2†], Haiyang Hu[2], Haiqi Liu[2], Jingwen Xiong[3], Xiangjin Gong[3], Hao Chi[2*], Qibiao Wu[1*] and Guanhu Yang[4*]

[1]Faculty of Chinese Medicine, and State Key Laboratory of Quality Research in Chinese Medicine, Macau University of Science and Technology, Macao, Macao SAR, China, [2]Clinical Medical College, Southwest Medical University, Luzhou, China, [3]Department of Sports Rehabilitation, Southwest Medical University, Luzhou, China, [4]Department of Specialty Medicine, Ohio University, Athens, OH, United States

Extracellular vesicles (EVs) have been proven to play a significant immunoregulatory role in many chronic diseases, such as cancer and immune disorders. Among them, EVs derived from NK cells are an essential component of the immune cell functions. These EVs have been demonstrated to carry a variety of toxic proteins and nucleic acids derived from NK cells and play a therapeutic role in diseases like malignancies, liver fibrosis, and lung injury. However, natural NK-derived EVs (NKEVs) have certain limitations in disease treatment, such as low yield and poor targeting. Concurrently, NK cells exhibit characteristics of memory-like NK cells, which have stronger proliferative capacity, increased IFN-γ production, and enhanced cytotoxicity, making them more advantageous for disease treatment. Recent research has shifted its focus towards engineered extracellular vesicles and their potential to improve the efficiency, specificity, and safety of disease treatments. In this review, we will discuss the characteristics of NK-derived EVs and the latest advancements in disease therapy. Specifically, we will compare different cellular sources of NKEVs and explore the current status and prospects of memory-like NK cell-derived EVs and engineered NKEVs.

KEYWORDS

NK cell, NK cell-derived exosome, engineered EVs, chronic disease, microvesicles, therapy, surface modification

1 Introduction

Extracellular vesicles are vesicles enclosed by phospholipid bilayers secreted by all cell types, so they can be found in tissue culture supernatants and biological fluids, such as blood, saliva, breast milk, cerebrospinal fluid, and malignant ascites (1). Based on the different formation processes, EVs were divided into three groups: exosomes, microvesicles (microparticles) and apoptotic bodies. Among them, exosomes have received more attention. They are EVs with a size range of 50~200nm (2). They bud inward from the limiting membrane of endosomes to form multivesicular bodies (MVBs). Subsequently, MVBs fuse with the plasma membrane to release exosomes into the extracellular space. The surface of exosomes is enriched with tetraester proteins, such as CD63, CD81 and CD9. Although exosomes have no final and specific surface markers, they are a combination of expression markers and lack of specific intracellular protein expression, meeting the minimum requirements of the current exosome definition. Due to the overlap size between the three populations, surface markers, and the lack of proteins restricted to specific populations,it has been a challenge to distinguish exosomes from microvesicles (3); At present, researchers collectively refer to three different types of vesicles as EVs.

Natural killer (NK) cells are natural lymphocytes that fight infection and kill tumor cells, mainly in the peripheral blood, bone marrow, lymph nodes, and spleen (4). Based on the recognition of activating or inhibitory receptors and stress-induced ligands, NK cells not only enhance cytokine production and cell killing, but also provide immune self-tolerance and negative feedback mechanisms, and perform the three major functions of immune surveillance, immune response, and immune memory (5, 6).

Another mechanism of NK cell involves rapidly killing target cells through the slow Fas-FasL-dependent pathway, or via the utilization of intracellular lytic granules releasing proteins such as granzymes and perforin (7). The formidable antineoplastic potential of NK cells has been effectively leveraged in numerous clinical trials, employing autologous or allogeneic NK cells, as well as chimeric antigen receptor (CAR)-modified NK cells, in the concerted effort to combat hematologic malignancies (8–10). Nonetheless, the therapeutic application of NK cells in the context of solid tumors poses a more intricate challenge, primarily attributed to their constrained capacity for infiltrating neoplastic tissues (11, 12). Considering the unique biocompatibility and higher penetration ability of EVs, NK cell-derived EVs may be the key to overcoming this challenge.

In the burgeoning domain of targeted drug delivery, the realm of nanotechnology has emerged as a pivotal contributor, notably through the advancement of intelligent carriers. Among these carriers, systems predicated on EVs have garnered considerable and pervasive attention (12). EVs are crucial mediators in many physiological processes, and EVs derived from NK cells can inherit bioactive molecules and some membrane proteins from parent cells, playing a role in immune surveillance and cytotoxicity. They also serve as carrier systems that effectively target solid tumor cells, playing a significant role in the treatment of cancer, metabolic, and neurodegenerative diseases (13, 14). However, achieving efficient and precise drug delivery for specific applications of NKEVs presents significant challenges. Engineering of isolated NKEVs through genetic engineering or chemical modifications can effectively enhance their targeting ability, homing and chemotaxis, as well as their immunomodulatory and anti-tumor capabilities. Although NK cell-derived extracellular vesicles have not yet entered clinical trials, they have become an important research focus (14).

2 Basic biology of NK cell-derived extracellular vesicles

2.1 Components and mechanisms of action

2.1.1 Cytotoxic proteins

Enriched cytotoxic proteins from NK cell sources are a typical characteristic of NKEVs and a key mechanism by which NKEVs exert cytotoxic effects. These proteins mainly include perforin (PFN), granzyme A (GzmA), granzyme B (GzmB), and granulysin (GNLY) (Figure 1).

Perforin is a pore-forming protein that can create pores on the endosome membrane, releasing granzyme B into the target cell, subsequently inducing target cell death through apoptosis (15). The levels of perforin in NKEVs are exceptionally high, several times to several tens of times higher than other cytotoxic proteins (16).

GzmA is a serine protease that induces cysteine-dependent apoptosis (17). Upon entering the nucleus of target cells, GzmA cleaves the SET complex, shifting it from DNA repair to DNA damage (18). As integral constituents of the SET complex, SET and HMG2 proteins assume the role of substrates for GzmA in the context of programmed cell death. Evidently, the degradation of SET and HMG2B becomes apparent in CHLA255 and SupB15 cells upon treatment with NKEVs (16). Additionally, GzmA orchestrates the activation of a pathway intricately linked to mitochondrial stress. Upon internalization into target cells, GzmA instigates oxidative stress reactions, instigating mitochondrial depolarization and a surge in reactive oxygen species (ROS). This cascade, in turn, facilitates the aggregation of the SET complex within the cellular nucleus (19, 20). Consequently, GzmA elicits a distinctive and parallel cell death pathway, operating autonomously of caspases.

GzmB is possibly the most active member of the granzyme family. After entering the cytoplasm of target cells, GzmB activates initiator caspases, caspase-8 and caspase-10, following two pathways to promote cell apoptosis (17). One is directly processing of caspase-3, 7 by GzmB, thus promoting cell apoptosis. The other pathway is related to mitochondrial-associated caspase cascades. GzmB promotes the release of cytochrome C (cyt c) from the mitochondrial membrane interstitial space into the cytoplasm to bind to caspase- 9 and form apoptotic vesicles by truncating BID, which indirectly promotes the activation of caspase-3, 7 (16, 21, 22). Wu et al. found that NK-EVs can induce the release of cytochrome c from neuroblastoma cells, confirming the mechanism by which GzmB in NKEVs exerts its cytotoxicity. GzmB and GNLY may also induce

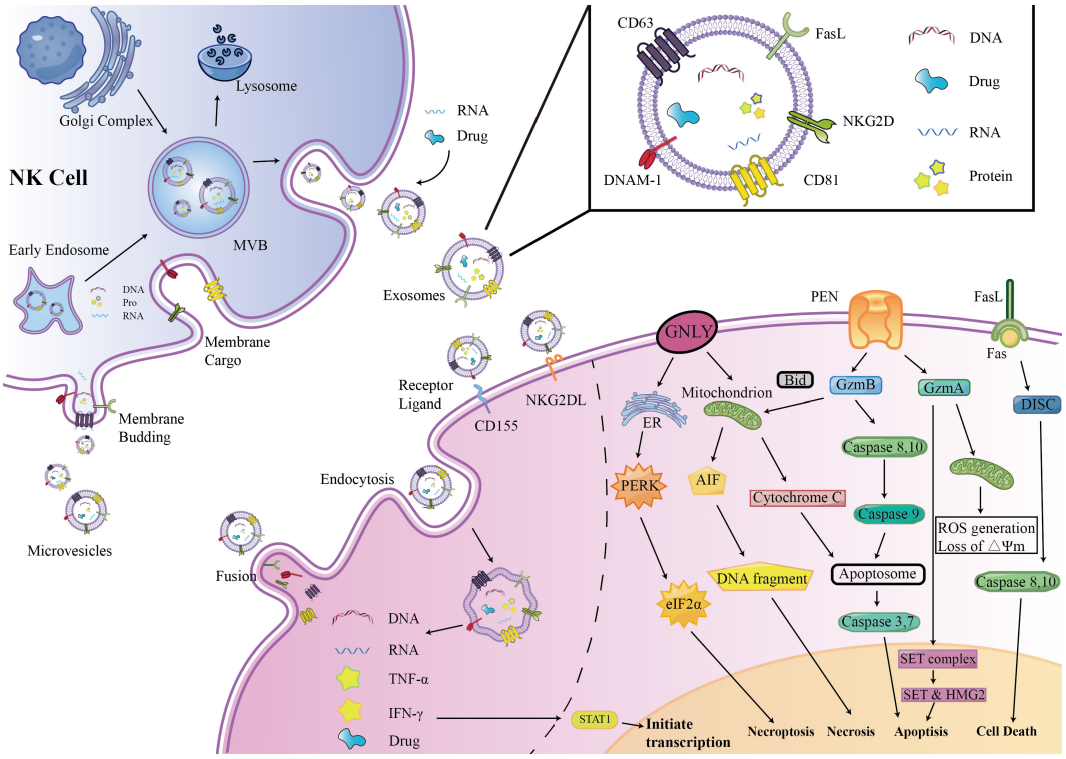


FIGURE 1
NKEVs biogenesis, secretion, content, uptake mechanism and Cytotoxic effects. EVs were divided into three groups: exosomes, microvesicles (microparticles) and apoptotic bodies. NK cell-derived EVs express activation receptors eg. NKG2D and DNAM1, and express molecules involved in cytotoxicity, e.g., perforin-granzyme mediated fusion, receptor–ligand mediated reaction, granulysin mediated electrostatic interaction.

cell death in target cells through endoplasmic reticulum stress via NK-EVs (16).

FasL is a type II transmembrane protein of the tumor necrosis factor superfamily, which interacts with receptors on the target cell membrane, such as Fas or CD95, leading to receptor trimerization. Subsequently, a death-inducing signaling complex (DISC) is formed, recruiting and activating caspase-8,10 proenzymes, promoting cell apoptosis through two pathways (23, 24). In one pathway, a large amount of caspase-8 proenzymes is recruited, activating the caspase-3 and caspase-7 pathways. In the other pathway, a small amount of caspase-8 cleaves BID, activating the mitochondria-related apoptosis pathway (25, 26).

The involvement of FasL in the cytotoxicity exerted by NKEVs has engendered substantial discourse within the scientific community. Divergent perspectives posit potential mechanisms, with one school of thought implicating classical receptor-ligand interactions facilitated by FasL-expressing NK92-cell-derived EVs. This notion gains empirical support as these vesicles demonstrate time- and dose-dependent cytotoxicity against melanoma (27) and hepatocellular carcinoma cells (28). Concurrently, an alternate mechanism is proposed involving the endocytic pathway, wherein target cells internalize NKEVs carrying soluble FasL. Notably, the enrichment of FasL in NK-EVsIL-15 has been identified, and both BLI and MTT assays corroborate that NK-EVIL-15-mediated cell death is, in part, associated with the presence of FasL (29). However, a counter perspective contends that FasL might not substantially contribute to cytotoxicity, as some studies suggest its content in EV preparations ranks lowest among cytotoxic proteins. This viewpoint gains further support from protein correlations and Fas antibody blocking experiments, which collectively imply that FasL might not play a decisive role in the observed cytotoxic effects (16, 30).

2.1.2 Cytokines

NKEV can inherit a series of cytokines produced by NK cells, such as interferon IFN-γ and TNF-α, and thus interact immunologically with other cells.

IFN-γ is a soluble dimeric cytokine and is a type II interferon with antiviral, antitumor, and immunomodulatory properties. Upon activation of IFN-γ receptor, a cascade is initiated wherein JAK1 and JAK2 undergo phosphorylation. The resultant phosphorylated STAT1 subsequently assembles into homodimers, translocating to the cell nucleus. In this nuclear milieu, these phosphorylated homodimers efficaciously exert their regulatory effects (31). Ample investigations have substantiated the presence of IFN-γ within EVs originating from NK cells. The consequential release of IFN-γ in close proximity to target cells by NKEVs has been demonstrated to curtail the proliferation and migration of endothelial cells, as supported by diverse studies (32, 33).

TNF-α is an inflammatory cytokine that plays a crucial role in immune regulation, cell proliferation, cell death, and morphogenesis through multiple signaling pathways (34). TNF-α has been found in EVs originating from diverse immune cells, including dendritic cells and macrophages (35, 36). Zhu et al. were

the first to discover TNF-α in NK cell-derived EVs and confirmed the relevance of TNF-α to the cytotoxicity mediated by NKEVs in melanoma cells (27).

2.1.3 Activating receptors

NKG2D, a type II transmembrane C-type lectin-like activating receptor, assumes significance as it forms homodimers and finds ubiquitous expression not only on NK cells but also on $CD8^+$ T cells and a limited subset of $CD4^+$ T cells (27). Research has shown that NKG2D is highly expressed on the membrane of NKEVs and is frequently used as a marker for EVs derived from NK cells (32). While the specific role of NKG2D on NKEVs is not yet clear, it has been confirmed that co-culturing cancer cells with NKEVs results in a significant decrease in apoptosis when anti-NKG2D antibodies is used (30).

DNAM-1, or CD226, represents a natural cytotoxicity receptor with broad expression encompassing T cells and a majority of quiescent NK cells. This receptor assumes a pivotal role in governing NK cell adhesion, cytotoxicity, and the facilitation of immune synapse formation (37, 38). Pace et al. blocked DNAM1 on the EV surface to inhibit the cytotoxic effects of NKEV; furthermore, DNAM1 may be ligand-bound to act through caspase-induced apoptosis (37, 39). However, in the apoptosis experiment using HCT116 spheroids, blocking DNAM-1 did not affect the apoptosis of recipient cells, possibly due to variations in ligand types and quantities on different target cells (30).

2.2 NKEVs regulate immune cells

In addition to cytotoxicity, NKEVs also exert immunomodulatory effects.The activation, inhibition of various immune cells and immune related modulators are directly or indirectly regulated by NKEV. Federici et al. observed that NKEVs exhibit the capacity to directly activate T cells. Moreover, these vesicles demonstrate the dual capability of inducing T cell proliferation, achieved either through direct stimulation or indirectly by elevating the expression of co-stimulatory molecules on monocytes (40). Jia et al. confirmed that NK-derived EVs can promote M1 polarization of macrophages, inhibit M2 polarization, thereby reducing bacterial load in mouse lung tissue to mitigate *Pseudomonas aeruginosa*-induced lung injury, and also decrease the percentage of neutrophils and lymphocytes in mouse lung tissue (41). Furthermore, the influence of NKEVs extends to both direct and indirect modulation of the function and activity of parent cells. Notably, an augmentation in the proportion of $CD56^+$ NK cells is observed as an exemplar of the impact exerted by NKEVs (40). Not only does the number of NK cell subtypes change, but also their cytotoxicity and content are affected. NK cells subjected to treatment with NKEVs manifest not only a marked augmentation in cytotoxicity but also a concurrent elevation in the release of tumor necrosis factor (TNF) and perforin (42). Even more interestingly, various cytokine genes involved in regulating NK cell proliferation, cytotoxicity, and migration, such as the ligands for the chemokine receptor CXCR3, including CXCL9, CXCL10, and CXCL11, are significantly upregulated (42).

Furthermore, through some specific treatments of NKEVs, their immunomodulatory abilities can be enhanced. In a recent investigation, hydrophilic siRNA and hydrophobic photosensitizer Ce 6 were employed to modify NK-derived exosomes through light-activated silencing of NK (LASNEO). This innovative approach resulted in the induction of substantial photodynamic therapeutic effects, facilitated by the generation of reactive oxygen species (ROS) subsequent to laser irradiation. Notably, this intervention prompted the polarization of M1 tumor-associated macrophages and the maturation of dendritic cells within the tumor microenvironment (TME).Furthermore, the targeted application of siRNAs against PLK1 or PD-L1 elicited potent gene silencing in cancer cells. Intriguingly, the consequential downregulation of PD-L1 contributed to the restoration of immune surveillance by $CD4^+$ T cells and $CD8^+$ T cells within the TME. LASNEO displayed excellent anti-tumor effects by recruiting various types of immune cells (43).

2.3 EVs produced by memory-like NK cells

NK cells were previously believed to lack immune memory. However, increasing evidence suggests that NK cells can generate specific memory responses, acquiring "memory-like" functional characteristics, resulting in enhanced functional activity (44). Traditionally, the acquisition of memory-like traits by NK cells ensues in response to activation signals emanating from both target cells and the surrounding microenvironment. A case in point is the phenomenon observed in tissues, where resident decidual NK cells have the capacity to engender a distinctive and enriched NK cell subset during recurring pregnancies. This subset augmentation culminates in an enhanced production of IFN-γ and vascular endothelial growth factor (VEGF), thereby potentially contributing to improved placental development (45). Upon infection with mouse cytomegalovirus (MCMV),(NK cells in mice undergo a transition, acquiring adaptive immune features (46). Cultivating NK cells in the presence of artificial antigen-presenting cells (aAPC) and K562-mb IL-21 proves to be a transformative milieu, resulting in substantial expansion and activation of these cells. This orchestrated response is notably accompanied by a marked increase in the production of NK-EVs (47). Furthermore, EVs produced by memory-like NK cells exhibit greater toxicity towards cancer cells (48).

Furthermore, these memory-like NK cells can also produce EVs, enhancing their functional activity. EVs derived from NK cells previously exposed to neuroblastoma cells (NB), which express NCRs and activation signals, can activate resting human NK cells, enhancing their NK-mediated anti-NB tumor response (49). NK cells exposed in advance to EVs derived from CML cells exhibit higher gene expression levels of caspase 3 and P53 compared to the untreated EVs group, showing stronger cytotoxic effects on tumor cells (48).

Federici et al. proposed that there is no significant difference in the quantity and expression of surface markers on EVs produced by

NK cells in the resting and activated states (50). However, current research indicates that memory-like NKEVs produced in response to cytokine stimulation appear to exhibit more effective cytotoxicity and anti-tumor effects.

Cytokine activation plays a crucial role in conferring memory-like characteristics to NK cells (51, 52). Stimulating NK cells with homeostatic and/or pro-inflammatory cytokines such as IL-2 and IL-15 enhances their effector functions, promotes anti-tumor immunity, and increases their persistence in the body (53).

In the study conducted by Zhu et al., it was discerned that the stimulation of an equivalent number of NK cells with IL-15 elicited a remarkable more than twofold augmentation in the overall production of extracellular vesicles (EVs). This increase extended not only to the quantity of EVs but also encompassed heightened protein content and particle numbers within these vesicles. Notably, IL-15-treated NKEVs (IL-15-treated NK-EVs) demonstrated a more potent tumor-targeting effect and an extended circulation period. These characteristics collectively resulted in a significant inhibition of the growth of heterotransplanted glioblastoma cells in murine models. Furthermore, the application of IL-15 was observed to correlate with an upregulation in the expression of Rab27a in NK cells. This observation suggests that IL-15 potentially exerts control over the cellular trafficking of Rab27a-specific cargoes, implicating a regulatory role in this particular pathway (29).

Enomoto et al. obtained similar conclusions when stimulating NK cells with IL-15, and IL-15 in combination with IL-21-induced EVs demonstrated stronger cytotoxic activity, even though the cytotoxicity of NK-92 cells was not enhanced under co-stimulation. This may be due to the NK-92 cells and the EVs they produce having different protein and RNA profiles, such as the enrichment of co-induced miR-146b and miR-23a, and the presence of CD226 (DNAM-1). Additionally, GZMB and GZMH were also co-induced by IL-15 and IL-21 (39).

In one study, it was revealed that the efficiency of EV production exhibited a notable increase when NK cells were subjected to co-stimulation with cytokines IL-15, IL-12, and IL-18, in comparison to stimulation with IL-15 alone. This heightened efficiency in co-stimulated EVs translated into a pronounced proclivity for spheroid apoptosis, particularly evident in the context of WM 9, OVCAR-3, and SK-RB-3 spheroids. Intriguingly, the cell lines WM9 and SK-RB-3, known for their resistance to NK cell-mediated killing, exhibited vulnerability to apoptosis induced by these co-stimulated EVs. This observation suggests a compelling prospect: that NK cell-derived extracellular vesicles possess the capability to target cells autonomously, irrespective of their donor cell origin (30).

Furthermore, EVs produced by stimulating NK cells with IL-1β did not show a significant change in total protein content. However, the expression of perforin significantly increased, and there was a dose-dependent enhancement of EVs in their inhibitory effect on endothelial cell proliferation and migration (33).

Moreover, it was observed that EVs generated through the stimulation of NK cells with IL-1β did not manifest a substantial alteration in their overall protein content (Table 1). However, the expression of perforin significantly increased, and there was a dose-dependent enhancement of EVs in their inhibitory effect on endothelial cell proliferation and migration.

3 NK cell source

There are various options for the sources of NK cells used to generate EVs (Figure 2A). Currently, common sources for primary NK cells include peripheral blood-derived NK cells (PB-NK), cord blood-derived NK cells (CB-NK), and splenic NK cells from mice. In addition, immortalized NK cell lines, such as the NK-92 cell line and the NK3.3 cell line, are also commonly used. Recently, NK cells derived from induced pluripotent stem cells (iPSC) and highly efficient chimeric antigen receptor (CAR)-armed NK cells have demonstrated effective expansion capabilities, making them potential sources of EVs. NK cell-derived EVs from different sources exhibit distinct characteristics in terms of isolation and extraction, cytotoxicity, and safety (Figure 2C).

3.1 Primary NK cells

The number of NKEVs (NK cell-derived extracellular vesicles) in human blood is low, and there are many other cell-derived EVs, making isolation challenging (37, 50). Therefore, a preferable approach, compared to directly extracting NKEVs from blood, is to first obtain primary NK cells and then extract NKEVs. Human peripheral blood emerges as the most readily accessible source for procuring primary NK cells. Typically, single-nucleated cells are separated from the blood using density gradient centrifugation. NK cell populations are then obtained through flow cytometry (54). However, NK cells in peripheral blood are mature, and their viability decreases when frozen, making long-term storage difficult. Umbilical cord blood contains an abundant population of immature NK cells that are highly tolerant to freezing and exhibit good homing to the bone marrow (55). Furthermore, NK cells expanded from cord blood have higher proliferation capacity and lower cytotoxicity compared to NK cells obtained from peripheral blood (56–58). In a study by Luo et al., the successful isolation of cord blood mononuclear cells (CBMC) from cord blood was achieved through the implementation of density gradient centrifugation. Subsequently, NK cells were co-stimulated using IL-2 and irradiated K562-engineered cells. This tailored approach resulted in a noteworthy expansion of NK cells and facilitated the subsequent isolation of a substantial quantity of highly cytotoxic and high-quality NK-EVs (42). For commonly used laboratory animals like C57BL6 mice, obtaining blood samples is limited, and spleen tissue is often used as the source for NK cells. Spleen tissue is processed into a single-cell suspension through grinding, and then CD3- CD49b+ cell populations are obtained using flow cytometry to isolate NK cells (59).

TABLE 1 Extracellular vesicles produced by cytokine-activated memory-like NK cells.

Source of NK cells	Activation factor	Extraction method of Evs	Size Before using cytokines	Change in total number of Evs	Change in total amount of proteins	Functional changes	Effector molecules	Target cells	reference
NK-92MI	IL-15	Ultracentrifugation	106.9±21.6 nm (after use of IL-15118.2±20.3 nm)	More than twice as much as before	More than twice as much	Stronger anti-tumor activity *in vivo* and *ex vivo*, greater tumor targeting and longer circulation time *in vivo*	GZMB, perforin, and FasL were enriched; TRAIL, NKp30, and NKp44 increased	U87/MG, MDA-MB-231 and CAL-62	(29)
NK-92	IL-15	Ultracentrifugation	148.2 nm			Increase cytotoxicity	GZMB and GZMH slightly increased	K562, Jurkat, A549 and HeLa cells	(39)
	IL-21					No significant change in cytotoxicity			
	IL-15 and IL-21					Increase cytotoxicity	GZMB, GZMH, miR-146b and miR-23a enriched		
NK-92	IL-1β	Ultracentrifugation	210~490 nm		No increase	Inhibited endothelial cell migration, enhanced endothelial cell activation, and facilitated endothelial cell acquisition of pro-inflammatory and pro-coagulant phenotypes	Increased perforin and decreased granzyme B	EA.hy926 cell line endothelial cells	(33)
Primary NK cells	CD16	Test kit	60~125 nm (electron microscopy); 200 nm(particle size)			Weak cytotoxicity	Low levels of granzyme B and perforin	HCT116 colorectal cancer cells	(30)
	IL - 15			More than twice as much as CD16		Weaker than combined			
	IL-15, IL-12 and IL-18			More than IL-15 alone	2-fold higher than CD16-activated primary NK cells	Strongest cytotoxicity	Highest levels of granzyme B		
NK-92	IL-15			6-fold higher than CD16-activated primary NK cells	8-fold higher than CD16-activated primary NK cells	Stronger killing ability (weaker than primary NK)	High levels of granzyme A		
	IL-15, IL-12 and IL-18			7-fold higher than CD16-activated primary NK cells	4-fold higher than CD16-activated primary NK cells	Stronger killing ability (not as good asprimary NK)	Highest levels of granzyme A and high levels of granzyme B		
NKYG-1	IL-15			5-fold higher than CD16-activated primary NK cells	14-fold higher than CD16-activated primary NK cells	No cytotoxicity	Low levels of granzyme B and perforin		
	IL-15, IL-12 and IL-18			5-fold higher than CD16-activated primary NK cells	7-fold higher than CD16-activated primary NK cells				

10.3389/fimmu.2023.1328094

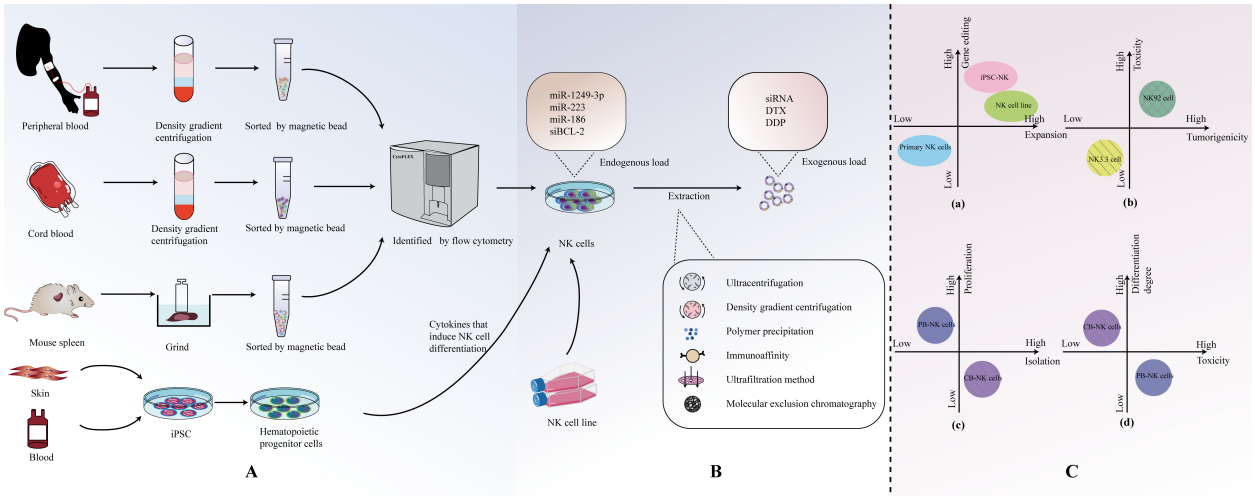


FIGURE 2
(A) Origin and isolation of NK cells. **(B)** Isolation of NKEVs. **(C)** Comparison of NK cell-derived EVS from different sources.

3.2 NK cell line

NK-92 is the most commonly used NK cell line. In comparison to the complex process of collecting NK cells from peripheral blood mononuclear cells (PBMCs) and activating them for seven days, NK-92EV (extracellular vesicles secreted by the NK-92 cell line) can be rapidly isolated and utilized for clinical immunotherapy (27, 50, 60). It is currently the only NK cell line approved by the FDA for clinical applications and is the most widely used NK cell line in experimental research. NK-92 is a NK tumor cell line derived from a non-Hodgkin's lymphoma patient (61), and EVs from these transformed/tumor cells may carry cargo specific to cells capable of altering receptor cells or causing adverse effects (62, 63). However, studies have indicated that the protein levels of cytotoxic proteins in EVs derived from PB-NK cells are generally higher than those from NK-92 sources (16).

NK3.3, a non-tumorogenic NK cell line originating from the peripheral blood of a healthy donor, presents a distinct advantage. This unique lineage alleviates the requirement for identifying and securing consent from numerous healthy peripheral blood donors, concurrently mitigating concerns related to donor variability. Importantly, the utilization of NK3.3 circumvents the potential introduction of oncogenic elements that may be associated with the use of transformed NK-92 cells. Furthermore, this cell line demonstrates the capacity for extensive expansion in substantial quantities, contributing to its utility in research and applications requiring large-scale production (64). Although research has shown that NK-92 cells are more effective than NK3.3 cells in lysing the K562 leukemia cell line, in Cochran et al.'s study, EVs sourced from NK3.3 cells have demonstrated the capacity to instigate morphological transformations and modulate protein expression patterns pertinent to apoptosis induction in diverse cancer cells. This consequential effect manifests as a potent inhibition of tumor proliferation, accompanied by robust cytotoxicity specifically targeted at K562 cells. Importantly, these discernible impacts on cancer cells do not extend to exert any influence on the growth or viability of non-tumorogenic normal cells (64, 65). Hence, NK3.3 emerges as a promising candidate with the potential to function as an efficacious, safe, and universally applicable immunotherapeutic agent.

3.3 iPSC and car-NK cells

NK cells derived from induced pluripotent stem cells (iPSC) have been shown to be superior in cellular therapy compared to primary NK cells and the NK-92 cell line. The therapeutic properties of iPSC-derived cardiomyocyte (66)-EVs, MSC (67)-EVs, and iPSC (68)-EVs have been confirmed. However, further research on their extracellular vesicles (EVs) has not been conducted yet, and this may be a highly promising research direction that deserves further exploration (69, 70). Moreover, the recent advancement in the creation of CAR-equipped NK cells, strategically designed to target specific tumor antigens, marks a sophisticated and potent avenue for EV sourcing. These EVs have the potential for higher specificity in targeting tumor cells (71).

4 Extraction of NKEVs

Currently, the methods for EVs are continually evolving and being updated. Commonly used methods include ultracentrifugation, density gradient centrifugation, polymer precipitation, immunoaffinity methods, ultrafiltration, and size exclusion chromatography (Figure 2B). In fact, the purity of isolated EVs often comes at the cost of sacrificing factors such as cost, yield, scalability, and therapeutic efficacy. If one aims to enrich more EVs in a specific isolation, it inevitably requires more time, labor, and cost (55, 72).

In recent years, some new extraction methods have gradually become research hotspots, such as microfluidic devices, which can

efficiently and precisely separate particles of micrometer or nanometer size in a given volume of fluid (73). Due to their miniaturization, integration, high-throughput capacity, and low time consumption, microfluidic devices hold great promise for improving recovery rates, reducing sample volumes, and shortening processing times. In consideration of the synergistic benefits arising from the integration of microfluidic devices and chemical release strategies, Kang et al. introduced a refined microfluidic platform incorporating anti- NK cell antibody functionality, denoted as the NK-go chip. This innovative platform leverages biocompatible graphene oxide for the capture of NK cell-derived exosomes (NK-Exos) during short-term culture. Additionally, it employs anti-CD63 magnetic beads (ExoBeads) to facilitate the subsequent recovery and purification processes. The study found that the highest exosome purity is obtained with a 12-hour incubation on the chip (74).

Wu et al. have developed a seesaw-motion bioreactor (SMB) system with continuous fluid flow, which not only expands the production of extracellular vesicles (EVs) by increasing the yield of EVs per cell but also achieves scalable EV production by increasing the working volume and cell density of the cell culture medium during continuous-flow cell culture. Through *in vivo* and *in vitro* experiments, it has been demonstrated that the toxicity of NKEVs produced in this manner is not significantly altered compared to static conditions (75).

5 Therapeutic potential of NKEVs-based delivery platforms for the treatment of chronic diseases

Over the past few years, exosomal therapies have made remarkable progress, and the ability to leverage cell-to-cell transfer of information is increasingly becoming a focus of chronic disease research. More importantly, EVs are loaded with a wide range of bioactive molecules from the parent cell, mainly drugs, lipids, proteins and nucleic acids (DNA, coding and non-coding RNA) (76) (Table 2). These loads can be introduced before or after exosome isolation. Pre-isolation loading methods Refers to the introduction of therapeutic molecules into parental cells prior to EV production so that they are encapsulated prior to EV biogenesis (82) (Table 3). Currently, NK cell-derived EVs have received extensive attention as delivery vectors for miRNAs or drugs, and there is a large scope for development in the transport of siRNAs. NKEVs emerge as inherently advantageous drug carriers, distinguished by their elevated biocompatibility, diminished immunogenicity, and the capacity to traverse the blood-brain barrier. Noteworthy in their role as cargo transporters, NKEVs exhibit intrinsic targeting capabilities and cytotoxicity during transit, thereby eliciting potent killing effects. Moreover, the regulatory influence of NKEVs extends to the modulation of signaling pathways within recipient cells, thereby orchestrating anti-tumor functions through the efficient delivery of cargo. This multifaceted potential positions NKEVs as a promising avenue for therapeutic interventions in chronic diseases, encompassing conditions such as cancer, diabetes, depression, and immune disorders (84).

5.1 NKEVs and breast cancer

The inclusions loading capacity and nano-size of EVs, coupled with the membrane proteins inherited from NK cells, so NKEV provides an important solution as a delivery strategy to efficiently deliver small molecule nucleic acids to breast cancer cells. As an illustrative instance, the NK cell line NK92MI underwent lentiviral transduction for the purpose of expressing small interfering RNAs targeting BCL-2 (siBCL-2) within EVs denoted as NKExos. This strategic modification ensured the encapsulation of siBCL-2 within NKExos without imposing a substantial impact on NK cell viability or effector function (78). Subsequently, siBCL-2NKExos targeting BCL-2 enhanced intrinsic apoptosis in breast cancer cells without affecting non-malignant cells. Meanwhile, NKEV can also deliver other non-nucleic acid cargoes. Han et al. extracted EVs from NK-92 cells and prepared paclitaxel PTX-NK-Exos delivery system by electroporation, which targeted human breast cancer MCF-7 cells, effectively inhibited the proliferation of tumor cells and induced apoptosis, thus exerting anti-tumor effects (77). Not only that, NKEV delivery of iron death inducer induced RSL3 leading to intracellular lipid peroxidation in breast cancer cells, resulting in iron death (85).

5.2 NKEVs and other cancers

A plethora of studies has substantiated the involvement of EVs in cancer development, underscoring their pivotal role in the regulation of tumor growth, invasion, and metastasis. These effects are attributed to the dynamic influence of EVs on the tumor microenvironment and their adept modulation of the immune response (86, 87). EVs derived from NK cells have a certain targeting ability. NKEVs can distinguish between transformed and non-transformed cells, and NK-EVs can effectively kill tumor cells. This highlights a potential and interesting application of NKEVs in cancer treatment (37). Kim et al. found that NKEVs have a strong targeting effect and significant toxicity on solid tumors in a primary liver cancer mouse model but do not affect the activity and apoptosis of normal liver cells (28). Currently, gliomas (LGG) have a poor prognosis with a lack of specific biomarkers. While there exists ongoing research aimed at constructing prognostic markers for lower-grade gliomas (LGG) linked to potential factors such as immunogenic cell death (88), inflammatory signaling (89), or long non-coding RNAs (90), the pervasive and inherent capability of exosomes to traverse the blood-brain barrier (BBB) presents an avenue of greater promise in the realms of diagnosis, targeted treatment, and prognosis assessment for brain diseases. As highlighted by Zhu et al., NK-EVs containing IL-15 exhibit heightened cytolytic activity against various human cancer cell lines, including glioblastoma, breast cancer, and thyroid cancer. This enhanced cytotoxicity is accompanied by an upregulation of

TABLE 2 Cargo loaded by NKEVs.

Source of NK cells	Extraction method of Evs	Engineering approach	Target cells	Function	reference
NK-92	Differential ultracentrifugation	Electroporation loading paclitaxel PTX	Human breast cancer MCF-7 cells	Increased cellular uptake of drugs, promoting apoptosis	(77)
PB-NK, CB-NK	Differential ultracentrifugation	Electroporation loading of cisplatin	Human ovarian cancer cell lines SKOV3, OV-90, COC1/DDP	Promoted apoptosis and improved drug resistance	(42)
NK-92MI	Differential ultracentrifugation	Electroporation loading of siRNA targeting PLK1 (siPLK1), modified with photosensitizer Ce6	Hepatocellular Carcinoma HepG2-Luc cell line, Colorectal carcinoma CT26 cell line, Murine macrophage RAW264.7 cell line	Promoted polarization of M1 tumor-associated macrophages and DC cell maturation	(43)
NK-92MI	Test kit	siBCL-2 transfection into NK cells by virus	ER+ breast cancer cells HEK293T cells	Activated mitochondria-dependent apoptosis	(78)
NK92-MI	Differential ultracentrifugation	miR-223 transfected into NK cells	Human hepatic stellate cells-LX-2	Targeted ATG7, inhibited TGF-β1-induced autophagy and attenuated TGF-β1-induced stellate cell activation	(79)
PB-NK	Differential ultracentrifugation		Human PC cells (MiaPaCa-2 and PANC-1)	Inhibited cell proliferation, migration and invasion	(80)
PB-NK	High Performance Liquid Chromatography	miR-186 transfected into NK cells	Neuroblastoma cell line	Inhibited tumorigenic potential of adult neuroblastoma and prevented TGFβ1-dependent inhibition of NK cells	(81)
PB-NK	Differential ultracentrifugation	Polyamidoamine hybridization of NKEVs with reproducible let-7a (membrane fusion)	Human adult neuroblastoma CHLA-255 cells (MDA-MB-231-luc, CHLA-255-luc cells)	Inhibited tumor growth	(54)

molecules associated with NK cell cytotoxicity. Importantly, NK-EVs do not exhibit significant toxicity against normal cells or murine models, emphasizing their potential as a safe and effective therapeutic modality (29).

However, the targeting mechanisms of NKEVs are not yet clear, but some believe that surface receptors on the vesicles such as TRAIL, NKp30, and NKp44, or adhesion molecules such as lymphocyte function-associated antigen (LFA)-1/intercellular adhesion molecule (ICAM)-1, are involved in the recognition and targeting of tumor cells (29, 91).

NKEV delivers miRNAs for the treatment of a variety of cancers, mainly including its mechanism of action includes direct binding to proteins, preventing their binding to receptors as ligands, and triggering downstream signaling, thus altering the activity of the target cells. Neviani et al. primarily utilized a liposomal preparation, anionic liposome nanoparticles (lypopolyplex nanoparticles, the NP) loaded with miR-186 mimics or controls, exposed to PBMC-isolated NK cells, and EVs isolated from NK cell supernatants delivered mature miRNAs targeting and impairing neuroblastoma cell survival and migration, while resisting NK cell inhibition, to achieve inhibition of the tumorigenic potential of the cells (81). Cytokines can also serve as miRNA targets. SUN et al. found that NK cells isolated from PBMC, which derived EVs, directly targeted IL-26 via miR-3607-3p, in which $LNM^{+}PC$ patients (pancreatic cancer patients with lymph node metastasis) had higher levels of IL-26 than the control group, and that IL-26 may play a role in the reduction of metastasis of tumor cells, thereby inhibiting their malignant transformation (80). Contemplating the gene-silencing efficacy inherent to siRNA (92), the intercellular transferability of EVs between tumor cells and immune cells (93), and the tumor-homing proficiency exhibited by NKEVs (94), it is plausible to envisage that NKEV possesses the potential to orchestrate the modulation of immune cell activity. This may be achieved through the mediation of siRNA, consequently impeding the initiation and advancement of tumors. NKEVs modified with the hydrophobic photosensitizer Ce 6, loaded with hydrophilic siRNAs through electroporation, and subsequently subjected to laser activation, instigated substantial photodynamic therapeutic effects. This intervention not only facilitated the polarization of M1 tumor-associated macrophages and the maturation of dendritic cells (DC) within the tumor microenvironment (TME) but also orchestrated the recruitment of various immune cell types. Impressively, these tailored NKEVs demonstrated outstanding efficacy in exerting anti-hepatic tumor cell effects (43).

Most delivery platforms for cancer treatment based on Nanoscale Extracellular Vesicles (NKEV) are centered around nucleic acid therapies, particularly miRNA. However, theoretically, there may be a broader range of applications. As one of the three major gynecological cancers, the treatment of ovarian cancer using radiotherapy and chemotherapy is hampered by issues such as drug resistance and long-term complications. Considering the toxicity and loading capacity of Nanoscale

TABLE 3 Summary of studies on the NKEV drug delivery platform.

Source of NK cells	Extraction method of Evs	Size	Markers of Evs	Use Pathway of Evs	Loading Substances	Loading Method	Target Cells	Mechanism and Function	reference
NK-92	Differential ultracentrifugation	80~110nm	CD63, protein Alix, TSG101	Intravenous injection	Paclitaxel (PTX)	Electroporation	Human breast cancer MCF-7 cells	Inhibited cell proliferation and induced apoptosis more significantly than free PTX	(77)
NK-92MI	Differential ultracentrifugation	0~150nm	ALIX, CD63		MiR-223	Cell transfection	Human hepatic stellate cells-LX-2	Targeted ATG7, inhibited TGF-β1-induced autophagy and attenuated stellate cell activation	(79)
NK-92MI	CD63 Triisosexual ™ exosome detection kit	115.8~128.9 nm	CD63		SiBCL-2	Cell transfection	Breast cancer cells(MEC-1, MCF-7, T-47D, SKBR3, MDA-MB-MB-231)	Promoted apoptosis	(78)
PB-NK	Differential ultracentrifugation		D63, TSG101	Intravenous injection	MiR-3607-3p	Liposome transfection	Human PC cells (MiaPaCa-2, PANC-1)	Targeted IL-26, inhibited cell proliferation, migration and invasion.	(80)
Mouse spleen CD3-CD49b + cells	Test kit	50-150 nm	CD81, CD63	Intravenous injection	Cy3-miR-207	Cell transfection	Astrocytes	Targeted Tril to exert antidepressant effects	(59)
PB-NK	Size exclusion chromatography (SEC)	92.45 nm	CD56 (CD81, Calnexin, TSG101)		MicroRNA (miR)-186		Neuroblastoma cell lines	Inhibited tumorigenic potential of neuronal neoplasms, prevented TGFβ1-dependent inhibition of NK cells	(81)
Mouse splenic lymphocytes	Ultracentrifugation	30~150 nm	HSP70, CD63, TSG101, CD9	Tail vein injection	MiR-1249-3p	Cell transfection	Adipocytes and hepatocytes (3T3-L1 adipocytes, AML12 adipocytes)	Targeted SKOR1, inhibit TLR4/NF-κB pathway, enhanced insulin sensitivity, reduced inflammation in adipocytes and hepatocytes	(83)
PB-NK, CB-NK	Differential ultracentrifugation	73.2 ± 28.5 nm	CD81, CD63, TSG101		Cisplatin	Electroporation	Human ovarian cancer cell lines (SKOV3, OV-90, COC1/DDP)	Killed OC cells and sensitized tumor cells to DDP	(42)
NK-92MI	Differential ultracentrifugation	120 nm	CD9, CD63, CD81, TSG101	Intratumoral injection	SiRNA targeting PLK1 (siPLK1)	Electroporation	Hepatocellular Carcinoma HepG2-Luc cell line, Colorectal carcinoma CT26 cell line, Murine macrophage RAW264.7 cell line	Promoted polarization of M1 tumor-associated macrophages and DC cell maturation	(43)

10.3389/fimmu.2023.1328094

Extracellular Vesicles (NKEVs), along with the effectiveness of immunotherapy, combining NKEVs with immunotherapy for anticancer treatment may have potential applications (95). Cisplatin can inhibit tumor proliferation. Luo et al. loaded cisplatin into eNK-EXO, enhancing drug sensitivity in cisplatin-resistant ovarian cancer cells. Additionally, it can activate NK cells in the immunosuppressive tumor microenvironment, ultimately achieving an anti-ovarian cancer cell effect (42). In conclusion, utilizing extracellular vesicles derived from natural killer (NK) cells to load chemotherapeutic agents can enhance the uptake by solid tumors, thereby achieving a more precise and effective drug delivery.

5.3 NKEVs and other chronic diseases

Due to the cell-permeable capacity of exosomes and their ability to cross the blood-brain barrier, NKEV may be a promising strategy for the treatment of psychiatric or metabolic chronic diseases. As an exemplar, EVs derived from NK cells, isolated from mouse spleens, exhibited the capacity to traverse the blood-brain barrier. These EVs were subsequently internalized by astrocytes, conveying miR-207, which directly targeted proteins interacting with TLR4-Tril complexes, consequently inhibiting the NF-κB signaling pathway. This orchestrated molecular modulation led to a diminished release of pro-inflammatory cytokines. Notably, such intervention translated into a reduction in stress-related symptoms, including locomotor retardation, thereby eliciting antidepressant effects in the mice (59). 2021 wang et al. After co-culturing mouse splenic lymphocyte NK cells transfected with miR-1249-3p with 3T3 - L1 adipocytes or AML12 cells, miR-1249-3p from EV of the former origin inhibited the NF-κB signaling pathway, with novel roles in insulin resistance mitigation and attenuation of inflammatory response, a common dysfunction in patients with type 2 diabetes (83). However, the role of NKEV in immune chronic diseases is easily overlooked, and it has been demonstrated that NKEV exhibits a dose-dependent killing effect for K562 cell lines or Jurkat cell lines derived from chronic granulocytic leukemia or acute T-cell leukemia (39).

6 NKEVs in clinical diagnosis and treatment

In addition to being widely recognized as a nano-scale carrier transport platform, they also have a wide range of applications in clinical diagnosis and treatment. From the perspective of NK cells, on one hand, NK cells, as important effector cells of the innate immune system, can rapidly identify and eliminate heterogeneous cells such as virus-infected cells, tumor cells, and respond to the early pathological conditions of the body. On the other hand, NK cells differentiated in different tissue types exhibit a high degree of heterogeneity, and even within the same organ or tissue, NK cells can have different phenotypic characteristics and functions in different functional states. The high complexity of cell differentiation within tissues makes NK cells a potential specific indicator of the body's pathological functional state, especially in the early stages of disease. At present, clinical data has shown that the abundance of NK cells in the TME predicts a better prognosis for patients with various cancers, such as hepatocellular carcinoma (HCC), melanoma, and others. Simultaneously, researchers have established a signature of NK cell (NRG)-related genes to assess the immunotherapeutic efficacy in head and neck squamous cell carcinoma (96). Because EVs can inherit various characteristics and specific biomolecules from parent cells, it is one of the reasons for the heterogeneity of EVs. Therefore, NKEVs inherit the potential of NK cells as specific biomarkers. As early as 2012, it was discovered that in pre-eclampsia, the number of microvesicles in peripheral blood would undergo changes, and among them, there were fewer microvesicles formed by NK cells (97). The correlation between the content of EVs released by NK cells in the blood and the development of the disease reveals the potential clinical application value of NKEVs in disease diagnosis and treatment.

In recent years, through the application of microfluidic systems for NKEV extraction, it has been discovered that changes in NKEV concentration are positively correlated with the number of circulating tumor cells (CTC) in non-small cell lung cancer (NSCLC) patients (74). CTCs, representing subclones with a high metastatic propensity, are used in liquid biopsies for cancer. They provide more information than traditional tissue biopsies through phenotype and molecular analysis and can serve as biological markers of interest in precision cancer treatment. In 2017, the National Comprehensive Cancer Network (NCCN) in the United States included circulating tumor cells (CTC) in the clinical guidelines for breast cancer malignant tumor staging (TNM staging). Given the diagnostic significance of CTC in cancer and the robust association between NKEV and CTC counts, a compelling prospect emerges—namely, that the identification of NKEVs could offer a personalized approach to disease diagnosis and treatment. This notion supplements and amplifies the predictive capacity of CTCs in delineating patient prognosis. In a noteworthy development, Deeptha et al. engineered a highly sensitive, highly specific, and straightforward GBM ImmunoProfiler platform. This platform harnessed an expandable ultrafast laser multiphoton ionization mechanism to scrutinize serum samples from glioblastoma (GBM) patients, enabling the tracking of NKEV expression in the circulation alongside immune checkpoint markers, namely PDL-1 and cytotoxic T-lymphocyte-associated protein 4 (CTLA4). The clinical validation of this cutting-edge technology furnishes robust evidence advocating for the utilization of NKEVs as a diagnostic and therapeutic tool in minimally invasive biopsies (98).

7 Discussion and future

NK cells are important immune cells in the body, and their EVs have similar characteristics to the parental cells. Compared with other immune cells, such as T cells, T cells need expensive and time-consuming engineering and expansion of T cell processes, as well as the therapeutic effect limitation caused by low expression of major

histocompatibility complex (MHC) on tumor cells. NK cell therapy is independent of antigen presentation and can be better controlled to reduce the risk of cytokine storm.

EV has many advantages, such as biocompatibility, blood-brain barrier penetration, small size, and suitable for infiltrating solid tumors (59, 81). The cytotoxicity of NK cells to a variety of tumors is inhibited by acidic extracellular pH, which inhibits the release of perforin/granzyme containing particles and fas/fasl interaction (50, 99). The promotion of EV accumulation and delivery is notably facilitated by acidity. Within the acidic tumor microenvironment, this distinctive milieu actively encourages the uptake of EVs by tumor cells. This phenomenon is driven by the compelling influence of low pH, which not only attracts EVs but also fosters the facilitation of membrane fusion processes (27, 100). Moreover, NK extracellular vesicles (NK exo) have natural tumor targeting ability and immune regulation ability, and are ideal molecular carriers, which can effectively transmit drugs or signal molecules to tumor cells or immune cells, thereby enhancing the anti-tumor effect. In addition, the exosome production of NK92 cells under hypoxia treatment increased (101); The hypoxic milieu proves conducive to both the accumulation and delivery of exosomes. This advantageous effect arises from the low oxygen concentration within the environment, a factor that attracts exosomes and facilitates their membrane fusion processes (102).

Even though NKEVs (NK cell-derived Extracellular Vesicles) have been shown to have some degree of specificity towards tumor sites, the mechanism of EVs targeting specific cells and the precise delivery of therapeutic EVs to target cells remain a yet-to-be-solved and critically important issue. Addressing this issue could enable us to utilize EVs as a means of delivering more therapeutic EVs to target cells and avoiding unwanted side effects. In recent years, engineered EVs have gradually come into focus, and surface modifications of EVs, including chemical modifications and genetic engineering, have significantly enhanced their tumor-targeting capabilities. The development of EV-nanomedicine co-delivery systems, such as liposomes, can further enhance the loading efficiency of EVs while ensuring their targeting specificity. This approach has also been validated in EVs derived from NK cells (54, 103). Microfluidics and lab-on-a-chip technologies make it possible to control the size of liposomes, and freeze-thaw cycles appear to be advantageous for the fusion of EVs with liposomes. These advancements make EV-nanomedicine delivery systems even more promising in terms of their potential for targeted drug delivery (104, 105).

Author contributions

HC: Conceptualization, Writing – review & editing. SH: Conceptualization, Data curation, Writing – original draft. LS: Data curation, Visualization, Writing – original draft. HH: Writing – original draft. HL: Writing – original draft. JX: Writing – original draft. XG: Writing – original draft. QW: Conceptualization, Writing – review & editing. GY: Conceptualization, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was supported by grants from the Science and Technology Development Fund, Macau SAR (file No.: 0098/2021/A2 and 0048/2023/AFJ).

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. Colombo M, Raposo G, Théry C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annu Rev Cell Dev Bi.* (2014) 30(1):255–89. doi: 10.1146/annurev-cellbio-101512-122326

2. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *J Extracell Vesicles.* (2018) 7(1):1535750. doi: 10.1080/20013078.2018.1535750

3. Witwer KW, Théry C. Extracellular vesicles or exosomes? On primacy, precision, and popularity influencing a choice of nomenclature. *J Extracell Vesicles.* (2019) 8 (1):1648167. doi: 10.1080/20013078.2019.1648167

4. Myers JA, Miller JS. Exploring the NK cell platform for cancer immunotherapy. *Nat Rev Clin Oncol* (2021) 18(2):85–100. doi: 10.1038/s41571-020-0426-7

5. Barrow AD, Martin CJ, Colonna M. The natural cytotoxicity receptors in health and disease. *Front Immunol* (2019) 10:909. doi: 10.3389/fimmu.2019.00909

6. Long EO. Negative signaling by inhibitory receptors: the NK cell paradigm. *Immunol Rev* (2008) 224:70–84. doi: 10.1111/j.1600-065X.2008.00660.x

7. Prager I, Watzl C. Mechanisms of natural killer cell-mediated cellular cytotoxicity. *J Leukocyte Biol* (2019) 105(6):1319–29. doi: 10.1002/JLB.MR0718-269R

8. Tanaka J, Miller JS. Recent progress in and challenges in cellular therapy using NK cells for hematological Malignancies. *Blood Rev* (2020) 44:100678. doi: 10.1016/j.blre.2020.100678

9. Schönfeld K, Sahm C, Zhang C, Naundorf S, Brendel C, Odendahl M, et al. Selective inhibition of tumor growth by clonal NK cells expressing an erbB2/HER2-specific chimeric antigen receptor. *Mol Ther* (2015) 23(2):330–8. doi: 10.1038/mt.2014.219

10. Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science* (2002) 295(5562):2097–100. doi: 10.1126/science.1068440

11. Rusakiewicz S, Semeraro M, Sarabi M, Desbois M, Locher C, Mendez R, et al. Immune infiltrates are prognostic factors in localized gastrointestinal stromal tumors. *Cancer Res* (2013) 73(12):3499–510. doi: 10.1158/0008-5472.CAN-13-0371

12. Gras Navarro A, Björklund AT, CheKenya M. Therapeutic potential and challenges of natural killer cells in treatment of solid tumors. *Front Immunol* (2015) 6:202.

13. Elsharkasy OM, Nordin JZ, Hagey DW, de Jong OG, Schiffelers RM, Andaloussi SE, et al. Extracellular vesicles as drug delivery systems: Why and how? *Adv Drug Deliver Rev* (2020) 159:332–43. doi: 10.1016/j.addr.2020.04.004

14. Herrmann IK, Wood MJA, Fuhrmann G. Extracellular vesicles as a next-generation drug delivery platform. *Nat Nanotechnol.* (2021) 16(7):748–59. doi: 10.1038/s41565-021-00931-2

15. Voskoboinik I, Smyth MJ, Trapani JA. Perforin-mediated target-cell death and immune homeostasis. *Nat Rev Immunol* (2006) 6(12):940–52. doi: 10.1038/nri1983

16. Wu CH, Li J, Li L, Sun J, Fabbri M, Wayne AS, et al. Extracellular vesicles derived from natural killer cells use multiple cytotoxic proteins and killing mechanisms to target cancer cells. *J Extracell Vesicles.* (2019) 8(1):1588538. doi: 10.1080/20013078.2019.1588538

17. Wen C, Seeger RC, Fabbri M, Wang L, Wayne AS, Jong AY. Biological roles and potential applications of immune cell-derived extracellular vesicles. *J Extracell Vesicles.* (2017) 6(1):1400370. doi: 10.1080/20013078.2017.1400370

18. Lieberman J. Granzyme A activates another way to die. *Immunol Rev* (2010) 235 (1):93–104. doi: 10.1111/j.0105-2896.2010.00902.x

19. Lucken-Ardjomande S, Martinou JC. Granzyme a, a stealth killer in the mitochondrion. *Cell* (2008) 133(4):568–70. doi: 10.1016/j.cell.2008.04.031

20. Martinvalet D, Zhu P, Lieberman J. Granzyme A induces caspase-independent mitochondrial damage, a required first step for apoptosis. *Immunity* (2005) 22(3):355–70. doi: 10.1016/j.immuni.2005.02.004

21. Cullen SP, Brunet M, Martin SJ. Granzymes in cancer and immunity. *Cell Death Differ* (2010) 17(4):616–23. doi: 10.1038/cdd.2009.206

22. Slee EA, Harte MT, Kluck RM, Wolf BB, Casiano CA, Newmeyer DD, et al. Ordering the cytochrome c-initiated caspase cascade: hierarchical activation of caspases-2, -3, -6, -7, -8, and -10 in a caspase-9-dependent manner. *J Cell Biol* (1999) 144(2):281–92. doi: 10.1083/jcb.144.2.281

23. Suda T, Takahashi T, Golstein P, Nagata S. Molecular cloning and expression of the Fas ligand, a novel member of the tumor necrosis factor family. *Cell* (1993) 75 (6):1169–78. doi: 10.1016/0092-8674(93)90326-L

24. Lavrik IN, Krammer PH. Regulation of CD95/Fas signaling at the DISC. *Cell Death Differ* (2012) 19(1):36–41. doi: 10.1038/cdd.2011.155

25. Luo X, Budihardjo I, Zou H, Slaughter C, Wang X. Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. *Cell* (1998) 94(4):481–90. doi: 10.1016/S0092-8674(00) 81589-5

26. Li H, Zhu H, Xu CJ, Yuan J. Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. *Cell* (1998) 94(4):491–501. doi: 10.1016/S0092-8674(00)81590-1

27. Zhu L, Kalimuthu S, Gangadaran P, Oh JM, Lee HW, Baek SH, et al. Exosomes derived from natural killer cells exert therapeutic effect in melanoma. *Theranostics* (2017) 7(10):2732–45. doi: 10.7150/thno.18752

28. Kim HY, Min HK, Song HW, Yoo A, Lee S, Kim KP, et al. Delivery of human natural killer cell-derived exosomes for liver cancer therapy: an *in vivo* study in subcutaneous and orthotopic animal models. *Drug Deliv.* (2022) 29(1):2897–911. doi: 10.1080/10717544.2022.2118898

29. Zhu L, Kalimuthu S, Oh JM, Gangadaran P, Baek SH, Jeong SY, et al. Enhancement of antitumor potency of extracellular vesicles derived from natural killer cells by IL-15 priming. *Biomaterials* (2019) 190–191:38–50. doi: 10.1016/j.biomaterials.2018.10.034

30. Aarsund M, Segers FM, Wu Y, Inngjerdingen M. Comparison of characteristics and tumor targeting properties of extracellular vesicles derived from primary NK cells or NK-cell lines stimulated with IL-15 or IL-12/15/18. *Cancer Immunol Immun* (2022) 71(9):2227–38. doi: 10.1007/s00262-022-03161-0

31. Jorgovanovic D, Song M, Wang L, Zhang Y. Roles of IFN-γ in tumor progression and regression: a review. *biomark Res* (2020) 8:49. doi: 10.1186/s40364-020-00228-x

32. Choi JW, Lim S, Kang JH, Hwang SH, Hwang KC, Kim SW, et al. Proteome analysis of human natural killer cell derived extracellular vesicles for identification of anticancer effectors. *Molecules* (2020) 25(21):5216. doi: 10.3390/molecules25215216

33. Markova K, Mikhailova V, Milyutina Y, Korenevsky A, Sirotskaya A, Rodygina V, et al. Effects of microvesicles derived from NK cells stimulated with IL-1β on the phenotype and functional activity of endothelial cells. *Int J Mol Sci* (2021) 22(24):13663.

34. Aggarwal BB. Signalling pathways of the TNF superfamily: a double-edged sword. *Nat Rev Immunol* (2003) 3(9):745–56. doi: 10.1038/nri1184

35. McDonald MK, Tian Y, Qureshi RA, Gormley M, Ertel A, Gao R, et al. Functional significance of macrophage-derived exosomes in inflammation and pain. *Pain* (2014) 155(8):1527–39. doi: 10.1016/j.pain.2014.04.029

36. Gao W, Liu H, Yuan J, Wu C, Huang D, Ma Y, et al. Exosomes derived from mature dendritic cells increase endothelial inflammation and atherosclerosis via membrane TNF-α mediated NF-κB pathway. *J Cell Mol Med* (2016) 20(12):2318–27. doi: 10.1111/jcmm.12923

37. Di Pace A, Tumino N, Besi F, Alicata C, Conti L, Munari E, et al. Characterization of human NK cell-derived exosomes: role of DNAM1 receptor in exosome-mediated cytotoxicity against tumor. *Cancers* (2020) 12(3):661.

38. Wang H, Qi J, Zhang S, Li Y, Tan S, Gao GF. Binding mode of the side-by-side two-IgV molecule CD226/DNAM-1 to its ligand CD155/Necl-5. *P Natl Acad Sci USA* (2019) 116(3):988–96. doi: 10.1073/pnas.1815716116

39. Enomoto Y, Li P, Jenkins LM, Anastasakis D, Lyons GC, Hafner M, et al. Cytokine-enhanced cytolytic activity of exosomes from NK Cells. *Cancer Gene Ther* (2022) 29(6):734–49. doi: 10.1038/s41417-021-00352-2

40. Federici C, Shahaj E, Cecchetti S, Camerini S, Casella M, Iessi E, et al. Natural-killer-derived extracellular vesicles: immune sensors and interactors. *Front Immunol* (2020) 11:262. doi: 10.3389/fimmu.2020.00262

41. Jia R, Cui K, Li Z, Gao Y, Zhang B, Wang Z, et al. NK cell-derived exosomes improved lung injury in mouse model of Pseudomonas aeruginosa lung infection. *J Physiol Sci* (2020) 70(1):50. doi: 10.1186/s12576-020-00776-9

42. Luo H, Zhou Y, Zhang J, Zhang Y, Long S, Lin X, et al. NK cell-derived exosomes enhance the anti-tumor effects against ovarian cancer by delivering cisplatin and reactivating NK cell functions. *Front Immunol* (2023) 13:1087689. doi: 10.3389/fimmu.2022.1087689

43. Zhang M, Shao W, Yang T, Liu H, Guo S, Zhao D, et al. Conscription of immune cells by light-activatable silencing NK-derived exosome (LASNEO) for synergetic tumor eradication. *Adv Sci* (2022) 9(22):2201135. doi: 10.1002/advs.202201135

44. O'Sullivan TE, Sun JC, Lanier LL. Natural killer cell memory. *Immunity* (2015) 43(4):634–45. doi: 10.1016/j.immuni.2015.09.013

45. Gamliel M, Goldman-Wohl D, Isaacson B, Gur C, Stein N, Yamin R, et al. Trained memory of human uterine NK cells enhances their function in subsequent pregnancies. *Immunity* (2018) 48(5):951–962.e5. doi: 10.1016/j.immuni.2018.03.030

46. Sun JC, Beilke JN, Lanier LL. Adaptive immune features of natural killer cells. *Nature* (2009) 457(7229):557–61. doi: 10.1038/nature07665

47. Jong AY, Wu CH, Li J, Sun J, Fabbri M, Wayne AS, et al. Large-scale isolation and cytotoxicity of extracellular vesicles derived from activated human natural killer cells. *J Extracellular Vesicles.* (2017) 6(1):1294368. doi: 10.1080/20013078.2017.1294368

48. Mohammadi F, Hashemi ZS, Forooshani RS, Alizadeh S. Bioactivity of exosomes derived from trained natural killer cells versus non-trained one: more functional and antitumor activity Vol. 2022. Pizzimenti S, editor. *Biomed Res Int* (2022) p. 1–11.

49. Shoae-Hassani A, Hamidieh AA, Behfar M, Mohseni R, Mortazavi-Tabatabaei SA, Asgharzadeh S. NK cell–derived exosomes from NK cells previously exposed to neuroblastoma cells augment the antitumor activity of cytokine-activated NK cells. *J Immunother.* (2017) 40(7):265–76. doi: 10.1097/CJI.0000000000000179

50. Lugini L, Cecchetti S, Huber V, Luciani F, Macchia G, Spadaro F, et al. Immune surveillance properties of human NK cell-derived exosomes. *J Immunol* (2012) 189 (6):2833–42. doi: 10.4049/jimmunol.1101988

51. Romee R, Rosario M, Berrien-Elliott MM, Wagner JA, Jewell BA, Schappe T, et al. Cytokine-induced memory-like natural killer cells exhibit enhanced responses against myeloid leukemia. *Sci Transl Med* (2016) 8(357):357ra123. doi: 10.1126/scitranslmed.aaf2341

52. Cooper MA, Elliott JM, Keyel PA, Yang L, Carrero JA, Yokoyama WM. Cytokine-induced memory-like natural killer cells. *Proc Natl Acad Sci USA* (2009) 106(6):1915–9. doi: 10.1073/pnas.0813192106

53. Hu W, Wang G, Huang D, Sui M, Xu Y. Cancer immunotherapy based on natural killer cells: current progress and new opportunities. *Front Immunol* (2019) 10:1205. doi: 10.3389/fimmu.2019.01205

54. Wang G, Hu W, Chen H, Shou X, Ye T, Xu Y. Cocktail strategy based on NK cell-derived exosomes and their biomimetic nanoparticles for dual tumor therapy. *Cancers* (2019) 11(10):1560. doi: 10.3390/cancers11101560

55. Atkinson SP. Stem cells translational medicine. *Stem Cells Transl Med* (2018) 7 (7):503–5. doi: 10.1002/sctm.18-0110

56. Luevano M, Daryouzeh M, Alnabhan R, Querol S, Khakoo S, Madrigal A, et al. The unique profile of cord blood natural killer cells balances incomplete maturation and effective killing function upon activation. *Hum Immunol* (2012) 73(3):248–57. doi: 10.1016/j.humimm.2011.12.015

57. Verneris MR, Miller JS. The phenotypic and functional characteristics of umbilical cord blood and peripheral blood natural killer cells. *Brit J Haematol* (2009) 147(2):185–91. doi: 10.1111/j.1365-2141.2009.07768.x

58. Sánchez-Martínez D, Azaceta G, Muntasell A, Aguiló N, Núñez D, Gálvez EM, et al. Human NK cells activated by EBV+ lymphoblastoid cells overcome anti-apoptotic mechanisms of drug resistance in haematological cancer cells. *Oncoimmunology* (2015) 4(3):e991613.

59. Li D, Wang Y, Jin X, Hu D, Xia C, Xu H, et al. NK cell-derived exosomes carry miR-207 and alleviate depression-like symptoms in mice. *J Neuroinflamm.* (2020) 17 (1):126. doi: 10.1186/s12974-020-01787-4

60. Fais S. NK cell-released exosomes: Natural nanobullets against tumors. *Oncoimmunology* (2013) 2(1):e22337. doi: 10.4161/onci.22337

61. Gong JH, Maki G, Klingemann HG. Characterization of a human cell line (NK-92) with phenotypical and functional characteristics of activated natural killer cells. *Leukemia* (1994) 8(4):652–8.

62. Hannafon B, Ding WQ. Intercellular communication by exosome-derived microRNAs in cancer. *Int J Mol Sci* (2013) 14(7):14240–69. doi: 10.3390/ijms140714240

63. Mashouri L, Yousefi H, Aref AR, mohammad AA, Molaei F, Alahari SK. Exosomes: composition, biogenesis, and mechanisms in cancer metastasis and drug resistance. *Mol Cancer.* (2019) 18(1):75. doi: 10.1186/s12943-019-0991-5

64. Cochran AM, Kornbluth J. Extracellular vesicles from the human natural killer cell line NK3.3 have broad and potent anti-tumor activity. *Front Cell Dev Biol* (2021) 9:698639. doi: 10.3389/fcell.2021.698639

65. Gunesch JT, Angelo LS, Mahapatra S, Deering RP, Kowalko JE, Sleiman P, et al. Genome-wide analyses and functional profiling of human NK cell lines. *Mol Immunol* (2019) 115:64–75. doi: 10.1016/j.molimm.2018.07.015

66. Santoso MR, Ikeda G, Tada Y, Jung JH, Vaskova E, Sierra RG, et al. Exosomes from induced pluripotent stem cell-derived cardiomyocytes promote autophagy for myocardial repair. *J Am Heart Assoc* (2020) 9(6):e014345. doi: 10.1161/JAHA.119.014345

67. Du Y, Li D, Han C, Wu H, Xu L, Zhang M, et al. Exosomes from Human-Induced Pluripotent Stem Cell-Derived Mesenchymal Stromal Cells (hiPSC-MSCs) Protect Liver against Hepatic Ischemia/ Reperfusion Injury via Activating Sphingosine Kinase and Sphingosine-1-Phosphate Signaling Pathway. *Cell Physiol Biochem* (2017) 43(2):611–25. doi: 10.1159/000480533

68. Liu S, Mahairaki V, Bai H, Ding Z, Li J, Witwer KW, et al. Highly purified human extracellular vesicles produced by stem cells alleviate aging cellular phenotypes of senescent human cells. *Stem Cells* (2019) 37(6):779–90. doi: 10.1002/stem.2996

69. Boyd-Gibbins N, Karagiannis P, Hwang DW, Kim SI. iPSCs in NK cell manufacturing and NKEV development. *Front Immunol* (2022) 13:890894. doi: 10.3389/fimmu.2022.890894

70. Karagiannis P, Kim SI. iPSC-derived natural killer cells for cancer immunotherapy. *Mol Cells* (2021) 44(8):541–8. doi: 10.14348/molcells.2021.0078

71. Gong Y, Klein Wolterink RGJ, Wang J, Bos GMJ, Germeraad WTV. Chimeric antigen receptor natural killer (CAR-NK) cell design and engineering for cancer therapy. *J Hematol Oncol* (2021) 14(1):73. doi: 10.1186/s13045-021-01083-5

72. Fu X, Song J, Yan W, Downs BM, Wang W, Li J. The biological function of tumor-derived extracellular vesicles on metabolism. *Cell Commun Signal* (2023) 21(1):150. doi: 10.1186/s12964-023-01111-6

73. Giancaterino S, Boi C. Alternative biological sources for extracellular vesicles production and purification strategies for process scale-up. *Biotechnol Adv* (2023) 63:108092. doi: 10.1016/j.biotechadv.2022.108092

74. Kang Y, Niu Z, Hadlock T, Purcell E, Lo T, Zeinali M, et al. On-chip biogenesis of circulating NK cell-derived exosomes in non-small cell lung cancer exhibits antitumoral activity. *Adv Sci* (2021) 8(6):2003747. doi: 10.1002/advs.202003747

75. Wu J, Wu D, Wu G, Bei HP, Li Z, Xu H, et al. Scale-out production of extracellular vesicles derived from natural killer cells via mechanical stimulation in a seesaw-motion bioreactor for cancer therapy. *Biofabrication* (2022) 14(4):045004. doi: 10.1088/1758-5090/ac7eeb

76. Lu S, Lu L, Liu Y, Li Z, Fang Y, Chen Z, et al. Native and engineered extracellular vesicles for wound healing. *Front Bioeng Biotechnol* (2022) 10:1053217. doi: 10.3389/fbioe.2022.1053217

77. Han D, Wang K, Zhang T. Natural killer cell-derived exosome-entrapped paclitaxel can enhance its anti-tumor effect. *Eur Rev Med Pharmacol Sci* 24(10):5703–13 (2020).

78. Kaban K, Hinterleitner C, Zhou Y, Salva E, Kantarci AG, Salih HR, et al. Therapeutic silencing of BCL-2 using NK cell-derived exosomes as a novel therapeutic approach in breast cancer. *Cancers* (2021) 13(10):2397. doi: 10.3390/cancers13102397

79. Wang L, Wang Y, Quan J. Exosomal miR-223 derived from natural killer cells inhibits hepatic stellate cell activation by suppressing autophagy. *Mol Med* (2020) 26(1):81. doi: 10.1186/s10020-020-00207-w

80. Zhou M. Natural killer cell-derived exosomal miR-3607-3p inhibits pancreatic cancer progression by targeting IL-26. *Front Immunol* (2019) 10.

81. Neviani P, Wise PM, Murtadha M, Liu CW, Wu CH, Jong AY, et al. Natural killer–derived exosomal miR-186 inhibits neuroblastoma growth and immune escape mechanisms. *Cancer Res* (2019) 79(6):1151–64. doi: 10.1158/0008-5472.CAN-18-0779

82. Tenchov R, Sasso JM, Wang X, Liaw WS, Chen CA, Zhou QA. Exosomes—Nature's lipid nanoparticles, a rising star in drug delivery and diagnostics. *ACS Nano.* (2022) 16(11):17802–46. doi: 10.1021/acsnano.2c08774

83. Wang Y, Li M, Chen L, Bian H, Chen X, Zheng H, et al. Natural killer cell-derived exosomal miR-1249-3p attenuates insulin resistance and inflammation in mouse models of type 2 diabetes. *Sig Transduct Target Ther* (2021) 6(1):409. doi: 10.1038/s41392-021-00805-y

84. Soriani A, Vulpis E, Cuollo L, Santoni A, Zingoni A. Cancer extracellular vesicles as novel regulators of NK cell response. *Cytokine Growth F R* (2020) 51:19–26. doi: 10.1016/j.cytogfr.2019.11.007

85. Tao B, Du R, Zhang X, Jia B, Gao Y, Zhao Y, et al. Engineering CAR-NK cell derived exosome disguised nano-bombs for enhanced HER2 positive breast cancer brain metastasis therapy. *J Control Release.* (2023) 363:692–706. doi: 10.1016/j.jconrel.2023.10.007

86. Wang X, Huang J, Chen W, Li G, Li Z, Lei J. The updated role of exosomal proteins in the diagnosis, prognosis, and treatment of cancer. *Exp Mol Med* (2022) 54(9):1390–400. doi: 10.1038/s12276-022-00855-4

87. Xiong J, Chi H, Yang G, Zhao S, Zhang J, Tran LJ, et al. Revolutionizing anti-tumor therapy: unleashing the potential of B cell-derived exosomes. *Front Immunol* (2023) 14:1188760. doi: 10.3389/fimmu.2023.1188760

88. Kuang Y, Jiang B, Zhu H, Zhou Y, Huang H, Li C, et al. Classification related to immunogenic cell death predicts prognosis, immune microenvironment characteristics, and response to immunotherapy in lower-grade gliomas. *Front Immunol* (2023) 14:1102094. doi: 10.3389/fimmu.2023.1102094

89. Cao Y, Zhu H, Chen Q, Huang H, Xie D, Li X, et al. Integrated analysis of inflammatory response subtype-related signature to predict clinical outcomes, immune status and drug targets in lower-grade glioma. *Front Pharmacol* (2022) 13:914667. doi: 10.3389/fphar.2022.914667

90. Cao Y, Zhu H, Tan J, Yin W, Zhou Q, Xin Z, et al. Development of an immune-related lncRNA prognostic signature for glioma. *Front Genet* (2021) 12:678436. doi: 10.3389/fgene.2021.678436

91. Jang SC, Kim OY, Yoon CM, Choi DS, Roh TY, Park J, et al. Bioinspired exosome-mimetic nanovesicles for targeted delivery of chemotherapeutics to Malignant tumors. *ACS Nano.* (2013) 7(9):7698–710. doi: 10.1021/nn402232g

92. Paunovska K, Loughrey D, Dahlman JE. Drug delivery systems for RNA therapeutics. *Nat Rev Genet* (2022) 23(5):265–80. doi: 10.1038/s41576-021-00439-4

93. Chen X, Chi H, Zhao X, Pan R, Wei Y, Han Y. Role of exosomes in immune microenvironment of hepatocellular carcinoma. *J Oncol* (2022) 2022:2521025. doi: 10.1155/2022/2521025

94. Wu F, Xie M, Hun M, She Z, Li C, Luo S, et al. Natural killer cell-derived extracellular vesicles: novel players in cancer immunotherapy. *Front Immunol* (2021) 12:658698. doi: 10.3389/fimmu.2021.658698

95. Gong X, Chi H, Strohmer DF, Teichmann AT, Xia Z, Wang Q. Exosomes: A potential tool for immunotherapy of ovarian cancer. *Front Immunol* (2022) 13:1089410. doi: 10.3389/fimmu.2022.1089410

96. Chi H, Xie X, Yan Y, Peng G, Strohmer DF, Lai G, et al. Natural killer cell-related prognosis signature characterizes immune landscape and predicts prognosis of HNSCC. *Front Immunol* (2022) 13:1018685. doi: 10.3389/fimmu.2022.1018685

97. Mikhailova VA, Ovchinnikova OM, Zainulina MS, Sokolov DI, Sel'kov SA. Detection of microparticles of leukocytic origin in the peripheral blood in normal pregnancy and preeclampsia. *B Exp Biol Med+.* (2014) 157(6):751–6. doi: 10.1007/s10517-014-2659-x

98. Ishwar D, Haldavnekar R, Das S, Tan B, Venkatakrishnan K. Glioblastoma associated natural killer cell EVs generating tumour-specific signatures: noninvasive GBM liquid biopsy with self-functionalized quantum probes. *ACS Nano.* (2022) 16(7):10859–77. doi: 10.1021/acsnano.2c03055

99. Fischer B, Müller B, Fischer KG, Baur N, Kreutz W. Acidic pH inhibits non-MHC-restricted killer cell functions. *Clin Immunol* (2000) 96(3):252–63. doi: 10.1006/clim.2000.4904

100. Parolini I, Federici C, Raggi C, Lugini L, Palleschi S, De Milito A, et al. Microenvironmental pH is a key factor for exosome traffic in tumor cells. *J Biol Chem* (2009) 284(49):34211–22. doi: 10.1074/jbc.M109.041152

101. Jiang Y, Jiang H, Wang K, Liu C, Man X, Fu Q. Hypoxia enhances the production and antitumor effect of exosomes derived from natural killer cells. *Ann Transl Med* (2021) 9(6):473–3. doi: 10.21037/atm-21-347

102. Patton MC, Zubair H, Khan MA, Singh S, Singh AP. Hypoxia alters the release and size distribution of extracellular vesicles in pancreatic cancer cells to support their adaptive survival. *J Cell Biochem* (2020) 121(1):828–39. doi: 10.1002/jcb.29328

103. Zhu L, Gangadaran P, Kalimuthu S, Oh JM, Baek SH, Jeong SY, et al. Novel alternatives to extracellular vesicle-based immunotherapy – exosome mimetics derived from natural killer cells. *Artif Cell Nanomed B* (2018) 46(sup3):166–79. doi: 10.1080/21691401.2018.1489824

104. Zhang F, Guo J, Zhang Z, Duan M, Wang G, Qian Y, et al. Application of engineered extracellular vesicles for targeted tumor therapy. *J BioMed Sci* (2022) 29(1):14. doi: 10.1186/s12929-022-00798-y

105. Maeki M, Kimura N, Sato Y, Harashima H, Tokeshi M. Advances in microfluidics for lipid nanoparticles and extracellular vesicles and applications in drug delivery systems. *Adv Drug Delivery Rev* (2018) 128:84–100. doi: 10.1016/j.addr.2018.03.008

frontiers | Frontiers in Immunology

TYPE Mini Review
PUBLISHED 08 January 2024
DOI 10.3389/fimmu.2023.1339669

OPEN ACCESS

EDITED BY
Chun Wai Mai,
UCSI University, Malaysia

REVIEWED BY
Zhijie Zhao,
Shanghai Jiao Tong University, China
Guichuan Lai,
Chongqing Medical University, China

*CORRESPONDENCE
Qing Wang
Doctorwq1983@163.com
Ting Zhang
cczthd18@gmail.com
Zhijian Peng
13912690058@163.com

†These authors have contributed equally to this work and share the first authorship

RECEIVED 16 November 2023
ACCEPTED 18 December 2023
PUBLISHED 08 January 2024

CITATION
Huang H, Chen P, Feng X, Qian Y, Peng Z, Zhang T and Wang Q (2024) Translational studies of exosomes in sports medicine – a mini-review. *Front. Immunol.* 14:1339669. doi: 10.3389/fimmu.2023.1339669

Translational studies of exosomes in sports medicine – a mini-review

Haoqiang Huang[1†], Peng Chen[2†], Xinting Feng[3†], Yinhua Qian[1], Zhijian Peng[1*], Ting Zhang[4*] and Qing Wang[1*]

[1]Department of Orthopaedics, Kunshan Hospital of Traditional Chinese Medicine, Kunshan, Jiangsu, China, [2]Department of Sports Medicine, Peking University Shenzhen Hospital, Shenzhen, China, [3]Department of Sports Medicine, Huashan Hospital, Fudan University, Shanghai, China, [4]Department of Integrative Medicine, Huashan Hospital, Fudan University, Shanghai, China

This review in sports medicine focuses on the critical role of exosomes in managing chronic conditions and enhancing athletic performance. Exosomes, small vesicles produced by various cells, are essential for cellular communication and transporting molecules like proteins and nucleic acids. Originating from the endoplasmic reticulum, they play a vital role in modulating inflammation and tissue repair. Their significance in sports medicine is increasingly recognized, particularly in healing athletic injuries, improving articular cartilage lesions, and osteoarthritic conditions by modulating cellular behavior and aiding tissue regeneration. Investigations also highlight their potential in boosting athletic performance, especially through myocytes-derived exosomes that may enhance adaptability to physical training. Emphasizing a multidisciplinary approach, this review underlines the need to thoroughly understand exosome biology, including their pathways and classifications, to fully exploit their therapeutic potential. It outlines future directions in sports medicine, focusing on personalized treatments, clinical evaluations, and embracing technological advancements. This research represents a frontier in using exosomes to improve athletes' health and performance capabilities.

KEYWORDS

exosomes, sports medicine, chronic diseases, injury recovery, immune regulation, translation

1 Introduction

Exosomes represent a specialized subset of extracellular vesicles, with dimensions typically ranging from 30 to 150 nanometers, that are elaborated and liberated by a broad spectrum of cells via the endoplasmic reticulum vesicle system (1–4). These nanostructures play a quintessential role in cellular communication, encapsulating and conveying an assortment of biomolecules, including proteins, lipids, RNA, and DNA (5–9). Their

interaction with recipient cells significantly modulates the recipient's cellular functions (9, 10). The ontogeny of exosomes commences within the endoplasmic reticulum vesicle system, where they originate as incipient endosomes in the cytoplasmic milieu. Subsequently, they progress to mature into multivesicular bodies (MVBs), which are repositories for myriad intraluminal vesicles that ultimately coalesce with the plasma membrane, culminating in the extracellular release of exosomes. This elaborate process is under the stringent regulation of a network of proteins and lipids (2, 4). Exosomes are taxonomically classified per their progenitor cell lineage, inherent biological functions, and distinctive surface markers, rendering them an intriguing focus of current biomedical research. Depending on the source cell, exosomes can be subclassified into various types, such as those derived from immune cells (e.g., exosomes produced by T cells, B cells, or dendritic cells), tumor cells (exosomes from tumor cells, typically associated with promoting tumor growth and metastasis), and stem cells (exosomes from various stem cells like mesenchymal stem cells and embryonic stem cells, noted for their robust tissue repair and regenerative capabilities). Additionally, based on their biological function, exosomes can be classified as pro-inflammatory or anti-inflammatory, immunomodulatory, pro-tumorigenic, or anti-tumorigenic, and more (11–14). The analysis of exosomal surface markers, such as CD63, CD81, and CD9, which are commonly regarded as universal markers, also aids in their categorization. Understanding the diverse classifications of exosomes is pivotal in comprehending their varying biological functions and potential clinical applications. Delving into the study of different types of exosomes opens new avenues and strategies for clinical treatments, particularly in the realms of sports medicine and regenerative medicine, where exosomal research holds broad application prospects and profound scientific significance (15–17). In the field of sports medicine, exosome research is emerging as a novel and vibrant area of study. Investigating the role of exosomes in cellular communication, as well as their potential in repairing sports injuries and enhancing athletic performance, is expected to provide new theoretical foundations and experimental evidence for the clinical translation of sports medicine (7, 10, 18, 19).

Sports medicine is an interdisciplinary field of research dedicated to exploring the dynamic interplay between physical activity and human health (7, 20–26). This domain is committed to the prevention and treatment of sports injuries and the enhancement of athletic performance (7, 12, 23, 27–30). The scope of sports medicine encompasses but is not limited to, disciplines such as physiology, biomechanics, sports psychology, and sports nutrition (13, 31–35). Professionals in sports medicine frequently collaborate with physical therapists, sports psychologists, nutritionists, and experts from related fields to provide comprehensive services to athletes and the general populace. The primary objectives of sports medicine include the prevention and treatment of sports injuries, achieved through a profound understanding of the mechanisms underlying these injuries. This understanding fosters the development of novel preventive strategies and therapeutic approaches aimed at reducing the incidence of injuries and accelerating the recovery process (20, 22, 27). Additionally, sports medicine focuses on optimizing athletic performance through scientifically validated training methods, appropriate nutritional supplementation, and psychological adjustment strategies. This support empowers athletes and fitness enthusiasts to achieve peak performance levels. Furthermore, sports medicine advocates for a healthy lifestyle, utilizing education and outreach to encourage public participation in regular physical activity, thereby fostering physical and mental well-being and preventing chronic diseases (25, 26, 29, 36). As technology advances and society evolves, sports medicine will continue to expand into new domains and technologies to meet the growing demands for sports and health.

The intersection and significance of exosomes in the realm of sports medicine primarily manifest in their potential contributions to the recovery from sports injuries and the enhancement of athletic performance. Exosomes, serving as critical vectors in intercellular communication, are carriers of functional molecules such as mRNA, microRNA, and specific proteins. These molecules are instrumental in the early diagnosis and targeted treatment of various diseases. Recent studies have illuminated the significant role of exosomes in sports medicine, demonstrating that physical exercise can influence the content of exosomes, thus revealing their crucial role in this field (37).

Firstly, exosomes show remarkable potential in the realm of sports injury recovery. Research has underscored the therapeutic value of exosomes in the treatment of joint cartilage damage and osteoarthritis (OA). Joint cartilage injury, a common clinical issue, can lead to joint dysfunction, significant pain, and secondary osteoarthritis. Exosomes, originating from the endoplasmic reticulum and actively participating in cellular communication under both physiological and pathological conditions, have gained considerable attention across various domains. The significance of exosomes in the progression of osteoarthritis and their therapeutic value in cartilage repair and osteoarthritis treatment are progressively being recognized. The functional differences between various types and sources of exosomes are determined by their specific contents, influencing their role in the onset and progression of osteoarthritis and the treatment value and future therapeutic design strategies related to cartilage injuries/osteoarthritis (38). Secondly, the improvement of athletic performance through exosomes is of notable significance. Exosomes secreted by skeletal muscle cells can bind or fuse with the plasma membranes of target cells or be endocytosed, thereby transferring their effective payloads. This exosome-mediated communication between cells and organs can be viewed as a mode of transportation for myokines, potentially impacting athletic performance and the body's adaptability to exercise (39). In summary, the intersection and importance of exosomes in sports medicine are primarily evident in their potential contributions to the recovery from sports injuries and the improvement of athletic performance. As research on exosomes continues to deepen and the field of sports medicine evolves, exosomes may emerge as a pivotal therapeutic strategy for facilitating sports injury recovery and enhancing athletic performance.

The purpose of this scholarly review is to meticulously interrogate the role and significance of exosomes within the domain of sports medicine, offering novel perspectives and

foundational support for their clinical application. The specific knowledge gaps that our review seeks to address are distinct from previous studies, with a particular emphasis on translational research and personalized treatment of exosomes in sports medicine rather than merely discussing related mechanistic studies.

2 Biological basis of exosomes

2.1 Biogenesis and release of exosomes

The genesis and extracellular dispensation of exosomes are governed by a sophisticated and nuanced cascade of cellular events, necessitating the orchestrated participation of myriad organelles and biomolecular constituents. Herein, we delineate the sequential phases of this process:

The origin of exosome biogenesis is situated within the cellular endosomal framework. The odyssey begins with the invagination of the cell's plasma membrane, leading to the genesis of late endosomes, also termed multivesicular bodies (MVBs). Within the confines of these MVBs, a secondary invagination transpires, culminating in the creation of intraluminal vesicles (ILVs). These nascent vesicles harbor an array of biomolecular entities, including proteins, lipids, and RNA moieties, derived from both the cytosol and the cell's exterior membrane. Subsequently, MVBs are trafficked to the periphery of the cell, coalescing with the plasma membrane and facilitating the liberation of ILVs into the extracellular milieu, at which juncture they assume the designation of exosomes (40).

The incipient phase of exosomal biogenesis is catalyzed by the cell's endosomal recycling apparatus. This process commences with the selective internalization of molecular constituents from the plasma membrane into the incipient endosomes. These early endosomes undergo a series of biochemical and biophysical processes to transform into late endosomes and subsequently into MVBs. The formation of ILVs within MVBs is accomplished through the inward budding of the cell membrane. MVBs are then transported to the cell membrane, where they fuse and release exosomes into the extracellular space (40, 41).

The release mechanisms of exosomes may be regulated by the Endosomal Sorting Complex Required for Transport (ESCRT) machinery, although there is some contention regarding this. During exosome biogenesis, several key proteins such as Alix, flotillin, and TSG101 have been identified as participants in the process. These proteins are likely intricately involved with the fusion of the cell membrane and the release of exosomes (42).

Exosome biogenesis and release involve interactions among multiple organelles and biomolecules, as well as a variety of biochemical and biophysical processes. The cargo of exosomes, including proteins and miRNAs, and their sorting and packaging, are integral components of the exosome biogenesis process. With those functions, exosomes can efficiently transport specific biomolecules to target cells and act as a key role in intercellular communication (43).

2.2 Contents of exosomes

The molecular cargo of exosomes mirrors their progenitor cellular milieu, encompassing a panoply of moleculcar constituents such as lipids, proteins, and nucleic acid sequences. The exosomal membrane is characterized by a lipid bilayer architecture, enriched with cholesterol, sphingomyelin, ceramide, and diglycerides (44). Exosomes are notably replete with an array of transmembrane proteins, including tetraspanins, antigen-presenting complexes, an assortment of glycoproteins, and molecules facilitating cellular adhesion; alongside a cadre of luminal proteins comprising heat shock proteins, elements of the cytoskeleton, components of the endosomal sorting complexes required for transport (ESCRT) machinery, membranous transporters, fusogenic proteins, and an array of growth factors and cytokines (44, 45). Beyond these proteins, exosomal cargo encompasses nucleic acids, inclusive of DNA, messenger RNA, and microRNA.

The repertoire of exosomal contents further extends to lipids, metabolic intermediates, as well as proteins intrinsic to the cytoplasm and cellular interface. These molecular entities are capable of assimilation by recipient cells, exerting functional modulation (41, 42). Despite the obscurity surrounding the physiological raison of exosomes, burgeoning studies delineate their quintessential role as conveyors in intercellular signaling, orchestrating the communicative network among disparate cell types (41, 43). Proteomic scrutiny of exosomes secreted across various cellular origins has elucidated a conserved set of proteins, thereby postulating exosomes as a bona fide secretory subcellular organelle, while also identifying unique protein signatures indicative of the discrete functional capacities engendered by exosomes from divergent cellular provenances.

2.3 Biological activity and function of exosomes

Exosomes are extracellular vesicles produced by all cells, responsible for intercellular communication. Carrying genetic information and proteins, they transport molecules from one cell to another via vesicular transport, influencing biological processes such as immune responses, cell proliferation, and neural signaling (46). The bioactive cargo of exosomes may provide prognostic information for a range of diseases, including chronic inflammation, cardiovascular and renal diseases, neurodegenerative disorders, lipid metabolism diseases, and tumors (42). They contain components secreted by their parent cells (e.g., proteins, DNA, and RNA) and can be taken up by distant cells, affecting cellular functions and behavior (46).

Exosomes are recognized as a ubiquitous intracorporeal conveyance mechanism, replete with multifunctionality, ferrying an array of nucleic acids, proteins, lipids, and metabolic byproducts. They fulfill a critical function as conduits for both proximal and distal intercellular discourse in both physiological and pathological

states (46). The myriad functions and biological activities of exosomes are pivotal in the realms of cellular and pathobiological sciences, especially in the domains of intercellular signaling, inflammatory mediation, and the pathogenesis of disease.

Through the transport and transference of diverse bioactive moieties such as growth factors, cytokines, and microRNAs, exosomes exert regulatory control over the functional dynamics and behavioral responses of target cells (12, 47). For example, they can orchestrate immune responses by conveying molecules with immunomodulatory efficacy, such as antigen-presenting complexes and immunosuppressive agents, thereby modulating the functional status of immune cells. In addition, exosomes can impinge upon cellular proliferation, motility, and phenotypic differentiation by transmitting growth factors and cytokines (48). Within the neural milieu, exosomes demonstrate substantial bioactivity, influencing neuronal viability and functionality through the delivery of neurotrophic factors and neurotransmitter-related molecules (49). Notably, exosomes are implicated in oncogenesis and tumorigenesis, shaping the biological characteristics of neoplastic cells and the architectonics of the tumor microenvironment by the translocation of oncogenic and invasive factors (50).

Many types of exosomes are utilized as well in sports medicine, such as bone marrow MSC exosomes, adipose stem cell exosomes, embryonic MSC exosomes, umbilical cord MSC exosomes, dental pulp stem cell exosomes, and so on. In this article, we will describe the relevant studies that have been reported.

3 Exosomes in sports medicine-related research

3.1 Role of exosomes in tissue repair and regeneration

3.1.1 Muscle injury

The reparative role of exosomes in myotrauma has garnered considerable scrutiny, with findings affirming their ability to instigate muscular tissue regeneration through multifarious mechanisms. Predominantly, exosomes expedite myotrauma remediation and restoration by stimulating myogenic proliferation, catalyzing the phenotypic maturation of tendinous cells, fostering neurite outgrowth, and facilitating the proliferation of Schwannian cells (51). Exosomes derived from platelet-enriched plasma and mesenchymal stromal cells have been observed to significantly expedite the recuperation of muscular functionality (51).

Exosomes exert their influence by attenuating cellular pyroptosis and ameliorating ischemic myopathy. Empirical evidence suggests that exosomes sourced from mesenchymal stromal cells (MSCs) harbor the therapeutic potential for myopathic injuries, endorsing myoblastic differentiation in patients with Duchenne Muscular Dystrophy and in murine models of MDX (52). Furthermore, exosomes emanating from C2C12 myoblasts have been implicated in the promotion of myofibrillar regeneration, expediting lipogenesis within injured myocytes, mitigating myofibrosis, and accelerating reparative processes, which are attributed to the exosomal mediation of satellite cell proliferation and fibro-adipogenic progenitor cell differentiation (53).

Exosomes isolated from human adipose-derived mesenchymal stromal cells (AD-MSCs) have demonstrated promising therapeutic implications for myogenic regeneration. These exosomes are postulated as efficacious modalities for regenerative therapy, potentially inaugurating novel avenues for myotrauma remediation (54). Additionally, exosomes from bone marrow stromal cells (BMSCs) have been documented to enhance muscular healing by promoting M2 macrophagic polarization, whereas pro-inflammatory C2C12-derived exosomes have been associated with M1 macrophagic polarization and the suppression of myogenic repair mechanisms (12, 27, 55).

3.1.2 Frozen shoulder

Adhesive Capsulitis (AC), commonly manifested as Shoulder Stiffness (SS), is a pervasive affliction characterized by aggravated pain and a diminished range of articular motion (56). Pathologically, AC is classified as an inflammatory and fibrotic disorder. Investigations have unveiled that exosomes from Bone Marrow Stromal Cells (BMSCs) can suppress the expression of TGFBR1 through the mediation of let-7a-5p, consequently impeding the progression of AC (14, 28). Exosomes have emerged as a significant therapeutic entity in the management of diverse fibrotic maladies, with exosomes from various sources and their molecular cargoes—such as miRNAs, lncRNAs, and proteins—being contemplated as targeted therapeutic interventions. These entities can influence an array of cellular types and signal transduction pathways implicated in fibrosis (57, 58).

3.1.3 Tendon injury

Exosomes hold significant potential in the treatment of tendon injuries, encompassing Achilles and rotator cuff injuries. In the realm of tendon injury therapy, exosomes function through multiple mechanisms. These primarily include the suppression of inflammatory responses, modulation of macrophage polarization, regulation of gene expression, remodeling of the cellular microenvironment, restructuring of the extracellular matrix, and promotion of angiogenesis (59).

In the context of rotator cuff injury repair, exosomes exhibit considerable therapeutic potential. Studies have found that Mesenchymal Stem Cells (MSCs) can enhance healing post-rotator cuff repair through the release of exosomes (60). Additionally, purified exosome products are being explored to improve the surgical outcomes of rotator cuff tendon-bone healing and to reduce postoperative re-tear rates. This is achieved by focusing on biological and biomechanical factors (61).

In the treatment of Achilles tendon injuries, exosomes have also demonstrated the ability to promote the healing of injured tendons. Specifically, exosomes from tendon stem cells have been found to facilitate tendon injury healing through mechanisms that balance the synthesis and degradation of the tendon extracellular matrix (62). Concurrently, given that poor outcomes in many soft tissue injuries (such as Achilles tendon ruptures, rotator cuff tears, and flexor tendon injuries) are attributed to macrophage-induced

inflammation, researchers are investigating exosome-based therapies to suppress inflammation and thereby improve the treatment outcomes of tendon injuries (10).

3.1.4 Tendon-bone healing

Exosomes have demonstrated significant therapeutic potential in tendon-bone healing, particularly in the healing process following Anterior Cruciate Ligament Reconstruction (ACLR). Research indicates that exosomes derived from Bone Marrow Stromal Cells (BMSCs) can facilitate tendon-bone healing by modulating the polarization of M1/M2 type macrophages, a mechanism that has been validated in rat models of ACLR (63). Typically, ACLR may fail due to the inability to regenerate normal tissue at the tendon-bone junction and the formation of fibrous scar tissue at this interface (64). However, the combination of BMSC-derived exosomes with cartilage fragments has been shown to enhance healing at the tendon-bone interface, thereby increasing the success rate of ACLR (65).

The role of exosomes in facilitating tendon-bone healing primarily encompasses (1) inhibition of inflammatory responses and regulation of macrophage polarization, (2) control of gene expression, remodeling of the cellular microenvironment, and restructuring of the extracellular matrix, and (3) promotion of angiogenesis (59). Although studies have observed the effects of BMSC-derived exosomes (BMSC-Exos) on tendon-bone healing post-ACLR in rats, both *in vivo* and *in vitro*, elucidating the possible mechanisms, it remains unclear whether BMSC-Exos can facilitate tendon-bone healing in humans post-ACLR. Additionally, some studies have explored the effects of exosomes on tendon-bone healing and osteogenesis at the tendon-bone junction using rat ACLR models. For instance, by locally injecting IONP-Exos, BMSC-Exos, or PBS into the surgical knee joint, the retention of exosomes at the surgical site was observed. It was found that exosomes can promote bone formation at the tendon-bone junction (66).

3.1.5 Arthritis

Exosomes have demonstrated potential value in the treatment of arthritis, primarily manifesting in alleviating cartilage damage, inhibiting bone overgrowth, and modulating immune responses. Certain types of exosomes show potential advantages in reducing inflammation and regulating immune responses, which could be significant for the treatment of disease models including Rheumatoid Arthritis (RA). Exosomes can inhibit the proliferation of T lymphocytes indicative of inflammation and induce other anti-inflammatory effects (67, 68). Additionally, exosomes are involved in numerous physiological and pathological processes, influencing the development of various diseases, including Osteoarthritis (OA), by regulating intercellular communication (69).

In the treatment of Rheumatoid Arthritis, exosomes can act as therapeutic carriers. These extracellular vesicles from mice cells affect biological mechanisms and signal transduction by transporting genetic and proteomic components (67). Exosomes also play a role in RA-related arthritis, where these specialized function extracellular vesicles are responsible for transporting autoantigens and mediators to distant cells (68).

3.1.6 Cartilage injury

Firstly, exosomes can promote cartilage repair and regeneration by regulating cell migration, proliferation, differentiation, and extracellular matrix synthesis. For example, exosomes from Mesenchymal Stem Cells (MSCs) can modulate immune responses, reduce cell apoptosis, enhance cell proliferation, and initiate the proliferation and migration of chondroprogenitor cells (19, 38). Additionally, exosomes can alleviate cartilage injury, reduce bone overgrowth, inhibit the production of M1 macrophages, and promote the generation of M2 macrophages, while also decreasing levels of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α, and increasing levels of the anti-inflammatory cytokine IL-10 (70).

In the treatment of rheumatoid arthritis, exosomes can be used as therapeutic carriers; they are extracellular vesicles in mice that influence biological mechanisms and signaling and can play a role by transporting genetic and protein components (67). Exosome also plays a role in rheumatoid arthritis, these specialized functioning extracellular vesicles are responsible for transporting self-antigens and mediators to distant cells (68).

3.2 Potential of exosomes in enhancing athletic performance

3.2.1 Metabolic regulation

Exosomes, small vesicles originating from the endoplasmic reticulum, circulate through blood and other bodily fluids, providing a unique platform for intercellular communication. Recent research highlights the pivotal role of exosomes in metabolic regulation during physical activity, especially in endurance exercises (39, 71, 72). Here is an overview of the role of exosomes in exercise-induced metabolic regulation.

Bioactive Molecules in Exosomes: During exercise, the bioactive molecules within exosomes, such as peptides and nucleic acids (collectively termed exerkines), undergo alterations. Studies indicate that endurance exercise induces the release of exosomes, particularly peptides and nucleic acids from skeletal muscle and other tissues (73, 74). These bioactive molecules can exert endocrine-like effects, impacting the pathophysiology of conditions like obesity and Type 2 Diabetes (73–75).

Intercellular Communication Role of Exosomes: Functioning as endocrine-like vesicles, exosomes can carry proteins, microRNAs, and other nucleic acids, facilitating communication between cells and tissues, and even among organs. This contributes to the formation of a coordinated metabolic network within the body (72, 74, 76).

Future Therapeutic Potential of Exosomes: Researchers hypothesize that future therapies for obesity and Type 2 Diabetes might involve the use of modified exosomes enriched with exerkines. These exosomes, through their contained bioactive molecules, could positively regulate metabolic health, offering new therapeutic possibilities for these metabolic diseases (73, 74, 76).

Release of Exosomes and Exercise Intensity: Interestingly, studies have also discovered that with increasing exercise intensity, the concentration of exosomes in circulation correspondingly rises.

This further suggests a critical role of exosomes in metabolic regulation during exercise (73, 77).

3.2.2 Anti-fatigue and antioxidant potential of exosomes

Recent research has unveiled the significance of exosomes in combating fatigue and oxidative stress (78–81). In the realm of anti-fatigue, exosomes are believed to improve cellular energy metabolism and enhance cells' resistance to fatigue and damage. The mechanistic repertoire of exosomes in cellular bioenergetics encompasses the enhancement of mitochondrial efficacy, the amplification of adenosine triphosphate (ATP) synthesis, and the optimization of oxidative phosphorylation efficiency (78, 79). Additionally, the exosomal content of select RNA species and proteins may actuate specific metabolic cascades, thereby underpinning recuperative processes subsequent to physical exertion (78, 80, 81).

In the context of antioxidative activity, exosomes possess proficiency in the sequestration and neutralization of reactive oxygen species (80–82). This antioxidative mechanism is predominantly ascribed to the presence of enzymatic constituents within exosomes, such as catalases, sulfiredoxins, and an array of redox-modulating molecules. These enzymes are adept at obliterating surplus free radicals, thereby mitigating oxidative stress and attenuating cellular damage. Moreover, exosomes exert a modulatory effect on intracellular antioxidant pathways, including the Nrf2 axis, thereby reinforcing the cellular defense against oxidative insults (82). The exosomal complement of bioactive RNA and proteins also fine-tunes the redox equilibrium, which bolsters their antioxidative properties.

Empirical investigations have corroborated that exosomal antioxidants, for instance, glutathione and superoxide dismutase, are capable of neutralizing excessive reactive species, ameliorating oxidative detriment (82, 83). This plays an instrumental role in diminishing muscular fatigue post-exercise and decelerating cellular senescence. Furthermore, exosomal antioxidants are pivotal in sustaining intracellular redox homeostasis, thus endorsing normative cellular operations (84, 85). Additionally, exosomes harbor an ensemble of anti-fatigue molecular entities such as heat shock proteins and antioxidative enzymes, orchestrating the cellular stress response and fostering recuperation (82, 86). In response to fatigue-inducing stimuli, cellular systems can escalate the release of these anti-fatigue proteins via exosomes, thereby enhancing endurance and resilience.

3.2.3 Enhancing athlete performance

Exosomes are implicated as pivotal entities in the mediation of myocyte repair, a process of particular pertinence to athletes undergoing rigorous training regimens (12, 52, 53, 87). In the aftermath of high-intensity exercise, athletes frequently endure microtraumas within muscular tissues, precipitating inflammation and consequent discomfort. Investigations have substantiated that exosomes possess the capability to encapsulate and convey growth factors, microRNAs, and an array of other bioactive compounds to myocytes experiencing trauma. This facilitates a cascade of cellular activities inclusive of proliferation, motility, and morphological specialization, thereby expediting the restoration and rejuvenation of compromised tissues. In addition, exosomes have been observed to potentiate athletic stamina. The myriad bioactive molecules harbored within exosomes are known to initiate a spectrum of metabolic processes that amplify the efficacy of mitochondrial oxidative phosphorylation (39, 88, 89). This suggests that in the milieu of sustained and intensive exertion, the myocytes of athletes are equipped to uphold an elevated rate of adenosine triphosphate (ATP) synthesis, thereby augmenting endurance.

In addition, exosomes can augment athletes' antioxidative capabilities. During exercise, increased oxygen consumption leads to the production of reactive oxygen species (ROS), which can cause cellular damage (8, 48, 82, 83). However, antioxidative enzymes and other molecules within exosomes can effectively scavenge these free radicals, protecting cells from damage and expediting the recovery process. Lastly, exosomes can modulate immune responses, reducing post-exercise inflammatory reactions. Specific proteins and RNA molecules within exosomes can influence the activation and secretion of immune cells, thereby inhibiting the production and release of inflammatory cytokines and reducing post-exercise inflammation (Figure 1) (50, 90, 91).

4 Perspectives and challenges

4.1 Technological innovation and optimization

Future research endeavors will continually drive innovation and optimization in exosome extraction, purification, and preparation techniques. This includes developing more efficient extraction methods, enhancing purification efficacy, and reducing production costs. Technological advancements will contribute to improving the quality and yield of exosome formulations, thereby increasing their feasibility for clinical applications (3, 92–94). Emerging technologies shaping exosome research include advanced nanoengineering approaches for precision therapeutics. Techniques like aptamer-guided targeting allow for the development of exosomal delivery systems that are more specific and effective. Additionally, microfluidic engineering and post-isolation modifications of exosomes are enhancing their application in nanomedicine. These innovations are crucial in refining exosome-based therapies, making them more targeted and efficient for use in sports medicine and beyond.

4.2 Multidisciplinary collaboration

Exosome research necessitates collaboration across multiple disciplines, including cell biology, molecular biology, clinical medicine, and engineering. Such collaboration will aid in a deeper understanding of the biological properties, mechanisms, and clinical applications of exosomes and help address various

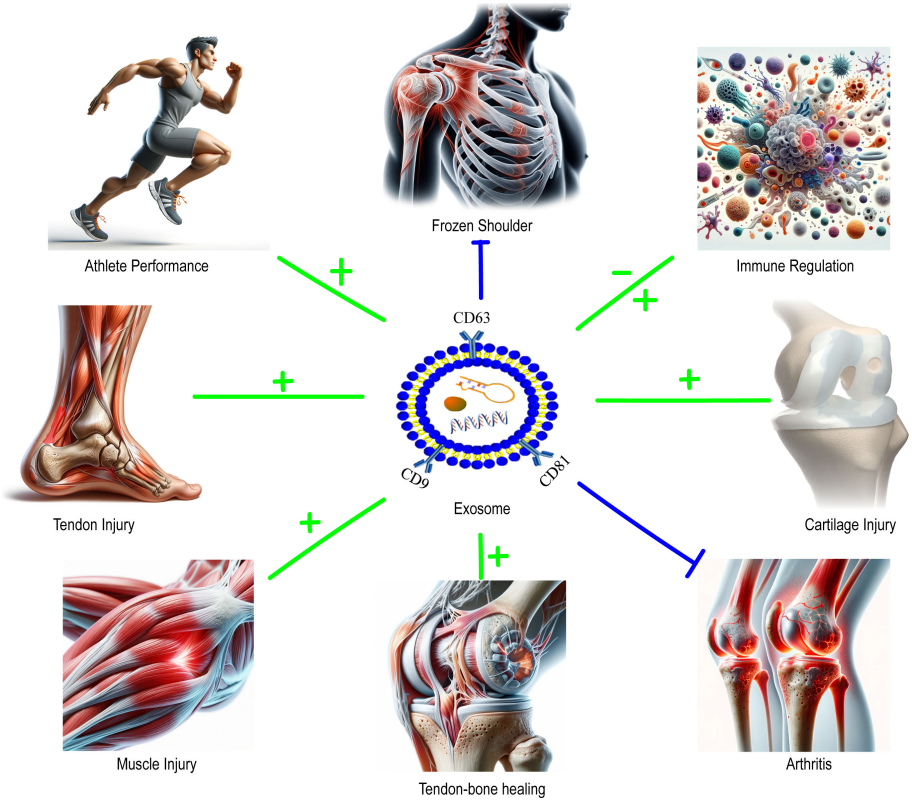


FIGURE 1
The role of exosomes on sports medicine-related issues.

challenges in exosome therapy. Cross-disciplinary teamwork will be a significant trend in future exosome research (95, 96). For example, bioengineering and nanoengineering techniques can be used to develop targeted exosomal delivery systems. These systems can be engineered with aptamers or chemical antibodies for precision medicine, enhancing the specificity and efficacy of exosome-based therapies (97–99).

4.3 Personalized therapy

The future development of exosome therapy will increasingly focus on personalized treatment strategies. Tailoring exosome formulations and treatment plans based on patient's genetic, molecular, and physiological characteristics can enhance therapeutic outcomes and minimize unnecessary side effects (100–102). The extent to which AI and machine learning can aid in the progression of personalized therapy needs to be explored further.

4.4 Clinical trials and regulatory approval

More clinical trials are needed in the future to validate the safety and efficacy of exosome therapy. Strict regulatory approval and compliance are essential to ensure patient safety and treatment reliability. Formulating clear clinical trial protocols and regulatory policies will be a crucial task moving forward (103, 104).

4.5 Long-term safety and efficacy monitoring

Monitoring the long-term safety and efficacy of exosome therapy poses one of the future challenges. As patients undergo prolonged exosome therapy, effective monitoring methods need to be established to assess long-term impacts and side effects. This will require large-scale patient follow-up studies and data analysis (105, 106).

4.6 Standardization of production and storage

To meet future clinical demands, standardized processes for the production and storage of exosome formulations are necessary. This includes ensuring consistency, stability, and purity of exosome formulations to meet diverse patient needs. Standardized processes will aid in enhancing the scalability and feasibility of exosome therapy (71, 107, 108).

4.7 Limitation and clinic-lab gap

In the field of exosome research in sports medicine, the translation of laboratory findings to clinical practice faces several challenges. These include ensuring the stability and consistent quality of exosome preparations, understanding the complex mechanisms of exosome-

cell interactions, and addressing safety concerns such as immune responses and potential long-term effects. Regulatory hurdles also play a significant role, as there is a need for standardized protocols and guidelines for exosome therapy. Moreover, ethical considerations, particularly in the context of enhancing athletic performance, must be thoroughly addressed. These challenges require multidisciplinary collaboration and advancements in both research methodologies and regulatory frameworks.

In summary, exosomes hold vast potential for clinical treatment in sports medicine-related diseases. However, future development must address challenges in technological innovation, multidisciplinary collaboration, personalized therapy, clinical trials and regulatory approval, long-term monitoring, and standardization of production. By overcoming these challenges, exosome therapy has the potential to offer more effective and safer treatment strategies for diseases in the field of sports medicine, improving patients' quality of life. This will require close collaboration among the scientific community, medical institutions, and governmental regulatory bodies to propel the future development of exosome therapy.

5 Summary

Research advancements in the field of sports medicine reveal the substantial potential of exosomes, which have already achieved some encouraging results in clinical translation. Here's a summary of the research progress and clinical translation potential of exosomes in sports medicine, emphasizing future research directions.

5.1 Research progress

Anti-inflammatory and Antioxidative Effects: Exosomes, rich in various anti-inflammatory factors and antioxidants, emerge as powerful tools for treating exercise-related diseases. Accompanying sports injuries are inflammation and oxidative stress, where exosomes can facilitate healing and recovery by inhibiting inflammatory responses and reducing oxidative damage.

Tissue Repair: Growth factors and signaling molecules in exosomes have the potential to promote tissue repair and regeneration. This is particularly vital for the treatment of sports-related muscle, skeletal injuries, and cartilage damage. Exosomes can activate stem cells and aid in repairing damaged tissues.

Optimization of Athletic Performance: Some studies have also explored the application of exosomes in enhancing athletic performance. Growth factors and proteins within exosomes can stimulate muscle growth and repair, improving muscle strength and endurance, and thereby aiding in enhancing athletic performance.

5.2 Clinical translation potential

Treating Sports Injuries: Exosome therapy can be employed in treating sports-related injuries such as muscle strains, fracture healing, and cartilage repair. It can accelerate the healing process, and reduce pain and inflammation, thereby shortening recovery time.

Joint Health: For joint diseases like osteoarthritis, exosome therapy shows potential in anti-inflammatory and joint-protective actions. Injecting exosomes into damaged joints can alleviate pain and improve joint functionality.

Cardiovascular Rehabilitation: Antioxidants and cardioprotective factors in exosomes aid in cardiovascular rehabilitation. They can reduce cardiac damage, improve cardiac function, and lower the risk of cardiovascular diseases.

In summary, the research progress and clinical translational potential of exosomes in sports medicine are exciting, but further in-depth studies and clinical validation are still needed. Future research directions should focus on an in-depth understanding of the mechanism, individualized treatment, more clinical trials, and the establishment of standardized preparation and quality control processes to fully explore the application prospects of exosomes in the field of sports medicine.

Author contributions

HH: Writing – original draft, Writing – review & editing. PC: Writing – original draft, Writing – review & editing. XF: Investigation, Writing – original draft. YQ: Data curation, Investigation, Writing – original draft. ZP: Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing. TZ: Conceptualization, Data curation, Funding acquisition, Supervision, Writing – original draft. QW: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by 1. Suzhou Clinical Key Disease Diagnosis and Treatment Technology Special Project (LCZX202127); 2.Kunshan High-level Medical Talent Program Project (Kunshan Health [2019] No. 6); 3.Project of National Clinical Research Base of Traditional Chinese Medicine (JD2023SZ23). Medical Research Foundation of Guangdong Province (B2021198).

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. Estébanez B, Jiménez-Pavón D, Huang C, Cuevas MJ, González-Gallego J. Effects of exercise on exosome release and cargo in *in vivo* and ex vivo models: A systematic review. *J Cell Physiol* (2021) 236:3336–53. doi: 10.1002/jcp.30094

2. Pegtel DM, Gould SJ. Exosomes. *Annu Rev Biochem* (2019) 88:487–514. doi: 10.1146/annurev-biochem-013118-111902

3. Cong M, Tan S, Li S, Gao L, Huang L, Zhang H-G, et al. Technology insight: Plant-derived vesicles-How far from the clinical biotherapeutics and therapeutic drug carriers? *Adv Drug Delivery Rev* (2022) 182:114108. doi: 10.1016/j.addr.2021.114108

4. Wang J, Li X, Wang S, Cui J, Ren X, Su J. Bone-targeted exosomes: strategies and applications. *Adv Heal Mater* (2023) 12(18):e2203361. doi: 10.1002/adhm.202203361

5. Liu Y, Weng W, Gao R, Liu Y. New insights for cellular and molecular mechanisms of aging and aging-related diseases: herbal medicine as potential therapeutic approach. *Oxid Med Cell Longev* (2019) 2019:4598167. doi: 10.1155/2019/4598167

6. 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

7. Chen Y, Luo Z, Sun Y, Zhou Y, Han Z, Yang X, et al. The effect of denture-wearing on physical activity is associated with cognitive impairment in the elderly: A cross-sectional study based on the CHARLS database. *Front Neurosci* (2022) 16:925398. doi: 10.3389/fnins.2022.925398

8. Wang Y, Zhao R, Liu D, Deng W, Xu G, Liu W, et al. Exosomes derived from miR-214-enriched bone marrow-derived mesenchymal stem cells regulate oxidative damage in cardiac stem cells by targeting caMKII. *Oxid Med Cell Longev* (2018) 2018:4971261. doi: 10.1155/2018/4971261

9. Ying W, Riopel M, Bandyopadhyay G, Dong Y, Birmingham A, Seo JB, et al. Adipose tissue macrophage-derived exosomal miRNAs can modulate *in vivo* and *in vitro* insulin sensitivity. *Cell* (2017) 171:372–384.e12. doi: 10.1016/j.cell.2017.08.035

10. Iyer SR, Scheiber AL, Yarowsky P, Henn RF, Otsuru S, Lovering RM. Exosomes isolated from platelet-rich plasma and mesenchymal stem cells promote recovery of function after muscle injury. *Am J Sports Med* (2020) 48:2277–86. doi: 10.1177/0363546520926462

11. Chen Y, Sun Y, Luo Z, Lin J, Qi B, Ying C, et al. Potential mechanism Q4 underlying exercise upregulated circulating blood exosome miR-215-5p to prevent necroptosis of neuronal cells and a model for early diagnosis of alzheimer's disease. *Front Aging Neurosci* (2022) 14, 1–15. doi: 10.3389/fnagi.2022.860364

12. Luo Z, Qi B, Sun Y, Chen Y, Lin J, Qin H, et al. Engineering bioactive M2 macrophage-polarized, anti-inflammatory, miRNA-based liposomes for functional muscle repair: from exosomal mechanisms to biomaterials. *Small* (2022) 18:2201957. doi: 10.1002/smll.202201957

13. Feng X, Peng Z, Yuan L, Jin M, Hu H, Peng X, et al. Research progress of exosomes in pathogenesis, diagnosis, and treatment of ocular diseases. *Front Bioeng Biotechnol* (2023) 11:1100310. doi: 10.3389/fbioe.2023.1100310

14. 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

15. Forterre A, Jalabert A, Berger E, Baudet M, Chikh K, Errazuriz E, et al. Proteomic analysis of C2C12 myoblast and myotube exosome-like vesicles: A new paradigm for myoblast-myotube cross talk? *PloS One* (2014) 9:e84153. doi: 10.1371/journal.pone.0084153

16. Puno MR, Weick EM, Das M, Lima CD. SnapShot: the RNA exosome. *Cell* (2019) 179:282–282.e1. doi: 10.1016/j.cell.2019.09.005

17. Pomatto M, Gai C, Negro F, Cedrino M, Grange C, Ceccotti E, et al. Differential therapeutic effect of extracellular vesicles derived by bone marrow and adipose mesenchymal stem cells on wound healing of diabetic ulcers and correlation to their cargoes. *Int J Mol Sci* (2021) 22:1–26. doi: 10.3390/ijms22083851

18. Wang C, Hu Q, Song W, Yu W, He Y. Adipose stem cell–derived exosomes decrease fatty infiltration and enhance rotator cuff healing in a rabbit model of chronic tears. *Am J Sports Med* (2020) 48:1456–64. doi: 10.1177/0363546520908847

19. 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

20. 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

21. Lin J, Yang X, Liu S, Luo Z, Chen Q, Sun Y, et al. Long non-coding RNA MFAT1 promotes skeletal muscle fibrosis by modulating the miR-135a-5p-Tgfbr2/Smad4 axis as a ceRNA. *J Cell Mol Med* (2021) 25:4420–33. doi: 10.1111/jcmm.16508

22. 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

23. Wang C, Sun Y, Ding Z, Lin J, Luo Z, Chen J. Influence of femoral version on the outcomes of hip arthroscopic surgery for femoroacetabular impingement or labral tears: A systematic review and meta-analysis. *Orthop J Sport Med* (2021) 9:232596712110091. doi: 10.1177/23259671211009192

24. Luo Z, Zhang T, Chen S. Exercise prescription: pioneering the "Third pole" for clinical health management. *Research* (2023) 6:1–4. doi: 10.34133/research.0284

25. Luo Z, Wan R, Liu S, Feng X, Peng Z, Wang Q, et al. Mechanisms of exercise in the treatment of lung cancer – a mini-review. *Front Immunol* (2023) 14:1244764. doi: 10.3389/fimmu.2023.1244764

26. 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

27. Luo Z, Lin J, Sun Y, Wang C, Chen J. Bone marrow stromal cell-derived exosomes promote muscle healing following contusion through macrophage polarization. *Stem Cells Dev* (2021) 30:135–48. doi: 10.1089/scd.2020.0167

28. 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

29. Luo Z, He Z, Qin H, Chen Y, Qi B, Lin J, et al. Exercise-induced IL-15 acted as a positive prognostic implication and tumor-suppressed role in pan-cancer. *Front Pharmacol* (2022) 13:1053137. doi: 10.3389/fphar.2022.1053137

30. Wan R, Zhang H, Liu S, Jiao C, Chen H, Luo Z, et al. Role of fibro-adipogenic progenitors in skeletal muscle aging. *Aging Pathobiol Ther* (2023) 5:72–8. doi: 10.31491/apt.2023.06.116

31. Qin H, Luo Z, Sun Y, He Z, Qi B, Chen Y, et al. Low-intensity pulsed ultrasound promotes skeletal muscle regeneration via modulating the inflammatory immune microenvironment. *Int J Biol Sci* (2023) 19:1123–45. doi: 10.7150/ijbs.79685

32. Qin H, Du L, Luo Z, He Z, Wang Q, Chen S, et al. The therapeutic effects of low-intensity pulsed ultrasound in musculoskeletal soft tissue injuries: Focusing on the molecular mechanism. *Front Bioeng Biotechnol* (2022) 10:1080430. doi: 10.3389/fbioe.2022.1080430

33. Lin J, Luo Z, Liu S, Chen Q, Liu S, Chen J. Long non-coding RNA H19 promotes myoblast fibrogenesis via regulating the miR-20a-5p-Tgfbr2 axis. *Clin Exp Pharmacol Physiol* (2021) 48:921–31. doi: 10.1111/1440-1681.13489

34. Hu T, Luo Z, Li K, Wang S, Wu D. Zanthoxylum nitidum extract attenuates BMP-2-induced inflammation and hyperpermeability. *Biosci Rep* (2020) 40(10): BSR20201098. doi: 10.1042/BSR20201098

35. Chen Y, Sun Y, Xu Y, Lin WW, Luo Z, Han Z, et al. Single-cell integration analysis of heterotopic ossification and fibrocartilage developmental lineage: endoplasmic reticulum stress effector xbp1 transcriptionally regulates the notch signaling pathway to mediate fibrocartilage differentiation. *Oxid Med Cell Longev* (2021) 2021:1–29. doi: 10.1155/2021/7663366

36. Chen P, Wang D, Shen H, Yu L, Gao Q, Mao L, et al. Physical activity and health in Chinese children and adolescents: expert consensus statement (2020). *Br J Sports Med* (2020) 54:1321–31. doi: 10.1136/bjsports-2020-102261

37. Li Y, Han C, Wang J, Zhou J, Liang C, Ranganna K, et al. Exosomes mediate the beneficial effects of exercise. *Adv Exp Med Biol* (2017) 1000:333–53. doi: 10.1007/978-981-10-4304-8_18

38. Zhou Q, Cai Y, Jiang Y, Lin X. Exosomes in osteoarthritis and cartilage injury: advanced development and potential therapeutic strategies. *Int J Biol Sci* (2020) 16:1811–20. doi: 10.7150/ijbs.41637

39. Aoi W, Tanimura Y. Roles of skeletal muscle-derived exosomes in organ metabolic and immunological communication. *Front Endocrinol (Lausanne)* (2021) 12:697204. doi: 10.3389/fendo.2021.697204

40. Krylova SV, Feng D. The machinery of exosomes: biogenesis, release, and uptake. *Int J Mol Sci* (2023) 24:1337. doi: 10.3390/ijms24021337

41. Hessvik NP, Llorente A. Current knowledge on exosome biogenesis and release. *Cell Mol Life Sci* (2018) 75:193–208. doi: 10.1007/s00018-017-2595-9

42. Zhang Y, Liu Y, Liu H, Tang WH. Exosomes: biogenesis, biologic function and clinical potential. *Cell Biosci* (2019) 9:19. doi: 10.1186/s13578-019-0282-2

43. Xie S, Zhang Q, Jiang L. Current knowledge on exosome biogenesis, cargo-sorting mechanism and therapeutic implications. *Membranes (Basel)* (2022) 12:498. doi: 10.3390/membranes12050498

44. Isola AL, Chen S. Exosomes: the messengers of health and disease. *Curr Neuropharmacol* (2017) 15:157–65. doi: 10.2174/1570159x14666160825160421

45. Fazaeli H, Kalhor N, Naserpour L, Davoodi F, Sheykhhasan M, Hosseini SKE, et al. A comparative study on the effect of exosomes secreted by mesenchymal stem cells derived from adipose and bone marrow tissues in the treatment of osteoarthritis-induced mouse model. *BioMed Res Int* (2021) 2021:9688138. doi: 10.1155/2021/9688138

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

47. Mori MA, Ludwig RG, Garcia-Martin R, Brandão BB, Kahn CR. Extracellular miRNAs: from biomarkers to mediators of physiology and disease. *Cell Metab* (2019) 30:656–73. doi: 10.1016/j.cmet.2019.07.011

48. Yamaguchi T, Izumi Y, Nakamura Y, Yamazaki T, Shiota M, Sano S, et al. Repeated remote ischemic conditioning attenuates left ventricular remodeling via

exosome-mediated intercellular communication on chronic heart failure after myocardial infarction. *Int J Cardiol* (2015) 178:239–46. doi: 10.1016/j.ijcard.2014.10.144

49. Ghamloush F, Ghayad SE, Rammal G, Fahs A, Ayoub AJ, Merabi Z, et al. The PAX3-FOXO1 oncogene alters exosome miRNA content and leads to paracrine effects mediated by exosomal miR-486. *Sci Rep* (2019) 9:1–12. doi: 10.1038/s41598-019-50592-4

50. Nabet BY, Qiu Y, Shabason JE, Wu TJ, Yoon T, Kim BC, et al. Exosome RNA unshielding couples stromal activation to pattern recognition receptor signaling in cancer. *Cell* (2017) 170:352–366.e13. doi: 10.1016/j.cell.2017.06.031

51. Wan R, Hussain A, Behfar A, Moran SL, Zhao C. The therapeutic potential of exosomes in soft tissue repair and regeneration. *Int J Mol Sci* (2022) 23:3869. doi: 10.3390/ijms23073869

52. Wang Z, Yang J, Sun X, Sun X, Yang G, Shi X. Exosome-mediated regulatory mechanisms in skeletal muscle: a narrative review. *J Zhejiang Univ Sci B* (2023) 24:1–14. doi: 10.1631/jzus.B2200243

53. Ji S, Ma P, Cao X, Wang J, Yu X, Luo X, et al. Myoblast-derived exosomes promote the repair and regeneration of injured skeletal muscle in mice. *FEBS Open Bio* (2022) 12:2213–26. doi: 10.1002/2211-5463.13504

54. Byun S-E, Sim C, Chung Y, Kim HK, Park S, Kim DK, et al. Skeletal muscle regeneration by the exosomes of adipose tissue-derived mesenchymal stem cells. *Curr Issues Mol Biol* (2021) 43:1473–88. doi: 10.3390/cimb43030104

55. 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

56. Itoi E, Arce G, Bain GI, Diercks RL, Guttmann D, Imhoff AB, et al. Shoulder stiffness: current concepts and concerns. *Arthrosc J Arthrosc Relat Surg Off Publ Arthrosc Assoc North Am Int Arthrosc Assoc* (2016) 32:1402–14. doi: 10.1016/j.arthro.2016.03.024

57. Liu Y, Zheng Y, Yang Y, Liu K, Wu J, Gao P, et al. Exosomes in liver fibrosis: The role of modulating hepatic stellate cells and immune cells, and prospects for clinical applications. *Front Immunol* (2023) 14:1133297. doi: 10.3389/fimmu.2023.1133297

58. Qin X-J, Zhang J-X, Wang R-L. Exosomes as mediators and biomarkers in fibrosis. *biomark Med* (2020) 14:697–712. doi: 10.2217/bmm-2019-0368

59. Zou M, Wang J, Shao Z. Therapeutic potential of exosomes in tendon and tendon-bone healing: A systematic review of preclinical studies. *J Funct Biomater* (2023) 14:299. doi: 10.3390/jfb14060299

60. Connor DE, Paulus JA, Dabestani PJ, Thankam FK, Dilisio MF, Gross RM, et al. Therapeutic potential of exosomes in rotator cuff tendon healing. *J Bone Miner Metab* (2019) 37:759–67. doi: 10.1007/s00774-019-01013-z

61. Ren Y, Zhang S, Wang Y, Jacobson DS, Reisdorf RL, Kuroiwa T, et al. Effects of purified exosome product on rotator cuff tendon-bone healing *in vitro* and *in vivo*. *Biomaterials* (2021) 276:121019. doi: 10.1016/j.biomaterials.2021.121019

62. Wang Y, He G, Guo Y, Tang H, Shi Y, Bian X, et al. Exosomes from tendon stem cells promote injury tendon healing through balancing synthesis and degradation of the tendon extracellular matrix. *J Cell Mol Med* (2019) 23:5475–85. doi: 10.1111/jcmm.14430

63. Li Z, Li Q, Tong K, Zhu J, Wang H, Chen B, et al. BMSC-derived exosomes promote tendon-bone healing after anterior cruciate ligament reconstruction by regulating M1/M2 macrophage polarization in rats. *Stem Cell Res Ther* (2022) 13:295. doi: 10.1186/s13287-022-02975-0

64. Sun Y, Chen W, Hao Y, Gu X, Liu X, Cai J, et al. Stem cell–conditioned medium promotes graft remodeling of midsubstance and intratunnel incorporation after anterior cruciate ligament reconstruction in a rat model. *Am J Sports Med* (2019) 47:2327–37. doi: 10.1177/0363546519859324

65. Zhang C, Jiang C, Jin J, Lei P, Cai Y, Wang Y. Cartilage fragments combined with BMSCs-Derived exosomes can promote tendon-bone healing after ACL reconstruction. *Mater Today Bio* (2023) 23:100819. doi: 10.1016/j.mtbio.2023.100819

66. Wu X-D, Kang L, Tian J, Wu Y, Huang Y, Liu J, et al. Exosomes derived from magnetically actuated bone mesenchymal stem cells promote tendon-bone healing through the miR-21-5p/SMAD7 pathway. *Mater Today Bio* (2022) 15:100319. doi: 10.1016/j.mtbio.2022.100319

67. Singh A, Behl T, Sehgal A, Singh S, Sharma N, Naqwi M, et al. Exploring the role of exosomes in rheumatoid arthritis. *Inflammopharmacology* (2023) 31:119–28. doi: 10.1007/s10787-022-01100-0

68. Heydari R, Koohi F, Rasouli M, Rezaei K, Abbasgholinejad E, Bekeschus S, et al. Exosomes as rheumatoid arthritis diagnostic biomarkers and therapeutic agents. *Vaccines* (2023) 11:687. doi: 10.3390/vaccines11030687

69. Ni Z, Zhou S, Li S, Kuang L, Chen H, Luo X, et al. Exosomes: roles and therapeutic potential in osteoarthritis. *Bone Res* (2020) 8:25. doi: 10.1038/s41413-020-0100-9

70. Wang R, Xu B. TGF-β1-modified MSC-derived exosomal miR-135b attenuates cartilage injury via promoting M2 synovial macrophage polarization by targeting MAPK6. *Cell Tissue Res* (2021) 384:113–27. doi: 10.1007/s00441-020-03319-1

71. Villatoro AJ, Alcoholado C, Martín-Astorga MC, Fernández V, Cifuentes M, Becerra J. Comparative analysis and characterization of soluble factors and exosomes from cultured adipose tissue and bone marrow mesenchymal stem cells in canine species. *Vet Immunol Immunopathol* (2019) 208:6–15. doi: 10.1016/j.vetimm.2018.12.003

72. Castaño C, Mirasierra M, Vallejo M, Novials A, Párrizas M. Delivery of muscle-derived exosomal miRNAs induced by HIIT improves insulin sensitivity through down-regulation of hepatic FoxO1 in mice. *Proc Natl Acad Sci* (2020) 117:30335–43. doi: 10.1073/pnas.2016112117

73. Safdar A, Saleem A, Tarnopolsky MA. The potential of endurance exercise-derived exosomes to treat metabolic diseases. *Nat Rev Endocrinol* (2016) 12:504–17. doi: 10.1038/nrendo.2016.76

74. Safdar A, Tarnopolsky MA. Exosomes as mediators of the systemic adaptations to endurance exercise. *Cold Spring Harb Perspect Med* (2018) 8:a029827. doi: 10.1101/cshperspect.a029827

75. Song M, Han L, Chen F, Wang D, Wang F, Zhang L, et al. Adipocyte-derived exosomes carrying sonic hedgehog mediate M1 macrophage polarization-induced insulin resistance via ptch and PI3K pathways. *Cell Physiol Biochem* (2018) 48:1416–32. doi: 10.1159/000492252

76. Isaac R, Reis FCG, Ying W, Olefsky JM. Exosomes as mediators of intercellular crosstalk in metabolism. *Cell Metab* (2021) 33:1744–62. doi: 10.1016/j.cmet.2021.08.006

77. Ma C, Wang J, Liu H, Chen Y, Ma X, Chen S, et al. Moderate exercise enhances endothelial progenitor cell exosomes release and function. *Med Sci Sport Exerc* (2018) 50:2024–32. doi: 10.1249/MSS.0000000000001672

78. Huang Q, Wu M, Wu X, Zhang Y, Xia Y. Muscle-to-tumor crosstalk: The effect of exercise-induced myokine on cancer progression. *Biochim Biophys Acta - Rev Cancer* (2022) 1877:188761. doi: 10.1016/j.bbcan.2022.188761

79. Mytidou C, Koutsoulidou A, Zachariou M, Prokopi M, Kapnisis K, Spyrou GM, et al. Age-Related Exosomal and Endogenous Expression Patterns of miR-1, miR-133a, miR-133b, and miR-206 in Skeletal Muscles. *Front Physiol* (2021) 12:708278. doi: 10.3389/fphys.2021.708278

80. Fulzele S, Mendhe B, Khayrullin A, Johnson M, Kaiser H, Liu Y, et al. Extracellular vesicles secreted from mouse muscle cells suppress osteoclast formation: Roles of mitochondrial energy metabolism. *Bone* (2017) 20:1–3. doi: 10.1016/j.stem.2016.12.009

81. Fulzele S, Mendhe B, Khayrullin A, Johnson M, Kaiser H, Liu Y, et al. Muscle-derived miR-34a increases with age in circulating extracellular vesicles and induces senescence of bone marrow stem cells. *Aging (Albany NY)* (2019) 11:1791–803. doi: 10.18632/aging.101874

82. Wang T, Jian Z, Baskys A, Yang J, Li J, Guo H, et al. MSC-derived exosomes protect against oxidative stress-induced skin injury via adaptive regulation of the NRF2 defense system. *Biomaterials* (2020) 257:120264. doi: 10.1016/j.biomaterials.2020.120264

83. Fahs A, Ramadan F, Ghamloush F, Ayoub AJ, Ahmad FA, Kobeissy F, et al. Effects of the oncoprotein PAX3-FOXO1 on modulation of exosomes function and protein content: Implications on oxidative stress protection and enhanced plasticity. *Front Oncol* (2020) 10:1784. doi: 10.3389/fonc.2020.01784

84. Mytidou C, Koutsoulidou A, Katsioloudi A, Prokopi M, Kapnisis K, Michailidou K, et al. Muscle-derived exosomes encapsulate myomiRs and are involved in local skeletal muscle tissue communication. *FASEB J* (2021) 35:1–21. doi: 10.1096/fj.201902468RR

85. Lu J, Yang X, He C, Chen Y, Li C, Li S, et al. Rejuvenation of tendon stem/progenitor cells for functional tendon regeneration through platelet-derived exosomes loaded with recombinant Yap1. *Acta Biomater* (2023) 161:80–99. doi: 10.1016/j.actbio.2023.02.018

86. Guescini M, Maggio S, Ceccaroli P, Battistelli M, Annibalini G, Piccoli G, et al. Extracellular vesicles released by oxidatively injured or intact C2C12 myotubes promote distinct responses converging toward myogenesis. *Int J Mol Sci* (2017) 18:2488. doi: 10.3390/ijms18112488

87. Yan B, Zhang Y, Liang C, Liu B, Ding F, Wang Y, et al. Stem cell-derived exosomes prevent pyroptosis and repair ischemic muscle injury through a novel exosome/circHIPK3/ FOXO3a pathway. *Theranostics* (2020) 10:6728–42. doi: 10.7150/thno.42259

88. Goulielmaki E, Ioannidou A, Tsekrekou M, Stratigi K, Poutakidou IK, Gkirtzimanaki K, et al. Tissue-infiltrating macrophages mediate an exosome-based metabolic reprogramming upon DNA damage. *Nat Commun* (2020) 11:42. doi: 10.1038/s41467-019-13894-9

89. Miao C, Zhang W, Feng L, Gu X, Shen Q, Lu S, et al. Cancer-derived exosome miRNAs induce skeletal muscle wasting by Bcl-2-mediated apoptosis in colon cancer cachexia. *Mol Ther - Nucleic Acids* (2021) 24:923–38. doi: 10.1016/j.omtn.2021.04.015

90. Conceição M, Forcina L, Wiklander OPB, Gupta D, Nordin JZ, Vrellaku B, et al. Engineered extracellular vesicle decoy receptor-mediated modulation of the IL6 trans-signalling pathway in muscle. *Biomaterials* (2021) 266:120435. doi: 10.1016/j.biomaterials.2020.120435

91. Wang H, Wang B, Zhang A, Hassounah F, Seow Y, Wood M, et al. Exosome-mediated miR-29 transfer reduces muscle atrophy and kidney fibrosis in mice. *Mol Ther* (2019) 27:571–83. doi: 10.1016/j.ymthe.2019.01.008

92. Wu S-T, Chen L, Kang W-W, Liu Q, Gao M-T, Li D-S, et al. Recent developments of fluorescence imaging technology in the second near-infrared window. *BioMed Eng Commun* (2022) 1:3. doi: 10.53388/bmec2022003

93. Zhuang Y, Fu Y, Huang S, Gong S. The application of intelligent sensors in medical research: a review. *BioMed Eng Commun* (2023) 2:17. doi: 10.53388/BMEC2023017.Executive

94. Wu F-C, Ju G-Q, Zhou L-H, Yuan F-W. CRISPR system contributes to deciphering the pharmaceutical compounds from TCM. *BioMed Eng Commun* (2023) 2:8. doi: 10.53388/bmec2023008

95. Hu X, Li F, Zeng J, Zhou Z, Wang Z, Chen J, et al. Noninvasive low-frequency pulsed focused ultrasound therapy for rheumatoid arthritis in mice. *Research* (2022) 2022:0013. doi: 10.34133/research.0013

96. Barreto C, Jandus A. Role of natural products in combating cancer. *Cancer Insight* (2022) 1:35–52. doi: 10.58567/ci01010003

97. Yue J, Chen Z-S, Xu X-X, Li S. Functions and therapeutic potentials of exosomes in osteosarcoma. *Acta Mater Med* (2022) 1:552–62. doi: 10.15212/amm-2022-0024

98. Wei T, Thakur SS, Liu M, Wen J. Oral delivery of glutathione: antioxidant function, barriers and strategies. *Acta Mater Med* (2022) 1:177–92. doi: 10.15212/amm-2022-0005

99. Gao Z, Zheng S, Kamei K, Tian C. Recent progress in cancer therapy based on the combination of ferroptosis with photodynamic therapy. *Acta Mater Med* (2022) 1:411–26. doi: 10.15212/amm-2022-0025

100. Dong N, Lv H, Liu C, Zhang P. Research progress in 3D-printed medicinal tablets. *Acta Mater Med* (2022) 1:154–63. doi: 10.15212/amm-2021-0010

101. Xie X, Hu L, Xue H, Xiong Y, Panayi AC, Lin Z, et al. Prognosis and treatment of complications associated with COVID-19: a systematic review and meta-analysis. *Acta Mater Med* (2022) 1:124–37. doi: 10.15212/amm-2022-0002

102. Li J, Chen J, Bai H, Wang H, Hao S, Ding Y, et al. An overview of organs-on-chips based on deep learning. *Research* (2022) 2022:9869518. doi: 10.34133/2022/9869518

103. Zhao T, Yang Z, Yu J, Lu J, Li L, Xu X, et al. Comparing long-term outcomes of entecavir and tenofovir disoproxil fumarate in liver transplant patients. *Adv Gut Microbiome Res* (2022) 2022:1–9. doi: 10.1155/2022/4779960

104. Xie H, Zhang J, Gu Q, Yu Q, Xia L, Yao S, et al. Cohort profile: A prospective study of gut microbiota in patients with acute ischemic stroke. *Adv Gut Microbiome Res* (2023) 2023:1–9. doi: 10.1155/2023/3944457

105. Cook MJ, O'Brien TJ, Berkovic SF, Murphy M, Morokoff A, Fabinyi G, et al. Prediction of seizure likelihood with a long-term, implanted seizure advisory system in patients with drug-resistant epilepsy: A first-in-man study. *Lancet Neurol* (2013) 12:563–71. doi: 10.1016/S1474-4422(13)70075-9

106. Netzer K, Balmith M, Thabile B. An appraisal of the regulatory policies governing the use of herbal traditional medicine. *Tradit Med Res* (2021) 6:57. doi: 10.53388/tmr20211013249

107. Chen C, Yao X, Xu Y, Zhang Q, Wang H, Zhao L, et al. Dahuang Zhechong Pill suppresses colorectal cancer liver metastasis via ameliorating exosomal CCL2 primed pre-metastatic niche. *J Ethnopharmacol* (2019) 238:111878. doi: 10.1016/j.jep.2019.111878

108. Sengupta V, Sengupta S, Lazo A, Woods P, Nolan A, Bremer N. Exosomes derived from bone marrow mesenchymal stem cells as treatment for severe COVID-19. *Stem Cells Dev* (2020) 29:747–54. doi: 10.1089/scd.2020.0080

frontiers | Frontiers in Immunology

TYPE Mini Review
PUBLISHED 08 January 2024
DOI 10.3389/fimmu.2023.1325530

OPEN ACCESS

EDITED BY
Zhiwen Luo,
Fudan University, China

REVIEWED BY
Zhen Sun,
First Affiliated Hospital of Anhui Medical University, China
Jianying Tian,
Ningxia Medical University, China

*CORRESPONDENCE
Xiaojie Lu
9862019541@jiangnan.edu.cn
Xiaomin Zheng
9862023245@jiangnan.edu.cn

†These authors have contributed equally to this work

RECEIVED 21 October 2023
ACCEPTED 15 December 2023
PUBLISHED 08 January 2024

CITATION
Ye Y, Gao M, Shi W, Gao Y, Li Y, Yang W, Zheng X and Lu X (2024) The immunomodulatory effects of mesenchymal stem cell-derived extracellular vesicles in Alzheimer's disease.
Front. Immunol. 14:1325530.
doi: 10.3389/fimmu.2023.1325530

The immunomodulatory effects of mesenchymal stem cell-derived extracellular vesicles in Alzheimer's disease

Yang Ye[1,2†], Mingzhu Gao[2,3†], Wentao Shi[2], Yan Gao[2], Yilu Li[2], Wenhui Yang[2], Xiaomin Zheng[1*] and Xiaojie Lu[2,3*]

[1]Research Institute for Reproductive Health and Genetic Diseases, Wuxi Maternity and Child Health Care Hospital, Wuxi School of Medicine, Jiangnan University, Wuxi, China, [2]Neuroscience Center, Wuxi School of Medicine, Jiangnan University, Wuxi, China, [3]Central Hospital of Jiangnan University, Wuxi No.2 People's Hospital, Wuxi, China

Neuroinflammation has been identified as another significant pathogenic factor in Alzheimer's disease following Aβ amyloid deposition and tau protein hyperphosphorylation, activated in the central nervous system by glial cells in response to injury-related and pathogen-related molecular patterns. Moderate glial cell activity can be neuroprotective; however, excessive glial cell activation advances the pathology of Alzheimer's disease and is accompanied by structural changes in the brain interface, with peripheral immune cells entering the brain through the blood-brain barrier, creating a vicious circle. The immunomodulatory properties of mesenchymal stem cells (MSCs) are primarily conveyed through extracellular vesicles (EVs). MSC-EVs participate in chronic inflammatory and immune processes by transferring nucleic acids, proteins and lipids from the parent cell to the recipient cell, thus MSC-EVs retain their immunomodulatory capacity while avoiding the safety issues associated with living cell therapy, making them a promising focus for immunomodulatory therapy. In this review, we discuss the modulatory effects of MSC-EVs on Alzheimer's disease-associated immune cells and the mechanisms involved in their treatment of the condition. We have found a clinical trial of MSC-EVs in Alzheimer's disease treatment and outlined the challenges of this approach. Overall, MSC-EVs have the potential to provide a safe and effective treatment option for Alzheimer's disease by targeting neuroinflammation.

KEYWORDS

neuroinflammation, Alzheimer's disease, mesenchymal stem cells, extracellular vesicles, central nervous immune system, peripheral immune system

Introduction

Alzheimer's disease (AD) is a neurodegenerative condition characterized by cognitive decline, memory impairments, and motor abnormalities that impact language, behavior, and visuospatial orientation (1). With the growth of the economy and the increasing average age of the population, the incidence of Alzheimer's disease is on the rise. According to the World Health Organization's 2019 report, there are approximately 55 million individuals worldwide affected by Alzheimer's, a number projected to reach around 139 million by 2050 (2, 3). The exact pathogenesis of Alzheimer's disease remains unclear, although it is widely believed to be influenced by factors such as aging, genetics, environment, and nutrition (4). Over the past few decades, the neuropathological diagnostic criteria for AD have focused on the presence of extracellular Aβ amyloid deposits known as neuritic plaques and intracellular tau protein hyperphosphorylation referred to as neurofibrillary tangles (NFTs) (5). However, therapeutic compounds tested for AD have failed to yield significant results (6), and there is mounting evidence suggesting that neuroinflammation, as a third pathological mechanism, precedes the formation of amyloid Aβ and tau protein hyperphosphorylation (7–9). Neuroinflammation refers to the presence of inflammation in the central nervous system, where glial cells are activated to respond to damage (10, 11), playing a role in neuroprotection (12). However, with the development of AD, glial cells are excessively activated, leading to an increase in pro-inflammatory cytokines, ultimately resulting in neuroinflammation and neurotoxicity (10), and further exacerbating the pathology of Aβ and tau through various mechanisms.

Mesenchymal stem cells (MSCs) are pluripotent stem cells with the capacity for self-renewal and multidirectional differentiation and are derived from numerous tissues in the body, including bone marrow, fat, muscle, lung, etc (13). Extracellular vesicles (EVs) are multifunctional intercellular messengers. They are cell-derived nano-sized double-membrane structures that contain proteins, lipids, RNA, metabolites, growth factors, and cytokines. As a cell-free bio-entity, MSC-EVs have garnered significant attention as a promising therapeutic candidate, exhibiting comparable or even superior efficacy when compared to MSCs themselves (14). In recent years, MSC-EVs have shown tremendous therapeutic potential in various diseases (15–18), including cardiovascular diseases, tumors, chronic kidney diseases, liver fibrosis, autoimmune diseases, and of course, neurological disorders such as stroke, Parkinson's disease, and Alzheimer's disease. In this comprehensive analysis, we delved into the alterations that occur in the innate and adaptive immune system in Alzheimer's disease. In addition, we have explored the immunomodulatory role of MSC-EVs, especially targeting immune cells, and the relevant therapeutic mechanisms for AD. Finally, we look forward to the future with anticipation, contemplating the potentials and obstacles of MSC-EVs for clinical applications in AD.

The immunomodulatory effects of MSC-EVs on CNS innate immune cells

It is widely believed that MSCs exert their therapeutic effects in various diseases by means of immunomodulation and tissue regeneration. This is achieved through the secretion of paracrine factors, including a class of membranous vesicles known as extracellular vesicles (EVs) (19). EVs are released into the extracellular environment by both healthy and apoptotic cells. Among the three primary subtypes of EVs, namely exosomes (exo), microvesicles (MVs), and apoptotic bodies, exosomes are the most abundant, ranging in size from 40 to 120 nm (19–21) (Table 1). To identify and distinguish MSC-EVs, various techniques are employed, include Electron microscopy, Nanoparticle tracking analysis (NTA), Flow cytometry, Western blotting and RNA/protein analysis (Table 2). MSC- EVs possess a diverse array of immunomodulatory properties, primarily targeting key components of the innate and adaptive immune systems, such as T and B lymphocytes, macrophages, dendritic cells, neutrophils, and natural killer cells (22). Many studies have confirmed that extracellular vesicles play an important role in intercellular communication. They transport bioactive lipids, mRNA, miRNA, lncRNA, and other paracrine messenger molecules, as well as genomic DNA, mitochondrial DNA, and various types of proteins

TABLE 1 The characterization of different types of extracellular vesicles.

Characteristic	Exosomes	Microvesicles	Apoptotic bodies
Size(nm)	40-120	100-1000	50-4000
Morphology	Homogenous cup-shape	Heterogeneous irregular	Heterogeneous irregular
Origin	Endosomal	Plasma membrane	Apoptotic cells
Proteins	CD63, CD81, CD9, annexins, heat-shock proteins, Alix, Tsg101, clathrin, caveolins, integrins, TfRs	Integrins, flotillins, selectins, CD40, metalloproteinases	Histones
Lipids	Lysobisphosphatidic acid, cholesterol, ceramide, sphingomyelin and low concentration of phosphatidylserine	High amount of cholesterol, sphingomyelin, ceramide, high concentration of phosphatidylserine	High concentration of phosphatidylserine
Nucleic acids	mRNA and miRNA	mRNA and miRNA	mRNA, miRNA, fragments of DNA

TABLE 2 Techniques and Methods to identified MSC-EVs.

Technique	Method	Identified
Electron microscopy	allows for the visualization of the vesicles and their characteristic size and morphology	distinguish exosomes from other types of extracellular vesicles
NTA	uses laser light scattering to measure the size and concentration of particles in a sample	determine the size distribution of MSC-EVs and estimate their concentration
Flow cytometry	used to analyze the surface markers of MSC-EVs	labeling the vesicles with specific antibodies against known exosomal markers to provide information about the protein composition of the vesicles
Western blotting	used to detect specific proteins in MSC-EVs	by probing for exosomal markers to confirm the identity of the vesicles as exosomes
RNA/ protein analysis	MSC-EVs can be isolated and their RNA and protein content can be analyzed	RNA sequencing and proteomics can provide information about the cargo carried by the vesicles, which can help in their identification and characterization

(23). This process of establishing intercellular communication through the transfer of bioactive molecules can alter the activity of cells under physiological and pathological conditions (24).

Neuroinflammation and CNS innate immunity in AD

Neuroinflammation has been demonstrated to be a major factor in the pathogenesis and progression of AD, activated by damage-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs) (7, 25). Cells contain five major pattern recognition receptors (PRRs), including Toll-like receptors (TLRs), retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), C-type lectin receptors (CLRs) and melanoma 2 (AIM2)-like receptors (ALRs), responsible for recognizing DAMPs and PAMPs, inducing inflammatory signaling pathways and immune responses that induce cell death to eliminate infected cells (26). The inflammatory response in the CNS is predominantly mediated by glial cells, including microglia and astrocytes. During the early stages of AD, microglia and astrocytes, which are innate immune cells, assume a neuroprotective role (12). However, as the disease progresses, glial cells become excessively activated and secrete substantial amounts of pro-inflammatory cytokines, thereby exacerbating neuroinflammation and further contributing to Aβ and tau protein deposition (10, 27). Consequently, this leads to synaptic damage, neuronal processes impairment, disruption of the blood-brain barrier (BBB), and infiltration of certain peripheral immune cells into the brain (28). Hence, an appropriate immune response aids in the clearance of Aβ and Tau deposits, while an excessive immune response fosters neuroinflammatory brain damage (29).

Microglia

As innate immune cells of the central nervous system, microglia are inactive and quiescent in the healthy brain, monitoring the surrounding neuronal environment and other glial cell communication (30). However, microglia are activated in pathological conditions such as neurodegenerative diseases, strokes and tumor invasion (31). Initially, activated microglia have an active role in the clearance of Aβ through phagocytosis; over a period of time, sustained activation also leads to a decrease in the enzymatic activity of microglia to degrade Aβ and a decrease in the efficiency of binding and phagocytosis of Aβ (32). The resultant pro-inflammatory cytokines also reduce the phagocytic activity of microglia, and they may also convert microglia to a pro-inflammatory phenotype (33, 34). In addition, pro-inflammatory microglia increase phosphorylation of tau, exacerbating the pathology of tau (35).

Microglia are able to progress towards a pro-inflammatory phenotype after sensing DAMPs and PAMPs through PRRs such as TLRs, RLRs and NLRs (36), which are at highly expressed in microglia in AD and cause inflammatory responses and pro-inflammatory cytokine secretion through PRRs signaling (37, 38). Under normal conditions, microglia clear Aβ by using surface receptors (CD14, TLR2, TLR4, α6β1 integrin, CD47) and scavenger receptors (CD36) (39, 40); with the TLR2, TLR4 and TLR4 coreceptor CD14 playing a major role (41). However, TLR2 and TLR4 in chronically activated microglia induce the production of Aβ (41) and lose the ability of Aβ elimination (42, 43). Related literature has reported that TLR2-deficient microglia cause phenotypic changes in microglia that reduce Aβ-triggered inflammatory activation and enhance phagocytosis of Aβ (44), and TLR2/4-deficient mice exhibit better neurocognitive and behavioral patterns in response to Aβ1-42 peptide than wild-type mice (45). Thus innate immune activation of microglia is implicated in AD pathogenesis.

Inflammasomes are multi-protein complexes involving intracytoplasmic pattern recognition receptors (PRRs) assembled with receptor proteins (NLR or ALR protein family), junctional proteins (ASC), and effector proteins (caspases) as the underlying structure, and are an essential component of the innate immune system, capable of recognizing PAMPs and DAMPs. A series of studies by Prof. Heneka's team revealed that NLRP3 can be activated by persistently activated microglia in the APP/PS1 mouse model, thereby mediating caspase-1 activation and elevated expression levels of the inflammatory factor IL-1β, and that inhibition of NLRP3 activity reduces Aβ load and decreases the production of pro-inflammatory cytokines and cognitive impairment (46). Furthermore, the team revealed the pathological relationship between NLRP3 and tau and showed that inhibition of NLRP3 function was able to regulate tau kinase and phosphatase thereby reducing tau hyperphosphorylation and aggregation (47). Thus, deposition of Aβ leads to the pathological development of tau, in which NLRP3 provides a key role.

In addition, activated NLRP3 promotes the oligomerization of ASC to form large intracellular macromolecular aggregates, termed ASC spots. ASC spots have been reported to be released into the extracellular space and propagate inflammatory responses via prion-like transport mediated by phagocytosis in neighbouring macrophages (48). Friker et al. showed that in AD mice, ASC expression was increased and interacted extracellularly with Aβ to form an intensely toxic ASC-Aβ complex that was capable of causing scorch death of microglia and preventing the clearance of Aβ by microglia (49). However, the detailed molecular mechanisms underlying the release of intracellular ASC spots into the extracellular space, and their role in neuroinflammation, remain unknown.

Activation of the microglia-associated PRRs signaling pathway induce the secretion of pro-inflammatory cytokines that prompt microglia to clear Aβ, but the release of pro-inflammatory cytokine and activation of inflammasome caused by excessive microglia activation further contributes to AD pathology.

Astrocyte

Astrocytes are the most common glial cells in the brain (50) and play an important role in regulating blood flow, maintaining the blood-brain barrier (BBB), providing energy metabolites to neurons, regulating extracellular ion homeostasis and modulating synaptic activity (51). Astrocytes express numerous receptors for PAMPs and DAMPs known to trigger innate immune responses, particularly TLRs, including TLR4 (52), in response to activators of innate immune responses (53). In contrast, astrocytes exhibit a response in response to CNS injury and disease that is often termed astroglial cell reactivity (54). Reactive astrocytes are an integral part of the innate immunity of the central nervous system. Similar to microglia, reactive astrocytes are divided into pro-inflammatory A1 and immunomodulatory (neuroprotective) A2 subsets (55). Pro-inflammatory reactive astrocytes upregulate complement cascade genes and induce pro-inflammatory factors such as IL-1β and TNF-α, while neuroprotective reactive astrocytes upregulate and support neuronal growth with a range of neurotrophic factors (56). Professor Barres' research has shown that reactive astrocytes A1 lose the function of resting astrocytes to form synapses and produce toxic effects on neurons. In addition, as synaptic loss is also a characteristic feature of AD, Barres et al. also found that in AD, nearly 60% of astrocytes in the prefrontal cortex (the active site of the disease) are in the A1 condition and drive the disease progression in AD due to the high toxicity of A1 to neurons and oligodendrocytes (57).

Although the innate immune sensing of astrocytes is not well understood, many studies have shown that astrocytes and microglia regulate each other's functions by secreting cytokines. On the one hand, the inflammatory factors TNF-α, IL-1 and C1q secreted by activated microglia induce the transformation of resting astrocytes into neurotoxic reactive astrocytes A1 (57); on the other hand, the large amount of IL-3 secreted by astrocytes is able to bind to the IL-3α receptor aberrantly expressed by microglia in AD disease and is capable of regulating microglia to perform the clearance of Aβ function (58). Thus, the interaction between astrocytes and microglia may become a new therapeutic direction.

MSC-EVs inhibit glial cell activity

As previously mentioned, the excessive activation of glial cells exacerbates the neuroinflammatory pathology of Alzheimer's disease. Numerous *in vitro* and *in vivo* experiments have demonstrated that the extracellular vesicles derived from mesenchymal stem cells (MSCs) inhibit the activity of glial cells, thereby reducing the expression of pro-inflammatory cytokines and alleviating neuroinflammation. Mao Ding et al. discovered that extracellular vesicles from human umbilical cord MSCs regulate the levels of inflammatory cytokines by modulating the activity of microglial cells *in vitro*. Injection of extracellular vesicles derived from human umbilical cord MSCs into AD mouse models has been shown to improve cognitive impairment and promote the clearance of Aβ. Additionally, there is a decrease in the number of inflammatory microglial cells, an increase in the levels of immunoregulatory microglial cells, a reduction in the levels of pro-inflammatory cytokines (IL-1β and TNF-α) in the peripheral blood and brain of mice, and an elevation in the levels of anti-inflammatory cytokines (IL-10 and TGF-β) (59). In addition, the Mesenchymal stem cell-derived exosomes can also reduce the activity of astrocytes. In a study on exosomes derived from hypoxia-preconditioned MSCs (PC-MSCs), injection of PC-MSC exosomes significantly improved the learning and memory abilities of APP/PS1 mice compared to exosomes from normoxic MSCs. The activity of microglia and astrocytes was reduced, plaque deposition and Aβ levels were decreased, and the expression of growth-related protein 43, synaptophysin 1, and IL-10 was increased. The levels of neuroglial fibrillary acidic protein, ionized calcium-binding adapter molecule 1, TNF-α, IL-1β, as well as the activation of STAT3 and NF-κB, all sharply decreased. This may be attributed to the higher expression of miR-21 in PC-MSC exosomes (60). Some studies have indicated that the levels of miRNA-21 significantly decrease in the presence of chronic inflammation and cellular apoptosis. However, the mesenchymal stem cells in the extracellular matrix exhibit high levels of miRNA-21, which contribute to the reduction of inflammation and cellular apoptosis (61). Therefore, the extracellular vesicles released by mesenchymal stem cells containing miRNA can inhibit the activity of immune cells and induce their phenotypic transformation into anti-inflammatory. Vascular dementia (VaD) is another common cause of dementia, following Alzheimer's disease. In the establishment of a VaD rat model through bilateral carotid artery ligation, there is an increase in inflammatory microglial cells. HUCMSC-Evs, by activating the PI3K/AKT/Nrf2 pathway, suppresses the activity of inflammatory microglial cells, inflammation, and oxidative stress, thereby protecting the neural function of VaD rats (62).

The immunomodulatory effects of MSC-EVs on peripheral immune cells

Peripheral immune cell infiltration in AD

As mentioned above, excessive protein deposition in AD triggers a shift in glial cells towards an inflammatory phenotype, the release of pro-inflammatory cytokines and complement, causing hyperactivation of glial cells and a vicious cycle of neurodegeneration. In this state, structural or biological changes occur at the brain interface, allowing peripheral immune cells to infiltrate the brain parenchyma through the blood-brain barrier (63), choroid plexus (64, 65) or meninges (66, 67), exacerbating the pathological development of AD. Single cell sequencing has shown that peripheral immune cells include myeloid cells such as natural killer cells (NK cells), polymorphonuclear neutrophils (PMNs), monocytes/macrophages, dendritic cells (68), and adaptive immune cells such as T cells (69) and B cells (70, 71). Due to the unclear role of dendritic cells in AD, as well as the controversial results regarding how MSC-EVs regulate B cells. Here we focused on macrophages and T cells.

Monocytes/macrophages

In AD, damage to the central nervous system leads to increased permeability of the BBB, favoring infiltration of peripheral monocytes. Aβ has been shown to induce the release of chemokines, such as monocyte chelator proteins (MCPs), capable of attracting monocytes. Pro-inflammatory cytokines such as IL-6 and TNF-a produced by monocytes to enhance their phagocytosis of Aβ (72). A recent study has shown that inflammatory monocytes/macrophages are elevated in cell cultures stimulated by Aβ in AD patients. These cells express TLR2, TLR4, IL-6 and CCR2, which in turn can facilitate the migration of monocytes/macrophages across the BBB into the brain. Research has shown that patients with AD and mild cognitive impairment (MCI) exhibit higher expression of TLR3 and TLR8 in monocytes/macrophages, as well as production of IL-23. Additionally, AD monocytes/macrophages also possess independent MHC-II/Aβ42 complexes. These findings suggest that monocytes/macrophages in AD exhibit inflammatory characteristics and are involved in both innate and adaptive immune responses through TLR stimulation. Furthermore, they may present Aβ peptides in an MHC-restricted manner (73). In the presence of soluble or mildly aggregated Aβ, there is an increase in T cell proliferation and pro-inflammatory cytokine secretion. These observations indicate that Aβ may not only act as an antigen but also as a more widespread positive regulator of peripheral adaptive immune responses. When activated T cells cross the blood-brain barrier and enter the brain, they can also modulate adaptive immune responses within the brain (74). In parallel, alterations in the monocyte/macrophage subpopulation were observed in AD (75, 76), but whether this alteration is due to a shift in the monocyte phenotype or the gradual death of classical monocytes remains to be further investigated.

T cell

Lymphocytes are an indispensable component of the adaptive immune system, and mounting evidence suggests that adaptive immune cells play a crucial role in the pathophysiology of neurodegenerative diseases such as Alzheimer's disease (AD). T cells infiltrate the central nervous system during the onset of AD, promoting neuroinflammation (69, 77–79). On the one hand, helper T cells cross the blood-brain barrier and interact with glial cells, triggering immune and inflammatory responses, ultimately leading to neuroinflammation and neuronal damage. Browne et al. (80). found a significant presence of T cells, particularly Aβ-specific Th1 cells, in the brains of APP/PS1 mice, which increased activation of microglia and Aβ deposition through the production of IFN-γ, resulting in cognitive impairment. *In vitro* experiments conducted by McQuillan et al. (81). also demonstrated that Aβ-specific Th1 and Th17 cells induce glial cells to produce pro-inflammatory cytokines, while Th2 cells attenuate this effect. The aforementioned study elucidates that the regulation of T cell activation on microglia is contingent upon their cellular phenotype. Furthermore, T cell activation can also promote the activation and proliferation of glial cells, thereby exacerbating the inflammatory response. Earlier work by Yong et al. (82) demonstrated that IFN-γ produced by T cells induces proliferation of astrocytes *in vitro* and facilitates reactive astrogliosis in the brain. Currently, IL-17 produced by Th17 cells has been repeatedly confirmed as an effective stimulant for astrocytes. IL-17 stimulation activates inducible nitric oxide synthase (83), regulates the expression of macrophage inflammatory protein-1α (MIP-1α) through the PI3K/Akt and NF-κB pathways (84), and enhances the IL-6 signaling pathway in astrocytes (85). On the other hand, the infiltration of cytotoxic T cells is associated with the deterioration of AD (69, 77, 78). A recent study discovered the presence of clonally expanded CD8+ cells in the cerebrospinal fluid of AD patients, with TEMRA being the predominant subset (69). These cells are associated with immune memory and can release inflammatory factors and cytotoxic molecules. Furthermore, the cytotoxic effector genes of these cells are highly expressed in the hippocampus of AD patients. Additionally, the levels of TEMRA cells in the peripheral blood of AD patients show a negative correlation with cognitive levels, as well as a negative correlation with central memory T cells (TCM) and effector memory T cells (TEM). This suggests that adaptive immune cells may also play a role in Alzheimer's disease, and CD8+ T cells may impact neurodegeneration and/or cognitive impairment in AD.

Other immune cells

NK cells are potent cytotoxic effectors against infected pathogens and tumor cells (86, 87). They play a crucial role in bridging the innate and adaptive immune systems by secreting cytokines and interacting with other immune cells. Compared to healthy elderly individuals of matching age, the distribution of NK cells in AD patients remains unchanged. However, in the early stages of AD, specifically in cases of amnestic mild cognitive impairment (aMCI), NK cells are activated and exhibit stronger

activity (88). For instance, increased production of granule enzyme B and pro-inflammatory cytokines (TNFα, IFNγ) has been observed in aMCI subjects, contrasting with NK cells in confirmed cases of mild AD (mAD) (87). The activated state of NK cells may be a congenital immune response to cope with unidentified challenges, which may include viral or microbial agents. Furthermore, this activation state may contribute to the occurrence of neuroinflammation. Therefore, the protective role of NK cells may no longer be effective in the progression from aMCI to AD, and NK cells could potentially be considered as biomarkers for the early stages of AD.

At the same time, polymorphonuclear neutrophils (PMN), as frontline immune cells, also participate in the early stages of AD. The functional changes of these cells during different stages of AD pathology may be associated with pathological stimuli (89). CD177 expression was increased in mAD but not in healthy individuals or aMCI patients. Expression of CD14 and CD16 was lower in the PMN of patients with mAD compared with controls, whereas it was unchanged in patients with aMCI. Only the PMN of aMCI patients expressed lower levels of CD88. The production of inflammatory cytokines (TNFα, IL-6, IL-1β, IL-12p70) and chemokines (MIP-1α, MIP-1β, IL-8) in response to LPS stimulation was very low in patients with aMCI and virtually absent in patients with mAD. TLR2 is only expressed at lower levels in aMCI. We therefore suggest that since AD may be the result of a pathogen challenge, neutrophils at the front line will fight the pathogen and instruct other immune cells to intervene. In this way, neutrophils may be involved in the earliest stages of AD pathogenesis.

Although the extracellular vesicles of MSCs have shown potential therapeutic effects in immune regulation, further research is needed to understand their role in modulating immune cells in AD. AD is a neurodegenerative disorder that is associated with abnormal activation of the immune system and inflammatory responses. Therefore, understanding the regulatory effects of MSCs extracellular vesicles on immune cells in Alzheimer's disease is of great significance in uncovering the mechanisms of disease progression and developing new treatment strategies.

The mechanism of MSC-EVs in treating AD

The immunomodulatory effects of MSC-EVs on immune cells mainly manifest in inhibiting glial cell activity, reducing the expression of inflammatory factors, thereby alleviating neuroinflammatory reactions; inhibiting the proliferation and differentiation of lymphocytes, promoting the differentiation of lymphocytes into anti-inflammatory subtypes; and inducing macrophages to transition from a pro-inflammatory phenotype to an anti-inflammatory phenotype. In addition, the MSC-EVs in the treatment of Alzheimer's disease also includes the clearance of Aβ, neuroprotective effects and as a potential drug delivery vehicle.

The clearance of Aβ

Aβ is a hallmark pathological protein of AD, which are believed to be associated with neuronal damage and death. Once they exceed the clearance capacity of neuroglial cells, abnormal accumulation will lead to gradual decline in memory and cognitive dysfunction. It has been proven that clearing pathogenic proteins is beneficial for treating AD (90). MSCs-EVs can reduce the deposition of Aβ in the body through several different ways. Firstly, by inhibiting the expression of neutral sphingomyelinase-2 (nSMase2), the secretion function of pathological cells in AD patients can be reduced. This leads to a decrease in pathological exosomes and ultimately lowers the level of Aβ in the brain (91). Additionally, the reduction of nSMase2 can inhibit the conversion of sphingomyelin to ceramide, thereby increasing the level of sphingomyelin and promoting the secretion of exosomes from normal neurons. These exosomes can induce conformational changes in Aβ deposits, transforming them into fiber tissue without causing toxic effects on brain tissue. Surrounding microglial cells can uptake and degrade these fiber tissues, thereby reducing the amount of Aβ (92). Moreover, the surface of exosomes is rich in glycosphingolipids, which facilitate the binding of Aβ to exosomes. This characteristic enables exosomes to effectively serve as carriers for adsorbing Aβ and accelerating its removal from the body (93). Secondly, neprilysin (NEP) and insulin-degrading enzyme (IDE), as well as zinc metallopeptidase, are believed to be involved in the degradation of Aβ in the brain (94). As early as 2000, researchers injected radiolabeled synthetic Aβ peptides into the hippocampus of rats and observed that endogenous NEP could subsequently proteolytically degrade the peptides (95). In mice with NEP or IDE deficiencies, endogenous Aβ levels increased in a gene-dose-dependent manner (96, 97). Thirdly, research has found that in the human body, enkephalin is one of the enzymes in brain tissue that is most effective in breaking down and absorbing Aβ (98). Experiments have shown that when fat MSCs exosomes are added to the environment of AD model cells with high expression of Aβ-related proteins, the amount of Aβ detected in the cells and surrounding environment significantly decreases. This is due to the fact that fat MSCs exosomes are rich in enkephalinase levels that exceed the average (99). Hence, the crucial role of MSC-EVs in Aβ degradation highlights their potential in Alzheimer's disease treatment.

Neuroprotective effects of MSC-Evs

Another pathological hallmark of AD is synaptic dysfunction, which is directly associated with cognitive impairment. The experimental results from Mariana et al. (100) show that MSCs and their exosomes can protect hippocampal neurons and related synapses from damage caused by oxidative stress reactions resulting from Aβ deposition. Cui et al. (60) summarized the experimental results and speculated that MSC exosomes may improve learning and memory abilities in APP/PS1 double transgenic AD model mice by improving the function of damaged synapses and immune

regulation at the site of injury. They found that exosomes extracted from mesenchymal stem cells subjected to hypoxic preconditioning significantly enhanced the expression of synaptic proteins (synapsin 1 and PSD95). The expression levels of synaptic proteins can to some extent reflect the function of synapses, and synapsin 1 and PSD95 are synaptic proteins involved in neural signal transmission and maintaining synaptic integrity. Another experiment showed that after fusion with neural cells, MSC exosomes can transfer miR-133b into neurons, promoting axonal repair and reducing neural damage caused by modeling. Additionally, MSC exosomes are rich in miR-17-92, and increasing their exogenous content can promote the generation of oligodendrocytes and axonal growth. In a transient cerebral ischemia mouse model, intravenous injection of exosomes with high expression of miR-17-92 enhanced neuronal plasticity and axonal growth speed compared to injection of normal MSC exosomes, achieving the effect of promoting neural function recovery (101).

Potential drug delivery vehicle

The lipid bilayer structure of exosomes gives them excellent biocompatibility, supporting the loading of hydrophobic or hydrophilic drugs (102). Mesenchymal stem cell-derived exosomes can directly bind to membrane receptors through their exosomal exosome, allowing their contents to be internalized into target cells, or deliver bioactive substances to target cells through fusion with the plasma membrane. In addition, exosomes can easily cross the blood-brain barrier (BBB) and increase the concentration of drugs in the brain (103). Furthermore, exosome administration can avoid some complications, including intracranial infection, non-specific absorption, and drug toxicity, due to the low immunogenicity of exosomes (104). Previous studies have shown that exosomes can deliver drugs to the brains of AD mice (104). Furthermore, by using peptide-modified exosomes expressing the membrane protein Lamp2b, exosomes produced by engineered dendritic cells can bind to neuron-specific rabies virus glycoprotein (RVG) peptide, improving the cognitive function of AD transgenic mice (105).

Blood exosomes as biomarkers of Alzheimer's disease

In addition to potential therapeutic value, EVs can also serve as biomarkers, which is important in clinical applications. In particular blood exosomes, which are EVs secreted by living cells into the circulating blood, are regarded as a relatively noninvasive novel tool for monitoring brain physiology and disease states, and brain-derived exosomes in peripheral blood is an ideal biomarker for AD. A meta-analysis described the diagnostic performance of biomarkers of blood exosomes in AD (Registration No. CRD4200173498) (106). The findings revealed that individuals with preclinical Alzheimer's disease, mild cognitive impairment, and Alzheimer's disease exhibited elevated levels of core biomarkers, including Aβ1-42, P-T181-tau, P-S396-tau, and T-tau, in blood neuron-derived exosomes. Furthermore, there was an increase in molecules associated with other risk factors, such as C1q for neuroinflammation, P-S312-IRS-1 for metabolism disorder, HGF for neurotrophic deficiency, VEGF-D for vascular injury, and cathepsin D for autophagy-lysosomal system dysfunction. At the genetic level, the differential expression of REST, a transcription-related factor, and miR-132, a microRNA, also influenced RNA splicing, transport, and translation. These findings confirm the potential of the aforementioned core molecules and additional risk-related factors in blood exosomes as candidate biomarkers for preclinical and clinical Alzheimer's disease. Consequently, these findings support the further development of exosome biomarkers for a clinical blood test for Alzheimer's disease.

Application of MSC-EVs in clinical practice and their advantages and limitations

There is presently an ongoing clinical trial, led by Ruijin Hospital affiliated with Shanghai Jiao Tong University, which aims to assess the safety and efficacy of utilizing allogeneic adipose-derived mesenchymal stem cells in patients with Alzheimer's disease (www.clinicaltrials.gov). Although the clinical trial is still awaiting results, the therapeutic efficacy of MSC-EVs has shown promising outcomes in other conditions, such as pre-eclampsia (NCT03562715) and chronic ulcers (NCT04134676).

The advantages of MSC-EVs mainly lie in the following aspects: (1) The nanoscale MSC-EVs reduce vascular obstruction and are more capable of penetrating the blood-brain barrier (107); (2) MSC-EVs cannot replicate, avoiding uncontrolled division and reducing the risk of tumor formation during proliferation (108), as well as preventing mutations and DNA damage caused by cell transplantation (109); (3) MSC-EVs have low immunogenicity, making allogeneic applications possible (110); (4) Mesenchymal stem cells can produce a large amount of EVs, whose composition remains unchanged, facilitating storage and suitable for large-scale production (111). Apart from these advantages, the clinical application of MSC-EVs, especially in the context of AD, still faces certain limitations, primarily including: (1) the current methods for extracting MSC-EVs are time-consuming and inefficient, necessitating further exploration and research into efficient extraction methods that can be effectively applied in clinical settings; (2) Due to the different composition of cytokines in mesenchymal stem cell-derived extracellular vesicles from different sources, the clinical application relies on time-saving, cost-effective, and efficient methods. Further research is needed for the development of effective biomarkers for extracellular vesicles; (3) the specific mechanisms by which MSC-EVs regulate immune responses, promote Aβ degradation, and enhance axonal growth remain unclear and require further experimental investigation; (4) due to the complex biological composition of MSC-EVs, their safety when applied in animal models and the

significance of specific therapeutic molecules within MSC-EVs warrant further attention.

Conclusion

As a progressive neurodegenerative disease, Alzheimer's disease currently lacks a cure. Previous research on the pathogenesis of Alzheimer's disease has primarily focused on the abnormal accumulation of neurofibrillary tangles (NFTs) and amyloid plaques (Aβ). However, clinical trials targeting this mechanism have ended in failure, indicating that NFTs and Aβ are not the primary causes of Alzheimer's disease. In recent years, studies have discovered that excessive immune response in the central nervous system may be a significant factor in protein deposition. In this pathological state, peripheral immune cells gather in the brain parenchyma through a compromised blood-brain barrier, further exacerbating the progression of Alzheimer's disease. Mesenchymal stem cell-derived extracellular vesicles (MSC-EVs), as a cell-free therapy, have demonstrated excellent immunomodulatory effects on both central nervous system immune cells and peripheral immune cells. They have also shown two major benefits in Alzheimer's disease: clearing protein deposits and neuroprotection (Figure 1). Compared to the MSC - Evs as drug delivery carrier alone, directly isolated MSC-EVs retain natural substances and surface markers, which can minimize immune rejection and other potential complications. Engineering vesicles can by modifying the composition of vesicle or surface characteristics, load specific drugs or therapeutic molecules, so as to realize precise targeting and controlled release, but the engineering process can be complex and may alter the natural properties of the vesicles. The choice between direct isolation of MSC-EVs or preparation of engineered vesicles as therapeutic interventions depends on the specific application and desired outcomes. Further research and clinical trials are needed to determine which approach is more effective and practical in different therapeutic contexts. Currently, there is an ongoing study investigating the safety and efficacy of MSC-EVs in treating Alzheimer's disease. It is believed that in the near future, further exploration of its therapeutic mechanisms and optimization of

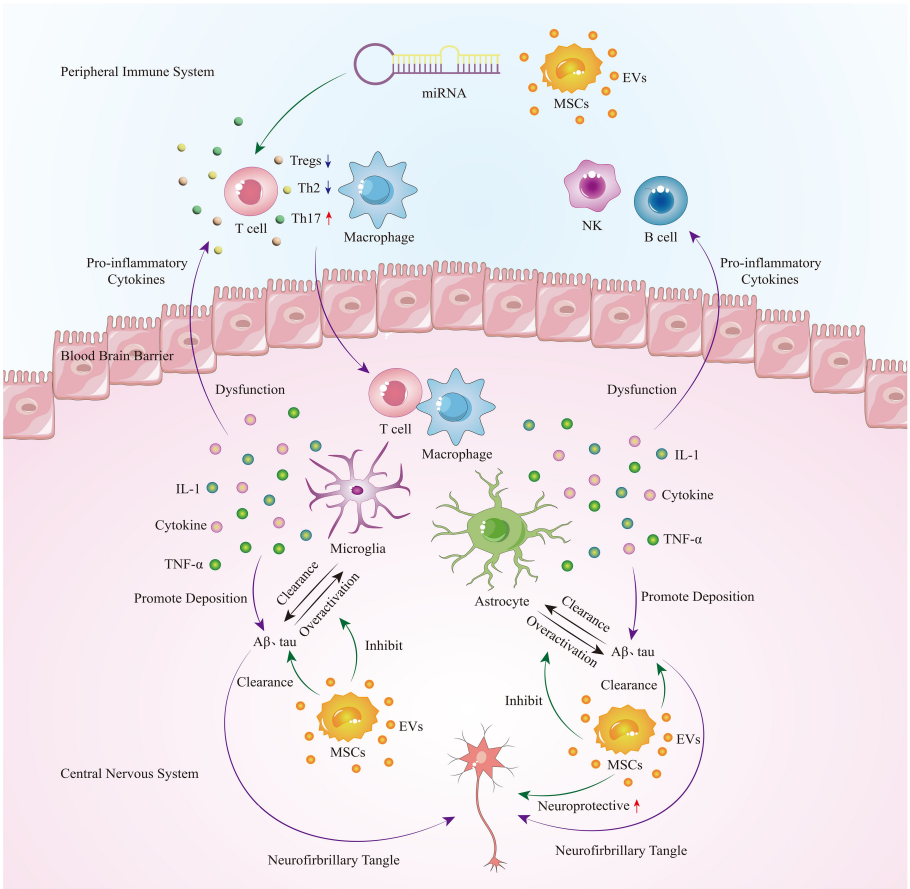


FIGURE 1
In the early stages of AD, immune cells, microglia, and astrocytes in the central nervous system are activated to clear protein deposits. However, as the disease progresses, glial cells become overactivated, leading to the secretion of a large number of pro-inflammatory cytokines. This not only exacerbates protein deposition but also damages the blood-brain barrier, allowing peripheral immune cells such as T cells and macrophages to infiltrate the brain, further exacerbating neuroinflammation and causing a vicious cycle. Extracellular vesicles derived from mesenchymal stem cells can regulate peripheral immune cells, inhibit overactive glial cells, and play a therapeutic role in Alzheimer's disease by promoting neuroprotection and clearing protein deposits.

treatment strategies will provide more effective treatment options for Alzheimer's disease patients.

Author contributions

YY: Funding acquisition, Writing – original draft, Writing – review & editing. MG: Writing – original draft. WS: Writing – review & editing. YG: Writing – review & editing. YL: Writing – review & editing. WY: Writing – review & editing. XZ: Supervision, Writing – review & editing. XL: Conceptualization, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was supported by Wuxi Health Commission Project (Nos. Q202111).

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

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Lane CA, Hardy J, Schott JM. Alzheimer's disease. *Eur J Neurol* (2018) 25(1):59–70. doi: 10.1111/ene.13439

2. Cova I, Markova A, Campini I, Grande G, Mariani C, Pomati S. Worldwide trends in the prevalence of dementia. *J Neurol Sci* (2017) 379:259–60. doi: 10.1016/j.jns.2017.06.030

3. Serge G, Pedro R, José AM. Journey through the diagnosis of dementia. *World Alzheimer Report 2021*. (2021). Available at: https://www.alzint.org/resource/world-alzheimer-report-2021.

4. Armstrong RA. Risk factors for Alzheimer's disease. *Folia Neuropathol* (2019) 57 (2):87–105. doi: 10.5114/fn.2019.85929

5. Long JM, Holtzman DM. Alzheimer disease: an update on pathobiology and treatment strategies. *Cell* (2019) 179(2):312–39. doi: 10.1016/j.cell.2019.09.001

6. Plascencia-Villa G, Perry G. Status and future directions of clinical trials in Alzheimer's disease. *Int Rev Neurobiol* (2020) 154:3–50. doi: 10.1016/bs.irn.2020.03.022

7. Leng F, Edison P. Neuroinflammation and microglial activation in Alzheimer disease: where do we go from here? *Nat Rev Neurol* (2021) 17(3):157–72. doi: 10.1038/s41582-020-00435-y

8. Calsolaro V, Edison P. Neuroinflammation in Alzheimer's disease: Current evidence and future directions. *Alzheimers Dement* (2016) 12(6):719–32. doi: 10.1016/j.jalz.2016.02.010

9. Eikelenboom P, van Exel E, Hoozemans JJ, Veerhuis R, Rozemuller AJ, van Gool WA. Neuroinflammation-an early event in both the history and pathogenesis of Alzheimer's disease. *Neurodegener Dis* (2010) 7(1-3):38–41. doi: 10.1159/000283480

10. Kwon HS, Koh SH. Neuroinflammation in neurodegenerative disorders: the roles of microglia and astrocytes. *Transl Neurodegener* (2020) 9(1):42. doi: 10.1186/s40035-020-00221-2

11. Bachiller S, Jiménez-Ferrer I, Paulus A, Yang Y, Swanberg M, Deierborg T, et al. Microglia in neurological diseases: A road map to brain-disease dependent-inflammatory response. *Front Cell Neurosci* (2018) 12:488. doi: 10.3389/fncel.2018.00488

12. Kinney JW, Bemiller SM, Murtishaw AS, Leisgang AM, Salazar AM, Lamb BT. Inflammation as a central mechanism in Alzheimer's disease. *Alzheimers Dement (N Y)* (2018) 4:575–90. doi: 10.1016/j.trci.2018.06.014

13. Spees JL, Lee RH, Gregory CA. Mechanisms of mesenchymal stem/stromal cell function. *Stem Cell Res Ther* (2016) 7(1):125. doi: 10.1186/s13287-016-0363-7

14. Andaloussi S EL, Mäger I, Breakefield XO, Wood MJ. Extracellular vesicles: biology and emerging therapeutic opportunities. *Nat Rev Drug Discovery* (2013) 12 (5):347–57. doi: 10.1038/nrd3978

15. Weng Z, Zhang B, Wu C, Yu F, Han B, Li B, et al. Therapeutic roles of mesenchymal stem cell-derived extracellular vesicles in cancer. *J Hematol Oncol* (2021) 14(1):136. doi: 10.1186/s13045-021-01141-y

16. Huang Y, Yang L. Mesenchymal stem cells and extracellular vesicles in therapy against kidney diseases. *Stem Cell Res Ther* (2021) 12(1):219. doi: 10.1186/s13287-021-02289-7

17. Psaraki A, Ntari L, Karakostas C, Korrou-Karava D, Roubelakis MG. Extracellular vesicles derived from mesenchymal stem/stromal cells: The regenerative impact in liver diseases. *Hepatology* (2022) 75(6):1590–603. doi: 10.1002/hep.32129

18. Xu F, Fei Z, Dai H, Xu J, Fan Q, Shen S, et al. Mesenchymal stem cell-derived extracellular vesicles with high PD-L1 expression for autoimmune diseases treatment. *Adv Mater* (2022) 34(1):e2106265. doi: 10.1002/adma.202106265

19. Rani S, Ryan AE, Griffin MD, Ritter T. Mesenchymal stem cell-derived extracellular vesicles: toward cell-free therapeutic applications. *Mol Ther* (2015) 23 (5):812–23. doi: 10.1038/mt.2015.44

20. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. *J Cell Biol* (2013) 200(4):373–83. doi: 10.1083/jcb.201211138

21. Hristov M, Erl W, Linder S, Weber PC. Apoptotic bodies from endothelial cells enhance the number and initiate the differentiation of human endothelial progenitor cells in *vitro*. *Blood* (2004) 104(9):2761–6. doi: 10.1182/blood-2003-10-3614

22. Lai P, Weng J, Guo L, Chen X, Du X. Novel insights into MSC-EVs therapy for immune diseases. *biomark Res* (2019) 7:6. doi: 10.1186/s40364-019-0156-0

23. Kalra H, Simpson RJ, Ji H, Aikawa E, Altevogt P, Askenase P, et al. Vesiclepedia: a compendium for extracellular vesicles with continuous community annotation. *PLoS Biol* (2012) 10(12):e1001450. doi: 10.1371/journal.pbio.1001450

24. Harting MT, Srivastava AK, Zhaorigetu S, Bair H, Prabhakara KS, Toledano Furman NE, et al. Inflammation-stimulated mesenchymal stromal cell-derived extracellular vesicles attenuate inflammation. *Stem Cells* (2018) 36(1):79–90. doi: 10.1002/stem.2730

25. Baufeld C, O'Loughlin E, Calcagno N, Madore C, Butovsky O. Differential contribution of microglia and monocytes in neurodegenerative diseases. *J Neural Transm (Vienna)* (2018) 125(5):809–26. doi: 10.1007/s00702-017-1795-7

26. Kanneganti TD. Intracellular innate immune receptors: Life inside the cell. *Immunol Rev* (2020) 297(1):5–12. doi: 10.1111/imr.12912

27. Hickman S, Izzy S, Sen P, Morsett L, El Khoury J. Microglia in neurodegeneration. *Nat Neurosci* (2018) 21(10):1359–69. doi: 10.1038/s41593-018-0242-x

28. Chen X, Holtzman DM. Emerging roles of innate and adaptive immunity in Alzheimer's disease. *Immunity* (2022) 55(12):2236–54. doi: 10.1016/j.immuni.2022.10.016

29. Pasparakis M, Vandenabeele P. Necroptosis and its role in inflammation. *Nature* (2015) 517(7534):311–20. doi: 10.1038/nature14191

30. Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* (2010) 330(6005):841–5. doi: 10.1126/science.1194637

31. Zhang G, Wang Z, Hu H, Zhao M, Sun L. Microglia in Alzheimer's disease: A target for therapeutic intervention. *Front Cell Neurosci* (2021) 15:749587. doi: 10.3389/fncel.2021.749587

32. Michelucci A, Heurtaux T, Grandbarbe L, Morga E, Heuschling P. Characterization of the microglial phenotype under specific pro-inflammatory and

anti-inflammatory conditions: Effects of oligomeric and fibrillar amyloid-beta. *J Neuroimmunol* (2009) 210(1-2):3–12. doi: 10.1016/j.jneuroim.2009.02.003

33. Tang Y, Le W. Differential roles of M1 and M2 microglia in neurodegenerative diseases. *Mol Neurobiol* (2016) 53(2):1181–94. doi: 10.1007/s12035-014-9070-5

34. Jimenez S, Baglietto-Vargas D, Caballero C, Moreno-Gonzalez I, Torres M, Sanchez-Varo R, et al. Inflammatory response in the hippocampus of PS1M146L/APP751SL mouse model of Alzheimer's disease: age-dependent switch in the microglial phenotype from alternative to classic. *J Neurosci* (2008) 28(45):11650–61. doi: 10.1523/JNEUROSCI.3024-08.2008

35. Lee DC, Rizer J, Selenica ML, Reid P, Kraft C, Johnson A, et al. LPS- induced inflammation exacerbates phospho-tau pathology in rTg4510 mice. *J Neuroinflammation* (2010) 7:56. doi: 10.1186/1742-2094-7-56

36. Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, et al. Neuroinflammation in Alzheimer's disease. *Lancet Neurol* (2015) 14(4):388–405. doi: 10.1016/S1474-4422(15)70016-5

37. Heneka MT, Kummer MP, Latz E. Innate immune activation in neurodegenerative disease. *Nat Rev Immunol* (2014) 14(7):463–77. doi: 10.1038/nri3705

38. McDonough A, Lee RV, Weinstein JR. Microglial interferon signaling and white matter. *Neurochem Res* (2017) 42(9):2625–38. doi: 10.1007/s11064-017-2307-8

39. Bamberger ME, Harris ME, McDonald DR, Husemann J, Landreth GE. A cell surface receptor complex for fibrillar beta-amyloid mediates microglial activation. *J Neurosci* (2003) 23(7):2665–74. doi: 10.1523/JNEUROSCI.23-07-02665.2003

40. Wilkinson K, El Khoury J. Microglial scavenger receptors and their roles in the pathogenesis of Alzheimer's disease. *Int J Alzheimers Dis* (2012) 2012:489456. doi: 10.1155/2012/489456

41. Reed-Geaghan EG, Savage JC, Hise AG, Landreth GE. CD14 and toll-like receptors 2 and 4 are required for fibrillar A{beta}-stimulated microglial activation. *J Neurosci* (2009) 29(38):11982–92. doi: 10.1523/JNEUROSCI.3158-09.2009

42. Hickman SE, Allison EK, El Khoury J. Microglial dysfunction and defective beta-amyloid clearance pathways in aging Alzheimer's disease mice. *J Neurosci* (2008) 28(33):8354–60. doi: 10.1523/JNEUROSCI.0616-08.2008

43. Thériault P, ElAli A, Rivest S. The dynamics of monocytes and microglia in Alzheimer's disease. *Alzheimers Res Ther* (2015) 7(1):41. doi: 10.1186/s13195-015-0125-2

44. Liu S, Liu Y, Hao W, Wolf L, Kiliaan AJ, Penke B, et al. TLR2 is a primary receptor for Alzheimer's amyloid β peptide to trigger neuroinflammatory activation. *J Immunol* (2012) 188(3):1098–107. doi: 10.4049/jimmunol.1101121

45. Vollmar P, Kullmann JS, Thilo B, Claussen MC, Rothhammer V, Jacobi H, et al. Active immunization with amyloid-beta 1-42 impairs memory performance through TLR2/4-dependent activation of the innate immune system. *J Immunol* (2010) 185(10):6338–47. doi: 10.4049/jimmunol.1001765

46. Heneka MT, Kummer MP, Stutz A, Delekate A, Schwartz S, Vieira-Saecker A, et al. NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. *Nature* (2013) 493(7434):674–8. doi: 10.1038/nature11729

47. Ising C, Venegas C, Zhang S, Scheiblich H, Schmidt SV, Vieira-Saecker A, et al. NLRP3 inflammasome activation drives tau pathology. *Nature* (2019) 575(7784):669–73. doi: 10.1038/s41586-019-1769-z

48. Franklin BS, Bossaller L, De Nardo D, Ratter JM, Stutz A, Engels G, et al. The adaptor ASC has extracellular and 'prionoid' activities that propagate inflammation. *Nat Immunol* (2014) 15(8):727–37. doi: 10.1038/ni.2913

49. Friker LL, Scheiblich H, Hochheiser IV, Brinkschulte R, Riedel D, Latz E, et al. β-amyloid clustering around ASC fibrils boosts its toxicity in microglia. *Cell Rep* (2020) 30(11):3743–54. doi: 10.1016/j.celrep.2020.02.025

50. Colombo E, Farina C. Astrocytes: key regulators of neuroinflammation. *Trends Immunol* (2016) 37(9):608–20. doi: 10.1016/j.it.2016.06.006

51. Oksanen M, Lehtonen S, Jaronen M, Goldsteins G, Hämäläinen RH, Koistinaho J. Astrocyte alterations in neurodegenerative pathologies and their modeling in human induced pluripotent stem cell platforms. *Cell Mol Life Sci* (2019) 76(14):2739–60. doi: 10.1007/s00018-019-03111-7

52. Gong T, Liu L, Jiang W, Zhou R. DAMP-sensing receptors in sterile inflammation and inflammatory diseases. *Nat Rev Immunol* (2020) 20(2):95–112. doi: 10.1038/s41577-019-0215-7

53. Farina C, Aloisi F, Meinl E. Astrocytes are active players in cerebral innate immunity. *Trends Immunol* (2007) 28(3):138–45. doi: 10.1016/j.it.2007.01.005

54. Katsouri L, Birch AM, Renziehausen AWJ, Zach C, Aman Y, Steeds H, et al. Ablation of reactive astrocytes exacerbates disease pathology in a model of Alzheimer's disease. *Glia* (2020) 68(5):1017–30. doi: 10.1002/glia.23759

55. Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH. Mechanisms underlying inflammation in neurodegeneration. *Cell* (2010) 140(6):918–34. doi: 10.1016/j.cell.2010.02.016

56. Liddelow SA, Barres BA. Reactive astrocytes: production, function, and therapeutic potential. *Immunity* (2017) 46(6):957–67. doi: 10.1016/j.immuni.2017.06.006

57. Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, et al. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature* (2017) 541(7638):481–7. doi: 10.1038/nature21029

58. McAlpine CS, Park J, Griciuc A, Kim E, Choi SH, Iwamoto Y, et al. Astrocytic interleukin-3 programs microglia and limits Alzheimer's disease. *Nature* (2021) 595(7869):701–6. doi: 10.1038/s41586-021-03734-6

59. Ding M, Shen Y, Wang P, Xie Z, Xu S, Zhu Z, et al. Exosomes isolated from human umbilical cord mesenchymal stem cells alleviate neuroinflammation and reduce amyloid-beta deposition by modulating microglial activation in Alzheimer's disease. *Neurochem Res* (2018) 43(11):2165–77. doi: 10.1007/s11064-018-2641-5

60. Cui GH, Wu J, Mou FF, Xie WH, Wang FB, Wang QL, et al. Exosomes derived from hypoxia-preconditioned mesenchymal stromal cells ameliorate cognitive decline by rescuing synaptic dysfunction and regulating inflammatory responses in APP/PS1 mice. *FASEB J* (2018) 32(2):654–68. doi: 10.1096/fj.201700600R

61. Cheng X, Zhang G, Zhang L, Hu Y, Zhang K, Sun X, et al. Mesenchymal stem cells deliver exogenous miR-21 via exosomes to inhibit nucleus pulposus cell apoptosis and reduce intervertebral disc degeneration. *J Cell Mol Med* (2018) 22(1):261–76. doi: 10.1111/jcmm.13316

62. Wang P, Yi T, Mao S, Li M. Neuroprotective mechanism of human umbilical cord mesenchymal stem cell-derived extracellular vesicles improving the phenotype polarization of microglia via the PI3K/AKT/Nrf2 pathway in vascular dementia. *Synapse* (2023) 77(4):e22268. doi: 10.1002/syn.22268

63. Sweeney MD, Sagare AP, Zlokovic BV. Blood-brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. *Nat Rev Neurol* (2018) 14(3):133–50. doi: 10.1038/nrneurol.2017.188

64. Shechter R, Miller O, Yovel G, Rosenzweig N, London A, Ruckh J, et al. Recruitment of beneficial M2 macrophages to injured spinal cord is orchestrated by remote brain choroid plexus. *Immunity* (2013) 38(3):555–69. doi: 10.1016/j.immuni.2013.02.012

65. Dani N, Herbst RH, McCabe C, Green GS, Kaiser K, Head JP, et al. A cellular and spatial map of the choroid plexus across brain ventricles and ages. *Cell* (2021) 184(11):3056–74. doi: 10.1016/j.cell.2021.04.003

66. Herisson F, Frodermann V, Courties G, Rohde D, Sun Y, Vandoorne K, et al. Direct vascular channels connect skull bone marrow and the brain surface enabling myeloid cell migration. *Nat Neurosci* (2018) 21(9):1209–17. doi: 10.1038/s41593-018-0213-2

67. Cugurra A, Mamuladze T, Rustenhoven J, Dykstra T, Beroshvili G, Greenberg ZJ, et al. Skull and vertebral bone marrow are myeloid cell reservoirs for the meninges and CNS parenchyma. *Science* (2021) 373(6553):eabf7844. doi: 10.1126/science.abf7844

68. Jordão MJC, Sankowski R, Brendecke SM, Sagar, Locatelli G, YH T, et al. Single-cell profiling identifies myeloid cell subsets with distinct fates during neuroinflammation. *Science* (2019) 363(6425):eaat7554. doi: 10.1126/science

69. Gate D, Saligrama N, Leventhal O, Yang AC, Unger MS, Middeldorp J, et al. Clonally expanded CD8 T cells patrol the cerebrospinal fluid in Alzheimer's disease. *Nature* (2020) 577(7790):399–404. doi: 10.1038/s41586-019-1895-7

70. Brioschi S, Wang WL, Peng V, Wang M, Shchukina I, Greenberg ZJ, et al. Heterogeneity of meningeal B cells reveals a lymphopoietic niche at the CNS borders. *Science* (2021) 373(6553):eabf9277. doi: 10.1126/science.abf9277

71. Phongpreecha T, Fernandez R, Mrdjen D, Culos A, Gajera CR, Wawro AM, et al. Single-cell peripheral immunoprofiling of Alzheimer's and Parkinson's diseases. *Sci Adv* (2020) 6(48):eabd5575. doi: 10.1126/sciadv

72. Malm T, Koistinaho M, Muona A, Magga J, Koistinaho J. The role and therapeutic potential of monocytic cells in Alzheimer's disease. *Glia* (2010) 58(8):889–900. doi: 10.1002/glia.20973

73. Larbi A, Pawelec G, Witkowski JM, Schipper HM, Derhovanessian E, Goldeck D, et al. Dramatic shifts in circulating CD4 but not CD8 T cell subsets in mild Alzheimer's disease. *J Alzheimers Dis* (2009) 17(1):91–103. doi: 10.3233/JAD-2009-1015

74. Jóźwik A, Landowski J, Bidzan L, Fülop T, Bryl E, Witkowski JM. Beta-amyloid peptides enhance the proliferative response of activated CD4CD28 lymphocytes from Alzheimer disease patients and from healthy elderly. *PloS One* (2012) 7(3):e33276. doi: 10.1371/journal.pone.0033276

75. Tian J, Dai SB, Jiang SS, Yang WY, Yan YQ, Lin ZH, et al. Specific immune status in Parkinson's disease at different ages of onset. *NPJ Parkinsons Dis* (2022) 8(1):5. doi: 10.1038/s41531-021-00271-x

76. Aguilar-Ruiz SR, Torres-Aguilar H, González-Domínguez É, Narváez J, González-Pérez G, Vargas-Ayala G, et al. Human CD16+ and CD16- monocyte subsets display unique effector properties in inflammatory conditions in vivo. *J Leukoc Biol* (2011) 90(6):1119–31. doi: 10.1189/jlb.0111022

77. Togo T, Akiyama H, Iseki E, Kondo H, Ikeda K, Kato M, et al. Occurrence of T cells in the brain of Alzheimer's disease and other neurological diseases. *J Neuroimmunol* (2002) 124(1-2):83–92. doi: 10.1016/s0165-5728(01)00496-9

78. Unger MS, Li E, Scharnagl L, Poupardin R, Altendorfer B, Mrowetz H, et al. CD8 + T-cells infiltrate Alzheimer's disease brains and regulate neuronal- and synapse-related gene expression in APP-PS1 transgenic mice. *Brain Behav Immun* (2020) 89:67–86. doi: 10.1016/j.bbi.2020.05.070

79. Shukla AK, McIntyre LL, Marsh SE, Schneider CA, Hoover EM, Walsh CM, et al. CD11a expression distinguishes infiltrating myeloid cells from plaque-associated microglia in Alzheimer's disease. *Glia* (2019) 67(5):844–56. doi: 10.1002/glia.23575

80. Browne TC, McQuillan K, McManus RM, O'Reilly JA, Mills KH, Lynch MA. IFN-γ Production by amyloid β-specific Th1 cells promotes microglial activation and

increases plaque burden in a mouse model of Alzheimer's disease. *J Immunol* (2013) 190(5):2241–51. doi: 10.4049/jimmunol.1200947

81. McQuillan K, Lynch MA, Mills KH. Activation of mixed glia by Abeta-specific Th1 and Th17 cells and its regulation by Th2 cells. *Brain Behav Immun* (2010) 24 (4):598–607. doi: 10.1016/j.bbi.2010.01.003

82. Yong VW, Moumdjian R, Yong FP, Ruijs TC, Freedman MS, Cashman N, et al. Gamma-interferon promotes proliferation of adult human astrocytes in vitro and reactive gliosis in the adult mouse brain in *vivo*. *Proc Natl Acad Sci U S A* (1991) 88 (16):7016–20. doi: 10.1073/pnas.88.16.7016

83. Trajkovic V, Stosic-Grujicic S, Samardzic T, Markovic M, Miljkovic D, Ramic Z, et al. Interleukin-17 stimulates inducible nitric oxide synthase activation in rodent astrocytes. *J Neuroimmunol* (2001) 119(2):183–91. doi: 10.1016/s0165-5728(01)00391-5

84. Yi H, Bai Y, Zhu X, Lin L, Zhao L, Wu X, et al. IL-17A induces MIP-1α expression in primary astrocytes via Src/MAPK/PI3K/NF-kB pathways: implications for multiple sclerosis. *J Neuroimmune Pharmacol* (2014) 9(5):629–41. doi: 10.1007/s11481-014-9553-1

85. Ma X, Reynolds SL, Baker BJ, Li X, Benveniste EN, Qin H. IL-17 enhancement of the IL-6 signaling cascade in astrocytes. *J Immunol* (2010) 184(9):4898–906. doi: 10.4049/jimmunol.1000142

86. Miller JS. Biology of natural killer cells in cancer and infection. *Cancer Invest* (2002) 20(3):405–19. doi: 10.1081/cnv-120001185

87. Le Page A, Bourgade K, Lamoureux J, Frost E, Pawelec G, Larbi A, et al. NK Cells are Activated in Amnestic Mild Cognitive Impairment but not in Mild Alzheimer's Disease Patients. *J Alzheimers Dis* (2015) 46(1):93–107. doi: 10.3233/JAD-143054

88. Richartz-Salzburger E, Batra A, Stransky E, Laske C, Köhler N, Bartels M, et al. Altered lymphocyte distribution in Alzheimer's disease. *J Psychiatr Res* (2007) 41(1-2):174–8. doi: 10.1016/j.jpsychires.2006.01.010

89. Le Page A, Lamoureux J, Bourgade K, Frost EH, Pawelec G, Witkowski JM, et al. Polymorphonuclear neutrophil functions are differentially altered in amnestic mild cognitive impairment and mild Alzheimer's disease patients. *J Alzheimers Dis* (2017) 60 (1):23–42. doi: 10.3233/JAD-170124

90. Yuyama K, Sun H, Usuki S, Sakai S, Hanamatsu H, Mioka T, et al. A potential function for neuronal exosomes: sequestering intracerebral amyloid-β peptide. *FEBS Lett* (2015) 589(1):84–8. doi: 10.1016/j.febslet.2014.11.027

91. Yuyama K, Sun H, Mitsutake S, Igarashi Y. Sphingolipid-modulated exosome secretion promotes clearance of amyloid-β by microglia. *J Biol Chem* (2012) 287 (14):10977–89. doi: 10.1074/jbc.M111.324616

92. Dinkins MB, Dasgupta S, Wang G, Zhu G, Bieberich E. Exosome reduction in *vivo* is associated with lower amyloid plaque load in the 5XFAD mouse model of Alzheimer's disease. *Neurobiol Aging* (2014) 35(8):1792–800. doi: 10.1016/j.neurobiolaging.2014.02.012

93. Yuyama K, Sun H, Sakai S, Mitsutake S, Okada M, Tahara H, et al. Decreased amyloid-β pathologies by intracerebral loading of glycosphingolipid-enriched exosomes in Alzheimer model mice. *J Biol Chem* (2014) 289(35):24488–98. doi: 10.1074/jbc.M114.577213

94. de Dios C, Bartolessis I, Roca-Agujetas V, Barbero-Camps E, Mari M, Morales A, et al. Oxidative inactivation of amyloid beta-degrading proteases by cholesterol-enhanced mitochondrial stress. *Redox Biol* (2019) 26:101283. doi: 10.1016/j.redox.2019.101283

95. Iwata N, Tsubuki S, Takaki Y, Watanabe K, Sekiguchi M, Hosoki E, et al. Identification of the major Abeta1-42-degrading catabolic pathway in brain parenchyma: suppression leads to biochemical and pathological deposition. *Nat Med* (2000) 6(2):143–50. doi: 10.1038/72237

96. Iwata N, Tsubuki S, Takaki Y, Shirotani K, Lu B, Gerard NP, et al. Metabolic regulation of brain Abeta by neprilysin. *Science* (2001) 292(5521):1550–2. doi: 10.1126/science.1059946

97. Farris W, Mansourian S, Chang Y, Lindsley L, Eckman EA, Frosch MP, et al. Insulin-degrading enzyme regulates the levels of insulin, amyloid beta-protein, and the beta-amyloid precursor protein intracellular domain in *vivo*. *Proc Natl Acad Sci U.S.A.* (2003) 100(7):4162–7. doi: 10.1073/pnas.0230450100

98. Yoon SS, Jo SA. Mechanisms of amyloid-β Peptide clearance: potential therapeutic targets for Alzheimer's disease. *Biomol Ther (Seoul)* (2012) 20(3):245–55. doi: 10.4062/biomolther.2012.20.3.245

99. Katsuda T, Tsuchiya R, Kosaka N, Yoshioka Y, Takagaki K, Oki K, et al. Human adipose tissue-derived mesenchymal stem cells secrete functional neprilysin-bound exosomes. *Sci Rep* (2013) 3:1197. doi: 10.1038/srep01197

100. de Godoy MA, Saraiva LM, de Carvalho LRP, Vasconcelos-Dos-Santos A, Beiral HJV, Ramos AB, et al. Mesenchymal stem cells and cell-derived extracellular vesicles protect hippocampal neurons from oxidative stress and synapse damage induced by amyloid-β oligomers. *J Biol Chem* (2018) 293(6):1957–75. doi: 10.1074/jbc.M117.807180

101. Xin H, Katakowski M, Wang F, Qian JY, Liu XS, Ali MM, et al. MicroRNA cluster miR-17-92 Cluster in Exosomes Enhance Neuroplasticity and Functional Recovery After Stroke in Rats. *Stroke* (2017) 48(3):747–53. doi: 10.1161/STROKEAHA.116.015204

102. Kim MS, Haney MJ, Zhao Y, Yuan D, Deygen I, Klyachko NL, et al. Engineering macrophage-derived exosomes for targeted paclitaxel delivery to pulmonary metastases: in *vitro* and in *vivo* evaluations. *Nanomedicine* (2018) 14 (1):195–204. doi: 10.1016/j.nano.2017.09.011

103. Qu M, Lin Q, Huang L, Fu Y, Wang L, He S, et al. Dopamine-loaded blood exosomes targeted to brain for better treatment of Parkinson's disease. *J Control Release* (2018) 287:156–66. doi: 10.1016/j.jconrel.2018.08.035

104. Wang H, Sui H, Zheng Y, Jiang Y, Shi Y, Liang J, et al. Curcumin-primed exosomes potently ameliorate cognitive function in AD mice by inhibiting hyperphosphorylation of the Tau protein through the AKT/GSK-3β pathway. *Nanoscale* (2019) 11(15):7481–96. doi: 10.1039/c9nr01255a

105. Cui GH, Guo HD, Li H, Zhai Y, Gong ZB, Wu J, et al. RVG-modified exosomes derived from mesenchymal stem cells rescue memory deficits by regulating inflammatory responses in a mouse model of Alzheimer's disease. *Immun Ageing* (2019) 16:10. doi: 10.1186/s12979-019-0150-2

106. Liu WL, Lin HW, Lin MR, Yu Y, Liu HH, Dai YL, et al. Emerging blood exosome-based biomarkers for preclinical and clinical Alzheimer's disease: a meta-analysis and systematic review. *Neural Regener Res* (2022) 17(11):2381–90. doi: 10.4103/1673-5374.335832

107. Gorabi AM, Kiaie N, Barreto GE, Read MI, Tafti HA, Sahebkar A. The therapeutic potential of mesenchymal stem cell-derived exosomes in treatment of neurodegenerative diseases. *Mol Neurobiol* (2019) 56(12):8157–67. doi: 10.1007/s12035-019-01663-0

108. Reza-Zaldivar EE, Hernández-Sapiéns MA, Minjarez B, Gutiérrez-Mercado YK, Márquez-Aguirre AL, Canales-Aguirre AA. Potential effects of MSC-derived exosomes in neuroplasticity in Alzheimer's disease. *Front Cell Neurosci* (2018) 12:317. doi: 10.3389/fncel.2018.00317

109. Phinney DG, Pittenger MF. Concise review: MSC-derived exosomes for cell-free therapy. *Stem Cells* (2017) 35(4):851–8. doi: 10.1002/stem.2575

110. Stremersch S, De Smedt SC, Raemdonck K. Therapeutic and diagnostic applications of extracellular vesicles. *J Control Release* (2016) 244(Pt B):167–83. doi: 10.1016/j.jconrel.2016.07.054

111. Yeo RW, Lai RC, Zhang B, Tan SS, Yin Y, Teh BJ, et al. Mesenchymal stem cell: an efficient mass producer of exosomes for drug delivery. *Adv Drug Delivery Rev* (2013) 65(3):336–41. doi: 10.1016/j.addr.2012.07.001

frontiers | Frontiers in Immunology

TYPE Review
PUBLISHED 08 February 2024
DOI 10.3389/fimmu.2024.1355845

OPEN ACCESS

EDITED BY
Chun Wai Mai,
UCSI University, Malaysia

REVIEWED BY
Noa B Martin-Cofreces,
Princess University Hospital, Spain
Giorgio Mangino,
Sapienza University of Rome, Italy

*CORRESPONDENCE
Jean-Philippe Herbeuval
jean-philippe.herbeuval@parisdescartes.fr
Thibaut Fourniols
thibaut.fourniols@everzom.com

†These authors have contributed equally to this work and share last authorship

RECEIVED 14 December 2023
ACCEPTED 24 January 2024
PUBLISHED 08 February 2024

CITATION
Wong C, Stoilova I, Gazeau F, Herbeuval J-P and Fourniols T (2024) Mesenchymal stromal cell derived extracellular vesicles as a therapeutic tool: immune regulation, MSC priming, and applications to SLE.
Front. Immunol. 15:1355845.
doi: 10.3389/fimmu.2024.1355845

Mesenchymal stromal cell derived extracellular vesicles as a therapeutic tool: immune regulation, MSC priming, and applications to SLE

Christophe Wong[1,2,3], Ivana Stoilova[2,3], Florence Gazeau[4], Jean-Philippe Herbeuval[2,3*†] and Thibaut Fourniols[1*†]

[1]EVerZom, Paris, France, [2]Centre National de la Recherche Scientifique (CNRS) Unité Mixte de Recherche (UMR) 8601, Université Paris Cité, Paris, France, [3]Chemistry and Biology, Modeling and Immunology for Therapy (CBMIT), Université Paris Cité, Paris, France, [4]Matière et Systèmes Complexes (MSC) UMR CNRS 7057, Université Paris Cité, Paris, France

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by a dysfunction of the immune system. Mesenchymal stromal cell (MSCs) derived extracellular vesicles (EVs) are nanometer-sized particles carrying a diverse range of bioactive molecules, such as proteins, miRNAs, and lipids. Despite the methodological disparities, recent works on MSC-EVs have highlighted their broad immunosuppressive effect, thus driving forwards the potential of MSC-EVs in the treatment of chronic diseases. Nonetheless, their mechanism of action is still unclear, and better understanding is needed for clinical application. Therefore, we describe in this review the diverse range of bioactive molecules mediating their immunomodulatory effect, the techniques and possibilities for enhancing their immune activity, and finally the potential application to SLE.

KEYWORDS

extracellular vesicles, secretome, immune regulation, mesenchymal stromal cell, priming, systemic lupus erythematosus

1 Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease, associated with multiorgan damage and variable clinical manifestations (1). SLE is a multifactorial disorder, in which the phenotype is modulated by a combination of genetic, epigenetic, environmental, hormonal and immunoregulatory factors. SLE is characterized by a dysfunction of the immune system, notably a presence of autoreactive T cells and hyperactive B cells, leading to a loss of tolerance, production of autoantibodies against self-antigens formation and deposition of immune complexes, as well as a sustained systemic inflammation. SLE is classified as interferonopathy, since type I interferons (IFN-

I) play a crucial role in the development of the disease (2). The chronic production of IFN-I, especially IFN-α, is a key characteristic of SLE and contributes to the autoimmune process (3). IFN-I stimulates the activation of interferon producing cells, such as plasmacytoid dendritic cells (pDCs), which are responsible for elevated levels of IFN-α in blood plasma and organs (4). The excessive signaling of IFN-I results in the increased expression of various pro-inflammatory cytokines, chemokines, and markers of immune cell activation. This, in turn, contributes to the dysregulation of the immune system and the generation of autoantibodies. Additionally, prolonged exposure to IFN-I can boost the activation and survival of autoreactive B cells and encourage the differentiation of T cells into pro-inflammatory subsets, sustaining the autoimmune response (5). SLE manifests through periods of flares and remissions, with symptoms showing considerable variation among individuals. Treatment objectives revolve around managing symptoms, mitigating inflammation, and safeguarding against organ damage. Due to its clinical heterogeneity and complex pathogenesis, SLE remains hard to diagnose and the available treatments show limited efficiency.

Long considered as platelet debris, EVs have gained critical interest from the scientific community in the recent years (6). EVs are released by all cellular organisms and can be defined by their heterogeneity. Indeed, apart from their structural definition, a cell secreted particle enclosed by a lipidic bilayer, EVs differ from their sources, biogenesis, biophysical, biochemical characteristics and functional activity (7). A standard classification can be established based on the biogenesis of EVs: exosomes are generally small sized extracellular vesicles, from endosomal origin, released by multivesicular bodies (MVBs). Ectosomes can be defined as extracellular vesicles stemming from the plasma membrane. EV biogenesis pathways and mechanisms of interaction with target cells have been extensively reviewed (8, 9). EVs are heterogenous in size and have a diameter varying in the range of the nanometer. However, EVs are not the only nanometer-sized particles secreted by cells and there is still no specific markers allowing an efficient separation of EVs. Thus, guidelines have been set by the international community regarding ways of purifying and characterizing EVs. Transmembranal proteins such as tetraspanins, cytosolic proteins such as TSG101, and other cell-dependent proteins have been used to demonstrate the EV nature and to some extent the degree of purity of an EV preparation (10). EVs have been linked with a variety of cellular functions, and can affect other cells with their surface proteins or the cargo encapsulated by EVs. EVs carry diverse proteins, nucleic acids and lipids from their parent cell, which can in turn be delivered to the recipient cell. EVs have a fundamental role in the immune system and in immune-related diseases, highlighting their potential either as a new biomarker or a therapeutic tool (7).

In the field of mesenchymal stromal cells (MSCs), EVs have emerged as a potential avenue for cell-free therapy. MSCs have been used for their immunosuppressive capabilities, with utilization in clinical trials and availability on the market. Nonetheless, the use of MSCs can carry some drawbacks and EVs have been looked into as a cell-free alternative. More specifically, as EVs carry the same biological molecules as their parent cells, the effect of MSCs is partially mediated by them with paracrine actions. EVs have shown to have the same immunomodulatory and regenerative potential than their parent cells, and have thus become a promising alternative to MSCs themselves for therapies (11, 12). More broadly, the secretome embraces not only the previously mentioned EVs but also a variety of proteins, lipids, and nucleic acids. These components may be either inside or adsorbed to EVs, or freely present in the secretome (13). This added complexity of which fractions holds the therapeutical activity and the issues of standardization regarding means of production, concentration, and characterization in the field of EVs make any conclusion towards the effect of MSC derived EVs or MSC derived secretome a complex equation.

2 Multiparametric influence on the immunomodulatory potential of MSC-EVs

In order to understand which fractions hold the immunomodulatory potential, the next part focuses on having a critical look over the many parameters influencing MSC-EV immune activity. Some scientific papers use the term exosomes to describe their fractions, while they could be more accurately defined as EVs or even secretome. Various downstream processes such as size exclusion chromatography (SEC), polyethylene glycol (PEG) and ultracentrifugation broadly used by the scientific community separate selectively the content of the secretome (14), and alter the composition of the protein corona of EVs (15). The protein corona is a set of proteins and other molecules adsorbed to the EVs. These proteins can be bioactive and mediate part of the immunomodulatory potential (16, 17). All of this heterogeneity of methods brings at the same time strength to the immunomodulatory response of EVs as a global effect, but brings hardship as to determine what is driving this specific effect. A number of studies showed the difference of potency between the secretome, the EV free fraction, and the fractions containing EVs. Papait et al. showed that amniotic MSCs derived conditioned media (e.g secretome) and the EV free fraction (meaning the supernatant after ultracentrifugation) maintained their immunomodulatory potential by inhibition of T cell, promotion of Tregs, shifted monocytes towards M2 instead of M1, but also reduction of the maturation of dendritic cells. The EV fraction collected after ultracentrifugation had no effect even though these EVs were uptaken (18). Another study has found no effect of the three fractions on the inhibition of T cell proliferation (19). These differences in results might be from methodological differences, specifically in the dose parameter (e.g. protein amount in ug or particles concentration measured by Nanoparticles Tracking Analysis (NTA)), cell sources of EVs, or downstream processes. A recent study has shown that the immunomodulatory potential of

MSCs is independent of EVs, which runs counter to most results in the literature using EV-enriched fractions (20). González-Cubero et al. showed that EVs and soluble fractions from conditioned media promote an *in vitro* anti-inflammatory modulation in intervertebral disc degeneration in a "highly synergistic way", thus highlighting that the use of the whole secretome rather than isolated EVs might be more beneficial for therapy (21). While it is still hard to understand what exactly mediates the immunomodulatory potential, the effect might come from a synergistic combination of both the soluble factors from the secretome and the EVs.

Other factors have also been shown to have an impact on the immunomodulatory potential of EVs or secretome. Indeed, the donor of primary cells, the source of MSCs, the passage doubling number, and the production method (which will be addressed later on) can influence the secretome content and thus the potency of EVs (22–25). Immortalized MSCs might be a solution for reproducible batches of secretome/EVs for therapeutic use (26).

The characterization of the secretome of MSCs shows the presence of both pro and anti-inflammatory molecules. While the secretome of MSC-EVs has shown to have high levels of IL-6 and IL-8 (27), a recent study showed that the conditioned media derived from umbilical cord (UC)-MSC promoted anti-inflammatory macrophages polarization. This despite a mostly pro-inflammatory profile of cytokines, though the authors have only investigated surface markers of M2 macrophages (28). MSC-EVs contain nucleic acids, which may bind to TLR7 and 9 as foreign nucleic acids and trigger a pro-inflammatory cascade. However, this review hasn't found any reports of inflammation induced by MSC-EV-associated DNA (29).

The methods to assess EV immune potency are also critical. The dose of EV is differently calculated, either with particles concentration, protein concentration, or even EVs per receptor cells, which may result in some disparities in results (30). Some studies tend to isolate a specific cell type for their *in vitro* potency, though immune cells do not uptake homogenously EVs. Monocytes seem to uptake the highest proportion of MSC-EVs (18, 31, 32). Nonetheless, even though EVs are not uptaken by lymphocytes, studies on MSC-EVs and on EV-free fractions added to PBMCs have shown an immunosuppressive effect on T cells, thus independent of the uptake (18, 33, 34). Others have shown that MSC-EVs failed to suppress lymphocyte proliferation (35). The immunomodulatory effect of MSCs on B cells is independent of secreted EVs (36). This reinforces that the global immunomodulatory effect might not be entirely mediated by EVs, but also through soluble factors of the secretome. The effect of EVs on T cells could also be mediated in an indirect way, through the actions on EV-uptaking immune cells (37, 38).

Regarding the immunoregulatory effect of MSC-EVs on immune cells, some reviews have already discussed it thoroughly, either as a comprehensive overview (39), or more precisely in the case of SLE (40). Thus, in the next part, this review will focus on the immunomodulatory factors in the secretome of MSCs.

3 The immunomodulatory bioactive molecules of MSC-derived secretome and EVs

The immunomodulatory potential of MSC-EVs could be driven by a broad range of bioactive molecules, including proteins, nucleic acids, and lipids. Many evidences suggest the importance of specific

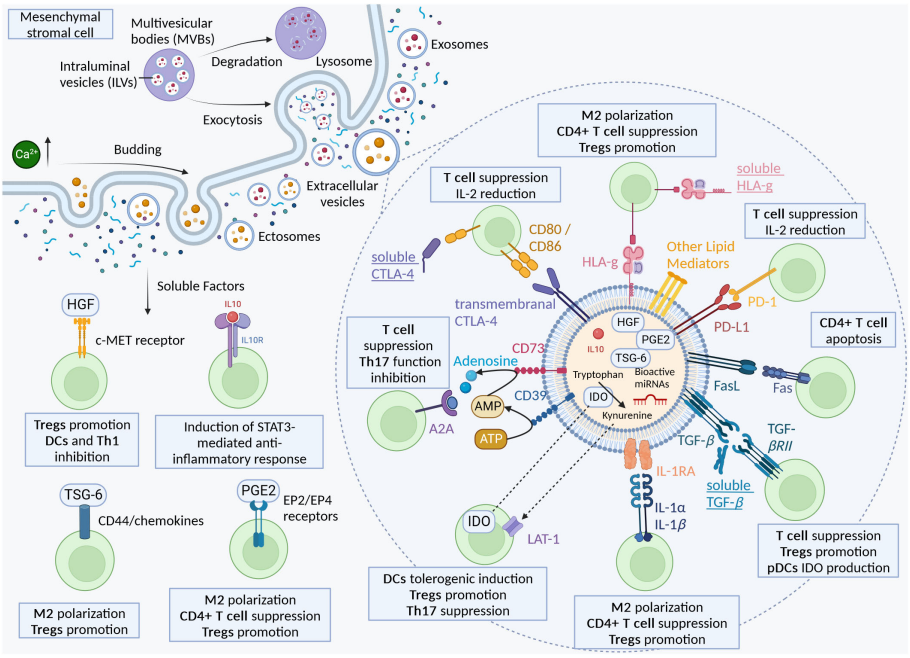


FIGURE 1
The immunomodulatory bioactive molecules of MSC-derived secretome and EVs.

bioactive proteins mediating the immunomodulatory effect of MSC-EVs. This review focuses on the supposed localization of the bioactive molecules, though it is important to remember that soluble proteins secreted by MSCs are also probably carried by EVs. The lack of studies comparing EV-free conditioned media and purified EV fractions makes the localization of all bioactive molecules a hard task. (Figure 1) is a proposition of the immunomodulatory bioactive molecules of MSC-derived secretome and EVs.

3.1 Soluble proteins secreted by MSCs

The immune regulation of MSCs can be mediated by soluble proteins secreted by MSCs.

IL-10 is an anti-inflammatory cytokine secreted by immune cells such as macrophages, dendritic cells, Th and Tregs, amongst others. IL-10 induces a strong immunosuppressive response in immune cells, targeting specific genes, cytokines and chemokines production. An in-depth focus on IL-10 and its effects has already been reviewed (41). IL-10 is also secreted by MSCs and mediates part of MSC-EVs immunomodulatory activity. Jiang et al. showed cardioprotective effects of MSC-EVs using *in vivo* models of pigs, which were "largely blunted" after IL-10 knockdown (IL10KD). Using *in vitro* models, IL10KD MSC-EVs achieved less inhibition of T-cell proliferation than control MSC-EVs (42). These results are in accordance with Eirin et al. observation on their model of kidney inflammation, showing an IL-10 dependent immunomodulation of MSC-EVs (43). MSC-EVs derived from MSC overexpressing IL-10 showed higher concentrations of IL-10, enhanced the suppressive effect of these EVs on Th1 and Th17 and upregulated Tregs *in vitro* (44). IL-10 thus mediates the immunosuppressive effect of MSCs.

Hepatocyte growth factor (HGF) is secreted by MSCs, which can mediate immunosuppressive effects. The effect of HGF in the scope of MSCs has already been described (45, 46). Chen et al. showed that knockdown of HGF secretion by MSCs abrogate the suppression of T cell proliferation, and monocytes cultured with HGF alone or MSCs can secrete high levels of IL-10 through ERK1/2 pathway (47). In the case of MSC-EVs, treatment with MSC derived "microvesicles" reduced IL-6 production and increased IL-10 production in the conditioned media of endothelial cells, which was reverted after knockdown of HGF in MSCs. Notably, MSC-derived conditioned media had a higher regulative activity than microvesicles (48).

Tumor necrosis factor-inducible gene 6 protein (TSG-6) is a protein implicated in the immunomodulatory effects of MSCs. TSG-6 is a multifunctional protein with anti-inflammatory properties, and can interact with a broad variety of ligands such as chemokines. TSG-6 is overexpressed in a pro-inflammatory environment (49). Chaubey et al. showed the presence of TSG-6 in UC-MSC-EVs was linked to the therapeutic efficiency in their model of mouse lung disease (50). TSG-6 in canine MSC-EVs played a key role in the downregulation of pro-inflammatory cytokines, the polarization of M1 to M2 and the increase of Tregs in the colon (51). Human bone marrow(BM)-MSC derived EVs containing TSG-6 decreased pro-inflammatory cytokines in scar tissues, inhibited collagen deposition, thus suppressing scar formation. This effect was enhanced using BM-MSC modified to overexpress TSG-6, and reverted to the normal after knockdown, showing a TSG-6 dependent effect of MSC-EVs (52). Lu et et al. showed that AD-MSC derived EVs had therapeutic effects in their model of spinal cord ischemia reperfusion injury by transmitting TSG-6 (53). Other studies have shown the importance of TSG-6 in MSC conditioned media for immunomodulatory activity (54, 55).

3.2 Proteins adsorbed to the corona & membrane proteins

IL-1 receptor antagonist (IL-1RA) has also been found in the secretome of MSCs. IL-1α and IL-1β, potent inflammatory cytokines can bind to IL-1R, eliciting a MyD88-dependent inflammatory cascade. On the other hand, IL-1RA can bind without triggering any downstream signaling, therefore acting as a potent antagonist of IL-1a and IL-1β and shutting down immune responses. An in-depth report of the actions of MSC-derived IL-1RA can be found (56). Kou et al. showed that IL-1RA was found in the supernatant of cultured mouse MSCs after centrifugation at 3,000 g and 20,000 g, and not in the pelleted fraction representing the bigger EVs, but was found in was in the EV pelleted fraction they call "small EVs" after ultracentrifugation at 120 000g. Using various methods of microscopy, they showed that IL-1RA was carried by EVs on their surface. They subsequently demonstrated that IL-1RA associated EV release was controlled by Fas through binding with Fap-1 and Cav-1 and upregulated when the MSCs were treated with TNF-α (57).

Transforming Growth Factor Beta (TGF-β) is a cytokine carried by MSC-EVs (58). TGF-β can bind on its receptor TGF-βRII, which triggers a downstream cascade targeting a variety of growth factors and inflammatory cytokines. TGF-β1 can be found either bounded to the plasma membrane, or in soluble form (59). TGF-β has a pleiotropic function on the regulation of immune cells. TGF-β suppresses T cells while promoting Tregs, regulates B cell activation, promotes expression of IDO in pDCs, inhibits DC function, amongst other actions (60, 61). TGF-β has been studied in MSC-EVs regarding its immunosuppressive activity. Alvarez et al. showed that TGF-β1 was primarily present in MSC-EV fraction, compared to EV free supernatant and that the immunomodulatory activity of MSC-EVs on CD4+ T cells is partially mediated by TGF-β1 (62). This same conclusion has been advanced by another group in a canine model (34). Kim et al. showed that amongst other molecules, TGF-β had a significant influence on the immunomodulatory properties of MSC-EVs in their model of cornea. Silencing of TGF-β1 resulted in the loss of MSC-EV suppressive effects, while overexpression resulted in more effective EVs in the suppression of T-cell receptor IL-2 and IFN-γ secretion in activated splenocytes (25). Song et al. used MSC-EVs produced in 3D with exogenous TGF-β3, which resulted in higher levels of TGF-β1 compared to non-treated 3D MSC-EVs, and higher immunomodulatory activity of treated EVs (63).

CD73 is one of the three conventional surface markers to identify MSCs (64). But it is also one of the enzyme of purinergic signaling, responsible for transforming Adenosine monophosphate (AMP) into Adenosine (Ado), a nucleoside known for its immunosuppressive role on T cells and Th17 cells (65). Indeed, CD73+ engineered UC-MSC derived EVs reduced concentration of ATP while increasing the levels of adenosine compared to non-engineered EVs. These engineered EVs improved the functional recovery after spinal cord injury, improving the polarization from M1 to M2 phenotype, but also downregulated more the pro-inflammatory cytokines after spinal cord injury compared to the native EVs, while it also upregulated more the anti-inflammatory cytokines such as IL-10 (66). Another study has shown that conditioning of MSCs with pro-inflammatory cytokines promoted the expression of CD73 in EVs, and that these EVs reprogram macrophages from M1 to M2 phenotype (67). Although MSCs and hence their EVs express CD39 (at a low level) and CD73, a study has shown that efficient adenosine production from ATP requires cooperation with activated T-cells expressing CD39 (68). The co-culture in the previous study significantly increased the expression of CD39 in MSCs and of CD73 in T-cells, supporting the previous findings of Saldanha-Araujo et al. (69). In the case of SLE, patients show a silenced activity of CD73 and CD38 in B cells, resulting in decrease of production of anti-inflammatory adenosine (70). MSC-EVs may thus constitute a potential therapeutic approach for the treatment of SLE due to their high expression of CD73. Other methods of overexpressing this enzyme could be also used to further improve the treatment.

3.2.1 Immune checkpoints

Cytotoxic T lymphocyte antigen 4 (CTLA-4) is an important regulator of T cell activation (71). It can bind to CD80 and CD86, resulting in an inhibition response rather than a stimulatory one by CD28. Furthermore, CTLA-4 can also bind to DCs, resulting in a downregulation of CD80 and CD86 (72). CTLA-4 expressed on Tregs stabilizes the interaction with T cells allowing for the Treg mediated suppression of T cell (73). CTLA-4 has been shown to be expressed by MSCs under different isoforms. CTLA-4 can be found as a transmembrane protein or can also be secreted. Secreted CTLA-4 by BM-MSCs has been significantly increased under hypoxic conditions. The authors have also shown a CTLA-4 mediated inhibitory effect of the secretion of TNF-α induced by PHA of PBMCs (74). As far as we know, there are no studies directly showing the presence of CTLA-4 in the membrane of MSC-EVs. As CTLA-4 is expressed in MSCs, their derived EVs could potentially carry it.

On the contrary, PD-L1 has been found on the surface of MSC-EVs. PD-L1 binds to the receptor programmed death-1 (PD-1), expressed on T cell surface, leading to an inhibition of their activation (75, 76). Wu et al. have shown that PD-L1 overexpressing MSC-EVs have enhanced therapeutic activity compared to native EVs in a model of LPS-induced pneumonia in mice (77). Other teams also used PD-L1 overexpressing MSC-EVs, showing an increased therapeutic activity in their *in vitro* and *in vivo* models. Notably, the use of anti-PD-L1 antibody reverted the effect to the level of wild type MSC-EVs, suggesting a PD-L1/PD-1 dependent immunosuppression (76–79). Pro-inflammatory and hypoxia MSC conditioning resulted in higher levels of IDO and PD-L1 in EVs, resulting in higher immunomodulatory activity (80). In regard to SLE, CD4+/CD25+/Foxp3+ Tregs from SLE patients expressed significantly lower amounts of PD-L1 compared to healthy patients (81).

HLA-G is a non-classic major histocompatibility complex (MHC) molecule which can mediate immunosuppression. HLA-G can bind to receptors expressed in immune cells, such as CD158d, CD85j, CD85d, CD8 and CD160, and can polarize macrophages towards an M2 phenotype, inhibits the proliferation of T cells, induces Tregs, inhibits the maturation of DCs amongst other actions. There are seven different isoforms, with four membrane bound (HLA-G1, G2, G3, and G4), and three soluble isoforms (HLA-G5, G6, and G7) (82–85). A limited number of studies have examined the impact of human leukocyte antigen G (HLA-G) in MSC-EVs. Selmani et al. showed that HLA-G5 can be found in the secretome of BM-MSCs. BM-MSCs secreted HLA-G5 in an IL-10 dependent manner. Interestingly, they noted a decrease of HLA-G expression over passages, but HLA-G5 content in supernatants was not affected. They further demonstrated that HLA-G5 is necessary for the suppression of allogenic T-cell and the expansion of Tregs, and the inhibition of "NK-cell mediated cytolysis and interferon-y secretion" (86). HLA-G has been found in "high levels" of MSC-EVs of four bone marrow donors, purified by PEGylation and ultracentrifugation (87). This could indicate that HLA-G can indeed be found in the secretome, but also linked with MSC-EVs. In the same vein, HLA-G, in both its membrane and soluble isoforms, has also been identified in UC-MSCs, without specifying their EVs (88).

3.2.2 Apoptose inducing ligands

FasL or CD178 or CD95 Ligand induces apoptotic death upon binding with its receptor Fas. Interestingly, FasL induces cell death only in its membrane-bound form (mFasL) while its soluble form (sFasL) binds to Fas without induction of the proapoptotic signaling pathway, thus competing with its membrane-bound form (89). sFasL is found after cleavage of mFasL by metalloproteinases (MMPs). sFasL can trigger inflammatory pathways such as NF-κB (90). As a matter of fact, MMPs are found in the secretome of MSCs after priming with IL-1β (91), and might cleave FasL into sFasL at the surface of MSCs and their EVs, which might in turn compete with FasL. It is not known in the detail in the case of MSCs and EVs how this dynamic between pro-inflammatory sFasL, MMPs and pro-apoptotic mFasL plays out regarding the immunomodulatory potential of MSC-EVs. Some studies have shown a FasL dependent apoptotic effect using MSCs, but little has been done with MSC-EVs. Vacaru et al. used modified MSCs overexpressing FasL which showed improved death induction in CD4+ and CD8+ T cells, but has not looked into the effect of EVs

(92). Akiyama et al. showed that bone marrow MSCs induced T-cell apototosis via Fas/FasL pathway. Recruitment of T cells was induced by secretion of Fas-regulated monocyte chemotactic protein 1 (MCP-1) (93). Wang et al. induced apoptotic EVs in mouse MSCs, and showed that these EVs used FasL to induce apoptosis in Multiple myeloma cells *in vivo* in mice (94).

3.3 EV cargos

Indoleamine 2,3-dioxygenase (IDO), is an immunosuppressive enzyme leading to the degradation of tryptophan to kynurenine and other metabolites, resulting in stopping T-cell proliferation, induction and activation of regulatory T cells (95). Kynurenine produced by DCs can be taken up by T cells through the large neutral amino acid transporter (LAT-1), and induces FoxP3 expression, resulting in Tregs differentiation. Kynurenine also inhibits the expression of retinoic acid receptor-related orphan receptor-γt (ROR-γt), thus suppressing the differentiation of Th17 cells. Kynurenine can also induce tolerogenic phenotype in DCs (96). IDO activation in plasmacytoid dendritic cells (pDC) induce immune tolerance and inhibition of IFN-I production (97, 98). As SLE is characterized by an enhanced IFN-I production by pDC, IDO could be a key immune modulator of MSC-EVs in SLE treatment. In MSCs, IDO is present in MSC-EVs and in cells, but only after IFN-γ pre conditioning. Increased levels of IDO expression result in higher levels of Kynurenine, both in the cell and in the EVs. EVs unprimed with IFN-γ showed no immunomodulatory properties, while primed EVs suppressed T-cell and induced Treg cells (99). MSC-EVs from overexpressing IDO MSCs have shown to activate M2 polarization in an IDO dependent manner (100). Another study showed an effect of unprimed MSC-EVs on inhibition of PBMC proliferation, but no statistically significant increase of Tregs proportion. Treatment with MSC-EVs primed with IFN-γ and TGF-β showed a higher increase in the proportion of Tregs compared to conditions primed with only one molecule, or unprimed. This increased immunomodulatory activity was linked with a higher concentration of IDO and IL-10 (101). However, Serejo et al. showed that while IFN-γ pre-treatment of MSCs did increase the expression of IDO in both cells and EVs, it did not result in increased T-cell suppression of proliferation (102). Another study found that conditioned media from primed cells successfully suppressed T cell proliferation, while primed MSC-EVs, and unprimed CM and EVs had no effect (103). The authors hypothesize that the absence of immunomodulatory effect could be due to a difference in experimental methodology. These studies show that IFN-γ priming is needed for IDO expression, which could mediate the immunosuppressive effect of MSC-EVs.

Regarding the iNOS-NO axis, the immunosuppressive activity of MSCs from human, monkey and pig is mostly mediated through IDO rather than iNOS, whereas MSCs derived from rat, hamster and rabbit mostly use iNOS (104). For that reason, we are redirecting to another review which has already described in-depth the role of iNOS in the immunomodulatory potential of MSCs and their EVs (105).

3.3.1 miRNAs

The immunomodulatory potential of MSC-EVs could also be mediated through miRNAs. miRNA are non-coding small nucleic acid of around twenty nucleotides which interfere with mRNA translation. As such, when delivered to the immune cells, miRNAs can influence their function through the inhibition of transcription factors or targeting of specific pathways regarding maturation, activation of the immune cells (106). miRNAs can be transported and delivered to other cells by extracellular vesicles, which make them a potent molecule responsible for the immunomodulatory potential of MSC-EVs. MSC-EVs have been found to be enriched in various miRNAs, with differentially expressed miRNAs depending on the source of the MSC-EVs (107). This should be kept in mind looking at potential immunomodulatory mediated miRNAs action as they could be weakly enriched in a specific source of MSC-EVs.

Kim et al. identified let-7b-5p and miR-21-5p as key microRNAs mediating the immunomodulatory effect of MSC-EVs (25). miR-21-5p, identified as one of the most enriched miRNA in MSC-EVs, targets CCR7 resulting in attenuated DC migration and function (108). miR-146a has been shown by Song et al. to promote M1-M2 transition and plays a protective role in sepsis (109). miR-181c in UC-MSC-EVs repressed inflammation by suppressing TLR4 (110). miR-155 has been shown to significantly reduce the proliferation of activated PBMCs, by targeting miR-221 as a potential inflammation mediator (111). miR-223 in MSC-EVs could also restrain adhesion and migration of T cells (112). A lot of other miRNAs have been identified as a potential mediator of MSC-EVs immunomodulatory effects (113). A few studies have highlighted limitations of current RNA-sequencing methods, which could raise concerns over the reproducibility and comparability of sequencing data across library preparation platforms (114, 115). Nonetheless, Srinivasan et al. indicate that technical variability is smaller than biological variability regarding the use of small RNAs in EVs (116). miRNAs have thus shown to mediate a broad Immunosuppressive effect of MSC-EVs.

3.3.2 Lipid mediators

While the scientific community has been mainly focused on the immunomodulatory impact of miRNAs and various proteins, some bioactive lipids have been coming in light.

Lipid mediators are lipids derived from polyunsaturated fatty acid (PUFAs) who can promote inflammation or resolution. PUFAs can be modified by different types of enzymes into a diversity of bioactive lipids. Among them, specialized pro resolving mediators (SPMs) such as resolvins or maresins are known to be anti-inflammatory and play a key role in the resolution of inflammation. The regulatory effect of SPMs on immune cells has

already been described extensively (117, 118). Regulatory lipid mediators have been found in MSC-EVs (119–121), and their concentration can be increased by priming MSCs with PUFAs, or even with pro-inflammatory cytokines (121, 122). Enzymes of the many lipid mediators pathways are carried by MSC and their EVs. Cardiac MSCs and their EVs carry 5-LO and 15-LO, enzymes which play a role in transforming PUFAs in SPMs (123). Thus, these enzymes are able to modify dynamically the concentration in various SPMs inside EVs. PUFA supplementation of MSCs to improve the immunomodulatory potential has already been described in the literature (124, 125). Secretome of primed MSC with different types of PUFA promoted an immunoregulatory phenotype in macrophages (126). While lipid mediators may play a role in the immunomodulatory effect of MSC-EVs, little research has been done about them.

Prostaglandin E2 (PGE2) is a lipid mediator belonging to the prostaglandin family. PGE2 is produced by the enzymes COX-1 and COX-2 from the ω-3 PUFAs arachidonic acid (AA) and can bind on EP receptors expressed on the surface of immune cells (45). EP2 and EP4 receptors, upon binding, upregulates cAMP levels whereas EP3 downregulates it (127). PGE2 binds on EP2 and EP4 receptors and regulates T helper cells (128). PGE2 has shown to have a broad immunomodulatory effect. It promotes an anti-inflammatory phenotype in macrophages, has ambivalent effects on DCs depending on their development stage, suppresses T cell activation and promotes Treg cells (129, 130). PGE2 can also induce neutrophils to produce less pro-inflammatory lipid mediators and increase the production of anti-inflammatory lipid mediators such as Lipoxins (131). In prostate cancer cells, PGE2 has been found either to be carried with EVs but also secreted as a soluble factor (132). PGE2 is expressed in MSCs, carried by their EVs, and the cells can be primed with ω-3 PUFA to express more PGE2 (121). Conditioned media from human MSCs spheroids inhibited pro-inflammatory cytokines and increased the secretion of anti-inflammatory cytokines in LPS stimulated macrophages, in a PGE2 dependent manner, mediated by binding on the EP4 receptor (133). MSC-EVs from pluripotent stem cells isolated by anion-exchange chromatography were able to inhibit the activating effects of dendritic cells on group 2 innate lymphoid cell, mediated by PGE2 binding on EP2/EP4 (134). TNF-α and IFN-γ were reduced in activated splenocytes, partially through PGE2/COX2 (135). These studies show the importance of bioactive lipids in the therapeutic activity of MSC-EVs.

In the end, multiple mechanism of actions have been proposed, using *in vitro* and *in vivo* models. Taking all these studies into account, the immunomodulatory potential of MSC-EVs is probably mediated not by one specific pathway, but by a combination of all these bioactive molecules. Since these molecules are located in the secretome either as a soluble fraction, in the membrane of the EVs, or inside the EVs, it appears that managing to keep all these bioactive molecules as a part of the secretome downstream process will be key for keeping MSC-related potency.

Many studies discussed before used different techniques to enhance the quantity of immunomodulatory molecules in MSC-EVs. In the next part, we discuss the techniques to obtain MSC-EVs with a higher immunomodulatory potential.

4 MSC priming for enhanced immunomodulatory potential

MSCs have an important role in tissue repair and can be mobilized to the sites of tissue damage and inflammation. Thus, MSCs *in vivo* are often subject to inflammatory stimuli such as but not limited to, damage-associated molecular patterns (DAMPs), pathogen-associated molecular patterns (PAMPs), pro-inflammatory cytokines from activated immune cells, or hypoxia (136, 137). Attempts to recreate this environment *in vitro* have been used to increase the immunomodulatory potential of MSC-EVs. As a matter of fact, MSCs have high plasticity regarding their immunomodulatory potential: an anti-inflammatory environment might inhibit the immunosuppressive activity of MSCs, while a pro-inflammatory one will enhance it (138, 139). This can explain why some immunomodulatory molecules such as IDO are only produced under a pro-inflammatory environment. (Table 1) summarizes all the priming methods for MSC-EV enhanced immunomodulatory potential.

Priming or conditioning MSCs with different methods have shown to induce different EV release, membrane markers, differential uptake and activation of T cell subsets (167). Priming also modifies the miRNA and protein EV cargo (168–170). Importantly, the source of MSCs and the inter-donor variability within the same source of MSCs has an impact on the response to priming and thus the improvement of immunosuppressive effect of MSC-EVs. Peltzer et al. showed that PCA failed to discriminate groups between MSC- EVs from 5 donors without priming, with IFN-γ priming and hypoxia, regarding their differential miRNA expression, showing that inter-individual variability was stronger, especially regarding their response to priming (171). Gorgun et al. showed that while priming had a significant effect on the secretome of MSCs, it did not majorly affect the miRNA in their EVs (172). A recent study was able to discriminate hypoxia and normoxia group looking at their miRNA, possibly underlying a donor disparity between studies (173). Jin et al. showed that priming overcame the MSC inter-donor variability by looking at gene expression (174). Priming with a cocktail of cytokines resulted in two different responses in different donors of MSCs (140). These studies show that priming MSC has significative changes on the biophysical, biochemical and bioactive properties of MSC-EVs, but the changes might be hidden by donor- and source-dependent differences.

One way to recreate the inflammatory conditions of tissue damage is to target Toll-Like Receptors (TLRs), which recognize various types of molecules such as DAMPs and PAMPs. LPS-preconditioned MSC-EVs were more efficient in converting THP-1 to M2 phenotype *in vitro*, and in relieving inflammation *in vivo* than untreated MSC-EVs, notably through miR-let-7b (141). LPS-primed MSC-EVs were significantly more efficient at increasing M2 and reducing M1 polarization *in vitro*, and were more effective in

TABLE 1 MSC-priming for increased MSC derived secretome and EVs enhanced immunomodulatory potential.

Priming used	Cell Type	Bioactive molecule and mean of action	Immunoregulatory effect	Reference
IFN-γ (1000U/mL) TNF-α (1000U/mL) IL-1β (10ng/mL) 24h	Nasal mucosa and BM-MSC EVs	Increase of PD-L1, PD-L2, ICAM-1	Suppressed CD3+ T cells Enhanced therapeutic effects in GvHD mice	(140)
LPS (100ng/mL) 48h	UC-MSC EVs	miR-let-7b Regulation of TLR4/NF-κB/ STAT3/AKT signaling pathway	Increased M2 polarization	(141)
LPS	BM-MSC EVs	AKT1/AKT2 signaling	Decreased of pro-inflammatory cytokines levels Promoted M2 polarization Alleviated myocardial injury Reduced post-infarction inflammation	(142)
LPS (10ng to 10μg/mL) Poly (I:C) (100ng to 100μg/mL) 6h	UC-MSC EVs		Decreased of pro-inflammatory cytokines levels Promoted M2 polarization	(143)
LPS (100ng/mL 1ug/mL)	BM-MSC EVs		LPS-concentration dependent variation of macrophages markers	(144)
LPS (1μg/mL) 24h	Periodontal ligament MSC EVs	miR-433-3p TLR2/TLR4/NF-κB p65	Promoted M1 phenotype	(145)
LPS (10ng/mL) Poly (I:C) (1μg/mL) 1h	Human multipotent MSC		Increased expression of immune suppressive factors with LPS Pro inflammatory phenotype with Poly (I:C)	(146)
IFN-γ (10ng/mL) TNF-α (15ng/mL) 72h	Human multipotent MSC EVs	A20 TSG-6	Enhanced T cell suppression Increased levels of immunomodulatory proteins	(147)
IFN-γ (10ng/mL) 48h	Adipose MSC Secretome		Promoted M2 polarization	(148)
IL-1β (25ng/mL) 24h	Human BM-MSC EVs	miR-147b Inhibition of NF-kb pathway	Inhibited inflammatory factors expression in osteoarthritis cells	(149)
IL-1β (10ng/mL) 12h	Mouse MSC EVs	miR-21 targets PDCD4	Higher expression of immunosuppressive factors in MSCs Promoted M2 polarization Alleviated sepsis in mice	(150)
IFN-γ (100ng/mL) 24/48h	UC-MSC EVs		Loss of protection against ischemic acute kidney injury No differences in Treg induction	(151)
TGF-β (10ng/mL) IFN-γ (1000IU/mL) 72h	UC-MSC EVs	IDO, IL-10	Increased proportion of Tregs Higher levels in IDO, IL-10 and IFN-γ Similar levels of PBMC proliferation inhibition	(101)
Atorvastatin 1μgmol/L 48h	Mouse MSC EVs	lncRNA H19	Improved cardiac function Ameliorated fibrosis after myocardial infarction Reduced cardiac apoptosis and inflammation	(152)
IDO overexpression	Mouse BM-MSC EVs	IDO	Accelerated repair process after acute kidney injury Reduced fibrosis, inflammation Promoted M2 polarization	(100)
IL-10 overexpression	Human UC-MSC EVs	IL-10	Enhanced suppressive effect on Tcells Differentiation of Th1/Th17 cells Upregulated Tregs Ameliorated autoimmune uveitis	(44)
TSG-6 overexpression	Human BM-MSC EVs	TSG-6	Attenuated scar pathological injury Decreased inflammation	(52)
TGF-β, PTX3, let-7b-5p, miR-21-5p overexpression	Human MSC EVs	TGF-b, PTX3, let-7b-5p, miR-21-5p	Decreased inflammation from Th1 and TH17 cells	(25)

(Continued)

TABLE 1 Continued

Priming used	Cell Type	Bioactive molecule and mean of action	Immunoregulatory effect	Reference
			Suppressed TLR4 and TCR signaling in splenocytes	
CD73 overexpression	Human UC-MSC EVs	CD73, Promotion cAMP/PKA signaling pathway	Decreased inflammation and ATP Promoted M2 polarization Ameliorated recovery after spinal cord injury	(66)
PD-L1 overexpression	Mouse BM-MSC EVs	PD-L1 PD-1/PD-L1 pathway	Alleviated pneumonia Reduced levels of inflammation	(77)
PD-L1 overexpression	BM-MSC EVs	PD-L1	Prolonged allograft survival Increased Treg proportion and suppressive effect on T cell proliferation	(78)
PD-L1 overexpression	Mouse BM-MSC EVs	PD-L1	Inhibited immune cells activation Reduced inflammation in colon Ameliorated ulcerative colitis and psoriasis	(79)
miR-181a overexpression	Human UC-MSC EVs	miR-181a targeting c-Fos gene in PBMCs	Decreased levels of inflammation Increased Treg polarization Retarded ischemic damage *in vivo*	(153)
miR-126 overexpression	Mouse adipose MSC EVs	miR-126	Ameliorated functional recovery after stroke Inhibited microglial activation and inflammation after ischemic stroke	(154)
TRAIL overexpression	Human adult MSC EVs	TRAIL	Induced apoptosis in cancer cell lines	(155)
Hypoxia	Mouse adipose MSC EVs	lncRNA-Gm37494 upregulated	Promoted functional recovery after spinal cord injury Promoted M2 polarization and suppressed inflammation	(156)
Hypoxia	Bone MSC EVs	mIR-216a-5p enrichment TLR4/NF-κB/PI3K/AKT signaling cascades	Promoted M2 polarization Increased functional recovery after spinal cord injury	(157)
IFN-γ (50ng/mL) TNF-α (10ng/mL) IL-1β (10ng/mL) HIF-overexpression	Human dental pulp MSC EVs		Promoted M2 polarization Reduced inflammation and PBMC adhesion Ameliorated fibrosis Attenuated TNBS-induced colitis in mice	(80)
Hypoxia	Human adipose MSC EVs		Improved renal recovery after Ischemic injury Promoted M2 polarization	(158)
Spheroid	Human amnion MSC EVs		Inhibited activated PBMC proliferation	(159)
Spheroid	Human MSC secretome		Aggregation method influenced PGE2 secretion Suppressed T-cell Polarized M2 polarization Enhanced expression of immunomodulatory factors	(160)
Spheroid	Human adipose MSC		Protective effect against colitis Inhibited immune cell infiltration in colon	(161)
Spheroid TNF-α, IFN-γ (20 ng/mL)	Human UC-MSC EVs		Increased HGF levels in secretome Enhanced reduction of NF-kB and pro inflammatory cytokines expression Anti-apoptotic and anti-fibrotic effect	(162)
Spheroid	Human BM-MSC EVs		Lower kynurenine concentration Lower anti-inflammatory effect in lungs and lower anti-fibrotic effect	(163)
Aggregates in WAVE bioreactor	Human BM-MSC EVs		Higher miR-21-5p and miR-22-3p expression Higher inhibition of CD8+ T cell proliferation	(164)

(Continued)

TABLE 1 Continued

Priming used	Cell Type	Bioactive molecule and mean of action	Immunoregulatory effect	Reference
Hollow Fiber 3D culture	Human UC-MSC EVs		Improved renal function after kidney injury Reduced inflammatory factors Repressed T cell proliferation and macrophage infiltration	(165)
Hollow Fiber 3D culture	Human UC-MSC EVs		Decreased expression of inflammatory factors Improved cardiac function in acute myocardial infarction Promoted M2 polarization	(166)

relieving post-infarction inflammation in mice (142). Hwang et al. showed that TLR-3 and 4 primed MSCs secretomes were more successful in reducing pro-inflammatory cytokines from LPS-induced macrophages. EVs were key in increasing the percentage of M2 (143). Notably, Kink et al. showed that the concentration of LPS for priming MSC resulted in EVs having different effects on macrophage receptors expression, which might explain the effect of the next studies (144). Indeed, some studies show that TLR-priming does not always result in enhanced immunosuppressive effect. Recent results from Cui et al. showed that LPS primed MSC-EVs induced a M1 and not a M2 phenotype (145). Previous results from Waterman et al. showed a pro-inflammatory phenotype MSCs after TLR-4 priming, and anti-inflammatory phenotype after TLR-3 priming (146). All in all, these results show that priming through TLRs might be an interesting method to prime MSC-EVs, though some optimization of concentration of priming might be important to achieve the highest enhanced immunosuppressive effect.

A second way to prime MSCs is to use pro-inflammatory cytokines. A study by Cheng et al. showed that priming with IFN-γ and TNF-α resulted with higher suppression of T cell proliferation and induced a different protein profile with higher levels of anti-inflammatory proteins such as TSG-6 and A20 (147). Ragni et al. showed that IFN-γ priming changes the proteins secreted by MSCs and the miRNA content of their EVs, resulting in a diminution of M1 polarization (148). Priming with IL-1β resulted in higher anti-inflammatory activity of EVs, with a significant increase of mir-147b, which partially mediated the immunomodulatory effect of primed MSC-EVs in osteoarthritis cells (149). IL-1β priming on mouse MSC resulted in an enhanced macrophage polarization to M2 *in vivo* and *in vitro*, and a better therapeutic effect on septic mice by MSC-EVs. Equivalent priming also induced higher expression of miR-21, which mediated the effect of MSC-EVs on sepsis (150). Nonetheless, conditioning does not always result in better activity: priming with IFN-γ did not induce a better therapeutic effect in T-cell modulation activity, and induced a loss of protection against ischemic acute kidney injury (151). A few teams have also tried cocktails of different pro-inflammatory molecules, compared to one-molecule priming. A combination of IFN-γ and TGF-β priming resulted in a higher proportion of Treg cells after treatment with EVs, but also elevated levels of IFN-γ, IL-10 and IDO within these EVs. The combination of priming resulted in better immunomodulatory effect than EVs derived from untreated MSC, or MSCs treated with only one of the molecules (101). Hackel et al. showed a variation of response between MSCs when treated with multicytokine combination of IFN-γ, TNF-α and IL-1β. One group of 3 donors secreted more PD-L1 with the full priming compared to two-cytokines priming, and the other group of 3 other donors responded equally with 3 and 2-cytokine priming. Nonetheless, priming still induced higher levels of PD-L1 and PD-L2 compared to non-primed EVs. Higher therapeutic effects of primed MSC-EVs were mediated by PD-1 ligands (140). Priming MSCs with pro-inflammatory components has been shown to modify the whole secretome, elevating the level of anti-inflammatory molecules in the secretome and in EVs, thus mediating higher potency and therapeutical effects of MSC-EVs. Priming with higher number of cytokines seems to induce a higher immunomodulatory effect of EVs.

Other molecules have also been used to prime MSCs. Conditioning with Atorvastatin resulted in a better cardioprotective effect on infarcted rat heart, with a better inhibition of TNF-α and Il-6 in the tissue of the infarct zone (152).

Genetic modifications of MSC producing EVs can improve the immunomodulatory potential of MSC-EVs. A few studies already described above have targeted specific immunomodulatory proteins such as IDO (100), IL-10 (44), TSG-6 (52), TGF-β, PTX3, miR-let7-5p and miR-21-5p (25), CD73 (66), PD-L1 (77–79), in order to secrete more of these specific proteins and induce a better immunoregulatory activity of MSC-EVs. Genetic modifications can also target the expression of bioactive miRNAs. Overexpression of miRNA-181a induced better inhibition of the inflammatory response, increased the percentage of Tregs among PBMCs, and delayed ischemic damage *in vivo* (153). Overexpression of miRNA-126 promoted functional recovery after stroke by suppressing microglia activation (154). Genetic modifications can also target molecules which are not constitutively expressed by MSCs. A study has shown that modified MSCs for the expression of TRAIL secrete EVs with TRAIL, and are able to induce apoptosis in various cell lines (155).

Conditioning of MSC in hypoxia has also been shown to improve the immunomodulatory potential of MSC-EVs. Hypoxic environment leads to increased levels of hypoxia-inducible factors, notably HIF-1a, which regulates many physiological pathways, including angiogenesis (175). The effect of hypoxia on the immune-modulatory properties of BM-MSCs has already been reviewed (176). Extracellular vesicles derived from hypoxia conditioned MSCs were more effective in decreasing levels of pro-

inflammatory cytokines and in shifting microglia from M1 to M2 polarization, compared to non-treated MSC-derived EVs (156). Same results were observed by another group (157). Hypoxia was also shown to increase the expression of HGF (172), which we have already discussed the immunomodulatory effect. Gómez-Ferrer et al. showed that double-primed hypoxia + inflammation MSC-derived EVs were more efficient in repolarizing M1 to M2-like phenotype than single-primed inflammation MSC-EVs. These EVs promoted healing in a TNBS induced mouse colitis, partially through reduction of pro-inflammatory cytokines (80). Hypoxic UC-MSC-derived EVs inhibited more efficiently maturation of DCs (177). Hypoxia treated AD-MSC EVs were more efficient in reducing macrophage infiltration, reducing levels of Il-6, though MCP-1 levels were higher, compared to non-treated MSC-EVs, in renal tissue after ischemia reperfusion injury (158). Thus, hypoxia conditioning of MSCs could be a relevant technique to enhance the MSC-EV immunosuppressive functions for chronic diseases.

Finally, a few studies have reported higher potency with EVs derived from MSC cultured in 3D. 3D culture of MSCs englobes a great variety of techniques, including but not limited to microcarriers, diverse scaffolds, microgels, and spheroids, which can be then cultured in bioreactors or other types of vessels. 3D culture better mimics the natural cell conditions, and increases the levels of secreted angiogenic and immunomodulatory factors (178).

Bulati et al. were able to differentially cluster between IFN-γ primed and 3D spheroid cultured MSCs by looking at their miRNA EVs (179). Many studies have shown that MSCs have increased immunomodulatory potential after 3D spheroid culture (159–161). EVs derived from pro-inflammatory primed MSC spheroids had an enhanced anti-inflammatory effect by decreasing the expression of NF-κB, IL-8 and IL-6 in TNF-induced inflammation in HK2 cells, compared to 3D cultured only EVs and 2D cultured EVs (162). Nonetheless, Kusuma et al. have shown contrasting results where 3D spheroid cultured MSC-EVs produced significantly less IDO, and that overall, these EVs had a lower immunosuppressive and therapeutic potency than 2D MSC-EVs (163). Further studies are needed to understand the impact of MSC-EVs derived from spheroids.

Regarding MSC culture in bioreactors, 3D cultured in WAVE bioreactor MSC-EVs induced same decrease in M1 markers expression in macrophages than 2D MSC-EVS, but had enhanced suppression of CD8+ T cell proliferation (164). Hollow Fiber bioreactor systems have gained popularity for the 3D culture of MSC for EV research. 3D cultured MSC-EVs using this system were more efficient than 2D cultured MSC-EVs in alleviating acute kidney injury, notably by reducing inflammatory factors, repressing T cell and macrophage infiltration (165). Sun et al. also used the hollow fiber system, and found that 3D-MSC EVs exhibited a stronger anti-inflammatory effect on stimulated monocytes, but also in acute myocardial infarction rats (166). Thus, 3D culture in bioreactor seems to enhance the immune-modulatory potential of MSC-EVs.

Other than MSCs, other EVs have been showing immunosuppressive potential for a therapeutic approach. Namely, tumor cells like sarcoma cells evade the immune system, which is partially mediated by the release of EVs (180, 181). EVs derived from Ewing Sarcoma induced a pro-inflammatory response on myeloid cells, but impaired the maturation and function of dendritic cells (182). Droste et al. wrote an in-depth review about tumor derived EVs, their effect on immune cells and how *in vivo* animal models help understand the potential of these EVs (183).

Based on their broad immunosuppressive effects and the possibility to enhance them through a variety of techniques, MSC-EVs could be a promising therapeutic solution for the treatment of SLE.

5 EVs for diagnosis and treatment of SLE

5.1 EVs for diagnosis of SLE

Recent studies suggested that EVs open a new perspective for both diagnosis and treatment of SLE. Several teams reported differences between the EV profile of SLE patients and healthy controls. First, the number of total EV was found to vary. Most studies reported an increase of total EVs in SLE patients (184–189), while Nielsen et al. reported a decrease (190). Apart from the number of EV, their composition has also been shown to differ between SLE patients and healthy controls. Østergaard et al. outlined a decrease in the level of cytoskeletal, mitochondrial and organelle proteins contained in microparticles from SLE patients (191). Additionally, Chuang et al. recently reported an overexpression of Eosinophil Cationic Protein (ECP) in SLE T cell-derived EVs and demonstrated their pro-inflammatory property in a mouse model (192).

Aside from protein-containing EVs, attention was drawn to miRNA-containing EVs. Li et al. reported compared to healthy controls, an increase of miR-21 and miR-155, and a decrease of miR-146a in serum EVs (193). Additionally, the expression of miR-21 and miR-146a were negatively associated with respectively anti-SSA/RO antibodies and anti-dsDNA antibodies, which are important features of SLE pathogenesis. The decrease of miR-146a-containing exosomes in the serum of SLE patients was also demonstrated by Dong et al., who suggested that miR-146a is internalized into MSCs and contributes to MSC senescence in SLE patients by targeting the TRAF6/NF-κB pathway (194). Interestingly, Perez-Hernandez et al. had previously found an increase in the urinary miR-146a-containing exosomes in SLE patients (195), suggesting that the location of the EVs should be also taken into consideration. Furthermore, Tan et al. showed that exosomal miR-451a is downregulated in the serum of SLE patients and correlates with the SLE disease activity and renal damage, due to its implication in intercellular communication (196). It has also been demonstrated that microRNAs-containing exosomes, isolated from the plasma of SLE patients, can activate pDCs through the receptor TLR7 and induce excessive production of IFN-α, leading to a chronic state of inflammation in SLE (197). Overall, the EV profile of SLE patients seem to significantly differ from the one of

healthy controls, which outlines them as prominent biomarkers for SLE. Moreover, their presence in various body fluids, such as blood, urine and saliva, guarantee a facilitated access for diagnosis and could replace the rather invasive biopsies traditionally used for monitoring the disease progression.

5.2 MSC-EVs for treatment of SLE

Allogenic MSCs have already been used as a potential treatment for SLE (198, 199). As the broad immunosuppressive effect of MSCs is mediated by the secretome and EVs, they represent a potential alternative for the treatment of SLE (200). Multiple studies have already been carried using MRL/lpr mice model. Xie et al. have tested the effect of human umbilical cord MSCs and their EVs on a classical animal model of SLE. They have shown that UC-MSCs exert immunoregulatory effects on SLE, partially mediated by their EVs. UC-MSC-EVs were able to inhibit CD4+ T cells in their model, but lower amounts of TGF-β and IL-17 were found in the supernatant (201). Another study also using UC-MSC-EVs has shown an amelioration of SLE after EV administration in MRL/lpr mice by inducing M2 macrophages polarization and increasing regulatory T cell (202). BM-MSC-EVs promoted anti-inflammatory phenotype of macrophages, and induced recruitment of Tregs in murine lupus nephritis model. Notably, they showed the importance of miR-16 and miR-21 in the polarization of macrophages (203). Another study has compared tooth MSCs and their EVs to treat SLE in the same MRL/lpr mice model. The administration of EVs exerted a therapeutic effect on this model by rescuing the immune microenvironment. Furthermore, they have shown a decreased effect with the presence of RNase, hinting at the importance of RNA in the immunomodulatory potential of EVs (204). BM-MSC derived apoptotic vesicles ameliorated lupus in the same model, by suppressing activated CD4+ T cells (205).

Dou et al. showed that MSC-EVs reduced the expression of pro-inflammatory cytokines and promoted M2 polarization of macrophages, notably through tsRNA-21109. This same RNA is downregulated in SLE patients, which sheds light on possible means of action of MSC-EVs in SLE (206). Chen et al. investigated the effect of MSC-EVs in diffuse alveolar hemorrhage (DAH) mice, an uncommon but fatal complication of SLE. EVs alleviated symptoms of DAH, decreased the expression of pro-inflammatory factors and enhanced M2 polarization (207).

Tu et al. showed a lower expression of miR-19b, an imbalance between Th17 and Tregs, a much higher expression of pro-inflammatory cytokines in PBMCs from SLE patients. UC-MSC EVs treatment increased the expression of miR-19b, regulated the Th17/Tregs balance and reduced the expression of pro-inflammatory factors (208). The amount of B cells in SLE patients is significantly upregulated. UC-MSC-EVs promoted B cell apoptosis, inhibited overactivation and decreased the levels of pro-inflammatory cytokines, possibly through regulation of the upregulated miR-155 in SLE patients (209). Type I IFN release by pDCs is closely related to the severity of SLE. While MSCs have shown to reduce the release of IFN-α and inhibit the function of pDCs (210, 211), no studies regarding the impact of MSC-EVs have been carried. Overall, these studies show the therapeutic potential of MSC-EVs for the treatment of SLE.

6 Conclusion

The emerging field of EVs presents a promising avenue for therapeutic treatment. EVs carry a variety of membrane and soluble proteins, and play a key role in immune processes. Thus, EVs could be used for diagnostic as a biomarker, or a therapeutic tool. More specifically, MSC-EVs mediate a broad immunosuppressive effect, showcasing their potential as a cell-free therapy for SLE. Further techniques such as pre-conditioning of MSCs, genetic modification or EV engineering could enhance their immunomodulatory activity and could be applied to further therapeutic applications of EVs.

However, a more specific understanding of whether the immunoregulatory activity is mediated by EV-associated bioactive molecules, soluble factors, or both is needed. The impact of the many sources of heterogeneity in EV studies on these immune mediators should be investigated. The EV field still suffers from barriers such as standardization of isolation and characterization methods, Good Manufacturing Practice (GMP)-compliant large scale production, or specific guidelines for validation of EVs as a therapeutic tool, which in turn hinders the use of EVs for the treatment of SLE. Furthermore, regarding SLE, the role of EVs on pDC activation should be further investigated to understand their potential role on chronic production of IFN-I in SLE. Similarly, a fine characterization of pDC derived EVs content should be carried out prior considering the use of EVs as therapeutic strategy. The effect of MSC-EVs regarding the regulation of interferon production of pDCs should be investigated. Finally, methodological studies on the dosage and administration interval of MSC-EVs in SLE are still essential to advance their therapeutic development.

Author contributions

CW: Writing – original draft, Writing – review & editing. IS: Writing – original draft, Writing – review & editing. FG: Writing – original draft, Writing – review & editing. J-PH: Writing – original draft, Writing – review & editing. TF: Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. CW is a student from the FIRE PhD program funded by the Bettencourt Schueller foundation and the EURIP graduate program (ANR-17-EURE-0012). IS is a student from the MTCI PhD program, and this

work was supported by the Fondation pour la Recherche Médicale, grant number ECO202306017404.

Acknowledgments

All figures were created using Biorender.com.

Conflict of interest

CW and TF are employed by EVerZom.

The remaining 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

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Tsokos GC. Systemic lupus erythematosus. *New Engl J Med* (2011) 365(22):2110–21. doi: 10.1056/NEJMra1100359

2. Kaul A, Gordon C, Crow MK, Touma Z, Urowitz MB, Van Vollenhoven R, et al. Systemic lupus erythematosus. *Nat Rev Dis Primers.* (2016) 2(1):16039. doi: 10.1038/nrdp.2016.39

3. Banchereau J, Pascual V. Type I interferon in systemic lupus erythematosus and other autoimmune diseases. *Immunity* (2006) 25(3):383–92. doi: 10.1016/j.immuni.2006.08.010

4. Rodero MP, Decalf J, Bondet V, Hunt D, Rice GI, Werneke S, et al. Detection of interferon alpha protein reveals differential levels and cellular sources in disease. *J Exp Med* (2017) 214(5):1547–55. doi: 10.1084/jem.20161451

5. Dieudonné Y, Gies V, Guffroy A, Keime C, Bird AK, Liesveld J, et al. Transitional B cells in quiescent SLE: An early checkpoint imprinted by IFN. *J Autoimmunity.* (2019) 102:150–8. doi: 10.1016/j.jaut.2019.05.002

6. Couch Y, Buzàs EI, Vizio DD, Gho YS, Harrison P, Hill AF, et al. A brief history of nearly EV-erything – The rise and rise of extracellular vesicles. *J Extracell Vesicles.* (2021) 10(14):e12144. doi: 10.1002/jev2.12144

7. Buzas EI. The roles of extracellular vesicles in the immune system. *Nat Rev Immunol* (2023) 23(4):236–50. doi: 10.1038/s41577-022-00763-8

8. Meldolesi J. Exosomes and ectosomes in intercellular communication. *Curr Biol* (2018) 28(8):R435–44. doi: 10.1016/j.cub.2018.01.059

9. van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. *Nat Rev Mol Cell Biol* (2018) 19(4):213–28. doi: 10.1038/nrm.2017.125

10. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *J Extracell Vesicles.* (2018) 7(1):1535750. doi: 10.1080/20013078.2018.1535750

11. Witwer KW, Van Balkom BWM, Bruno S, Choo A, Dominici M, Gimona M, et al. Defining mesenchymal stromal cell (MSC)-derived small extracellular vesicles for therapeutic applications. *J Extracell Vesicles.* (2019) 8(1):1609206. doi: 10.1080/20013078.2019.1609206

12. Rani S, Ryan AE, Griffin MD, Ritter T. Mesenchymal stem cell-derived extracellular vesicles: toward cell-free therapeutic applications. *Mol Ther* (2015) 23(5):812–23. doi: 10.1038/mt.2015.44

13. Eleuteri S, Fierabracci A. Insights into the secretome of mesenchymal stem cells and its potential applications. *Int J Mol Sci* (2019) 20(18):4597. doi: 10.3390/ijms20184597

14. Sidhom K, Obi PO, Saleem A. A review of exosomal isolation methods: is size exclusion chromatography the best option? *Int J Mol Sci* (2020) 21(18):6466. doi: 10.3390/ijms21186466

15. Wolf M, Poupardin RW, Ebner-Peking P, Andrade AC, Blöchl C, Obermayer A, et al. A functional corona around extracellular vesicles enhances angiogenesis, skin regeneration and immunomodulation. *J Extracellular Vesicles.* (2022) 11(4):e12207. doi: 10.1002/jev2.12207

16. Heidarzadeh M, Zarebkohan A, Rahbarghazi R, Sokullu E. Protein corona and exosomes: new challenges and prospects. *Cell Communication Signaling* (2023) 21(1):64. doi: 10.1186/s12964-023-01089-1

17. Tóth EÁ, Turiák L, Visnovitz T, Cserép C, Mázló A, Sódar BW, et al. Formation of a protein corona on the surface of extracellular vesicles in blood plasma. *J Extracell Vesicles.* (2021) 10(11):e12140. doi: 10.1002/jev2.12140

18. Papait A, Ragni E, Cargnoni A, Vertua E, Romele P, Masserdotti A, et al. Comparison of EV-free fraction, EVs, and total secretome of amniotic mesenchymal stromal cells for their immunomodulatory potential: a translational perspective. *Front Immunol* (2022) 13:960909. doi: 10.3389/fimmu.2022.960909

19. Skovronova R, Scaccia E, Calcat-i-Cervera S, Bussolati B, O'Brien T, Bieback K. Adipose stromal cells bioproducts as cell-free therapies: manufacturing and therapeutic dose determine in *vitro* functionality. *J Trans Med* (2023) 21(1):723. doi: 10.1186/s12967-023-04602-9

20. Carceller MC, Guillén MI, Gil ML, Alcaraz MJ. Extracellular vesicles do not mediate the anti-inflammatory actions of mouse-derived adipose tissue mesenchymal stem cells secretome. *Int J Mol Sci* (2021) 22(3):1375. doi: 10.3390/ijms22031375

21. González-Cubero E, González-Fernández ML, Olivera ER, Villar-Suárez V. Extracellular vesicle and soluble fractions of adipose tissue-derived mesenchymal stem cells secretome induce inflammatory cytokines modulation in an in *vitro* model of discogenic pain. *Spine J* (2022) 22(7):1222–34. doi: 10.1016/j.spinee.2022.01.012

22. Kordelas L, Schwich E, Dittrich R, Horn PA, Beelen DW, Börger V, et al. Individual immune-modulatory capabilities of MSC-derived extracellular vesicle (EV) preparations and recipient-dependent responsiveness. *Int J Mol Sci* (2019) 20(7):1642. doi: 10.3390/ijms20071642

23. Madel RJ, Börger V, Dittrich R, Bremer M, Tertel T, Phuong NNT, et al. Independent human mesenchymal stromal cell-derived extracellular vesicle preparations differentially attenuate symptoms in an advanced murine graft-versus-host disease model. *Cytotherapy* (2023) 25(8):821–36. doi: 10.1016/j.jcyt.2023.03.008

24. Turlo AJ, Hammond DE, Ramsbottom KA, Soul J, Gillen A, McDonald K, et al. Mesenchymal stromal cell secretome is affected by tissue source and donor age. *Stem Cells* (2023) 41(11):1047–59. doi: 10.1093/stmcls/sxad060

25. Kim H, Lee MJ, Bae EH, Ryu JS, Kaur G, Kim HJ, et al. Comprehensive molecular profiles of functionally effective MSC-derived extracellular vesicles in immunomodulation. *Mol Ther* (2020) 28(7):1628–44. doi: 10.1016/j.ymthe.2020.04.020

26. Ramos YFM, Tertel T, Shaw G, Staubach S, de Almeida RC, Suchiman E, et al. Characterizing the secretome of licensed hiPSC-derived MSCs. *Stem Cell Res Ther* (2022) 13(1):434. doi: 10.1186/s13287-022-03117-2

27. Kiselevskii MV, VlasenkoRY, Stepanyan NG, Shubina I, Sitdikova SM, Kirgizov KI, et al. Secretome of mesenchymal bone marrow stem cells: is it immunosuppressive or proinflammatory? *Bull Exp Biol Med* (2021) 172(2):250–3. doi: 10.1007/s10517-021-05371-5

28. Peshkova M, Korneev A, Suleimanov S, Vlasova II, Svistunov A, Kosheleva N, et al. MSCs' conditioned media cytokine and growth factor profiles and their impact on macrophage polarization. *Stem Cell Res Ther* (2023) 14(1):142. doi: 10.1186/s13287-023-03381-w

29. Park KS, Bandeira E, Shelke GV, Lässer C, Lötvall J. Enhancement of therapeutic potential of mesenchymal stem cell-derived extracellular vesicles. *Stem Cell Res Ther* (2019) 10:288. doi: 10.1186/s13287-019-1398-3

30. Gupta D, Zickler AM, El Andaloussi S. Dosing extracellular vesicles. *Advanced Drug Delivery Rev* (2021) 178:113961. doi: 10.1016/j.addr.2021.113961

31. Eitan E, Green J, Bodogai M, Mode NA, Bæk R, Jørgensen MM, et al. Age-related changes in plasma extracellular vesicle characteristics and internalization by leukocytes. *Sci Rep* (2017) 7(1):1342. doi: 10.1038/s41598-017-01386-z

32. Khare D, Or R, Resnick I, Barkatz C, Almogi-Hazan O, Avni B. Mesenchymal stromal cell-derived exosomes affect mRNA expression and function of B-lymphocytes. *Front Immunol* (2018) 9:3053. doi: 10.3389/fimmu.2018.03053

33. Del Fattore A, Luciano R, Pascucci L, Goffredo BM, Giorda E, Scapaticci M, et al. Immunoregulatory effects of mesenchymal stem cell-derived extracellular vesicles on T lymphocytes. *Cell Transplant.* (2015) 24(12):2615–27. doi: 10.3727/096368915X687543

34. Crain SK, Robinson SR, Thane KE, Davis AM, Meola DM, Barton BA, et al. Extracellular vesicles from wharton's jelly mesenchymal stem cells suppress CD4 expressing T cells through transforming growth factor beta and adenosine signaling in a canine model. *Stem Cells Dev* (2019) 28(3):212–26. doi: 10.1089/scd.2018.0097

35. Gouveia de Andrade AV, Bertolino G, Riewaldt J, Bieback K, Karbanová J, Odendahl M, et al. Extracellular vesicles secreted by bone marrow- and adipose tissue-derived mesenchymal stromal cells fail to suppress lymphocyte proliferation. *Stem Cells Dev* (2015) 24(11):1374–6. doi: 10.1089/scd.2014.0563

36. Carreras-Planella L, Monguió-Tortajada M, Borràs FE, Franquesa M. Immunomodulatory effect of MSC on B cells is independent of secreted extracellular vesicles. *Front Immunol* (2019) 10:1288. doi: 10.3389/fimmu.2019.01288

37. Zhang B, Yeo RWY, Lai RC, Sim EWK, Chin KC, Lim SK. Mesenchymal stromal cell exosome–enhanced regulatory T-cell production through an antigen-presenting cell–mediated pathway. *Cytotherapy* (2018) 20(5):687–96. doi: 10.1016/j.jcyt.2018.02.372

38. Du YM, Zhuansun YX, Chen R, Lin L, Lin Y, Li JG. Mesenchymal stem cell exosomes promote immunosuppression of regulatory T cells in asthma. *Exp Cell Res* (2018) 363(1):114–20. doi: 10.1016/j.yexcr.2017.12.021

39. Xie M, Xiong W, She Z, Wen Z, Abdirahman AS, Wan W, et al. Immunoregulatory effects of stem cell-derived extracellular vesicles on immune cells. *Front Immunol* (2020) 11:13. doi: 10.3389/fimmu.2020.00013

40. Yang C, Sun J, Tian Y, Li H, Zhang L, Yang J, et al. Immunomodulatory effect of MSCs and MSCs-derived extracellular vesicles in systemic lupus erythematosus. *Front Immunol* (2021) 12:714832. doi: 10.3389/fimmu.2021.714832

41. Saraiva M, Vieira P, O'Garra A. Biology and therapeutic potential of interleukin-10. *J Exp Med* (2019) 217(1):e20190418. doi: 10.1084/jem.20190418

42. Jiang Y, Hong S, Zhu X, Zhang L, Tang H, Jordan KL, et al. IL-10 partly mediates the ability of MSC-derived extracellular vesicles to attenuate myocardial damage in experimental metabolic renovascular hypertension. *Front Immunol* (2022) 13:940093. doi: 10.3389/fimmu.2022.940093

43. Eirin A, Zhu XY, Puranik AS, Tang H, McGurren KA, van Wijnen AJ, et al. Mesenchymal stem cell–derived extracellular vesicles attenuate kidney inflammation. *Kidney Int* (2017) 92(1):114–24. doi: 10.1016/j.kint.2016.12.023

44. Li Y, Ren X, Zhang Z, Duan Y, Li H, Chen S, et al. Effect of small extracellular vesicles derived from IL-10-overexpressing mesenchymal stem cells on experimental autoimmune uveitis. *Stem Cell Res Ther* (2022) 13(1):100. doi: 10.1186/s13287-022-02780-9

45. Jiang W, Xu J. Immune modulation by mesenchymal stem cells. *Cell Proliferation.* (2020) 53(1):e12712. doi: 10.1111/cpr.12712

46. Volarevic V, Gazdic M, Simovic Markovic B, Jovicic N, Djonov V, Arsenijevic N. Mesenchymal stem cell-derived factors: Immuno-modulatory effects and therapeutic potential. *BioFactors* (2017) 43(5):633–44. doi: 10.1002/biof.1374

47. Chen PM, Liu KJ, Hsu PJ, Wei CF, Bai CH, Ho LJ, et al. Induction of immunomodulatory monocytes by human mesenchymal stem cell-derived hepatocyte growth factor through ERK1/2. *J Leukocyte Biol* (2014) 96(2):295–303. doi: 10.1189/jlb.3A0513-242R

48. Wang H, Zheng R, Chen Q, Shao J, Yu J, Hu S. Mesenchymal stem cells microvesicles stabilize endothelial barrier function partly mediated by hepatocyte growth factor (HGF). *Stem Cell Res Ther* (2017) 8(1):211. doi: 10.1186/s13287-016-0450-9

49. Day AJ, Milner CM. TSG-6: A multifunctional protein with anti-inflammatory and tissue-protective properties. *Matrix Biol* (2019) 78–79:60–83. doi: 10.1016/j.matbio.2018.01.011

50. Chaubey S, Thueson S, Ponnalagu D, Alam MA, Gheorghe CP, Aghai Z, et al. Early gestational mesenchymal stem cell secretome attenuates experimental bronchopulmonary dysplasia in part via exosome-associated factor TSG-6. *Stem Cell Res Ther* (2018) 9(1):173. doi: 10.1186/s13287-018-0903-4

51. An JH, Li Q, Ryu MO, Nam AR, Bhang DH, Jung YC, et al. TSG-6 in extracellular vesicles from canine mesenchymal stem/stromal is a major factor in relieving DSS-induced colitis. *PloS One* (2020) 15(2):e0220756. doi: 10.1371/journal.pone.0220756

52. Jiang L, Zhang Y, Liu T, Wang X, Wang H, Song H, et al. Exosomes derived from TSG-6 modified mesenchymal stromal cells attenuate scar formation during wound healing. *Biochimie* (2020) 177:40–9. doi: 10.1016/j.biochi.2020.08.003

53. Lu X, Lv C, Zhao Y, Wang Y, Li Y, Ji C, et al. TSG-6 released from adipose stem cells-derived small extracellular vesicle protects against spinal cord ischemia reperfusion injury by inhibiting endoplasmic reticulum stress. *Stem Cell Res Ther* (2022) 13(1):291. doi: 10.1186/s13287-022-02963-4

54. Song WJ, Li Q, Ryu MO, Ahn JO, Ha Bhang D, Chan Jung Y, et al. TSG-6 secreted by human adipose tissue-derived mesenchymal stem cells ameliorates DSS-induced colitis by inducing M2 macrophage polarization in mice. *Sci Rep* (2017) 7 (1):5187. doi: 10.1038/s41598-017-04766-7

55. Jha KA, Pentecost M, Lenin R, Gentry J, Klaic L, Del Mar N, et al. TSG-6 in conditioned media from adipose mesenchymal stem cells protects against visual deficits in mild traumatic brain injury model through neurovascular modulation. *Stem Cell Res Ther* (2019) 10:318. doi: 10.1186/s13287-019-1436-1

56. Harrell CR, Markovic BS, Fellabaum C, Arsenijevic N, Djonov V, Volarevic V. The role of Interleukin 1 receptor antagonist in mesenchymal stem cell-based tissue repair and regeneration. *BioFactors* (2020) 46(2):263–75. doi: 10.1002/biof.1587

57. Kou X, Xu X, Chen C, Sanmillan ML, Cai T, Zhou Y, et al. The Fas/Fap-1/Cav-1 complex regulates IL-1RA secretion in mesenchymal stem cells to accelerate wound healing. *Sci Transl Med* (2018) 10(432):eaai8524. doi: 10.1126/scitranslmed.aai8524

58. Han T, Song P, Wu Z, Xiang X, Liu Y, Wang Y, et al. MSC secreted extracellular vesicles carrying TGF-beta upregulate Smad 6 expression and promote the regrowth of neurons in spinal cord injured rats. *Stem Cell Rev Rep* (2022) 18(3):1078–96. doi: 10.1007/s12015-021-10219-6

59. Nolte M, Margadant C. Controlling immunity and inflammation through integrin-dependent regulation of TGF-β. *Trends Cell Biol* (2020) 30(1):49–59. doi: 10.1016/j.tcb.2019.10.002

60. Lodyga M, Hinz B. TGF-β1 – A truly transforming growth factor in fibrosis and immunity. *Semin Cell Dev Biol* (2020) 101:123–39. doi: 10.1016/j.semcdb.2019.12.010

61. Sanjabi S, Oh SA, Li MO. Regulation of the immune response by TGF-β: from conception to autoimmunity and infection. *Cold Spring Harb Perspect Biol* (2017) 9(6): a022236. doi: 10.1101/cshperspect.a022236

62. Álvarez V, Sánchez-Margallo FM, Macías-García B, Gómez-Serrano M, Jorge I, Vázquez J, et al. The immunomodulatory activity of extracellular vesicles derived from endometrial mesenchymal stem cells on CD4+ T cells is partially mediated by TGFbeta. *J Tissue Eng Regenerative Med* (2018) 12(10):2088–98. doi: 10.1002/term.2743

63. Song K, Dayem AA, Lee S, Choi Y, Lim KM, Kim S, et al. Superior therapeutic activity of TGF-β-induced extracellular vesicles against interstitial cystitis. *J Controlled Release.* (2022) 348:924–37. doi: 10.1016/j.jconrel.2022.06.045

64. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. *Int Soc Cell Ther position statement. Cytotherapy.* (2006) 8(4):315–7. doi: 10.1080/14653240600855905

65. Ferretti E, Horenstein AL, Canzonetta C, Costa F, Morandi F. Canonical and non-canonical adenosinergic pathways. *Immunol Lett* (2019) 205:25–30. doi: 10.1016/j.imlet.2018.03.007

66. Zhai X, Chen K, Yang H, Li B, Zhou T, Wang H, et al. Extracellular vesicles derived from CD73 modified human umbilical cord mesenchymal stem cells ameliorate inflammation after spinal cord injury. *J Nanobiotechnology.* (2021) 19(1):274. doi: 10.1186/s12951-021-01022-z

67. Watanabe Y, Fukuda T, Hayashi C, Nakao Y, Toyoda M, Kawakami K, et al. Extracellular vesicles derived from GMSCs stimulated with TNF-α and IFN-α promote M2 macrophage polarization via enhanced CD73 and CD5L expression. *Sci Rep* (2022) 12(1):13344. doi: 10.1038/s41598-022-17692-0

68. Kerkelä E, Laitinen A, Räbinä J, Valkonen S, Takatalo M, Larjo A, et al. Adenosinergic immunosuppression by human mesenchymal stromal cells requires co-operation with T cells. *Stem Cells* (2016) 34(3):781–90. doi: 10.1002/stem.2280

69. Saldanha-Araujo F, Ferreira FIS, Palma PV, Araujo AG, Queiroz RHC, Covas DT, et al. Mesenchymal stromal cells up-regulate CD39 and increase adenosine production to suppress activated T-lymphocytes. *Stem Cell Res* (2011) 7(1):66–74. doi: 10.1016/j.scr.2011.04.001

70. Hesse J, Siekierka-Harreis M, Steckel B, Alter C, Schallehn M, Honke N, et al. Profound inhibition of CD73-dependent formation of anti-inflammatory adenosine in B cells of SLE patients. *EBioMedicine* (2021) 73:103616. doi: 10.1016/j.ebiom.2021.103616

71. Halpert MM, Konduri V, Liang D, Chen Y, Wing JB, Paust S, et al. Dendritic cell-secreted cytotoxic T-lymphocyte-associated protein-4 regulates the T-cell response by downmodulating bystander surface B7. *Stem Cells Dev* (2016) 25(10):774–87. doi: 10.1089/scd.2016.0009

72. Bourque J, Hawiger D. Immunomodulatory bonds of the partnership between dendritic cells and T cells. *Crit Rev Immunol* (2018) 38(5):379–401. doi: 10.1615/CritRevImmunol.2018026790

73. Matheu MP, Othy S, Greenberg ML, Dong TX, Schuijs M, Deswarte K, et al. Imaging regulatory T cell dynamics and CTLA4-mediated suppression of T cell priming. *Nat Commun* (2015) 6(1):6219. doi: 10.1038/ncomms7219

74. Gaber T, Schönbeck K, Hoff H, Tran CL, Strehl C, Lang A, et al. CTLA-4 mediates inhibitory function of mesenchymal stem/stromal cells. *Int J Mol Sci* (2018) 19 (8):2312. doi: 10.3390/ijms19082312

75. Nirschl CJ, Drake CG. Molecular pathways: coexpression of immune checkpoint molecules: signaling pathways and implications for cancer immunotherapy. *Clin Cancer Res* (2013) 19(18):4917–24. doi: 10.1158/1078-0432.CCR-12-1972

76. Li M, Soder R, Abhyankar S, Abdelhakim H, Braun MW, Trinidad CV, et al. WJMSC-derived small extracellular vesicle enhance T cell suppression through PD-L1. *J Extracellular Vesicles.* (2021) 10(4):e12067. doi: 10.1002/jev2.12067

77. Wu Y, Wang H, Song A, Wang X, Ma Q, Yao C, et al. PD-L1-expressing extracellular vesicles for the treatment of pneumonia. *ACS Biomater Sci Eng.* (2023) 9 (11):6464–71. doi: 10.1021/acsbiomaterials.3c01173

78. Ou Q, Dou X, Tang J, Wu P, Pan D. Small extracellular vesicles derived from PD-L1-modified mesenchymal stem cell promote Tregs differentiation and prolong allograft survival. *Cell Tissue Res* (2022) 389(3):465–81. doi: 10.1007/s00441-022-03650-9

79. Xu F, Fei Z, Dai H, Xu J, Fan Q, Shen S, et al. Mesenchymal stem cell-derived extracellular vesicles with high PD-L1 expression for autoimmune diseases treatment. *Advanced Materials.* (2022) 34(1):2106265. doi: 10.1002/adma.202106265

80. Gómez-Ferrer M, Amaro-Prellezo E, Dorronsoro A, Sánchez-Sánchez R, Vicente Á, Cosín-Roger J, et al. HIF-overexpression and pro-inflammatory priming in human mesenchymal stromal cells improves the healing properties of extracellular vesicles in experimental crohn's disease. *Int J Mol Sci* (2021) 22(20):11269. doi: 10.3390/ijms222011269

81. Zhao L, Zhou X, Zhou X, Wang H, Gu L, Ke Y, et al. Low expressions of PD-L1 and CTLA-4 by induced CD4+CD25+ Foxp3+ Tregs in patients with SLE and their correlation with the disease activity. *Cytokine* (2020) 133:155119. doi: 10.1016/j.cyto.2020.155119

82. Li P, Wang N, Zhang Y, Wang C, Du L. HLA-G/sHLA-G and HLA-G-bearing extracellular vesicles in cancers: potential role as biomarkers. *Front Immunol* (2021) 12:791535. doi: 10.3389/fimmu.2021.791535

83. Rebmann V, König L, Nardi F da S, Wagner B, Manvailer LFS, Horn PA. The potential of HLA-G-bearing extracellular vesicles as a future element in HLA-G immune biology. *Front Immunol* (2016) 7:>173. doi: 10.3389/fimmu.2016.00173

84. Grange C, Camussi G. Immunosuppressive role of extracellular vesicles: HLA-G, an important player. *Ann Trans Med* (2017) 5(10):223–3. doi: 10.21037/atm.2017.03.61

85. Zoehler B, Fracaro L, Boldrini-Leite LM, da Silva JS, Travers PJ, Brofman PRS, et al. HLA-G and CD152 expression levels encourage the use of umbilical cord tissue-derived mesenchymal stromal cells as an alternative for immunosuppressive therapy. *Cells* (2022) 11(8):1339. doi: 10.3390/cells11081339

86. Selmani Z, Naji A, Zidi I, Favier B, Gaiffe E, Obert L, et al. Human leukocyte antigen-G5 secretion by human mesenchymal stem cells is required to suppress T lymphocyte and natural killer function and to induce CD4+CD25highFOXP3+ regulatory T cells. *Stem Cells* (2008) 26(1):212–22. doi: 10.1634/stemcells.2007-0554

87. Kordelas L, Rebmann V, Ludwig AK, Radtke S, Ruesing J, Doeppner TR, et al. MSC-derived exosomes: a novel tool to treat therapy-refractory graft-versus-host disease. *Leukemia* (2014) 28(4):970–3. doi: 10.1038/leu.2014.41

88. Ding DC, Chou HL, Chang YH, Hung WT, Liu HW, Chu TY. Characterization of HLA-G and related immunosuppressive effects in human umbilical cord stroma-derived stem cells. *Cell Transplant.* (2016) 25(2):217–28. doi: 10.3727/096368915X688182

89. Jang S, Krammer PH, Salgame P. Lack of proapoptotic activity of soluble CD95 ligand is due to its failure to induce CD95 oligomers. *J Interferon Cytokine Res* (2003) 23(8):441–7. doi: 10.1089/107999003322277856

90. Haymour L, Jean M, Smulski C, Legembre P. CD95 (Fas) and CD95L (FasL)-mediated non-canonical signaling pathways. *Biochim Biophys Acta (BBA) - Rev Cancer.* (2023) 1878(6):189004. doi: 10.1016/j.bbcan.2023.189004

91. Chen MS, Lin CY, Chiu YH, Chen CP, Tsai PJ, Wang HS. IL-1β-induced matrix metalloprotease-1 promotes mesenchymal stem cell migration via PAR1 and G-protein-coupled signaling pathway. *Stem Cells Int* (2018) 2018:e3524759. doi: 10.1155/2018/3524759

92. Vacaru AM, Dumitrescu M, Vacaru AM, Fenyo IM, Ionita R, Gafencu AV, et al. Enhanced suppression of immune cells *in vitro* by MSC overexpressing fasL. *Int J Mol Sci* (2020) 22(1):348. doi: 10.3390/ijms22010348

93. Akiyama K, Chen C, Wang D, Xu X, Qu C, Yamaza T, et al. Mesenchymal stem cell-induced immunoregulation involves fas ligand/fas-mediated T cell apoptosis. *Cell Stem Cell* (2012) 10(5):544–55. doi: 10.1016/j.stem.2012.03.007

94. Wang J, Cao Z, Wang P, Zhang X, Tang J, He Y, et al. Apoptotic extracellular vesicles ameliorate multiple myeloma by restoring fas-mediated apoptosis. *ACS Nano.* (2021) 15(9):14360–72. doi: 10.1021/acsnano.1c03517

95. Fallarino F, Grohmann U. Using an ancient tool for igniting and propagating immune tolerance: IDO as an inducer and amplifier of regulatory T cell functions. *Curr Med Chem* (2011) 18(15):2215–21. doi: 10.2174/092986711795656027

96. Stone TW, Williams RO. Modulation of T cells by tryptophan metabolites in the kynurenine pathway. *Trends Pharmacol Sci* (2023) 44(7):442–56. doi: 10.1016/j.tips.2023.04.006

97. Boasso A, Herbeuval JP, Hardy AW, Anderson SA, Dolan MJ, Fuchs D, et al. HIV inhibits CD4+ T-cell proliferation by inducing indoleamine 2,3-dioxygenase in plasmacytoid dendritic cells. *Blood* (2007) 109(8):3351–9. doi: 10.1182/blood-2006-07-034785

98. Chen W, Liang X, Peterson AJ, Munn DH, Blazar BR. The indoleamine 2,3-dioxygenase pathway is essential for human plasmacytoid dendritic cell-induced adaptive T regulatory cell generation1. *J Immunol* (2008) 181(8):5396–404. doi: 10.4049/jimmunol.181.8.5396

99. Romani R, Pirisinu I, Calvitti M, Pallotta MT, Gargaro M, Bistoni G, et al. Stem cells from human amniotic fluid exert immunoregulatory function via secreted indoleamine 2,3-dioxygenase1. *J Cell Mol Med* (2015) 19(7):1593–605. doi: 10.1111/jcmm.12534

100. Xie X, Yang X, Wu J, Tang S, Yang L, Fei X, et al. Exosome from indoleamine 2,3-dioxygenase-overexpressing bone marrow mesenchymal stem cells accelerates repair process of ischemia/reperfusion-induced acute kidney injury by regulating macrophages polarization. *Stem Cell Res Ther* (2022) 13:367. doi: 10.1186/s13287-022-03075-9

101. Zhang Q, Fu L, Liang Y, Guo Z, Wang L, Ma C, et al. Exosomes originating from MSCs stimulated with TGF-β and IFN-γ promote Treg differentiation. *J Cell Physiol* (2018) 233(9):6832–40. doi: 10.1002/jcp.26436

102. Serejo TRT, Silva-Carvalho AÉ, Braga LD de CF, Neves F de AR, Pereira RW, de Carvalho JL, et al. Assessment of the immunosuppressive potential of INF-γ Licensed adipose mesenchymal stem cells, their secretome and extracellular vesicles. *Cells* (2019) 8(1):22. doi: 10.3390/cells8010022

103. Torres Crigna A, Uhlig S, Elvers-Hornung S, Klüter H, Bieback K. Human adipose tissue-derived stromal cells suppress human, but not murine lymphocyte proliferation, via indoleamine 2,3-dioxygenase activity. *Cells* (2020) 9(11):2419. doi: 10.3390/cells9112419

104. Su J, Chen X, Huang Y, Li W, Li J, Cao K, et al. Phylogenetic distinction of iNOS and IDO function in mesenchymal stem cell-mediated immunosuppression in mammalian species. *Cell Death Differ* (2014) 21(3):388–96. doi: 10.1038/cdd.2013.149

105. Han Y, Yang J, Fang J, Zhou Y, Candi E, Wang J, et al. The secretion profile of mesenchymal stem cells and potential applications in treating human diseases. *Sig Transduct Target Ther* (2022) 7(1):1–19. doi: 10.1038/s41392-022-00932-0

106. Fernández-Messina L, Gutiérrez-Vázquez C, Rivas-García E, Sánchez-Madrid F, de la Fuente H. Immunomodulatory role of microRNAs transferred by extracellular vesicles. *Biol Cell* (2015) 107(3):61–77. doi: 10.1111/boc.201400081

107. Qiu G, Zheng G, Ge M, Wang J, Huang R, Shu Q, et al. Mesenchymal stem cell-derived extracellular vesicles affect disease outcomes via transfer of microRNAs. *Stem Cell Res Ther* (2018) 9(1):320. doi: 10.1186/s13287-018-1069-9

108. Reis M, Mavin E, Nicholson L, Green K, Dickinson AM, Wang XN. Mesenchymal stromal cell-derived extracellular vesicles attenuate dendritic cell maturation and function. *Front Immunol* (2018) 9:2538. doi: 10.3389/fimmu.2018.02538

109. Song Y, Dou H, Li X, Zhao X, Li Y, Liu D, et al. Exosomal miR-146a contributes to the enhanced therapeutic efficacy of interleukin-1β-primed mesenchymal stem cells against sepsis. *Stem Cells* (2017) 35(5):1208–21. doi: 10.1002/stem.2564

110. Li X, Liu L, Yang J, Yu Y, Chai J, Wang L, et al. Exosome derived from human umbilical cord mesenchymal stem cell mediates miR-181c attenuating burn-induced excessive inflammation. *eBioMedicine* (2016) 8:72–82. doi: 10.1016/j.ebiom.2016.04.030

111. Pers YM, Bony C, Duroux-Richard I, Bernard L, Maumus M, Assou S, et al. miR-155 contributes to the immunoregulatory function of human mesenchymal stem cells. *Front Immunol* (2021), 624024. doi: 10.3389/fimmu.2021.624024

112. Liu W, Zhou N, Liu Y, Zhang W, Li X, Wang Y, et al. Mesenchymal stem cell exosome-derived miR-223 alleviates acute graft-versus-host disease via reducing the migration of donor T cells. *Stem Cell Res Ther* (2021) 12(1):153. doi: 10.1186/s13287-021-02159-2

113. Liu H, Chen Y, Yin G, Xie Q. Therapeutic prospects of MicroRNAs carried by mesenchymal stem cells-derived extracellular vesicles in autoimmune diseases. *Life Sci* (2021) 277:119458. doi: 10.1016/j.lfs.2021.119458

114. Wright C, Rajpurohit A, Burke EE, Williams C, Collado-Torres L, Kimos M, et al. Comprehensive assessment of multiple biases in small RNA sequencing reveals significant differences in the performance of widely used methods. *BMC Genomics* (2019) 20(1):513. doi: 10.1186/s12864-019-5870-3

115. Godoy PM, Barczak AJ, DeHoff P, Srinivasan S, Etheridge A, Galas D, et al. Comparison of reproducibility, accuracy, sensitivity, and specificity of miRNA quantification platforms. *Cell Rep* (2019) 29(12):4212–4222.e5. doi: 10.1016/j.celrep.2019.11.078

116. Srinivasan S, Duval MX, Kaimal V, Cuff C, Clarke SH. Assessment of methods for serum extracellular vesicle small RNA sequencing to support biomarker development. *J Extracellular Vesicles.* (2019) 8(1):1684425. doi: 10.1080/20013078.2019.1684425

117. Perez-Hernandez J, Chiurchiù V, Perruche S, You S. Regulation of T-cell immune responses by pro-resolving lipid mediators. *Front Immunol* (2021) 12:768133. doi: 10.3389/fimmu.2021.768133

118. Pan G, Zhang P, Yang J, Wu Y. The regulatory effect of specialized pro-resolving mediators on immune cells. *Biomedicine Pharmacotherapy.* (2022) 156:113980. doi: 10.1016/j.biopha.2022.113980

119. Valkonen S, Holopainen M, Colas RA, Impola U, Dalli J, Käkelä R, et al. Lipid mediators in platelet concentrate and extracellular vesicles: Molecular mechanisms from membrane glycerophospholipids to bioactive molecules. *Biochim Biophys Acta Mol Cell Biol Lipids.* (2019) 1864(8):1168–82. doi: 10.1016/j.bbalip.2019.03.011

120. Sagini K, Costanzi E, Emiliani C, Buratta S, Urbanelli L. Extracellular vesicles as conveyors of membrane-derived bioactive lipids in immune system. *Int J Mol Sci* (2018) 19(4):1227. doi: 10.3390/ijms19041227

121. Pizzinat N, Ong-Meang V, Bourgailh-Tortosa F, Blanzat M, Perquis L, Cussac D, et al. Extracellular vesicles of MSCs and cardiomyoblasts are vehicles for lipid mediators. *Biochimie* (2020) 178:69–80. doi: 10.1016/j.biochi.2020.07.013

122. Holopainen M, Colas RA, Valkonen S, Tigistu-Sahle F, Hyvärinen K, Mazzacuva F, et al. Polyunsaturated fatty acids modify the extracellular vesicle membranes and increase the production of proresolving lipid mediators of human mesenchymal stromal cells. *Biochim Biophys Acta Mol Cell Biol Lipids.* (2019) 1864 (10):1350–62. doi: 10.1016/j.bbalip.2019.06.010

123. Ong-Meang V, Blanzat M, Savchenko L, Perquis L, Guardia M, Pizzinat N, et al. Extracellular vesicles produced by the cardiac microenvironment carry functional enzymes to produce lipid mediators *in situ*. *Int J Mol Sci* (2023) 24(6):5866. doi: 10.3390/ijms24065866

124. Abreu SC, Lopes-Pacheco M, da Silva AL, Xisto DG, de Oliveira TB, Kitoko JZ, et al. Eicosapentaenoic acid enhances the effects of mesenchymal stromal cell therapy in experimental allergic asthma. *Front Immunol* (2018) 9:1147. doi: 10.3389/fimmu.2018.01147

125. Silva JD, Lopes-Pacheco M, de Castro LL, Kitoko JZ, Trivelin SA, Amorim NR, et al. Eicosapentaenoic acid potentiates the therapeutic effects of adipose tissue-derived

mesenchymal stromal cells on lung and distal organ injury in experimental sepsis. *Stem Cell Res Ther* (2019) 10(1):264. doi: 10.1186/s13287-019-1365-z

126. Holopainen M, Impola U, Lehenkari P, Laitinen S, Kerkelä E. Human mesenchymal stromal cell secretome promotes the immunoregulatory phenotype and phagocytosis activity in human macrophages. *Cells* (2020) 9(9):2142. doi: 10.3390/cells9092142

127. Jin K, Qian C, Lin J, Liu B. Cyclooxygenase-2-Prostaglandin E2 pathway: A key player in tumor-associated immune cells. *Front Oncol* (2023) 13:1099811. doi: 10.3389/fonc.2023.1099811

128. Wei J, Ouyang X, Tang Y, Li H, Wang B, Ye Y, et al. ER-stressed MSC displayed more effective immunomodulation in RA CD4+CXCR5+ICOS+ follicular helper-like T cells through higher PGE2 binding with EP2/EP4. *Modern Rheumatol* (2020) 30 (3):509–16. doi: 10.1080/14397595.2019.1651446

129. Kulesza A, Paczek L, Burdzinska A. The role of COX-2 and PGE2 in the regulation of immunomodulation and other functions of mesenchymal stromal cells. *Biomedicines* (2023) 11(2):445. doi: 10.3390/biomedicines11020445

130. Kalinski P. Regulation of immune responses by prostaglandin E2. *J Immunol* (2012) 188(1):21–8. doi: 10.4049/jimmunol.1101029

131. Levy BD, Clish CB, Schmidt B, Gronert K, Serhan CN. Lipid mediator class switching during acute inflammation: signals in resolution. *Nat Immunol* (2001) 2 (7):612–9. doi: 10.1038/89759

132. Salimu J, Webber J, Gurney M, Al-Taei S, Clayton A, Tabi Z. Dominant immunosuppression of dendritic cell function by prostate-cancer-derived exosomes. *J Extracell Vesicles.* (2017) 6(1):1368823. doi: 10.1080/20013078.2017.1368823

133. Ylöstalo JH, Bartosh TJ, Coble K, Prockop DJ. Human mesenchymal stem/stromal cells (hMSCs) cultured as spheroids are self-activated to produce prostaglandin E2 (PGE2) that directs stimulated macrophages into an anti-inflammatory phenotype. *Stem Cells* (2012) 30(10):2283–96. doi: 10.1002/stem.1191

134. Liu XQ, Peng YQ, Huang LX, Li CG, Kuang PP, Chen DH, et al. Dendritic cells mediated by small extracellular vesicles derived from MSCs attenuated the ILC2 activity via PGE2 in patients with allergic rhinitis. *Stem Cell Res Ther* (2023) 14 (1):180. doi: 10.1186/s13287-023-03408-2

135. Harting MT, Srivastava AK, Zhaorigetu S, Bair H, Prabhakara KS, Toledano Furman NE, et al. Inflammation-stimulated mesenchymal stromal cell-derived extracellular vesicles attenuate inflammation. *Stem Cells* (2018) 36(1):79–90. doi: 10.1002/stem.2730

136. Szydlak R. Mesenchymal stem cells' homing and cardiac tissue repair. *Acta Biochim Pol* (2019) 66(4):483–9. doi: 10.18388/abp.2019_2890

137. Najar M, Krayem M, Merimi M, Burny A, Meuleman N, Bron D, et al. Insights into inflammatory priming of mesenchymal stromal cells: functional biological impacts. *Inflammation Res* (2018) 67(6):467–77. doi: 10.1007/s00011-018-1131-1

138. Wang Y, Chen X, Cao W, Shi Y. Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. *Nat Immunol* (2014) 15(11):1009–16. doi: 10.1038/ni.3002

139. Bernardo ME, Fibbe WE. Mesenchymal stromal cells: sensors and switchers of inflammation. *Cell Stem Cell* (2013) 13(4):392–402. doi: 10.1016/j.stem.2013.09.006

140. Hackel A, Vollmer S, Bruderek K, Lang S, Brandau S. Immunological priming of mesenchymal stromal/stem cells and their extracellular vesicles augments their therapeutic benefits in experimental graft-versus-host disease via engagement of PD-1 ligands. *Front Immunol* (2023) 14:1078551. doi: 10.3389/fimmu.2023.1078551

141. Ti D, Hao H, Tong C, Liu J, Dong L, Zheng J, et al. LPS-preconditioned mesenchymal stromal cells modify macrophage polarization for resolution of chronic inflammation via exosome-shuttled let-7b. *J Transl Med* (2015) 13:308. doi: 10.1186/s12967-015-0642-6

142. Xu R, Zhang F, Chai R, Zhou W, Hu M, Liu B, et al. Exosomes derived from pro-inflammatory bone marrow-derived mesenchymal stem cells reduce inflammation and myocardial injury via mediating macrophage polarization. *J Cell Mol Med* (2019) 23(11):7617–31. doi: 10.1111/jcmm.14635

143. Hwang S, Sung DK, Kim YE, Yang M, Ahn SY, Sung SI, et al. Mesenchymal Stromal Cells Primed by Toll-like Receptors 3 and 4 Enhanced Anti-Inflammatory Effects against LPS-Induced Macrophages via Extracellular Vesicles. *Int J Mol Sci* (2023) 24(22):16264. doi: 10.3390/ijms242216264

144. Kink JA, Forsberg MH, Reshetylo S, Besharat S, Childs CJ, Pederson JD, et al. Macrophages educated with exosomes from primed mesenchymal stem cells treat acute radiation syndrome by promoting hematopoietic recovery. *Biol Blood Marrow Transplantation.* (2019) 25(11):2124–33. doi: 10.1016/j.bbmt.2019.07.026

145. Cui S, Zhang Z, Cheng C, Tang S, Zhai M, Li L, et al. Small Extracellular Vesicles from Periodontal Ligament Stem Cells Primed by Lipopolysaccharide Regulate Macrophage M1 Polarization via miR-433-3p Targeting TLR2/TLR4/NF-κB. *Inflammation* (2023) 46(5):1849–58. doi: 10.1007/s10753-023-01845-y

146. Waterman RS, Tomchuck SL, Henkle SL, Betancourt AM. A new mesenchymal stem cell (MSC) paradigm: polarization into a pro-inflammatory MSC1 or an immunosuppressive MSC2 phenotype. *PloS One* (2010) 5(4):e10088. doi: 10.1371/journal.pone.0010088

147. Cheng A, Choi D, Lora M, Shum-Tim D, Rak J, Colmegna I. Human multipotent mesenchymal stromal cells cytokine priming promotes RAB27B-regulated secretion of small extracellular vesicles with immunomodulatory cargo. *Stem Cell Res Ther* (2020) 11(1):539. doi: 10.1186/s13287-020-02050-6

148. Ragni E, Perucca Orfei C, De Luca P, Mondadori C, Viganò M, Colombini A, et al. Inflammatory priming enhances mesenchymal stromal cell secretome potential as a clinical product for regenerative medicine approaches through secreted factors and EV-miRNAs: the example of joint disease. *Stem Cell Res Ther* (2020) 11(1):165. doi: 10.1186/s13287-020-01677-9

149. Kim M, Shin DI, Choi BH, Min BH. Exosomes from IL-1β-primed mesenchymal stem cells inhibited IL-1β- and TNF-α-mediated inflammatory responses in osteoarthritic SW982 cells. *Tissue Eng Regener Med* (2021) 18(4):525–36. doi: 10.1007/s13770-020-00324-x

150. Yao M, Cui B, Zhang W, Ma W, Zhao G, Xing L. Exosomal miR-21 secreted by IL-1β-primed-mesenchymal stem cells induces macrophage M2 polarization and ameliorates sepsis. *Life Sci* (2021) 264:118658. doi: 10.1016/j.lfs.2020.118658

151. Kilpinen L, Impola U, Sankkila L, Ritamo I, Aatonen M, Kilpinen S, et al. Extracellular membrane vesicles from umbilical cord blood-derived MSC protect against ischemic acute kidney injury, a feature that is lost after inflammatory conditioning. *J Extracellular Vesicles.* (2013) 2(1):21927. doi: 10.3402/jev.v2i0.21927

152. Huang P, Wang L, Li Q, Tian X, Xu J, Xu J, et al. Atorvastatin enhances the therapeutic efficacy of mesenchymal stem cells-derived exosomes in acute myocardial infarction via up-regulating long non-coding RNA H19. *Cardiovasc Res* (2020) 116 (2):353–67. doi: 10.1093/cvr/cvz139

153. Wei Z, Qiao S, Zhao J, Liu Y, Li Q, Wei Z, et al. miRNA-181a over-expression in mesenchymal stem cell-derived exosomes influenced inflammatory response after myocardial ischemia-reperfusion injury. *Life Sci* (2019) 232:116632. doi: 10.1016/j.lfs.2019.116632

154. Geng W, Tang H, Luo S, Lv Y, Liang D, Kang X, et al. Exosomes from miRNA-126-modified ADSCs promotes functional recovery after stroke in rats by improving neurogenesis and suppressing microglia activation. *Am J Transl Res* (2019) 11 (2):780–92.

155. Yuan Z, Kolluri KK, Gowers KHC, Janes SM. TRAIL delivery by MSC-derived extracellular vesicles is an effective anticancer therapy. *J Extracell Vesicles.* (2017) 6 (1):1265291. doi: 10.1080/20013078.2017.1265291

156. Shao M, Jin M, Xu S, Zheng C, Zhu W, Ma X, et al. Exosomes from long noncoding RNA-gm37494-ADSCs repair spinal cord injury via shifting microglial M1/M2 polarization. *Inflammation* (2020) 43(4):1536–47. doi: 10.1007/s10753-020-01230-z

157. Liu W, Rong Y, Wang J, Zhou Z, Ge X, Ji C, et al. Exosome-shuttled miR-216a-5p from hypoxic preconditioned mesenchymal stem cells repair traumatic spinal cord injury by shifting microglial M1/M2 polarization. *J Neuroinflammation.* (2020) 17 (1):47. doi: 10.1186/s12974-019-1653-7

158. Collino F, Lopes JA, Corrêa S, Abdelhay E, Takiya CM, Wendt CHC, et al. Adipose-derived mesenchymal stromal cells under hypoxia: changes in extracellular vesicles secretion and improvement of renal recovery after ischemic injury. *Cell Physiol Biochem* (2019) 52(6):1463–83. doi: 10.33594/000000102

159. Miceli V, Pampalone M, Vella S, Carreca AP, Amico G, Conaldi PG. Comparison of immunosuppressive and angiogenic properties of human amnion-derived mesenchymal stem cells between 2D and 3D culture systems. *Stem Cells Int* (2019) 2019:e7486279. doi: 10.1155/2019/7486279

160. Xie AW, Zacharias NA, Binder BYK, Murphy WL. Controlled aggregation enhances immunomodulatory potential of mesenchymal stromal cell aggregates. *Stem Cells Trans Med* (2021) 10(8):1184–201. doi: 10.1002/sctm.19-0414

161. Regmi S, Seo Y, Ahn JS, Pathak S, Acharya S, Nguyen TT, et al. Heterospheroid formation improves therapeutic efficacy of mesenchymal stem cells in murine colitis through immunomodulation and epithelial regeneration. *Biomaterials* (2021) 271:120752. doi: 10.1016/j.biomaterials.2021.120752

162. Kim JY, Rhim WK, Cha SG, Woo J, Lee JY, Park CG, et al. Bolstering the secretion and bioactivities of umbilical cord MSC-derived extracellular vesicles with 3D culture and priming in chemically defined media. *Nano Convergence.* (2022) 9(1):57. doi: 10.1186/s40580-022-00349-z

163. Kusuma GD, Li A, Zhu D, McDonald H, Inocencio IM, Chambers DC, et al. Effect of 2D and 3D culture microenvironments on mesenchymal stem cell-derived extracellular vesicles potencies. *Front Cell Dev Biol* (2022) 10:819726. doi: 10.3389/fcell.2022.819726

164. Yuan X, Sun L, Jeske R, Nkosi D, York SB, Liu Y, et al. Engineering extracellular vesicles by three-dimensional dynamic culture of human mesenchymal stem cells. *J Extracell Vesicles.* (2022) 11(6):e12235. doi: 10.1002/jev2.12235

165. Cao J, Wang B, Tang T, Lv L, Ding Z, Li Z, et al. Three-dimensional culture of MSCs produces exosomes with improved yield and enhanced therapeutic efficacy for cisplatin-induced acute kidney injury. *Stem Cell Res Ther* (2020) 11(1):206. doi: 10.1186/s13287-020-01719-2

166. Sun L, Ji Y, Chi B, Xiao T, Li C, Yan X, et al. A 3D culture system improves the yield of MSCs-derived extracellular vesicles and enhances their therapeutic efficacy for heart repair. *Biomedicine Pharmacotherapy.* (2023) 161:114557. doi: 10.1016/j.biopha.2023.114557

167. Andrews S, Maughon T, Marklein R, Stice S. Priming of MSCs with inflammation-relevant signals affects extracellular vesicle biogenesis, surface markers, and modulation of T cell subsets. *J Immunol Regenerative Med* (2021) 13:100036. doi: 10.1016/j.regen.2020.100036

168. Hyland M, Mennan C, Davies R, Wilson E, Tonge DP, Clayton A, et al. Extracellular vesicles derived from umbilical cord mesenchymal stromal cells show

enhanced anti-inflammatory properties via upregulation of miRNAs after pro-inflammatory priming. *Stem Cell Rev Rep* (2023) 19(7):2391–406. doi: 10.1007/s12015-023-10586-2

169. Rozier P, Maumus M, Maria ATJ, Toupet K, Jorgensen C, Guilpain P, et al. Lung fibrosis is improved by extracellular vesicles from IFNγ-primed mesenchymal stromal cells in murine systemic sclerosis. *Cells* (2021) 10(10):2727. doi: 10.3390/cells10102727

170. Hyland M, Mennan C, Wilson E, Clayton A, Kehoe O. Pro-inflammatory priming of umbilical cord mesenchymal stromal cells alters the protein cargo of their extracellular vesicles. *Cells* (2020) 9(3):726. doi: 10.3390/cells9030726

171. Peltzer J, Lund K, Goriot ME, Grosbot M, Lataillade JJ, Mauduit P, et al. Interferon-γ and hypoxia priming have limited effect on the miRNA landscape of human mesenchymal stromal cells-derived extracellular vesicles. *Front Cell Dev Biol* (2020) 8:581436. doi: 10.3389/fcell.2020.581436

172. Gorgun C, Ceresa D, Lesage R, Villa F, Reverberi D, Balbi C, et al. Dissecting the effects of preconditioning with inflammatory cytokines and hypoxia on the angiogenic potential of mesenchymal stromal cell (MSC)-derived soluble proteins and extracellular vesicles (EVs). *Biomaterials* (2021) 269:120633. doi: 10.1016/j.biomaterials.2020.120633

173. Zang H, Wang Z, Wu Q, Shi L, Chen G. Effect of hypoxia on the expression of microRNA in extracellular vesicles of human umbilical cord stem cells in *vitro*. *Cell Tissue Bank.* (2023) 24(4):769–78. doi: 10.1007/s10561-023-10095-z

174. Jin P, Zhao Y, Liu H, Chen J, Ren J, Jin J, et al. Interferon-γ and tumor necrosis factor-α Polarize bone marrow stromal cells uniformly to a th1 phenotype. *Sci Rep* (2016) 6(1):26345. doi: 10.1038/srep26345

175. Bai H, Wang Y, Zhao Y, Chen X, Xiao Y, Bao C. HIF signaling: A new propellant in bone regeneration. *Biomaterials Advances.* (2022) 138:212874. doi: 10.1016/j.bioadv.2022.212874

176. Fábián Z. The effects of hypoxia on the immune-modulatory properties of bone marrow-derived mesenchymal stromal cells. *Stem Cells Int* (2019) 2019:e2509606. doi: 10.1155/2019/2509606

177. Wu J, Huang QM, Liu Y, Zhou J, Tang WR, Wang XY, et al. Long-term hypoxic hUCMSCs-derived extracellular vesicles alleviates allergic rhinitis through triggering immunotolerance of their VEGF-mediated inhibition of dendritic cells maturation. *Int Immunopharmacology.* (2023) 124:110875. doi: 10.1016/j.intimp.2023.110875

178. Fuentes P, Torres MJ, Arancibia R, Aulestia F, Vergara M, Carrión F, et al. Dynamic culture of mesenchymal stromal/stem cell spheroids and secretion of paracrine factors. *Front Bioeng Biotechnol* (2022) 10:916229. doi: 10.3389/fbioe.2022.916229

179. Bulati M, Gallo A, Zito G, Busà R, Iannolo G, Cuscino N, et al. 3D culture and interferon-γ Priming modulates characteristics of mesenchymal stromal/stem cells by modifying the expression of both intracellular and exosomal microRNAs. *Biology* (2023) 12(8):1063. doi: 10.3390/biology12081063

180. Perut F, Roncuzzi L, Baldini N. The emerging roles of extracellular vesicles in osteosarcoma. *Front Oncol* (2019) 9:1342. doi: 10.3389/fonc.2019.01342

181. Evdokimova V, Gassmann H, Radvanyi L, Burdach SEG. Current state of immunotherapy and mechanisms of immune evasion in ewing sarcoma and osteosarcoma. *Cancers* (2023) 15(1):272. doi: 10.3390/cancers15010272

182. Gassmann H, Schneider K, Evdokimova V, Ruzanov P, Schober SJ, Xue B, et al. Ewing sarcoma-derived extracellular vesicles impair dendritic cell maturation and function. *Cells* (2021) 10(8):2081. doi: 10.3390/cells10082081

183. Droste M, Thakur BK, Eliceiri BP. Tumor-derived extracellular vesicles and the immune system—Lessons from immune-competent mouse-tumor models. *Front Immunol* (2020) 11:606859. doi: 10.3389/fimmu.2020.606859

184. Pereira J, Alfaro G, Goycoolea M, Quiroga T, Ocqueteau M, Massardo L, et al. Circulating platelet-derived microparticles in systemic lupus erythematosus: Association with increased thrombin generation and procoagulant state. *Thromb Haemost.* (2006) 95(01):94–9. doi: 10.1160/TH05-05-0310

185. Sellam J, Proulle V, Jüngel A, Ittah M, Miceli Richard C, Gottenberg JE, et al. Increased levels of circulating microparticles in primary Sjögren's syndrome, systemic lupus erythematosus and rheumatoid arthritis and relation with disease activity. *Arthritis Res Ther* (2009) 11(5):R156. doi: 10.1186/ar2833

186. Duval A, Helley D, Capron L, Youinou P, Renaudineau Y, Dubucquoi S, et al. Endothelial dysfunction in systemic lupus patients with low disease activity: evaluation by quantification and characterization of circulating endothelial microparticles, role of anti-endothelial cell antibodies. *Rheumatology* (2010) 49(6):1049–55. doi: 10.1093/rheumatology/keq041

187. Mobarrez F, Vikerfors A, Gustafsson JT, Gunnarsson I, Zickert A, Larsson A, et al. Microparticles in the blood of patients with systemic lupus erythematosus (SLE): phenotypic characterization and clinical associations. *Sci Rep* (2016) 6(1):36025. doi: 10.1038/srep36025

188. Lee JY, Park JK, Lee EY, Lee EB, Song YW. Circulating exosomes from patients with systemic lupus erythematosus induce a proinflammatory immune response. *Arthritis Res Ther* (2016) 18(1):264. doi: 10.1186/s13075-016-1159-y

189. López P, Rodríguez-Carrio J, Martínez-Zapico A, Caminal-Montero L, Suárez A. Circulating microparticle subpopulations in systemic lupus erythematosus are affected by disease activity. *Int J Cardiol* (2017) 236:138–44. doi: 10.1016/j.ijcard.2017.02.107

190. Nielsen CT, Østergaard O, Johnsen C, Jacobsen S, Heegaard NHH. Distinct features of circulating microparticles and their relationship to clinical manifestations in systemic lupus erythematosus: Plasma Microparticles in SLE. *Arthritis Rheumatism.* (2011) 63(10):3067–77. doi: 10.1002/art.30499

191. Østergaard O, Nielsen CT, Iversen LV, Tanassi JT, Knudsen S, Jacobsen S, et al. Unique protein signature of circulating microparticles in systemic lupus erythematosus. *Arthritis Rheumatism.* (2013) 65(10):2680–90. doi: 10.1002/art.38065

192. Chuang H, Chen M, Chen Y, Ciou Y, Hsueh C, Tsai C, et al. Induction of interferon-γ and tissue inflammation by overexpression of eosinophil cationic protein in T cells and exosomes. *Arthritis Rheumatol* (2022) 74(1):92–104. doi: 10.1002/art.41920

193. Li W, Liu S, Chen Y, Weng R, Zhang K, He X, et al. Circulating exosomal microRNAs as biomarkers of systemic lupus erythematosus. *Clinics* (2020) 75:e1528. doi: 10.6061/clinics/2020/e1528

194. Dong C, Zhou Q, Fu T, Zhao R, Yang J, Kong X, et al. Circulating exosomes derived-miR-146a from systemic lupus erythematosus patients regulates senescence of mesenchymal stem cells. *BioMed Res Int* (2019) 2019:1–10. doi: 10.1155/2019/6071308

195. Perez-Hernandez J, Forner MJ, Pinto C, Chaves FJ, Cortes R, Redon J. Increased urinary exosomal microRNAs in patients with systemic lupus erythematosus. *Alvarez ML editor. PloS One* (2015) 10(9):e0138618. doi: 10.1371/journal.pone.0138618

196. Tan L, Zhao M, Wu H, Zhang Y, Tong X, Gao L, et al. Downregulated serum exosomal miR-451a expression correlates with renal damage and its intercellular communication role in systemic lupus erythematosus. *Front Immunol* (2021) 12:630112. doi: 10.3389/fimmu.2021.630112

197. Salvi V, Gianello V, Busatto S, Bergese P, Andreoli L, D'Oro U, et al. Exosome-delivered microRNAs promote IFN-α secretion by human plasmacytoid DCs via TLR7. *JCI Insight* (2018) 3(10):e98204. doi: 10.1172/jci.insight.98204

198. Wang D, Zhang H, Liang J, Wang H, Hua B, Feng X, et al. A long-term follow-up study of allogeneic mesenchymal stem/stromal cell transplantation in patients with drug-resistant systemic lupus erythematosus. *Stem Cell Rep* (2018) 10(3):933–41. doi: 10.1016/j.stemcr.2018.01.029

199. Wen L, Labopin M, Badoglio M, Wang D, Sun L, Farge-Bancel D. Prognostic factors for clinical response in systemic lupus erythematosus patients treated by allogeneic mesenchymal stem cells. *Stem Cells Int* (2019) 2019:e7061408. doi: 10.1155/2019/7061408

200. Sharma J, Hampton JM, Valiente GR, Wada T, Steigelman H, Young MC, et al. Therapeutic development of mesenchymal stem cells or their extracellular vesicles to inhibit autoimmune-mediated inflammatory processes in systemic lupus erythematosus. *Front Immunol* (2017) 8:526. doi: 10.3389/fimmu.2017.00526

201. Xie M, Li C, She Z, Wu F, Mao J, Hun M, et al. Human umbilical cord mesenchymal stem cells derived extracellular vesicles regulate acquired immune response of lupus mouse in vitro. *Sci Rep* (2022) 12(1):13101. doi: 10.1038/s41598-022-17331-8

202. Sun W, Yan S, Yang C, Yang J, Wang H, Li C, et al. Mesenchymal stem cells-derived exosomes ameliorate lupus by inducing M2 macrophage polarization and regulatory T cell expansion in MRL/lpr mice. *Immunol Investigations.* (2022) 51(6):1785–803. doi: 10.1080/08820139.2022.2055478

203. Zhang M, Johnson-Stephenson TK, Wang W, Wang Y, Li J, Li L, et al. Mesenchymal stem cell-derived exosome-educated macrophages alleviate systemic lupus erythematosus by promoting efferocytosis and recruitment of IL-17+ regulatory T cell. *Stem Cell Res Ther* (2022) 13(1):484. doi: 10.1186/s13287-022-03174-7

204. Sonoda S, Murata S, Kato H, Zakaria F, Kyumoto-Nakamura Y, Uehara N, et al. Targeting of deciduous tooth pulp stem cell–derived extracellular vesicles on telomerase-mediated stem cell niche and immune regulation in systemic lupus erythematosus. *J Immunol* (2021) 206(12):3053–63. doi: 10.4049/jimmunol.2001312

205. Wang R, Hao M, Kou X, Sui B, Sanmillan ML, Zhang X, et al. Apoptotic vesicles ameliorate lupus and arthritis via phosphatidylserine-mediated modulation of T cell receptor signaling. *Bioactive Materials.* (2023) 25:472–84. doi: 10.1016/j.bioactmat.2022.07.026

206. Dou R, Zhang X, Xu X, Wang P, Yan B. Mesenchymal stem cell exosomal tsRNA-21109 alleviate systemic lupus erythematosus by inhibiting macrophage M1 polarization. *Mol Immunol* (2021) 139:106–14. doi: 10.1016/j.molimm.2021.08.015

207. Chen X, Wei Q, Sun H, Zhang X, Yang C, Tao Y, et al. Exosomes derived from human umbilical cord mesenchymal stem cells regulate macrophage polarization to attenuate systemic lupus erythematosus-associated diffuse alveolar hemorrhage in mice. *Int J Stem Cells* (2021) 14(3):331–40. doi: 10.15283/ijsc20156

208. Tu J, Zheng N, Mao C, Liu S, Zhang H, Sun L. UC-BSCs Exosomes Regulate Th17/Treg Balance in Patients with Systemic Lupus Erythematosus via miR-19b/KLF13. *Cells* (2022) 11(24):4123. doi: 10.3390/cells11244123

209. Zhao Y, Song W, Yuan Z, Li M, Wang G, Wang L, et al. Exosome derived from human umbilical cord mesenchymal cell exerts immunomodulatory effects on B cells from SLE patients. *J Immunol Res* (2023) 2023:e3177584. doi: 10.1155/2023/3177584

210. Chen M, Peng J, Xie Q, Xiao N, Su X, Mei H, et al. Mesenchymal stem cells alleviate moderate-to-severe psoriasis by reducing the production of type I interferon (IFN-I) by plasmacytoid dendritic cells (pDCs). *Stem Cells Int* (2019) 2019:e6961052. doi: 10.1155/2019/6961052

211. Hackstein H, Tschipakow I, Bein G, Nold P, Brendel C, Baal N. Contact-dependent abrogation of bone marrow-derived plasmacytoid dendritic cell differentiation by murine mesenchymal stem cells. *Biochem Biophys Res Commun* (2016) 476(1):15–20. doi: 10.1016/j.bbrc.2016.05.108

frontiers | Frontiers in Immunology

TYPE Original Research
PUBLISHED 20 February 2024
DOI 10.3389/fimmu.2024.1354479

OPEN ACCESS

EDITED BY
Chun Wai Mai,
UCSI University, Malaysia

REVIEWED BY
Jill Johnson,
Aston University, United Kingdom
Chunyan He,
Kunshan Hospital of Traditional Chinese Medicine, China

*CORRESPONDENCE
Nuno A. Silva
nunosilva@med.uminho.pt

†These authors have contributed equally to this work

RECEIVED 12 December 2023
ACCEPTED 07 February 2024
PUBLISHED 20 February 2024

CITATION
Lentilhas-Graça J, Santos DJ, Afonso J, Monteiro A, Pinho AG, Mendes VM, Dias MS, Gomes ED, Lima R, Fernandes LS, Fernandes-Amorim F, Pereira IM, de Sousa N, Cibrão JR, Fernandes AM, Serra SC, Rocha LA, Campos J, Pinho TS, Monteiro S, Manadas B, Salgado AJ, Almeida RD and Silva NA (2024) The secretome of macrophages has a differential impact on spinal cord injury recovery according to the polarization protocol.
Front. Immunol. 15:1354479.
doi: 10.3389/fimmu.2024.1354479

The secretome of macrophages has a differential impact on spinal cord injury recovery according to the polarization protocol

José Lentilhas-Graça[1,2,3], Diogo J. Santos[1,2†], João Afonso[1,2†], Andreia Monteiro[1,2], Andreia G. Pinho[1,2], Vera M. Mendes[3], Marta S. Dias[3,4], Eduardo D. Gomes[1,2], Rui Lima[1,2], Luís S. Fernandes[1,2], Fernando Fernandes-Amorim[1,2], Inês M. Pereira[1,2], Nídia de Sousa[1,2], Jorge R. Cibrão[1,2], Aline M. Fernandes[1,2], Sofia C. Serra[1,2], Luís A. Rocha[1,2], Jonas Campos[1,2], Tiffany S. Pinho[1,2], Susana Monteiro[1,2], Bruno Manadas[3], António J. Salgado[1,2], Ramiro D. Almeida[3,4] and Nuno A. Silva[1,2*]

[1]Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal, [2]ICVS/3B's Associate Lab, PT Government Associated Lab, Braga, Portugal, [3]CNC—Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal, [4]iBiMED-Institute of Biomedicine, Department of Medical Sciences, University of Aveiro, Aveiro, Portugal

Introduction: The inflammatory response after spinal cord injury (SCI) is an important contributor to secondary damage. Infiltrating macrophages can acquire a spectrum of activation states, however, the microenvironment at the SCI site favors macrophage polarization into a pro-inflammatory phenotype, which is one of the reasons why macrophage transplantation has failed.

Methods: In this study, we investigated the therapeutic potential of the macrophage secretome for SCI recovery. We investigated the effect of the secretome in vitro using peripheral and CNS-derived neurons and human neural stem cells. Moreover, we perform a pre-clinical trial using a SCI compression mice model and analyzed the recovery of motor, sensory and autonomic functions. Instead of transplanting the cells, we injected the paracrine factors and extracellular vesicles that they secrete, avoiding the loss of the phenotype of the transplanted cells due to local environmental cues.

Results: We demonstrated that different macrophage phenotypes have a distinct effect on neuronal growth and survival, namely, the alternative activation with IL-10 and TGF-β1 (M(IL-10+TGF-β1)) promotes significant axonal regeneration. We also observed that systemic injection of soluble factors and extracellular vesicles derived from M(IL-10+TGF-β1) macrophages promotes significant functional recovery after compressive SCI and leads to higher survival of spinal cord neurons. Additionally, the M(IL-10+TGF-β1) secretome supported the recovery of bladder function and decreased microglial activation, astrogliosis and fibrotic scar in the spinal cord. Proteomic analysis of the M(IL-10+TGF-β1)-derived

secretome identified clusters of proteins involved in axon extension, dendritic spine maintenance, cell polarity establishment, and regulation of astrocytic activation.

Discussion: Overall, our results demonstrated that macrophages-derived soluble factors and extracellular vesicles might be a promising therapy for SCI with possible clinical applications.

KEYWORDS

spinal cord injury, macrophages, secretome, neuroimmunology, neuroregeneration

Background

Spinal cord injury (SCI) is a devastating neurological disorder that strongly affects the physiological, psychological, and social behaviors of affected people. There is an urgent need to develop new therapeutic strategies for SCI repair (1). The spinal cord trauma, known as "primary injury", triggers a cascade of events, termed "secondary injury", leading to further neurological damage and contributing to regeneration failure after SCI (2). These include glutamate excitotoxicity, a potent and dysfunctional inflammatory response, release of molecules that inhibit axonal growth, and formation of a glial scar. From all these events, the defective immune response is one of the most important players in SCI pathophysiology. Circulating monocytes infiltrate the spinal cord and differentiate into macrophages in a multiphasic manner, where they should perform multiple functions involved in the wound healing process (3). It was recently demonstrated that the spleen releases the first monocytes that infiltrate the injured spinal cord (4). Moreover, Swirsky et al. characterized the splenic monocyte reservoir as a major source of the pro-inflammatory subtype during acute injury (5).

Macrophages can acquire a diverse spectrum of activation states with various functionalities. Macrophage activation can range from the most pro-inflammatory or classically activated phenotype to the anti-inflammatory/pro-repair or alternatively activated phenotype. Pro-inflammatory macrophages are important during the acute response to trauma and facilitate innate immunity to remove wound debris from the injury site. These macrophages release reactive oxygen species (ROS) and pro-inflammatory cytokines, such as IL-1β and TNF-α (6). Macrophages can acquire this phenotype *in vitro* by stimulating naïve macrophages with lipopolysaccharide (LPS) and IFN-γ (commonly known as M1). In contrast, alternatively activated macrophages secrete immunosuppressive cytokines, growth factors, and upregulate ECM components (e.g., IL-10, TGF-β1, and IGF-1) (7, 8). These macrophages exhibit tissue repair properties by promoting cell proliferation and maturation, tissue remodeling and stabilization, and adjusting and resolving inflammatory processes. These tasks are not performed by a single type of alternatively activated macrophage. Instead, they are subdivided into four distinct subtypes (commonly known as M2a, M2b, M2c, and M2d) that differ in cell surface markers, secreted cytokines, and biological functions (6). Herein, we focus on two alternatively activated macrophages, the M2a and M2c. The first can be obtained *in vitro* by stimulating naïve macrophages with IL-4 and IL-13, and their function is associated with a decrease in the inflammatory response, promotion of cell proliferation and migration, and facilitation of apoptosis. After SCI these cells fail to activate an appropriate pro-regenerative response (6). Whereas, the M2c macrophages have functions related to resolving inflammation, ECM synthesis, and promoting tissue maturation/repair. These cells can be obtained by activating naïve macrophages with TGF-β1 and IL-10. The significance of M2c cells in SCI repair remains largely unexplored because these cells do not populate the lesion site, impeding the initiation of the remodeling phase (6). Overall, the immune response at the initial stages after SCI resembles that in non-CNS injured tissues (9). However, pro-inflammatory macrophages quickly become the predominant cell type at the injury site (10), and pro-repair macrophages are unable to populate the injured tissue. The pro-inflammatory response is associated with fibrosis, oxidative damage, and neurodegeneration, contributing to wound healing failure (11).

Previous studies transplanted alternatively activated macrophages into the injured spinal cord to promote tissue repair and regeneration (12, 13). This therapeutic approach reached clinical testing, but failed to show any therapeutic effects (14). The reason behind this clinical trial failure may lie in the spinal cord microenvironment after injury. Indeed, a previous study reported that bone marrow-derived macrophages polarized *in vitro* by IL-4 failed to retain their typical markers when transplanted into the injured spinal cord (10). Moreover, it was demonstrated that intracellular accumulation of iron by macrophages induces a rapid switch from a pro-regenerative to a pro-inflammatory phenotype in spinal cord tissue (15). Thus, it is important to find alternative approaches for M2 macrophage transplantation. A possible alternative is to administer the secretome of macrophages instead of transplanting them into the SCI microenvironment. The secretome can be defined as the soluble factors, lipids, and

extracellular vesicles secreted by a cell, tissue, or organism into the extracellular space under defined time and conditions (16).

Herein, we explored whether systemic injections of secretome derived from different macrophage phenotypes have a therapeutic effect after SCI.

Materials and methods

Macrophages isolation and culture

Macrophages were obtained by differentiating monocytes extracted from the mouse spleens. C57BL/6 mice (~8 weeks old) were sacrificed by cervical dislocation, and their spleen was removed under aseptic conditions and kept on ice-cold VLE-RPMI 1640 (Merck KGaA) with 1% (v/v) penicillin-streptomycin (pen/strep, Gibco). The spleen was mechanically dissociated using two microscope slides until no major fragments were observed. The solution was centrifuged at 1200 rpm for 7 min and the supernatant was discarded. Ammonium-chloride-potassium (ACK) lysis solution was used to lyse erythrocytes (2mL/spleen). After adding HBSS (8mL/spleen, Gibco), centrifugation was performed, and the cell pellet was resuspended in RPMI for hematocytomer cell counting. Cells were plated at a density of 1 million cells/cm^2 in RPMI medium 1% (v/v) pen/strep (Gibco) for 3 h. The monocytes (≈10% of the total cells) are the first to adhere under serum starvation. After this time, the non-adherent cells were discarded and the medium was replaced by RPMI with 10% (v/v) fetal bovine serum (FBS, Millipore), 1% (v/v) pen/strep, and 50 ng/mL of macrophage colony-stimulating factor (M-CSF, Biolegend) to differentiate monocytes into macrophages. The cells were maintained at 37°C and 5% (v/v) CO_2 for a minimum of 7 days, with medium exchanges every 3/4 days. To achieve a pro-inflammatory phenotype, macrophages were stimulated with IFN-γ (20 ng/mL, Peprotech) and LPS (100 ng/mL, Sigma) for 24 h. One pro-regenerative phenotype was achieved by stimulation with IL-4 (20 ng/mL, Biolegend) and IL-13 (20 ng/mL, Peprotech), and the other phenotype was obtained with IL-10 (20 ng/mL, Peprotech) and TGF-β1 (20ng/mL, R&D Systems) stimulation. All polarizations were performed in RPMI with 10% FBS, 1% (v/v) pen/strep and 50 ng/mL of M-CSF.

The macrophage secretome was collected after each polarization. Briefly, cells were washed five times with PBS without Ca^{2+} and Mg^{2+} (Merck, KGaA), followed by two washes with RPMI 1% (v/v) pen/strep. After a 12-hour incubation with 16 mL (213ul/cm^2) of basal medium (RPMI) with 1% (v/v) pen/strep, the medium was collected, centrifuged at 1200 rpm for 5 min, and the supernatant was snap frozen with liquid nitrogen and stored at -80°C.

qPCR

Macrophage mRNA levels were analyzed using qPCR by extracting RNA from cells grown in T25 flasks. Briefly, 6 h after polarization, TripleXtractor (Grisp) was added to the flasks for 5 min. RNA was extracted and diluted in GRS PCR Grade Water (Grisp) following the manufacturer's instructions. cDNA was synthesized from 1 μg of RNA using the Xpert cDNA Synthesis Supermix (with gDNA eraser, Grisp) protocol. qPCR was performed on these samples using Xpert Fast SYBR blue mastermix (Grisp) with ROX reference dye. After mixing the mastermix with the respective primers (500 nM) and the cDNA on a PCR plate (Nerbe Plus), the reaction was performed on a 7500 Fast Real-Time PCR system (Applied Biosystems). The amplification was performed by heating at 95°C for 2 minutes succeeded by 40 cycles at 95°C for 5 s and 30 s at 60°C. Melt curve analysis was used to assess the specificity of the gene amplification. The primers used are listed in Table 1. The target genes were normalized to three reference genes: Gadph, Hprt and 18s. Fold-change levels were calculated using the 2-ΔΔct method relative to non-stimulated macrophages and normalized to the reference genes (17).

Axonal growth assay – dorsal root ganglia

Dorsal root ganglia (DRG) explants were used to study the impact of splenic macrophages on axonal growth. This assay was accomplished following a well-established protocol (18, 19). Briefly, DRG from thoracic regions of neonatal Wistar Han rat pups (P5-7) were removed and placed on ice-cold HBSS with 1% (v/v) pen/strep. Peripheral nerves attached to the DRG were removed, and the cleaned DRG were used. Two assays were performed. The first consisted of placing the DRG on top of a collagen extracellular matrix gel (3D culture), which was on top of polarized macrophages. Collagen gels were prepared by combining rat tail collagen type I (Corning) at a final concentration of 89.6% (v/v) with 10% (v/v) Dulbecco Modified Eagle Medium (DMEM, Gibco) 10x and 0.4%

TABLE 1 Primers for semi-quantitative Real Time-PCR.

Gene	Forward	Reverse
GAPDH	GGG CCC ACT TGA AGG GTG GA	TGG ACT GTG GTC ATG AGC CCT T
HPRT	GCT GGT GAA AAG GAC CTC T	CAC AGG ACT AGA ACA CCT GC
18s	GTA ACC CGT TGA ACC CCA TT	CCA TCC AAT CGG TAG TAG CG
iNOS	CTC GGA GGT TCA CCT CAC TGT	GCT GGA AGC CAC TGA CAC TT
TNF-α	GCC ACC ACG CTC TTC TGT CT	TGA GGG TCT GGG CCA TAG AAC
EGR2	TTG ACC AGA TGA ACG GAG TG	CCA GAG AGG AGG TGG AAG TG
IRF4	ACA GGA GCT GGA GGG ATT ATG	CTG TCA CCT GGC AAC CAT TT
ARG1	GTG TAC ATT GGC TTG CGA GA	GGT CTC TTC CAT CAC CTT GC
HIF1-α	GCA CTA GAC AAA GTT CAC CTG AGA	CGC TAT CCA CAT CAA AGC AA

(v/v) of sodium bicarbonate (7.5% (w/v), Sigma). After forming 30 uL gel droplets at 37°C and 5% (v/v) CO_2 for a minimum of 90 min, the gels were transferred to the macrophages' wells. The other assay consisted of direct placement of the DRG on top of polarized macrophages to study direct cellular interactions (2D culture). Both assays were performed in Neurobasal (Gibco) medium supplemented with 2% (v/v) B27 (Gibco), 2 mM L-glutamine (Invitrogen), 6 mg/mL D-glucose (Sigma), 1% (v/v) pen/strep, and 50 ng/mL of M-CSF with medium changes every two days and maintained at 37°C and 5% (v/v) CO_2 for four (3D) or three (2D) days. The cells were then fixed and immunocytochemistry was performed. The area occupied by the axons in each dorsal root ganglia explant was calculated using the ImageJ (NIH) plugin Neurite-J. Using confocal microscopy, the entire area with positive staining for Neurofilament was acquired. Then, the image was automatically translated to 8 bits and a binary mask was created with the aid of the "Analysis Particles" function which enables the correct segmentation of axonal structures based on an intensity-threshold image coupled with morphological parameters such as structure size and area. The mask generated can then be added as an input to the Neurite-J plugin.

Axonal growth assay –CNS-derived neuronal culture

Cortical neurons were dissected and isolated from Wistar rats E17 embryos as described previously (20). To physically and fluidically separate distal axons from cell bodies, neurons were plated in microfluidic chambers as described previously (21). Microfluidic chambers were assembled onto an ibiTreat low wall 50 mm µ-Dish (ibidi) and coated with poly-D-lysine (PDL) 0.1 mg/mL overnight at 37°C and 2 µg/mL laminin for 2 h at 37°C. Cortical neurons were plated in the somal compartment of microfluidic chambers at a density of 50,000 cells per chamber. Cells were maintained in a humidified 5% CO_2 incubator at 37°C and treated with 10 µM 5-Fluoro-2′-deoxyuridine (5'-FDU) on day 4 to inhibit glial cell proliferation.

On day 5, distal axons were submitted to a 20-hour starving and after which axons were treated with $M_{(IL\text{-}10+TGF\text{-}\beta1)}$-derived secretome or control medium. 25 µl of secretome was locally applied to the axonal compartment of the microfluidic chamber for 14 h. Neurobasal medium with 1% penicillin/streptomycin was used for control cultures. A higher volume of culture medium was maintained in the somal compartment to ensure fluidic isolation of the axonal compartment and, therefore, restrict the treatment to distal axons. After 14 h of local treatment, population-wide axonal growth was assessed by live-cell imaging microscopy.

Neurospheres derived from human induced neural stem cells

Neurospheres were generated by culturing human induced pluripotent stem cells (hiPSCs) *in vitro*nectin XF™ treated plates with mTeSR 1 (both from Stem Cell Technology). After 7 days, spontaneous differentiation was initiated by the generation of Embryoid Bodies (EBs). For that, cells were detached by using TrypLE™ Express Enzyme (ThermoFisher) and plate into low attachment 96 well plate in Advanced DMEM/12 supplemented with 15% (v/v) knockout serum replacement (KSR, ThermoFischer), 1% (v/v) non-essential amino acids (NEAA, ThermoFischer), 2% (v/v) glutamax (ThermoFischer), 2-mercaptoethanol (55 mM, ThermoFischer), and Y-27632 (5 mM, Rho-associated protein kinase inhibitor, StemCell Technology). The hole medium was changed every other day. On day 6, 6-9 EBs were transferred from 96 well plates to non-adherent plates (35 mm) and were cultured in Advanced DMEM/12 supplemented with 1% (v/v) non-essential amino acids, 1% (v/v) glutamax, 1% (v/v) of N2 supplement (ThermoFischer), and heparin (1µg/mL, Sigma-Aldrich) to induce neural differentiation. After 5 days, 6-9 neurospheres were plated into 24 well plates, pre-treated with poly-D-lysin/laminin (76 µg/mL, 20 µg/mL, respectively), and cultured in differentiation media: DMEM/F12: Neurobasal (1:1, both from ThermoFisher), 0.5% of N2 supplement, 1% (v/v) NEAA, 1% (v/v) glutamax, 55 mM 2-mercaptoethanol, 2% (v/v) B27 supplement (ThermoFischer), and insulin (2.5 µg/mL, Sigma). After 2 days, the culture medium was replaced by 500 µl of secretome. Cells were incubated for 2 days and fixed for further analysis using immunofluorescence.

Immunocytochemistry

Cells/DRG/Neurospheres were first incubated with 4% (v/v) PFA for 20 min, and then permeabilized with Triton-X100 0.2% diluted in PBS (PBS-T) for 5 minutes, at room temperature (RT). 10% FBS (Millipore) in PBS was used as a blocking solution for 1 h, followed by the addition of the primary antibodies for 2 h. For macrophages was used the rat anti-CD11b (1:100, BioLegend) and rabbit anti-iNOS (1:100, Abcam), for DRGS the mouse anti-neurofilament (1:200, Millipore) and for neurospheres the Anti-βIII Tubulin (1:100, mouse – Millipore). After washing, Alexa Fluor 488 goat anti-rat (1:1000, Invitrogen) and Alexa Fluor 594 goat anti-rabbit (1:1000, Invitrogen) secondary antibodies were added for another hour, diluted in blocking solution. Finally, the samples were counterstained with 40,6-diamidino-2-phenylindoledihydrochloride (DAPI) (1 µg/mL, Sigma) for 10 min and in the case of DRGs with and Phalloidin (1:500, Sigma) for 45 min at RT. Images were obtained using a confocal microscope (Olympus FV1000) for 3D cultures and an Olympus IX81 fluorescence microscope for 2D cultures. To calculate the axonal area, maximum distance reached by axons, and axonal arborization, ImageJ software was used, as previously described (22).

Live imaging of CNS-derived neurons

Live imaging was performed using a Zeiss LSM 880 microscope with an Airyscan and a Plan-Apo Chromat 20x/0.8 Ph2 objective. During live imaging cells were maintained in a 37°C and 5% CO_2 environment. A tiled phase-contrast image was obtained for each

condition immediately before treatment (t=0 h) and after 6, 10 and 14 hours of treatment.

Images were processed and quantified using ImageJ software version 1.51n. A region of interest (ROI) was chosen to encompass the entire length of the axonal compartment, and the same size ROI was used for all samples. The Feature J Hessian plugin was applied with the following settings: largest eigenvalue of the Hessian Tensor, smoothing scale = 2.0). The Local Threshold was adjusted to include all axons in the axonal network. A binary image was generated, and the Skeletonize (2D/3D) plugin was used to obtain a skeletonized image of the axonal network. Finally, the Analyze Skeleton (2D/3D) was applied with the following settings: prune cycle method=none, show detailed info. A Branch Information table was generated using the software, and the sum of all branch lengths was further calculated, giving the population-wide total axonal length. The results were normalized for t=0 under the respective treatment conditions.

Spinal cord injury surgery

All experiments were performed after obtaining consent from the ethical Subcommittee in Life and Health Sciences (SECVS; ID:018/2019, University of Minho) and were conducted in accordance with the local regulations on animal care and experimentation (European Union Directive 2010/63/EU). The ARRIVE guidelines for reporting animal research have been followed (23). C57BL/6J mice (Charles River) were maintained under sterile conditions and in light, humidity, and temperature-controlled rooms. Food and water were provided *ad libitum*. Animals were handled for 1 week prior to SCI surgery.

Spinal cord surgery was performed as previously described (24). Briefly, 42 C57BL/6J adult female mice (10-15 weeks age) were used in this study. Anesthesia was delivered intraperitoneally (ip) using Imalgene (ketamine, 75 mg/kg, Richter Pharma AG) and Dormitor (medetomidine, 1 mg/kg, Pfizer). Mice were shaved and disinfected with chlorohexidine. A dorsal midline incision was then made at the thoracic level (T5-T12). The paravertebral muscles were retracted, and the spinal process and laminar arc of T8-T9 were removed to expose the spinal cord. The spinal cord was compressed using fine forceps for 5 seconds. The wound was closed with 9 mm autoclip (Braintree Scientific), and anesthesia was reverted with Antisedan (atipamezole, Orion Corporation) applied subcutaneously. The injured animals were randomly divided into four experimental groups: 1) $M_{(INF-\gamma+LPS)}$ secretome (n=10); 2) $M_{(IL-4+IL-13)}$ secretome (n=11), 3) $M_{(IL-10+TGF-\beta1)}$ secretome (n=10), and 4) vehicle (RPMI medium with 1% pen/strep, n=11). Treatment was delivered by intraperitoneal injections (500 µl), and the first injections were administered 3, 6, 9, 14 days post-injury and once a week afterwards. Eight animals did not survive the experimental protocol.

In a separate cohort of animals, we employed the same protocol to induce spinal cord injury, and the same method and schedule to deliver the treatment. However, this time, we utilized (C57BL/6J x CBA)F1 mice expressing the Thy1-GFP transgene. Following spinal cord compression, the injured animals were randomly assigned to two experimental groups: 1) $M_{(IL-10+TGF-\beta1)}$ secretome (n=3), and 2) vehicle (RPMI medium with 1% pen/strep, n=3). Treatment was delivered by intraperitoneal injections (500 µl) as described above.

Post-operative care

After surgery and throughout all *in vivo* experiments, animals were closely monitored and cared for, as previously described (16). A solution containing the antibiotic enrofloxacin (Baytril, 5 mg/mL, Bayer), the analgesic buprenorphine (Bupaq, 0.05 mg/kg, Richer Pharma AG), vitamins (Duphalyte, Pfizer), and saline (0.09% NaCl) was administered subcutaneously twice a day until the animals showed autonomy and no infections detected. Manual bladder voiding was performed twice a day during the first week and once every day until sacrifice or spontaneous restoration of bladder control was achieved. Food pellets were provided on the cage floor during the first few days to allow easy access. Animals were also monitored for body temperature, correct scarring of the surgical incision, and recovery of general activities (grooming and nesting for example). Five days after surgery, the staples were removed, and the animals were regrouped to promote socialization and decrease anxiety and stress. Animals were monitored during the experiment for humane endpoints: wounds, autophagy behavior, or weight loss (>20% of their baseline weight).

Locomotor analysis

The BMS test was used to evaluate locomotor behavior (25), 3 days post-injury and once a week thereafter for 37 days. The mice were placed in an open arena for 4 min, and their locomotor function was evaluated by two independent observers who were blinded to the experimental groups. Each animal was scored on a scale ranging from 0 to 9. Animals presenting a BMS score greater than 1 in the first BMS assessment (3 dpi) was excluded because of incomplete spinal cord compression.

Bladder function

The bladders were manually voided and the animals were placed in the cage with water provided *ad libitum* overnight. Water weights in the cage bottles were measured before and after the experiment to assess water intake. Bladders were then voided into a beaker and the urine was weighed. The ratio between water intake and urine was calculated to assess bladder control in the different experimental groups. If the amount of urine was less than 0.1 g we considered that the animal regained total bladder control.

Von Frey

The Von Frey test was used to determine tactile sensitivity by measuring how much force is required to elicit movement of the

paw fingers, using the up-and-down method with Von Frey monofilaments, as previously described (26). The experimental setting consisted of placing the mice in an elevated mesh restrained inside a standard perforated box. Before the test started, each animal was habituated to the test conditions. A total of 9 monofilaments were used, ranging from 0.008 to 1.4 g. Both paws were stimulated with the central monofilament (0.16 g). If the animal moved the fingers of the paw, a weaker monofilament was used; otherwise, a stronger monofilament was applied. The test was performed until: 1) observed response to the 0.008 g monofilament, 2) no response to 1.4 g monofilaments, or 3) after a total of five measures around the threshold. 50% threshold was calculated using the formula:

$$50\,\%\ \text{threshold} = \frac{10^{(x\_f+k\delta)}}{10000}$$

Where xf is the value of the final monofilament used (log units), K is the tabular value for the pattern of positive/negative responses, and δ is the mean difference between stimuli (0.267).

Flow cytometry

Nine days post-injury, approximately 50 µL of blood was collected from the tail vein of the animals. Erythrocytes were depleted with ACK lysis solution. The cell pellet was then washed with FACS buffer (PBS, 10% BSA, 0.1% azide). $1x10^6$ cells were stained. The Fc portion was blocked using anti-mouse CD16/CD32 (Biolegend). Cell staining was performed by incubating a cocktail of antibodies for 30min at 4°C (Table 2). After washing, the cells were re-suspended in 200 µL FACS buffer. Precision counting beads (Biolegend) were added to the single-cell suspensions according to the manufacturer's instructions to calculate the final cell concentrations. Cells were acquired using an LSRII Flow Cytometer (BD Pharminogen) and analyzed using Flow Jo software version 10.4. The gating strategy used can be found in the Supplementary Data (Supplementary Figure S5).

Spinal cord collection, processing and immunohistochemistry

To understand the molecular and cellular effects of the different treatments on the spinal cord injury environment, an immunohistochemistry protocol to mark GFAP (astrocytes), Iba-1 (macrophages/microglia), PDGFR (fibrosis), and NeuN (mature neurons) was performed on mouse spinal cords. First, at 5 weeks post-injury mice were anesthetized and perfused with 20 mL of cold PBS and then with 4% PFA. A dorsal incision was made to remove the spinal cord with the vertebral column. The isolated spinal cords were then fixed with 4% PFA for 24h at 4°C. After, the tissue was placed on 30% saccharose solution until reaching saturation point, which was then cut into 1 cm fragments centered in the lesion site. Next, the spinal cords were embedded in optimal cutting temperature (OCT) solution and frozen in isopentane and liquid nitrogen. Using a Leica CM 1900 cryostat, the spinal cords were cut into transverse sections of 20 µm and mounted onto microscope slides (SuperFrost Plus) that were stored at -20°C for further use.

On the day of immunohistochemistry, slides with frozen sections were thawed at RT and cleaned with PBS to remove any remaining cryopreservation solution. This was followed by permeabilization with PBS-T 0.2% (v/v) for 10 min and a blocking solution of 5% (v/v) FCS in PBS-T 0.2% (v/v) for 30 min. An overnight incubation at 4°C was then performed with the following primary antibodies: rabbit anti-GFAP (1:200, DAKO), rabbit anti-Iba-1 (1:200, Wako), PDGFR (1:1000, Abcam), and rabbit anti-NeuN (1:200, D4G4O). The next day, after washing, the samples were incubated with Alexa Fluor 594 goat anti-rabbit (1:1000) (Abcam) secondary antibody for 3 h at RT. Cells were then counterstained with DAPI for 20 min before mounting the slides in Immu-Mount® (Thermo Scientific) for subsequent image analysis. A negative control (primary antibodies omitted) was performed to discard any background as positive staining (Supplementary Figure S6).

Imaging was performed using an Olympus Widefield Inverted Microscope IX81. GFAP staining was evaluated by measuring the

TABLE 2 Flow cytometry analysis summary of markers expressed on different cell populations.

Marker	Fluorochrome	Company	Target	Dilution
CD86	PerCpCy5.5	Biolegend	Myeloid cells	1/100
CD11b	PE	Biolegend	Myeloid cells	1/200
CD11c	BV 605	Biolegend	Mostly dendritic cells	1/100
NK 1.1	BV 510	Biolegend	Natural killer	1/100
CD19	FITC	Biolegend	B lymphocytes	1/200
CD3	APC	Biolegend	T lymphocytes	1/100
CD45	PeCy7	Biolegend	Leukocytes	1/200
Ly6C	BV711	Biolegend	Monocytes	1/100
Ly6G	BV650	Biolegend	Granulocytes	1/100
CD16/32	None	Biolegend	Fc Block	1/25

area of astrogliosis morphology, normalized to the total GFAP area. IBA-1 was evaluated by assessing the area of ramified macrophages/total microglia. Fibrosis was evaluated by assessing the area of PDGFR+ area normalized for total spinal cord area. The location of the spinocerebellar (SCT), rubrospinal (RST) and the corticospinal tracts (CST) were identified using the spinal cord atlas developed by Paxinos, Watson and Kayalioglu (27). The positive area for Thy1-GFP was calculated and divided for the total area of the tract in each spinal section. Positive Thy1-GFP and total areas were calculated using the plugin Neurite-J from the ImageJ (NIH) software as described above. NeuN staining was measured by counting the number of positive cells in laminae VIII and IX of both ventral horns.

Proteomics analysis

The secretome was first concentrated (×100) using ultracentrifugation with falcons with 5 kDa cut-off filter (Vivaspin, GE Healthcare). A protein precipitation step using TCA to a final concentration of 20% was performed, and protein pellets were re-suspended in 35µL of Laemmli sample buffer. Protein extracts from each sample were separated by SDS-PAGE for approximately 16 min at 110 V (Short-GeLC Approach) (1) and stained with Coomassie Brilliant Blue G-250. Each lane was divided into three separate gel fractions for a destaining step using a solution of 50 mM ammonium bicarbonate with 30% acetonitrile, followed by overnight protein digestion with trypsin. Peptide extraction from the gel was performed using solutions containing different percentages of acetonitrile (30, 50, and 98%) with 1% of formic acid. For protein identification, each fraction was analyzed separately, and for protein quantification, fractions from each sample were combined, and a single analysis per sample was performed by LC-MS/MS.

Samples were analyzed on a NanoLC™ 425 System (Eksigent) coupled to a Triple TOF™ 6600 mass spectrometer (Sciex) and the ionization source (ESI DuoSpray™ Source). The chromatographic separation was performed on a Triart C18 Capillary Column 1/32" (12 nm, S-3µm, 150 x 0.3 mm, YMC) and using a Triart C18 Capillary Guard Column (0.5 × 5 mm, 3 µm, 12nm, YMC) at 50°C. The flow rate was set to 5 µL/min, and mobile phases A and B were 5% DMSO plus 0.1% formic acid in water and 5% DMSO plus 0.1% formic acid in acetonitrile, respectively. The LC program was performed as follows: 5 – 30% of B (0 - 50 min), 30 – 98% of B (50 - 52 min), 98% of B (52 - 54 min), 98 - 5% of B (54 - 56 min), and 5% of B (56 - 65 min). The ionization source was operated in the positive mode set to an ion spray voltage of 5500 V, 25 psi for nebulizer gas 1 (GS1), 10 psi for nebulizer gas 2 (GS2), 25 psi for the curtain gas (CUR), and source temperature (TEM) at 100°C. For data-dependent acquisition (DDA) experiments, the mass spectrometer was set to scanning full spectra (m/z 350-2250) for 250 ms, followed by up to 100 MS/MS scans (m/z 100 – 1500). Candidate ions with a charge state between +1 and +5 and counts above the minimum threshold of 10 counts per second were isolated for fragmentation, and one MS/MS spectrum was collected before adding those ions to the exclusion list for 15 s (mass spectrometer operated by Analyst® TF 1.8.1, Sciex®). The rolling collision enErgy was used with a collision enErgy spread of 5. For SWATH experiments, the mass spectrometer was operated in a looped product ion mode and specifically tuned to a set of 42 overlapping windows, covering the precursor mass range of 350-1400 m/z. A 50 ms survey scan (350-2250 m/z) was acquired at the beginning of each cycle, and SWATH-MS/MS spectra were collected from 100-2250 m/z for 50 ms, resulting in a cycle time of 2.2 seconds.

Protein identification was performed using the ProteinPilot™ software (v5.0.2, Sciex) for each sample. The paragon method parameters were as follows: searched against the reviewed Mus musculus database from SwissProt, cysteine alkylation by acrylamide, digestion by trypsin, and gel-based ID. An independent False Discovery Rate (FDR) analysis using the target-decoy approach provided by Protein Pilot™, was performed to assess the quality of the identifications. SWATH data processing was performed using SWATH™ processing plug-in for PeakView™ (v2.0.01, Sciex®). Relative protein quantification was performed in all samples using information from the Ion-Library search. Quantification results were obtained for peptides with less than 1% of FDR for at least one of the samples by calculating the sum of up to five fragments/peptides. Relative peptide peak areas were normalized to the internal standard peak areas. Protein quantities were obtained by the sum of up to 15 peptides/proteins. Protein–protein interactions and network analysis was constructed using the online STRING database (https://string-db.org) version 11.5, depicting both functional and physical protein associations with a medium confidence level (0.4), and organized into clusters through k means clustering method. All identified proteins were then subjected to an over-representation analysis using the ConsensusPathDB. From a total of 368 proteins identified using LC-MS/MS, we focused the analysis on those that presented higher concentrations (fold changes of 2 or higher) between the two groups. These proteins were then grouped by function using the UniProt database and a heat map of their concentration was plotted with a cut off of 5 (ratios higher than 5 were color-expressed as 5). The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (28) partner repository with the dataset identifier PXD048453.

LEGENDplex

The concentration of relevant cytokines was evaluated in the secretomes of polarized macrophages using the LEGENDplex™ Mouse Macrophage/Microglia Panel kit according to manufacturer's instructions. The secretome was first concentrated (×10) using ultracentrifugation with falcons with 5 kDa cut-off filter (Vivaspin, GE Healthcare). Then, reagents were prepared from the stocks provided, and standard serial dilutions were prepared to generate a standard curve. Assay buffer (25µL) was added to standard and sample wells in a 1:1 ratio. 25µL of mixed beads

were added to each well, and the plate was incubated for 2 hours at RT with continuous agitation at 800 rpm. After a centrifugation of 250g for 5min, beads were washed with 1x wash buffer for 1min. 25µL of detection antibodies was added to each well, followed by 1 hour of incubation at RT with agitation at 800 rpm. 25µL of Streptavidin-phycoerythrin (SA-PE) was added directly to the previous solution, and the plate was incubated for 30 minutes at RT with agitation at 800 rpm. After a wash step with 150µL of 1x wash buffer, the samples were ready to read on the flow cytometer. For that, samples were vortexed, and 300 beads per analyte were acquired in a BD LSRII Flow Cytometer (BD, Pharminogen). The FCS files were analyzed using Biolegend's LEGENDplex™ data analysis software site. Concentration values were subsequently divided by 10 to account for the concentration step, providing an accurate representation of the actual cytokine concentration present in the secretome.

Statistical analysis

Statistical analyses were performed using GraphPad Prism software, version 8.0.1. The normality of the data was evaluated using the Shapiro-Wilk normality test. Gene expression, axonal regeneration *in vitro*, weight loss, bladder function, chronic pain, LEGENDplex, and flow cytometry data were analyzed using One-Way ANOVA followed by Tukey's multiple comparison test. Data from the BMS score, astrogliosis, fibrosis, spinal tracts area, axonal arborization, and ramified microglia were assessed by two-way ANOVA followed by Tukey's multiple comparison test. Live imaging data were assessed by unpaired, non-parametric t-test (Mann-Whitney test). Statistical significance was defined as $p < 0.05$ (95% confidence level). Data are presented as mean ± standard error (SEM).

Results

Monocytes isolation, differentiation and polarization

To successfully culture spleen-derived macrophages (Sp-MΦ), we isolated monocytes from the spleen and cultured them in the presence of macrophage colony-stimulating factor (M-CSF) to stimulate the survival, proliferation, and differentiation of monocytes into macrophages (Figure 1A). Using our protocol, we were able to obtain a highly enriched culture (97% purity) of Sp-MΦ (Figure 1B). Without M-CSF, it was impossible to establish and maintain the cells (Supplementary Figure S1A), indicating that M-CSF is essential for the Sp-MΦ culture.

To polarize macrophages into different phenotypes, we stimulated macrophages for 24h with 20 ng/mL of IFN-γ plus 100 ng/mL of LPS (classical activation) or with 20 ng/mL of IL-4 plus 20 ng/mL of IL-13 or 20 ng/mL of IL-10 plus 20 ng/mL of TGF-β1 (alternative activation). With immunocytochemistry it was possible to confirm that the classical activation leads to the polarization of 89% of the macrophages (Supplementary Figure S1B). Moreover, proteomics analysis of the secreted proteins of each macrophage population revealed that out of 487 proteins identified, 81 were

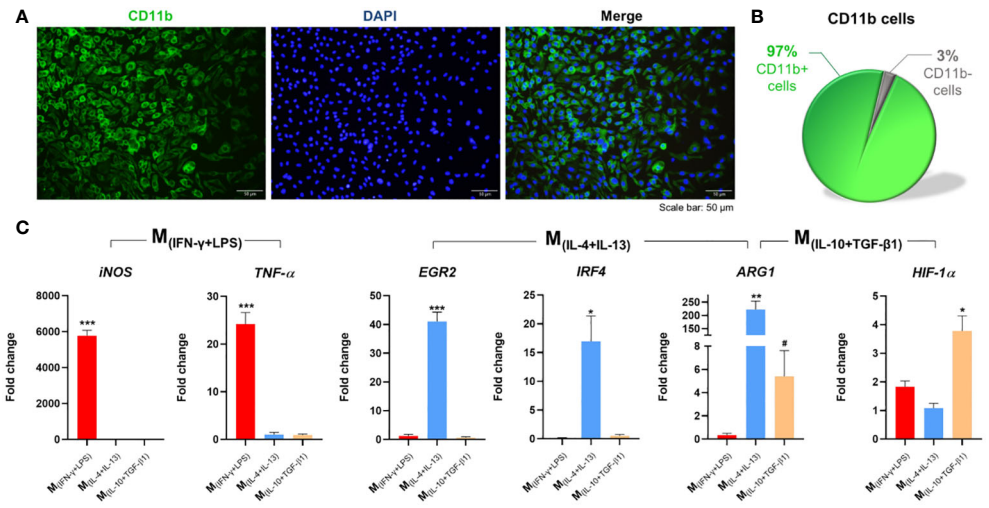


FIGURE 1
Isolation, differentiation, and polarization of macrophages. **(A)** Splenic monocytes cultured with macrophage colony-stimulating factor (M-CSF) for 7 days differentiated into macrophages; **(B)** with a culture purity of 97%. **(C)** Macrophages stimulated for 6 h with INF-γ and LPS significantly overexpressed *iNOS* (2, 7 df, $p<0.0001$) and *TNF-α* (2, 7 df, $p<0.0001$). Macrophages stimulated with IL-4 and IL-13 significantly overexpressed *EGR2* (2, 6 df, $p<0.0001$), *IRF4* (2, 6 df, $p=0.0216$), and Arg-1 ($p=0.0020$); and Macrophages stimulated with IL-10 and TGF-β1 significantly overexpressed ARG1 ($p=0.0357$), and *HIF-1α*.(2, 8 df, $p=0.0032$). Target genes were normalized to three reference genes: GADPH, HPRT and 18s. Fold-change levels were calculated by the 2-ΔΔct method related to non-stimulated macrophages. In immunocytochemistry photomicrographs macrophages were quantified using the anti-CD11b antibody (green) and nuclei were stained with DAPI (blue). One Way ANOVA followed with Tukey *post-hoc* test was used for statistical analysis. Arg-1 data were analyzed using the Mann Whitney test because normality was not achieved using the Shapiro-Wilk test. Data is presented as mean ± standard error (SEM). df= degrees of freedom, * or #- $p< 0.05$; **- $p< 0.01$; ***- $p< 0.001$. Scale bar =50 µm. n=3. 2 independent experiments were performed.

exclusive secreted by $M_{(INF-\gamma+LPS)}$ macrophages, 35 by $M_{(IL-4+IL-13)}$, and 90 by $M_{(IL-10+TGF-\beta1)}$ macrophages (Supplementary Figure S1C). Using the Protein Analysis Through Evolutionary Relationships (PANTHER) tool, we further demonstrated distinctions in the protein classes among these populations of proteins (Supplementary Figure S1D). Metabolite interconversion enzymes were identified as a common protein class between the different macrophage populations, but as can be observed by the pie charts, the protein class or the percentage of proteins in different classes varied considerably among each cell phenotype (Supplementary Figure S1D). Additionally, the phenotypes of each macrophage population was also confirmed by gene expression analysis. qPCR revealed that Sp-MΦ are easily polarized in vitro; namely, when macrophages were stimulated with IL-4+IL-13, they significantly overexpressed EGR2 and IRF4, and these genes were not overexpressed when macrophages were stimulated with IL-10+TGF-β1 (Figure 1C). The ARG1 gene was significantly overexpressed in the two populations of macrophages with alternative activation, however more overexpressed in the $M_{(IL-4+IL-13)}$ phenotype than in the $M_{(IL-10+TGF-\beta1)}$ macrophages. In contrast, the IL-10+TGF-β1 stimulation protocol led to a significant increase in HIF-1α expression, and these gene was not overexpressed with the IL-4+IL-13 stimuli. Using gene expression, we also confirm that macrophages under classic activation stimuli significantly overexpressed iNOS and TNF-α genes (Figure 1C). All these genes are known to be specifically overexpressed in these phenotypes (29). These results showed that we were able to obtain three different subsets of macrophages, one with classical activation ($M_{(INF-\gamma+LPS)}$) and two with alternative activation ($M_{(IL-10+TGF-\beta1)}$; $M_{(IL-4+IL-13)}$).

IL-10 and TGF-β1 activation promotes higher axonal growth

The effects of each macrophage subtype on axonal growth were then investigated. Spleen-derived macrophages polarized into $M_{(INF-\gamma+LPS)}$, $M_{(IL-10+TGF-\beta1)}$ or $M_{(IL-4+IL-13)}$ were co-cultured with DRGs growing in three dimensions (Figure 2). DRGs cultured without macrophages were used as baseline. The results showed that DRGs co-cultured with $M_{(IL-10+TGF-\beta1)}$ and $M_{(IL-4+IL-13)}$ macrophages had significantly higher axonal arborization than those co-cultured with $M_{(INF-\gamma+LPS)}$ or than basal levels (Figure 2A). DRGs co-cultured with $M_{(IL-10+TGF-\beta1)}$ and $M_{(IL-4+IL-13)}$ macrophages also presented significantly longer axons (Figure 2B) than those co-cultured with $M_{(INF-\gamma+LPS)}$. Concerning the total axonal area, only $M_{(IL-10+TGF-\beta1)}$ condition showed significant differences from baseline (Figure 2C). We also performed a similar experiment with DRGs growing in two dimensions, which did not allow axonal growth in depth, but enabled direct contact between macrophages and DRGs

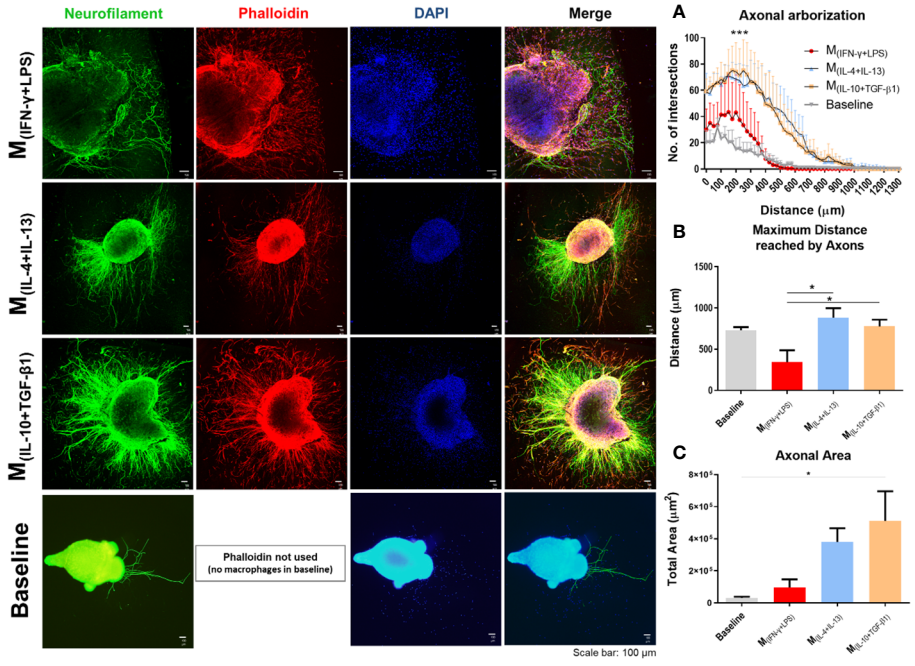


FIGURE 2
Classical ($M_{(INF-\gamma+LPS)}$) or alternative ($M_{(IL-4+IL-13)}$; $M_{(IL-10+TGF-\beta1)}$) activated macrophages co-cultured with dorsal root ganglia (DRGs) in 3D collagen hydrogels. DRGs were stained with Neurofilament (green), Macrophages and DRGs stained with Phalloidin (red) and nuclei counterstained with DAPI (blue). **(A)** DRGs co-cultured with $M_{(IL-4+IL-13)}$ and $M_{(IL-10+TGF-\beta1)}$ macrophages had significantly higher axonal arborization (3, 12 df, $p<0.0001$) and **(B)** significantly longer axons (3, 14 df, $p=0.0172$) than $M_{(INF-\gamma+LPS)}$ group and basal levels. **(C)** $M_{(IL-10+TGF-\beta1)}$ condition also showed significant higher axonal area than basal levels (3, 14 df, $p= 0.0292$). Statistical analysis for axonal arborization employed two-way ANOVA followed by Tukey's multiple comparisons test, while total area and distance were analyzed using one-way ANOVA followed by Tukey's test. Data is presented as mean ± standard error (SEM). df= degrees of freedom, *- $p< 0.05$; ***- $p< 0.001$. Scale bar =100 µm; $M_{(IFN-\gamma+LPS)}$ n= 3; $M_{(IL-4+IL-13)}$ n=5; $M_{(IL-10+TGF-\beta1)}$ n=5. 2 independent experiments were performed.

(Supplementary Figure S2). Interestingly, under these conditions, only the DRGs co-cultured with $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ macrophages presented significantly higher axonal arborization (Supplementary Figure S2A) than those co-cultured with the other subtypes of macrophages, these DRGs also have significantly longest neurite (Supplementary Figure S2B), and higher axonal area (Supplementary Figure S2C) than $M_{(IL\text{-}4+IL\text{-}13)}$ macrophages. It is important to point out that without the collagen matrix, axonal growth is significantly reduced, and not even the direct contact of the macrophages compensates for the absence of the 3D matrix.

The neuronal effects of the molecules and extracellular vesicles secreted by the different subtypes of splenic macrophages were also tested using human-derived neurospheres obtained from iPSCs. Neurospheres were allowed to differentiate into neurons for two days and then cultured with the secretome derived from each macrophage subtype (Figure 3A). It proved challenging to establish the baseline level of neuronal growth devoid of secreted factors as attempts to culture human neurospheres solely in basal medium were unsuccessful, leading to detachment from the culture plates and rendering meaningful analysis unfeasible. Nonetheless, we conducted a positive control using the regular culture medium to provide a comparative reference point. The total axonal area divided by the number of neurospheres was analyzed as described for the DRGs (see materials and methods section). As expected, the positive control group presented an overall neuronal area higher than all the groups, but notably it was only significantly different when compared with the $M_{(INF\text{-}\gamma+LPS)}$ and $M_{(IL\text{-}4+IL\text{-}13)}$ groups, but not with the $M_{(IL\text{-}10+TGF\text{-}\beta1)}$-derived secretome (Figure 3B). Results also demonstrated that the $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ secretome significantly promoted more axon preservation/regeneration than $M_{(IL\text{-}4+IL\text{-}13)}$ secretome (Figure 3B). Both subtypes are pro-regenerative; however, our in vitro results showed that the $M_{(IL\text{-}10+TGF\text{-}\beta1)}$-derived secretome has higher regenerative capabilities. For this reason, we then tested only the secretome derived from this sub-population in CNS-derived neurons. Primary cortical neurons were plated in the soma compartment of microfluidic chambers (Figures 4A, B), and neuronal growth was live imaged (Supplementary Video 1) in the axonal compartment for 14h (Figures 4C, D). The results demonstrated that $M_{(IL\text{-}10+TGF\text{-}\beta1)}$-derived secretome promoted significant axonal regeneration compared with the control medium (Figure 4E).

$M_{(IL\text{-}10+TGF\text{-}\beta1)}$ derived secretome promotes functional recovery *in vivo*

In vitro experiments demonstrated that the soluble factors and extracellular vesicles secreted by macrophages may have therapeutic potential for neural repair. Therefore, we tested whether intraperitoneal injections (500 µL) of macrophage-derived secretome could be used as a therapy for spinal cord injury. Forty-two mice were subjected to compression SCI and 3, 6, 9, and 14 days post-injury (and then once a week up to 28 dpi) were treated with secretome derived from different macrophage subtypes (Figure 5A). During the experimental protocol, all animals lost weight without significant differences between groups (Figure 5B). To evaluate motor function, we performed the BMS test, in which a higher score indicates higher motor recovery. We found that mice treated with $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ secretome had significantly higher BMS scores than those treated with the vehicle or $M_{(IL\text{-}4+IL\text{-}13)}$ (Figure 5C). Only animals treated with this pro-regenerative cocktail ($M_{(IL\text{-}10+TGF\text{-}\beta1)}$ secretome) were able to perform weight-supported plantar stepping, while the other treatment regimens only led to extensive ankle movement recovery without weight support. Interestingly, in the first 2/3 weeks post-injury, mice treated with the pro-inflammatory cocktail ($M_{(INF\text{-}\gamma+LPS)}$ secretome) presented a functional recovery very close to those treated with the $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ secretome, indicating that this pro-inflammatory cocktail may be beneficial in the early phase. However, continuing with $M_{(INF\text{-}\gamma+LPS)}$ secretome treatment, the functional recovery stabilized, and the therapeutic effect disappeared (Figure 5C), indicating that the non-resolving nature of chronic exposure to this pro-inflammatory cocktail is detrimental. Four weeks post-injury, we performed the von Frey filament test to assess the mechanical sensitivity function of the animals. We did not detect any statistical differences; however, mice treated with the pro-inflammatory cocktail ($M_{(INF\text{-}\gamma+LPS)}$ secretome) had lower values, indicating that this treatment may lead to hypersensitivity. In contrast, the vehicle and $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ secretome groups showed higher values in the von Frey filament test (Figure 5D), indicating less hypersensitivity. We also analyzed mouse autonomic function, namely bladder function, using the ration between water intake and amount of urine in the bladder. Bladder recovery is an important priority for people living with SCI (30). Our results showed that mice treated with the $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ secretome had a significant recovery

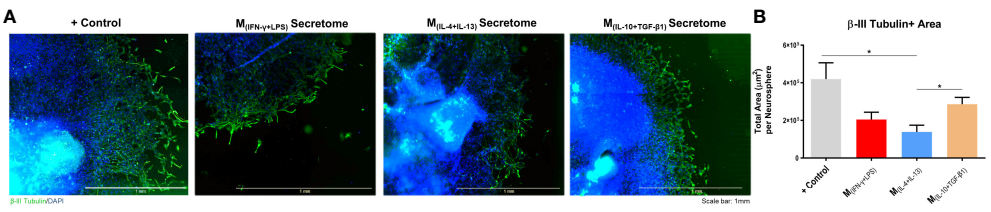


FIGURE 3
Axonal area of differentiated Neural Stem Cells obtained from human induced Pluripotent Stem Cells. **(A)** Axonal area was stained using anti-βIII tubulin antibody (green) and nuclei counterstained with DAPI (blue); **(B)** Statistical analysis demonstrated that the factors secreted by $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ macrophages are able to significantly preserve/regenerate the differentiated neurons (2, 18, p=0.0364) than the $M_{(IL\text{-}4+IL\text{-}13)}$-secreted factors. One Way ANOVA followed by Tukey *post-hoc* test was used for statistical analysis. Data is presented as mean ± standard error (SEM). *- p< 0.05; $M_{(IFN\text{-}\gamma+LPS)}$ n=6; $M_{(IL\text{-}4+IL\text{-}13)}$ n=9; $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ n=6; +Ct n=9. Scale bar=1 mm. 2 independent experiments were performed.

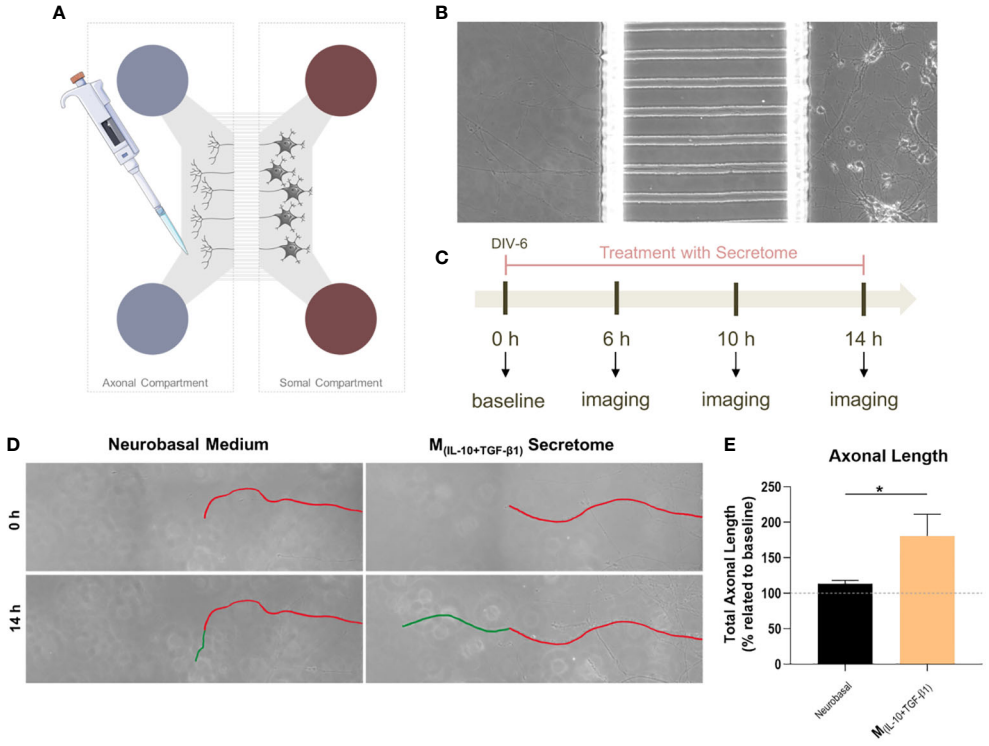


FIGURE 4
The effect of $M_{(IL\text{-}10+TGF\text{-}\beta1)}$-derived secretome on the CNS neurons. **(A)** Schematic representation of the microfluidic chambers used. **(B)** Brightfield images of the axonal and somal compartments of the microfluidic chambers. **(C)** Schematic representation of the workflow used. **(D)** Brightfield images of axons growing under the effect of the soluble factors and extracellular vesicles secreted by $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ macrophages or Vehicle (Neurobasal Medium). In red is represented the length of the axon at baseline and in green after 14h of live imaging. **(E)** The secretome of $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ macrophages promotes significantly axonal regeneration (p= 0.0286). Statistical significance tested by unpaired, non-parametric t-test (Mann-Whitney test). Data is presented as mean ± standard error (SEM). *- p< 0.05; n=4, DIV= days *in vitro*. 2 independent experiments were performed.

of bladder control compared to those treated with vehicle and $M_{(INF\text{-}\gamma+LPS)}$ secretome (Figure 5E). This preclinical trial demonstrated that the therapeutic effect of the molecules and extracellular vesicles secreted by the different subtypes of macrophages varies depending on the phenotype, even when using two pro-regenerative phenotypes.

$M_{(IL\text{-}10+TGF\text{-}\beta1)}$ derived secretome modulates pathophysiological events leading to neuronal survival *in vivo*

To understand the effect of the secretome on the immune response, we collected blood from all groups nine days post-injury and used healthy mice as controls. Flow cytometry was used to verify the inflammatory profile of leukocytes in circulation, which could infiltrate the injured spinal cord. Analysis revealed that mice treated with vehicle, $M_{(INF\text{-}\gamma+LPS)}$ and $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ secretome had a significantly higher frequency of myeloid cells in circulation (Figure 6A). Mice treated with the $M_{(INF\text{-}\gamma+LPS)}$ secretome had a significantly higher frequency of monocytes than the $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ secretome (Figure 6B). Mice treated with vehicle or $M_{(INF\text{-}\gamma+LPS)}$ secretome had a significantly higher frequency of $Ly6C^{high}$ monocytes (Figure 6C). It is noteworthy that the $Ly6C^{high}$ monocytes are prone to become pro-inflammatory macrophages (31). $M_{(INF\text{-}g+LPS)}$ also presented significantly more Ly6Clow-med monocytes in circulation (Figure 6D). All animals with SCI had significantly more circulating neutrophils (Figure 6E). Concerning the rest of myeloid cells, $M_{(INF\text{-}g+LPS)}$ also presented significant increase (Figure 6F). No differences were observed between the groups for B cells (Figure 6G). $M_{(INF\text{-}\gamma+LPS)}$ and vehicle-treated mice had a significantly lower frequency of T cells (Figure 6H). Of note, the number of animals used in the flow cytometry analysis varies from that used in functional recovery data because we opted to spare some animals during this sub-acute phase due to their weakened state. To conduct flow cytometry of circulating leukocytes blood collection was necessary, we decide to prioritize the well-being of the animals avoiding unnecessary risks of losing mice.

Thirty-eight days post-injury, the animals were sacrificed and the spinal cords were collected for histological analysis. IBA-1antibody was used to study the morphology of microglia and distinguish between ramified and amoeboid microglia (Figure 7A). Rostral-caudal analysis of the spinal cord showed that mice treated with the pro-regenerative cocktail $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ had a significantly higher percentage of ramified microglia than mice treated with the pro-inflammatory cocktail $M_{(INF\text{-}\gamma+LPS)}$, or the $M_{(IL\text{-}4+IL\text{-}13)}$-secreted factors (Figure 7B).

The GFAP antibody was used to analyze astrogliosis. Areas of clustered GFAP overstaining were considered astrogliosis (Figure 7C). The analysis revealed that mice treated with the pro-regenerative cocktail, $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ secretome, had significantly

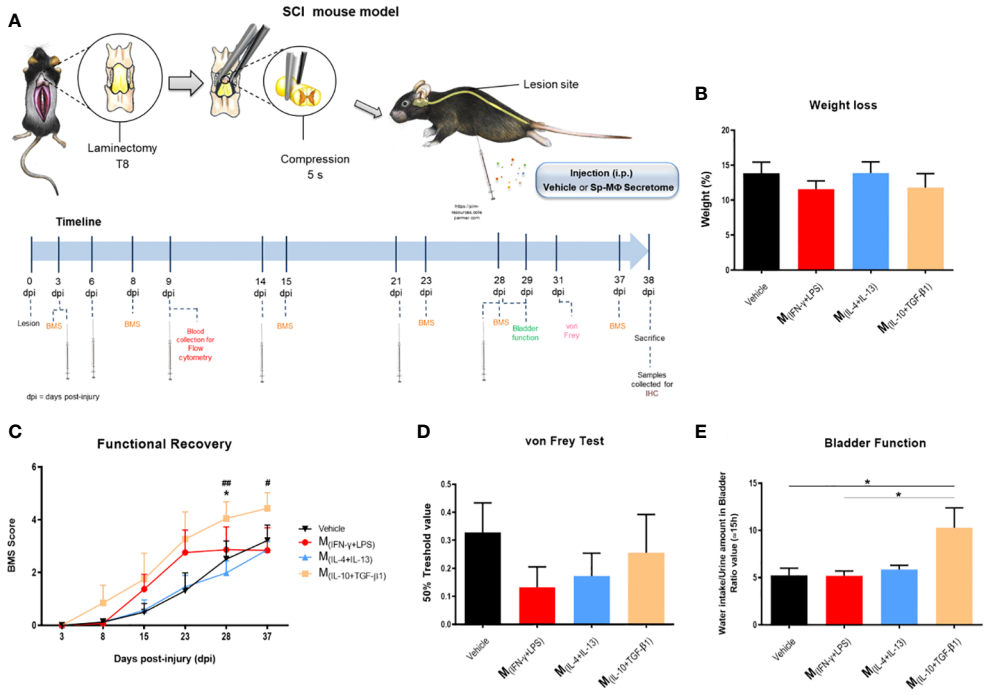


FIGURE 5
Pre-clinical evaluation of macrophages derived secretome using a SCI compression model. **(A)** Schematic layout of the *in vivo* testing. **(B)** The treatment had no effect on weight of the animals 38 dpi (3, 25 df, p=0.6013). **(C)** Animals treated with $M_{(IL\text{-}10+TGF\text{-}\beta1)}$-derived secretome presented significantly better functional scores than the other treatment groups, namely than the Vehicle (3, 25 df, p=0.0465) and $M_{(IL\text{-}4+IL\text{-}13)}$ group (3, 25 df, p= 0.0047) at 28 days and the $M_{(IL\text{-}4+IL\text{-}13)}$ at 37 days (3, 25 df, p= 0.0359). **(D)** No significantly differences were observed on the hypersensitivity of the animals 38 dpi, however, $M_{(INF\text{-}\gamma+LPS)}$-treated mice presented a tendency to be more hypersensitive (3, 25 df, p=0.5097). **(E)** Animals treated with $M_{(IL\text{-}10+TGF\text{-}\beta1)}$-derived secretome presented significant recovery of the bladder function when assessed 38 dpi (3, 22 df, p= 0.0137). Two-way repeated measure ANOVA was used to analyze statistical differences on the BMS data and One-Way ANOVA was used to analyze statistical differences on the other functional tests followed by the multiple comparison test Tukey. Data is presented as mean ± standard error (SEM). * or #- p< 0.05; ##- p< 0.01; df= degrees of freedom, Vehicle n=8; $M_{(INF\text{-}\gamma+LPS)}$ n=7; $M_{(IL\text{-}4+IL\text{-}13)}$ n=8; $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ n=6. 1 independent experiment was performed.

lower astrogliosis (Figure 7D), which indicate that these animals presented diminished scar.

Neurons from the ventral horns, namely from lamina VIII and IX, were counted using an anti-NeuN antibody (Supplementary Figure S3A). Mice treated with $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ secretome have significantly more neurons than animals treated with the pro-inflammatory-derived secretome (Supplementary Figure S3B). Finally, concerning fibrosis, the rostral-caudal analysis did not detect significant differences in the PDGFR+ area (Supplementary Figure S3C) between the treated groups when all areas of the spinal cord were analyzed (Supplementary Figure S3D); however, caudally to the injury epicenter, mice treated with the $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ secretome have significantly less fibrosis than the other treatments (Supplementary Figure S3E).

$M_{(IL\text{-}10+TGF\text{-}\beta1)}$ derived secretome preserved ascending and descending spinal tracts after SCI

Considering the functional and histological outcomes obtained from our pre-clinical trial, we executed a subsequent in vivo protocol with a focused objective: to assess the therapeutic efficacy of M(IL-10+TGF-β1) secretome specifically in the preservation of spinal tracts critical for locomotion. These tracts include the corticospinal tract (CST), rubrospinal tract (RST), and spinocerebellar tract (SCT). Within this cohort of animals, we employed mice harboring the Thy1-GFP transgene, and the percentage of positive area for Thy1-GFP in each distinct spinal tract was calculated (Figure 8A). The analysis encompassed a range spanning 600 µm to 2000 µm in both rostral and caudal directions from the epicenter. The vicinity of the epicenter was excluded from the analysis due to challenges in pinpointing the exact location of the spinal tracts. Results demonstrated that the administration of $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ secretome significantly contributes to the preservation of the spinocerebellar tract caudally to the epicenter (Figure 8B), both the rostral and caudal portions of the rubrospinal tract (Figure 8C), and the preservation of the corticospinal tract (Figure 8D) in the rostral region. Significant differences were also observed between the rostral and caudal regions, however, only in the motor tracts (RST and CST). Specifically, the rostral regions exhibited a markedly higher extent of neuronal preservation in comparison to the caudal regions (Figures 8C, D).

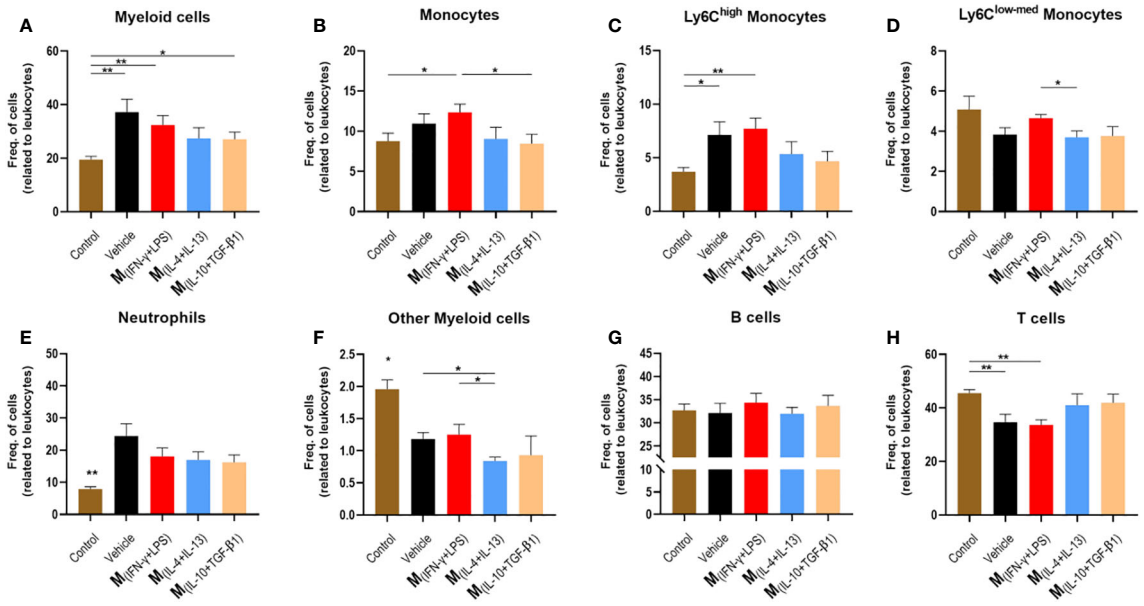


FIGURE 6
Leukocytes in circulation 9 days post injury. Blood was collected from the tail vein and process for analysis using flow cytometry. **(A)** Animals treated with vehicle, $M_{(INF-\gamma+LPS)}$ and $M_{(IL-10+TGF-\beta1)}$-derived secretome had significantly higher myeloid cells than control mice (4, 17 df, p=0.0240). **(B)** Animals treated with $M_{(INF-\gamma+LPS)}$-derived secretome had significantly higher frequency of monocytes than $M_{(IL-10+TGF-\beta1)}$ secretome group and control (4, 17 df, p=0.0457). **(C)** Animals treated with vehicle or $M_{(INF-\gamma+LPS)}$-derived secretome had a significantly higher frequency of $Ly6C^{high}$ monocytes (4, 17 df, p=0.0282) than control mice. **(D)** $M_{(INF-\gamma+LPS)}$ group also had significantly more $Ly6C^{med+low}$ monocytes than $M_{(IL-4+IL-13)}$-treated mice (4, 17 df, p=0.0457). **(E)** Animals without a SCI had significantly lower frequency of Neutrophils (4, 17 df, p=0.0055). **(F)** $M_{(IL-4+IL-13)}$-treated mice had significantly less other myeloid cells than vehicle and $M_{(INF-\gamma+LPS)}$-treated animals (4, 17 df, p=0.0358). **(G)** No differences were observed for B Cells (4, 17 df, p=0.8721) and **(H)** $M_{(INF-\gamma+LPS)}$ and vehicle-treated mice had significantly lower frequency of T cells (4, 17 df, p=0.0011) than control mice. One-Way ANOVA was used to analyze statistical differences followed by the multiple comparison test Tukey. Data is presented as mean ± standard error (SEM). *- p< 0.05; **- p< 0.01; df= degrees of freedom, Control n= 5; Vehicle n=5; $M_{(INF-\gamma+LPS)}$ n=4; $M_{(IL-4+IL-13)}$ n=5; $M_{(IL-10+TGF-\beta1)}$ n=4. 1 independent experiment was performed.

$M_{(IL-10+TGF-\beta1)}$ secretome present molecules involve with anti-inflammatory, phagocytosis and tissue repair/ remodeling processes

In order to understand which proteins secreted by the different phenotypes of macrophages could be important for the differences observed both in vitro and in vivo, we identified and quantified the proteins produced by the macrophages using both the bead-based immunoassay LEGENDplex and liquid chromatography with mass spectrometry (LC-MS/MS). LC-MS/MS allows a broader and non-target analysis; however, it may not detect small and low-concentrated proteins, such as cytokines and chemokines. For this reason, we complemented LC-MS/MS analysis with the immunoassay LEGENDplex. The results demonstrated that pro-inflammatory cytokines such as TNF-α, G-CSF and IL12p40 were present almost only in the secretome of $M_{(INF-\gamma+LPS)}$ and were significantly different from the other groups (Supplementary Figure S4). These results were expected because these cytokines are characteristic of proinflammatory macrophages. Additionally, the cytokine/hormone G-CSF was also significantly elevated in the $M_{(INF-\gamma+LPS)}$ secretome (Supplementary Figure S4). In turn, TGF-β1, a cytokine with anti-inflammatory properties, was present in higher quantities in the $M_{(IL-10+TGF-\beta1)}$ and $M_{(IL-4+IL-13)}$ subsets; however, only in the pro-regenerative phenotype $M_{(IL-10+TGF-\beta1)}$ that this cytokine reached significant differences (Supplementary Figure S4).

The macrophage subsets that presented interesting results both in vivo and in the LegendPlex assay were the $M_{(IL-10+TGF-\beta1)}$ and $M_{(INF-\gamma+LPS)}$ phenotypes, and therefore, a detailed proteomics analysis was only performed in the secretome derived from these two populations. From a total of 452 proteins identified, we focused the analysis on those that presented higher concentrations (fold changes of 2 or higher) between the two groups. These proteins were grouped by function using the UniProt database, and the results revealed that 14 out of 17 pro-inflammatory proteins were overconcentrated in the $M_{(INF-\gamma+LPS)}$ secretome, and 3 out of 4 anti-inflammatory proteins were overconcentrated in the $M_{(IL-10+TGF-\beta1)}$ secretome (Figure 9A). Moreover, the $M_{(IL-10+TGF-\beta1)}$ secretome was also enriched in proteins involved in phagocytosis (9 out of 10) and in proteins involved in tissue repair/remodeling (7 out of 8) (Figure 9A). These results were expected because the $M_{(INF-\gamma+LPS)}$ and $M_{(IL-10+TGF-\beta1)}$ subsets are classified as pro-inflammatory and anti-inflammatory/repairing, respectively. Finally, protein–protein interaction network analysis was constructed using the online STRING database depicting both functional and physical protein associations and the results revealed that the secretome of $M_{(INF-\gamma+LPS)}$ contains proteins from just one cluster, which can be considered a cluster related to the inflammatory process, since these proteins are involved in antigen processing and presentation of peptide antigen, T cell-mediated cytotoxicity, and complement activation (Figure 9B). In contrast, the $M_{(IL-10+TGF-\beta1)}$ secretome contained proteins from three different clusters, a cluster of proteins

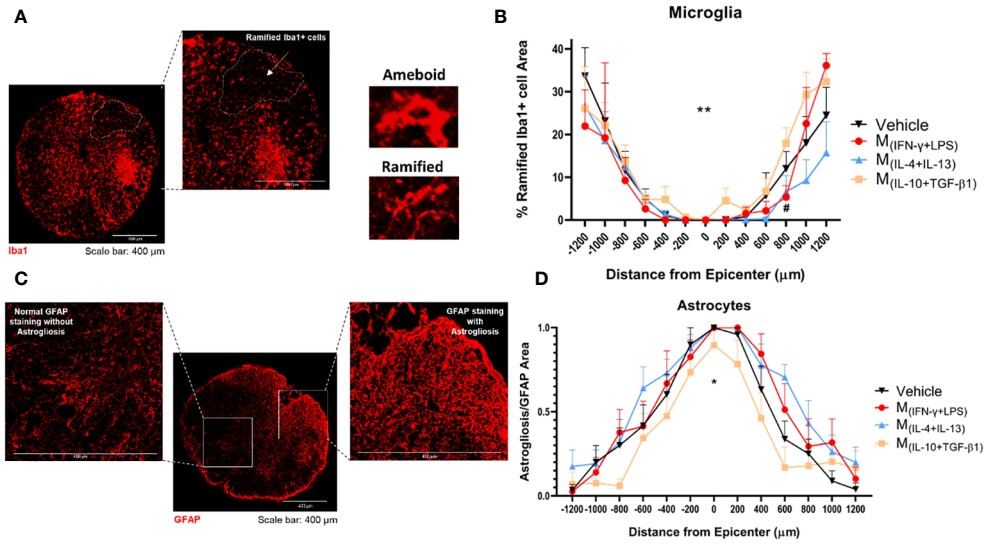


FIGURE 7
Histological analysis of the spinal cord 38 dpi. **(A)** Representative image of microglia from $M_{(IL-10+TGF-\beta1)}$-treated group, cells were stained using the antibody anti-IBA1 (red) and the area of the ramified microglia was analyzed. **(B)** Rostral-caudal analysis demonstrated that the animals treated with $M_{(IL-10+TGF-\beta1)}$-derived secretome presented overall significantly more ramified microglia than $M_{(IL-4+IL-13)}$ group (3, 239 df, p=0.0058) and presented more ramified microglia than the $M_{(INF-\gamma+LPS)}$ group at 800 µm caudal to the injury (3, 239 df, p=0.0469). **(C)** Representative image of astrocytes from vehicle-treated group, cells were stained with anti-GFAP antibody (red) and astrogliosis were analyzed by quantification of the area of clustered GFAP overstaining (areas impossible to distinguish individual astrocytes). **(D)** Rostral-caudal analysis demonstrated that mice treated with $M_{(IL-10+TGF-\beta1)}$-derived secretome had significantly less astrogliosis than the animals treated with $M_{(IL-4+IL-13)}$ or $M_{(INF-\gamma+LPS)}$ secretome (3, 231 df p<0.0001). Differences in both microglia and astrocytes analysis were detected using two-way ANOVA followed by Tukey's multiple comparisons test. A total of 284 spinal cord slices were observed to analyze astrogliosis and 301 slices to microglia. Data is presented as mean ± standard error (SEM). * or #- p< 0.05; **- p< 0.01; Vehicle n=7; $M_{(INF-\gamma+LPS)}$ n=6; $M_{(IL-4+IL-13)}$ n=8; $M_{(IL-10+TGF-\beta1)}$ n=6. 1 independent experiment was performed.

more related to metabolic processes (Cluster 1), with proteins that participate in Ganglioside and Glycosphingolipid catabolic processes (Figure 9B). Two other clusters were identified, with proteins that participate in relevant biological and cellular processes, such as astrocyte activation involved in immune response, regulation of dendritic spine maintenance, and regulation of response to wounding (Cluster 2), and proteins that play a role in axon extension and central nervous system neuron development (Cluster 3), some of which may be responsible for the improvements observed in vivo (Figure 9B).

Discussion

After injury, the immune system is fundamental for promoting adequate tissue repair and regeneration. However, it is well known that the immune response after SCI is dysfunctional and is an important contributor to the secondary damage observed after primary injury. Several therapeutic approaches have been designed to shut down the immune response after SCI; however, more important than shutting it down is to transform a dysfunctional response into a regenerative one. After SCI, splenic and bone marrow-derived monocytes infiltrate the lesion site and differentiate into macrophages (4). There is abundant literature exploring bone marrow-derived monocytes in an SCI context (10, 32–34), however, less is known about splenic monocytes. The spleen is not just important for erythrocyte recycling and immune response to pathogens. After injury, immune cells in the spleen become rapidly activated and mobilize to sites of damaged tissue. This activation and mobilization was first observed after myocardial ischemia and also demonstrated after SCI (5, 24). Splenic monocytes infiltrated the spinal cord in the acute phase of the injury, peaking at 7 days, whereas bone marrow-derived monocytes only infiltrated the cord 1 week after injury (4). Although the spleen has been characterized as the major source of pro-inflammatory monocytes after SCI (4), in ischemic brain injury models, splenic monocytes have been demonstrated to be key effector cells that modulate meningeal and parenchymal immune responses and limit ischemic injury, leading to improved functional outcomes (35). This indicates a complex interplay between the recruited splenic monocytes and the tissue microenvironment that finally determines the macrophage phenotype.

For these reasons, in this work, we aimed to study and further characterize splenic-derived macrophages in an SCI context, as this cell population may play a key role in tissue repair.

In this study, we used a protocol that led to a highly pure (97%) culture of primary splenic macrophages without the need to use cell sorting or magnetic beads separation kits. It is difficult to compare our purity with other protocols in the literature because the vast majority of studies do not disclaim this value (36–38) or use macrophage cell lines instead of primary cells (39). We demonstrated that splenic monocytes are similar to monocytes from other origins in terms of plasticity and are easily polarized into pro-inflammatory or pro-regenerative phenotypes. Moreover, we demonstrated that different splenic macrophage phenotypes have distinct effects on axonal growth and neuroprotection.

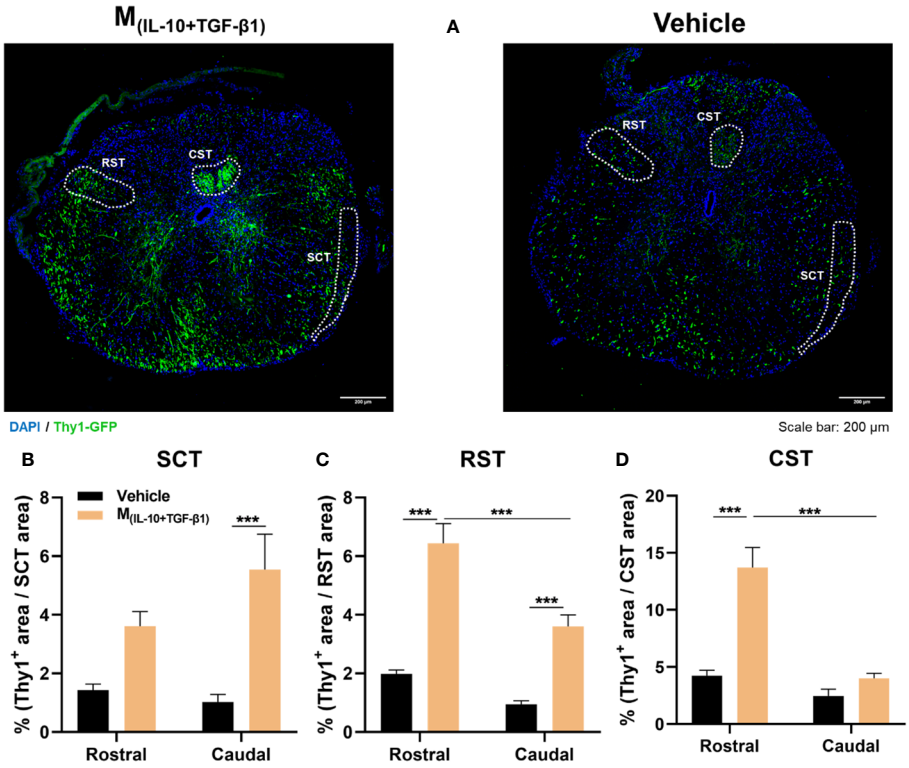


FIGURE 8
Histological analysis of ascending and descending spinal tracts 38 dpi. **(A)** Representative images of Thy1-GFP animals from $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ and Vehicle-treated group. The positive area for Thy1-GFP (green) was calculated and divided for the total area of the tract in each spinal section. The analysis encompassed a range spanning 600 μm to 2000 μm in both rostral and caudal directions from the epicenter. **(B)** The secretome derived from $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ macrophages significantly promoted higher neuronal preservation of spinocerebellar tract (SCT) in the caudal region (3, 65 df, p=0.0007) when compared with vehicle treatment. **(C)** Animals treated with $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ secretome also revealed a higher preservation of the rubrospinal tract (RST) both in the rostral (3, 64 df, p<0.0001) and in the caudal region (3, 64 df, p=0.0010). Moreover, the treatment effect was significantly higher in the rostral region than in the caudal (3, 64 df,<0.0001). **(D)** Likewise, the $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ secretome significantly preserved the corticospinal tract (CST) descending axons, namely rostrally from the epicenter (3, 74 df, p<0.0001), and this preservation was significantly higher in the rostral than in the caudal region (3, 74 df, p<0.0001). Two-way ANOVA followed by Tukey's multiple comparisons test was used to analyze statistical differences. A total of 163 spinal cord slices were analyzed. Data is presented as mean ± standard error (SEM). ***- p< 0.001. Vehicle n=3; $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ n=3. 1 independent experiment was performed.

Namely, classical activation (pro-inflammatory) has a detrimental impact, whereas alternative activation promotes axonal regeneration and neuroprotection. To the best of our knowledge, these biological effects were first described in our work for spleen-derived macrophages; however, these effects were also previously demonstrated in bone marrow-derived macrophages (10, 40). It is important to point out that the vast majority of the research in the literature only studied one type of alternative activation of macrophages (using IL-4); herein, we showed that activation with TGF-β1 and IL-10 has significantly superior biological effects than activation with IL-4 and IL-13, not only *in vitro* but also in an *in vivo* SCI model.

As previously mentioned, the microenvironment at the SCI site favors predominant and sustained macrophage polarization into a pro-inflammatory phenotype, which is detrimental to tissue repair (15). Some authors have investigated the therapeutic effect of transplanting alternatively activated macrophages into the damaged spinal cord to balance the ratio between pro- and anti-inflammatory macrophages at the injury site (12, 32). However, clinical trials have failed to demonstrate a significant therapeutic effect. Clinical results did not support the treatment of acute SCI with autologous incubated macrophage therapy (14). The reason behind this disappointing result may be that transplanted macrophages fail to retain their pro-regenerative phenotype when transplanted into the injured spinal cord (10). Kroner and colleagues demonstrated that intracellular accumulation of iron by macrophages induces a rapid switch from a pro-regenerative to a pro-inflammatory phenotype in the spinal cord tissue (15). Therefore, in this study, we decided to inject the soluble factors and extracellular vesicles produced by macrophages (secretome) instead of transplanting the cells. Herein, we explored whether systemic injections of secretomes derived from different macrophage phenotypes have a therapeutic effect after SCI. We tested the complete secretome rather than separating the soluble and vesicular fractions, because our previous evidence demonstrated that for SCI repair, the secretome as a whole is advantageous over the individual fractions (41). The local immune response after SCI is known to be dysfunctional; however, SCI also leads to the systemic dysregulation of the immune response. For instance, it was demonstrated that SCI

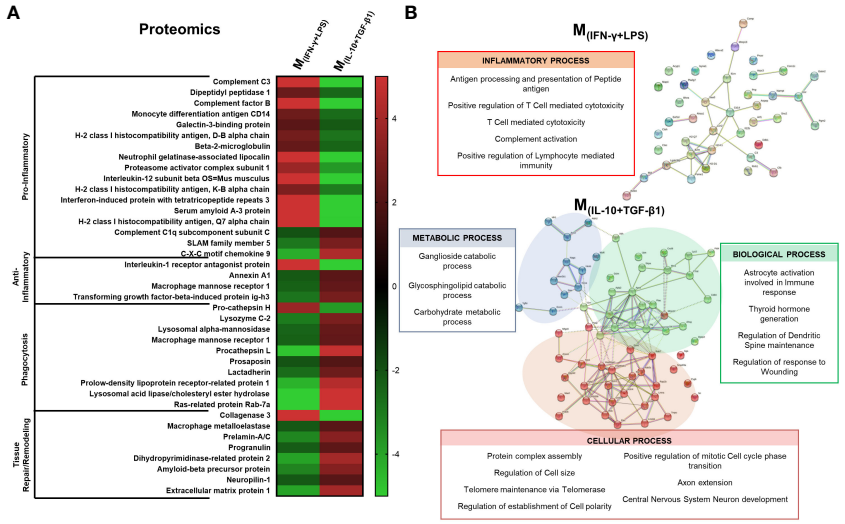


FIGURE 9
Proteomic analysis by LC-MS/MS focused on the proteins that presented higher concentration in the secretome. **(A)** 14 out of 17 pro-inflammatory proteins were overconcentrated in $M_{(INF-\gamma+LPS)}$-derived secretome; 3 out of 4 anti-inflammatory proteins were overconcentrated in $M_{(IL-10+TGF-\beta1)}$ secretome; $M_{(IL-10+TGF-\beta1)}$ secretome was enriched in proteins involved on phagocytosis (9 out of 10) and in proteins involved in tissue repair/remodeling (7 out of 8). **(B)** Cluster analysis using the STRING database revealed that the secretome of $M_{(INF-\gamma+LPS)}$ macrophages only presented proteins related to the inflammatory process (antigen processing and presentation of peptide antigen, T cell mediated cytotoxicity and complement activation); $M_{(IL-10+TGF-\beta1)}$ macrophages secreted proteins were classified into three main clusters: Cluster 1 - proteins related with metabolic process; Cluster 2 - proteins that participate in biological processes, such as, astrocyte activation, involved in immune response, regulation of dendritic spine maintenance and regulation of response to wounding; and Cluster 3 - proteins that play a role in cellular processes such as axon extension and central nervous system neuron development. Proteins were identified using the KEGG Orthology database. 1 independent experiment was performed.

could promote pro-inflammatory responses that damage peripheral organs (42, 43). Moreover, our group previously demonstrated that the infiltration of neutrophils into the injured spinal cord is affected by neural communication between the spinal cord and the spleen (24). The combined factors of local environment and systemic dysregulation of the immune response led us to choose the systemic administration of secretome instead of local administration or local transplantation of macrophages. In this way, we not only avoided losing the phenotype of the transplanted cells due to local environmental cues, but we are also able to modulate/prime immune cells even before they infiltrate the spinal cord. Notably, in our experimental animal model, the blood-spinal cord barrier (BSCB) is disrupted due to the mechanical compression, allowing the systemic-injected molecules to reach the spinal cord tissue. However, it is crucial to acknowledge that, even with this scenario, the majority of systemically delivered secretome is directed towards peripheral organs such as the liver, lungs, and spleen (44–46). Moreover, in some clinical scenarios, the BSCB may remain intact, in these situations intrathecal administration may be necessary.

In this study, we observed that the $M_{(IL-10+TGF-\beta1)}$-derived secretome is the most effective treatment in promoting functional recovery after compressive SCI. Additionally, factors and extracellular vesicles secreted by $M_{(IL-10+TGF-\beta1)}$ also supported the recovery of bladder function. Regain of bladder control is an important functional priority for persons living with SCI (30, 47). Interestingly, up to 3 weeks post-injury, treatment with the pro-inflammatory secretome, $M_{(INF-\gamma+LPS)}$, had a similar therapeutic effect to the $M_{(IL-10+TGF-\beta1)}$ secretome; however, the continued injection of molecules derived from the pro-inflammatory phenotype was shown to be detrimental in the long term. In line with this observation, previous research performed by Freria and colleagues demonstrated that preconditioning microglia with LPS injection before ischemic SCI elicits reactive spinal cord microglia and confers neuroprotection, leading to functional recovery (48). Indeed, a pro-inflammatory response seems to be necessary, at least in the acute phase or before injury; however, our results show that if this pro-inflammatory stimulus continues over time, the therapeutic effect ceases and becomes disadvantageous. We also observed that animals treated with the pro-inflammatory secretome tend to have more neuropathic pain. This data is in accordance with the current literature demonstrating that inflammation in the spinal cord leads to mechanical allodynia (49, 50). Microglia activation in the spinal cord is critical for developing pain hypersensitivity through the production of pro-inflammatory cytokines, chemokines and extracellular proteases (51). Activated microglia directly interacts with nociceptors and interneurons by modulating cell surface receptors and ion channels (52).

The identification and quantification of the molecules on the secretome were studied using flow cytometry, through the Legendplex immunoassay kit, and proteomic analysis using LC-MS/MS. Proteomics data were further examined using the STRING database, a web-based open resource that analyzes all known and predicted associations between proteins, including physical and functional interactions (53). Cluster analysis of the $M_{(INF-\gamma+LPS)}$-derived secretome revealed that only one class of proteins was functionally enriched. Namely, proteins associated with a pro-inflammatory response, such as molecules related to positive

regulation of T cells cytotoxicity and lymphocytes, mediate immunity, as well as complement activation molecules and proteins involved in antigen processing and presentation of peptide antigen. The immunoassay also revealed that the cytokines TNF-α, IL-12p40, and G-CSF were enriched in the $M_{(INF-\gamma+LPS)}$ secretome. On the other hand, analysis of the $M_{(IL-10+TGF-\beta1)}$-derived secretome showed that these macrophages secrete a wide variety of proteins structured in three main functional clusters: 1) proteins involved in phagocytosis; 2) proteins involved in tissue remodeling/response to wounding; and 3) proteins with anti-inflammatory properties. Moreover, STRING analysis identified clusters of proteins on the $M_{(IL-10+TGF-\beta1)}$ secretome involved in axon extension, dendritic spine maintenance, establishment of cell polarity, and regulation of astrocytic activation. Looking for individual proteins enriched in the $M_{(IL-10+TGF-\beta1)}$ secretome, it is possible to find some proteins with a known effect after SCI. For instance, it was demonstrated that Anexinn 1a administration decreased caspase-3 and IL-1β expression, reduced tissue damage, and protected axons of long descending pathways *in vivo* (54). In this context, the presence of Anexinn 1a within the secretome likely contributed to the preservation of long descending and ascending spinal tract. Our findings underscore the capacity of the $M_{(IL-10+TGF-\beta1)}$ secretome to significantly support the structural integrity of crucial neuronal tracts, including the ascending spinocerebellar tract (SCT) and the descending rubrospinal (RST) and corticospinal tracts (CST). Notably, these tracts assume pivotal roles in locomotion. For instance, the significance of SCT neurons in orchestrating the genesis and perpetuation of locomotor behavior in both neonatal and adult mice has been previously described (55). SCT neurons exhibit inherent rhythmogenic attributes and intricate circuit connectivity with spinal interneurons within the locomotor central pattern generator (55). Moreover, the indispensability of this neuronal pathway for motor function restoration in human individuals afflicted with spinal cord injuries has been well documented (56–58).

Likewise, the RST plays a multifaceted role in various components of dexterous motor functions. Disruptions within the RST give rise to deficits in intricate motor tasks such as reaching and grasping, as well as stepping movements (59). Evidently, the structural soundness of the RST is indispensable for limb coordination during activities encompassing food retrieval and ambulation. Equally pivotal, the contribution of CST neurons to voluntary movement has been extensively elucidated (60, 61), as has the paramount importance of this spinal tract in effecting motor recovery in SCI patients (62, 63). It is important to note that a higher degree of neuronal preservation was observed within regions that continue to receive afferent neuronal input. Consequently, the rostral portions of the descending tract demonstrate a superior level of neuronal preservation compared to their caudal counterparts due to the enduring reception of supraspinal information. In contrast, the ascending tract exhibits an inverse relationship, wherein higher preservation is evident in caudal regions due to the persistence of afferent input.

Progranulin is another protein enriched in the $M_{(IL-10+TGF-\beta1)}$ secretome, which may play a key role in repairing the injured spinal cord. Progranulin deficiency has been demonstrated to promote neuroinflammation and apoptosis and exacerbate damage (64). Moreover, progranulin protects lysosomal function and enhances the autophagic flux of microglia, allowing these cells to acquire an anti-inflammatory phenotype (65) and modulate the expression of GFAP, thereby decreasing the pro-inflammatory activation of astrocytes (66, 67). Indeed, previous studies have demonstrated that microglia respond rapidly to pathological stimuli, influencing then the fate of astrocytes (68, 69). Additional, using single-cell RNA sequencing, Brennan and colleagues revealed that microglia play a pivotal role in controlling stereotypical astrocyte-specific functions triggered by SCI, including upregulation of inflammatory genes, lipid processing, cell adhesion, and proliferation (70). Pro-inflammatory microglia release IL-1β, TNF-α, and complement component 1 subcomponent q (C1q), inducing the formation of inflammatory reactive astrocytes, commonly referred to as A1. Conversely, anti-inflammatory microglia promote the induction of pro-regenerative astrocytes, known as A2, thereby mitigating inflammation and exerting neuroprotective effects (68). Our histological analysis revealed that systemic injections of $M_{(IL-10+TGF-\beta1)}$ secretome resulted in fewer amoeboid microglia and reduced astrogliosis in the spinal cord tissue 5 weeks post-injury. The factors present in the secretome likely influenced the microglial phenotype, leading to decreased astrogliosis.

TGF-β1 is elevated in the $M_{(IL-10+TGF-\beta1)}$ secretome; however, its role after SCI is more controversial. Some studies have stated that TGF-β1 might have a detrimental role after SCI (71, 72), while others have shown that it may have a therapeutic role (73, 74). One study described TGF-β1 as an inducer and promoter of fibroblasts distribution and fibrotic scar formation (72). However, in this study we specifically analyzed the fibrotic scar and observed a significantly reduction of fibrosis on $M_{(IL-10+TGF-\beta1)}$-treated animals; therefore, the systemic administration or the presence of other molecules on the secretome seems to inhibit this effect of TGF-β1 on fibrosis. One possible explanation for this finding is that it may be an indirect effect mediated by the modified microglia, similar to the mechanism observed in astrogliosis. It was demonstrated that microglia activated with anti-inflammatory factors can attenuate neuroinflammation-induced scarring by rescuing the expression of Arf and Rho GAP adapter protein 3 (75). Additionally, transplantation of neonatal microglia and single-cell RNA sequencing studies have highlighted the crucial role of microglia in scar-free healing (76). It is also important to point out that PDGFR+ cells may play a multifaceted role after spinal cord injury, with conflicting findings reported in the literature. As a major pericyte marker, PDGFRβ has been associated with the proliferation of scar-forming cells (77). Studies suggest that inhibiting the proliferation of PDGFRβ+ pericytes reduces fibrotic scar formation by fibroblasts, thereby promoting axon regeneration and functional recovery following SCI (78). On the contrary, evidence also indicates a positive role for PDGFRβ+ pericytes in sealing the lesion core after SCI, aiding in injury containment and protecting neural tissue (77, 79). However, it was demonstrated that PDGFR+ cells that contribute to normal tissue healing and regeneration return to their physiological niche, and that their prolonged presence in the tissue resulted in tissue fibrosis and aberrant healing (80). Our analysis was performed 38 days after

injury, which may indicate that these cells are contributing to tissue fibrosis instead of tissue healing.

Finally, Dihydropyrimidinase-related protein 2, also known as Collapsin Response Mediator Protein-2 (CRMP2), is recognized for its affinity for tubulin heterodimers and functions in regulating the microtubule network, playing an important role in neuronal polarity establishment and axonal guidance (81). Several authors have identified CRMP2 as a crucial molecule for axonal regeneration (82, 83). The presence of this protein in the $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ secretome may be crucial for explaining the regeneration observed when using DRGs. *In vivo* CRMP2 was also identified as a contributor to the maintenance of spinal-cord regenerative ability (84), playing a key role in promoting axonal regeneration and leading to functional motor improvements (85). Recently, the function of CRMP2 was also described in human cells. The GADD45G/p38 MAPK/CDC25B signaling pathway promotes dephosphorylation of phosphorylated CRMP2 which in turn facilitates microtubule polymerization and leads to neurite outgrowth in human neurons (86).

Identifying the mechanism of action of our therapeutic approach is challenging; most likely, several proteins and extracellular vesicles have a distinct therapeutic action over time. Nonetheless, future experiments will focus on blocking some of the most promising candidates to understand whether the beneficial effects of the $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ secretome have one or several origins. In the first week after SCI, most of the monocytes circulating in the blood will be derived from the spleen reservoir (4), so in a putative clinical situation there is no need to obtain monocytes from the spleen of the person with SCI, a sample of blood will work. However, in future experiments, we will also have to test whether the $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ secretome obtained from monocytes isolated from blood has the same therapeutic action as those obtained directly from the spleen. Finally, in this study, we started the treatment 3 days after injury, which means that in a clinical scenario patients need to receive injections of the allogeneic-derived secretome. For autologous treatment, we will need to assess whether the $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ secretome maintains its therapeutic effect when administered at least 10 days post-injury.

Conclusions

In this study, we demonstrated that different splenic macrophage phenotypes secrete factors and extracellular vesicles with distinct therapeutic effects. We conclude that systemic injection of the $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ secretome is the most effective treatment in promoting functional motor recovery after compressive SCI. Additionally, the $M_{(IL\text{-}10+TGF\text{-}\beta1)}$ secretome supported the recovery of bladder function. Proteomic analysis showed that these macrophages secrete a wide variety of proteins involved in axon extension, dendritic spine maintenance, establishment of cell polarity, and regulation of astrocytic activation. The results presented herein are promising, and additional research is needed to optimize and characterize this therapy so that it can be translated to clinical use.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD048453.

Ethics statement

The animal study was approved by ethical Subcommittee in Life and Health Sciences (SECVS; ID:018/2019, University of Minho). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

JL-G: Formal analysis, Investigation, Writing – original draft. DS: Formal analysis, Investigation, Writing – review & editing. JA: Formal analysis, Investigation, Writing – review & editing. AM: Writing – review & editing. AP: Formal analysis, Investigation, Writing – review & editing. VM: Formal analysis, Investigation, Writing – review & editing. MD: Formal analysis, Investigation, Writing – review & editing. EG: Investigation, Writing – review & editing. RL: Investigation, Writing – review & editing. LF: Formal analysis, Investigation, Writing – review & editing. FF-A: Formal analysis, Investigation, Writing – review & editing. IP: Investigation, Writing – review & editing. NdS: Investigation, Writing – review & editing. JRC: Investigation, Writing – review & editing. AF: Investigation, Writing – review & editing. SS: Investigation, Writing – review & editing. LR: Investigation, Writing – review & editing. JC: Formal analysis, Investigation, Writing – review & editing. TP: Investigation, Writing – review & editing. SM: Investigation, Writing – review & editing. BM: Formal analysis, Writing – review & editing. AS: Funding acquisition, Writing – review & editing. RA: Formal analysis, Investigation, Writing – review & editing. NS: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work has been funded by National funds, through the Foundation for Science and Technology (FCT) - project UIDB/50026/2020 (DOI 10.54499/UIDB/50026/2020), UIDP/50026/2020 (DOI 10.54499/UIDP/50026/2020), LA/P/0050/2020 (DOI 10.54499/LA/P/0050/2020) and EXPL/MED-PAT/0931/2021. Financial support was also provided by Prémios Santa Casa Neurociências–Prize Melo e

Castro for Spinal Cord Injury Research (MC-18-2021) and by Wings For Life Spinal Cord Research Foundation (WFL-PT-14/23). Imaging acquisition was performed in the LiM facilities of iBiMED and at the ICVS Scientific Microscopy Platform, both are members of the national infrastructure PPBI - Portuguese Platform of Bioimaging (PPBI-POCI-01-0145-FEDER-022122).

Acknowledgments

We would also like to acknowledge the FCT for the Scientific Employment Stimulus to NAS and SM (CEECIND/04794/2017 and CEECIND/01902/2017) and for grants to JLG (PD/BD/127820/2016), MSD(SFRH/BD/141092/2018). José Graça would like to acknowledge the PhDOC – Inter-University Doctoral Program in Ageing and Chronic Disease.

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.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2024.1354479/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Splenic macrophages characterization. **(A)** MCS-F is essential for the survival and proliferation and differentiation of splenic monocytes into macrophages. **(B)** After 24h of polarization with the pro-inflammatory molecules LPS and INF-γ, 89% of the macrophages expressed iNOS. **(C)** A total of 487 proteins identified, 81 were exclusive to the secretome of $M_{(INF-\gamma+LPS)}$ macrophages, 35 to $M_{(IL-4+IL-13)}$, and 90 to $M_{(IL-10+TGF-\beta1)}$. **(D)** Using the PANTHER tool was possible to identified metabolite interconversion enzymes (dark blue) as a common protein class between the different macrophage populations, but as can be observed by the pie charts, the protein class and the percentage of proteins in different classes varied considerably among each cell phenotype. Anti-CD11b antibody was used to identify macrophages (green), anti-iNOS antibody was used to confirm the polarization (red) and nuclei was counterstained with DAPI (blue). Scale bar =50 µm. 2 independent experiments were performed for the *in vitro* data and 1 independent experiment for proteomics analysis.

SUPPLEMENTARY FIGURE 2
Classical ($M_{(INF-\gamma+LPS)}$) or alternative ($M_{(IL-4+IL-13)}$; $M_{(IL-10+TGF-\beta1)}$) activated macrophages co-cultured with dorsal root ganglia (DRGs) in 2D. DRGs stained with Neurofilament (green), Macrophages and DRGs stained with Phalloidin (red) and nuclei counterstained with DAPI (blue). **(A)** DRGs co-cultured with $M_{(IL-10+TGF-\beta1)}$ macrophages had significantly higher axonal arborization (3, 17 df, p<0.0001) and **(B)** significantly longer axons (p=0.0247) than the than $M_{(IL-4+IL-13)}$. **(C)** $M_{(IL-10+TGF-\beta1)}$ also had significant more axonal area (0.0240) than the $M_{(IL-4+IL-13)}$. Two way ANOVA followed by Tukey *post-hoc* test was used for axonal arborization analysis and Kruskal-Wallis test followed by Dunn's multiple comparisons test was used for longer distance and axonal area analysis. Data is presented as mean ± standard error (SEM). *- p< 0.05; ***- p< 0.001. Scale bar =200 µm; $M_{(INF-\gamma+LPS)}$ n=5; $M_{(IL-4+IL-13)}$ n=4; $M_{(IL-10+TGF-\beta1)}$ n=5. 2 independent experiments were performed.

SUPPLEMENTARY FIGURE 3
Histological analysis of the spinal cord. **(A)** Representative image of gray matter neurons from $M_{(IL-10+TGF-\beta1)}$-treated group, cell bodies were measured by counting the number of positive NeuN cells (red) in laminae VIII and IX of both ventral horns. **(B)** Rostral-caudal analysis demonstrated that the secretome derived from $M_{(IL-10+TGF-\beta1)}$ cells significantly promoted neuronal survival at the ventral horns (3, 253 df, p= 0.0438) when compared with $M_{(IFN-\gamma+LPS)}$. **(C)** Representative image of fibrotic scar from vehicle-treated group, anti-PDGFRβ antibody (red) was used to analyze fibrosis in the spinal cord. **(D)** Although there are not significant differences in PDGFRβ+ total area between treated groups, **(E)** rostral caudal analysis show that mice treated with $M_{(IL-10+TGF-\beta1)}$-derived secretome had significantly less fibrosis caudally to the injury area (3, 49 df, p= 0.0370). ANOVA followed by the Tukey *post-hoc* test was used to analyze statistical differences. A total of 312 spinal cord slices were observed to analyze neuronal survival and 134 slices (53 for the caudal calculation) for fibrosis. Data is presented as mean ± standard error (SEM). *- p< 0.05. Vehicle n=7; $M_{(INF-\gamma+LPS)}$ n=6; $M_{(IL-4+IL-13)}$ n=8; $M_{(IL-10+TGF-\beta1)}$ n=6. 1 independent experiment was performed.

SUPPLEMENTARY FIGURE 4
LEGENDplex immunoassay. The pro-inflammatory cytokines TNF-α, G-CSF and IL12p40 were significantly concentrated on the secretome of $M_{(INF-\gamma+LPS)}$. The cytokine/hormone G-CSF was also significantly concentrated on the $M_{(INF-\gamma+LPS)}$-derived secretome. TGF-β1, a cytokine with anti-inflammatory properties was significantly concentrated in the $M_{(IL-10+TGF-\beta1)}$-derived secretome. Data was analyzed using the two-way ANOVA (2, 130 df, p<0.0001) followed by the Tukey's multiple comparisons test. Data is presented as mean ± standard error (SEM). *- p< 0.05; ***- p< 0.001, $M_{(IFN-\gamma+LPS)}$ n=4; $M_{(IL-4+IL-13)}$ n=6; $M_{(IL-10+TGF-\beta1)}$ n=6. Concentration values plotted in the graph were divided by 10 to account for the concentration step performed before the analysis. The values for CXCL-1, IL-12p70, and IL-10 were below the limit of detection and were consequently excluded from the analysis. 1 independent experiment was performed.

SUPPLEMENTARY FIGURE 5
Gating strategy used for flow cytometry analysis of mice blood cells. Doublets were excluded by FSC-A *vs* FSC-H scatter. Blood total cells were gated by SSC-A *vs* FSC-A scatter. Leukocytes were gated by CD45+ cells and on this population lymphocytes and myeloid cells were distinguished by CD11b expression. In lymphocytes population, CD3+CD19- cells were defined as T cells and CD3-CD19+ cells were defined as B cells. In myeloid cell population, Ly6G *vs* Ly6C allowed the selection of neutrophils (Ly6G+Ly6C+) and monocytes (Ly6G-Ly6C+). The selection of eosinophils *vs* monocytes Ly6C intermediate *vs* monocytes Ly6C high was made based on Ly6C *vs* SSC-A gating.

SUPPLEMENTARY FIGURE 6
Negative control fluorescence images of the Alexa Fluor 594 goat anti-rabbit antibody (red) both at the injury site and 800µm from the injury site. DAPI (blue) was used as structural marker.

References

1. Silva NA, Sousa N, Reis RL, Salgado AJ. From basics to clinical: a comprehensive review on spinal cord injury. *Prog Neurobiol* (2014) 114:25–57. doi: 10.1016/j.pneurobio.2013.11.002

2. Silva NA, Sousa RA, Pires AO, Sousa N, Salgado AJ, Reis RL. Interactions between Schwann and olfactory ensheathing cells with a starch/polycaprolactone scaffold aimed at spinal cord injury repair. *J BioMed Mater Res A* (2012) 100:470–6. doi: 10.1002/jbm.a.33289

3. Milich LM, Ryan CB, Lee JK. The origin, fate, and contribution of macrophages to spinal cord injury pathology. *Acta neuropathologica* (2019) 137:785–97. doi: 10.1007/s00401-019-01992-3

4. Blomster LV, Brennan FH, Lao HW, Harle DW, Harvey AR, Ruitenberg MJ. Mobilisation of the splenic monocyte reservoir and peripheral CX(3)CR1 deficiency adversely affects recovery from spinal cord injury. *Exp Neurol* (2013) 247:226–40. doi: 10.1016/j.expneurol.2013.05.002

5. Swirski FK, Nahrendorf M, Etzrodt M, Wildgruber M, Cortez-Retamozo V, Panizzi P, et al. Identification of splenic reservoir monocytes and their deployment to inflammatory sites. *Science* (2009) 325:612–6. doi: 10.1126/science.1175202

6. Gensel JC, Zhang B. Macrophage activation and its role in repair and pathology after spinal cord injury. *Brain Res* (2015) 1619:1–11. doi: 10.1016/j.brainres.2014.12.045

7. Galli SJ, Borregaard N, Wynn TA. Phenotypic and functional plasticity of cells of innate immunity: macrophages, mast cells and neutrophils. *Nat Immunol* (2011) 12:1035–44. doi: 10.1038/ni.2109

8. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol* (2008) 8:958–69. doi: 10.1038/nri2448

9. Shechter R, Schwartz M. CNS sterile injury: just another wound healing? *Trends Mol Med* (2013) 19:135–43. doi: 10.1016/j.molmed.2012.11.007

10. Kigerl KA, Gensel JC, Ankeny DP, Alexander JK, Donnelly DJ, Popovich PG. Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord. *J Neurosci* (2009) 29:13435–44. doi: 10.1523/JNEUROSCI.3257-09.2009

11. Monteiro S, Salgado AJ, Silva NA. Immunomodulation as a neuroprotective strategy after spinal cord injury. *Neural Regener Res* (2018) 13:423–4. doi: 10.4103/1673-5374.228722

12. Schwartz M, Lazarov-Spiegler O, Rapalino O, Agranov I, Velan G, Hadani M. Potential repair of rat spinal cord injuries using stimulated homologous macrophages. *Neurosurgery* (1999) 44:1041–5. doi: 10.1097/00006123-199905000-00057

13. Schwartz M, Yoles E. Macrophages and dendritic cells treatment of spinal cord injury: from the bench to the clinic. *Acta Neurochir Suppl* (2005) 93:147–50. doi: 10.1007/3-211-27577-0_25

14. Lammertse DP, Jones LA, Charlifue SB, Kirshblum SC, Apple DF, Ragnarsson KT, et al. Autologous incubated macrophage therapy in acute, complete spinal cord injury: results of the phase 2 randomized controlled multicenter trial. *Spinal Cord* (2012) 50:661–71. doi: 10.1038/sc.2012.39

15. Kroner A, Greenhalgh AD, Zarruk JG, Passos dos Santos R, Gaestel M, David S. TNF and increased intracellular iron alter macrophage polarization to a detrimental M1 phenotype in the injured spinal cord. *Neuron* (2014) 83:1098–116. doi: 10.1016/j.neuron.2014.07.027

16. Pinho AG, Cibrão JR, Silva NA, Monteiro S, Salgado AJ. Cell secretome: basic insights and therapeutic opportunities for CNS disorders. *Pharm (Basel)* (2020) 13(2):31. doi: 10.3390/ph13020031

17. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* (2001) 25:402–8. doi: 10.1006/meth.2001.1262

18. Gomes ED, Mendes SS, Leite-Almeida H, Gimble JM, Tam RY, Shoichet MS, et al. Combination of a peptide-modified gellan gum hydrogel with cell therapy in a lumbar spinal cord injury animal model. *Biomaterials* (2016) 105:38–51. doi: 10.1016/j.biomaterials.2016.07.019

19. Gomes ED, Mendes SS, Assuncao-Silva RC, Teixeira FG, Pires AO, Anjo SI, et al. Co-transplantation of adipose tissue-derived stromal cells and olfactory ensheathing cells for spinal cord injury repair. *Stem Cells* (2018) 36:696–708. doi: 10.1002/stem.2785

20. Pinto MJ, Pedro JR, Costa RO, Almeida RD. Visualizing K48 ubiquitination during presynaptic formation by ubiquitination-induced fluorescence complementation (UiFC). *Front Mol Neurosci* (2016) 9:43. doi: 10.3389/fnmol.2016.00043

21. Pinto MJ, Alves PL, Martins L, Pedro JR, Ryu HR, Jeon NL, et al. The proteasome controls presynaptic differentiation through modulation of an on-site pool of polyubiquitinated conjugates. *J Cell Biol* (2016) 212:789–801. doi: 10.1083/jcb.201509039

22. Rocha LA, Gomes ED, Afonso JL, Granja S, Baltazar F, Silva NA, et al. *In vitro* evaluation of ASCs and HUVECs co-cultures in 3D biodegradable hydrogels on neurite outgrowth and vascular organization. *Front Cell Dev Biol* (2020) 8:489. doi: 10.3389/fcell.2020.00489

23. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *BMC veterinary Res* (2020) 16:242. doi: 10.1186/s12917-020-02451-y

24. Monteiro S, Pinho AG, Macieira M, Serre-Miranda C, Cibrao JR, Lima R, et al. Splenic sympathetic signaling contributes to acute neutrophil infiltration of the injured spinal cord. *J Neuroinflamm* (2020) 17:282. doi: 10.1186/s12974-020-01945-8

25. Basso DM, Fisher LC, Anderson AJ, Jakeman LB, McTigue DM, Popovich PG. Basso Mouse Scale for locomotion detects differences in recovery after spinal cord injury in five common mouse strains. *J Neurotrauma* (2006) 23:635–59. doi: 10.1089/neu.2006.23.635

26. de Sousa N, Pinho AG, Monteiro S, Liberato V, Santos DJ, Campos J, et al. Acute Baclofen administration promotes functional recovery after spinal cord injury. *Spine J* (2022) 23(3):379–91. doi: 10.1016/j.spinee.2022.09.007

27. Watson C, Paxinos G, Kayalioglu G, Heise C. Chapter 16 - atlas of the mouse spinal cord. In: Watson C, Paxinos G, Kayalioglu G, editors. *The Spinal Cord*. Academic Press, San Diego (2009). p. 308–79.

28. Perez-Riverol Y, Bai J, Bandla C, García-Seisdedos D, Hewapathirana S, Kamatchinathan S, et al. The PRIDE database resources in 2022: a hub for mass spectrometry-based proteomics evidences. *Nucleic Acids Res* (2022) 50:D543–d552. doi: 10.1093/nar/gkab1038

29. Lu J, Cao Q, Zheng D, Sun Y, Wang C, Yu X, et al. Discrete functions of M2a and M2c macrophage subsets determine their relative efficacy in treating chronic kidney disease. *Kidney Int* (2013) 84:745–55. doi: 10.1038/ki.2013.135

30. Lo C, Tran Y, Anderson K, Craig A, Middleton J. Functional priorities in persons with spinal cord injury: using discrete choice experiments to determine preferences. *J Neurotrauma* (2016) 33:1958–68. doi: 10.1089/neu.2016.4423

31. Kalinski AL, Yoon C, Huffman LD, Duncker PC, Kohen R, Passino R, et al. Analysis of the immune response to sciatic nerve injury identifies efferocytosis as a key mechanism of nerve debridement. *eLife* (2020) 9:e60223. doi: 10.7554/eLife.60223

32. Arai K, Harada Y, Tomiyama H, Michishita M, Kanno N, Yogo T, et al. Evaluation of the survival of bone marrow-derived mononuclear cells and the growth factors produced upon intramedullary transplantation in rat models of acute spinal cord injury. *Res Vet Sci* (2016) 107:88–94. doi: 10.1016/j.rvsc.2016.05.011

33. Kim SJ, Ko WK, Jo MJ, Arai Y, Choi H, Kumar H, et al. Anti-inflammatory effect of Tauroursodeoxycholic acid in RAW 264.7 macrophages, Bone marrow-derived macrophages, BV2 microglial cells, and spinal cord injury. *Sci Rep* (2018) 8:3176. doi: 10.1038/s41598-018-21621-5

34. Norden DM, Faw TD, McKim DB, Deibert RJ, Fisher LC, Sheridan JF , et al. Bone marrow-derived monocytes drive the inflammatory microenvironment in local and remote regions after thoracic spinal cord injury. *J Neurotrauma* (2019) 36:937–49. doi: 10.1089/neu.2018.5806

35. Garcia-Bonilla L, Brea D, Benakis C, Lane DA, Murphy M, Moore J, et al. Endogenous protection from ischemic brain injury by preconditioned monocytes. *J Neurosci* (2018) 38:6722–36. doi: 10.1523/JNEUROSCI.0324-18.2018

36. Bauer C, le Saux O, Pomozi V, Aherrahrou R, Kriesen R, Stolting S, et al. Etidronate prevents dystrophic cardiac calcification by inhibiting macrophage aggregation. *Sci Rep* (2018) 8:5812. doi: 10.1038/s41598-018-24228-y

37. Chao H-H, Li L, Gao X, Wang C, Yue W. CXCL12 expression in aborted mouse uteri induced by IFN-γ: Potential anti-inflammatory effect involves in endometrial restoration after abortion in mice. *Gene* (2019) 700:38–46. doi: 10.1016/j.gene.2019.02.089

38. Yu ZY, Chen DW, Tan CR, Zeng GH, He CY, Wang J, et al. Physiological clearance of Abeta by spleen and splenectomy aggravates Alzheimer-type pathogenesis. *Aging Cell* (2022) 21:e13533. doi: 10.1111/acel.13533

39. Li Y, Li X, Chen X, Sun X, Liu X, Wang G, et al. Qishen Granule (QSG) Inhibits Monocytes Released From the Spleen and Protect Myocardial Function via the TLR4-MyD88-NF-kappaB p65 Pathway in Heart Failure Mice. *Front Pharmacol* (2022) 13:850187. doi: 10.3389/fphar.2022.850187

40. Zhang K, Lu WC, Zhang M, Zhang Q, Xian PP, Liu FF, et al. Reducing host aldose reductase activity promotes neuronal differentiation of transplanted neural stem cells at spinal cord injury sites and facilitates locomotion recovery. *Neural Regener Res* (2022) 17:1814–20. doi: 10.4103/1673-5374.330624

41. Pinho AG, Cibrao JR, Lima R, Gomes ED, Serra SC, Lentilhas-Graca J, et al. Immunomodulatory and regenerative effects of the full and fractioned adipose tissue derived stem cells secretome in spinal cord injury. *Exp Neurol* (2022) 351:113989. doi: 10.1016/j.expneurol.2022.113989

42. Bao F, Bailey CS, Gurr KR, Bailey SI, Rosas-Arellano MP, Dekaban GA, et al. Increased oxidative activity in human blood neutrophils and monocytes after spinal cord injury. *Exp Neurol* (2009) 215:308–16. doi: 10.1016/j.expneurol.2008.10.022

43. Gris D, Hamilton EF, Weaver LC. The systemic inflammatory response after spinal cord injury damages lungs and kidneys. *Exp Neurol* (2008) 211:259–70. doi: 10.1016/j.expneurol.2008.01.033

44. Aimaletdinov AM, Gomzikova MO. Tracking of extracellular vesicles' Biodistribution: new methods and approaches. *Int J Mol Sci* (2022) 23(19):11312. doi: 10.3390/ijms231911312

45. Driedonks T, Jiang L, Carlson B, Han Z, Liu G, Queen SE, et al. Pharmacokinetics and biodistribution of extracellular vesicles administered intravenously and intranasally to Macaca nemestrina. *J extracellular Biol* (2022) 1 (10):e59. doi: 10.1002/jex2.59

46. Wiklander OPB, Nordin JZ, O'Loughlin A, Gustafsson Y, Corso G, Mäger I, et al. Extracellular vesicle in vivo biodistribution is determined by cell source, route of administration and targeting. (2015) 4:26316. doi: 10.3402/jev.v4.26316

47. Anderson KD. Targeting recovery: priorities of the spinal cord-injured population. *J Neurotrauma* (2004) 21:1371–83. doi: 10.1089/neu.2004.21.1371

48. Freria CM, Brennan FH, Sweet DR, Guan Z, Hall JC, Kigerl KA, et al. Serial systemic injections of endotoxin (LPS) elicit neuroprotective spinal cord microglia through IL-1-dependent cross talk with endothelial cells. *J Neurosci* (2020) 40:9103. doi: 10.1523/JNEUROSCI.0131-20.2020

49. Narita M, Kuzumaki N, Narita M, Kaneko C, Hareyama N, Miyatake M, et al. Chronic pain-induced emotional dysfunction is associated with astrogliosis due to cortical δ-opioid receptor dysfunction. (2006) 97:1369–78. doi: 10.1111/j.1471-4159.2006.03824.x

50. Hains BC, Saab CY, Klein JP, Craner MJ, Waxman SG. Altered sodium channel expression in second-order spinal sensory neurons contributes to pain after peripheral nerve injury. (2004) 24:4832–9. doi: 10.1523/JNEUROSCI.0300-04.2004%

51. Tansley S, Uttam S, Ureña Guzmán A, Yaqubi M, Pacis A, Parisien M, et al. Single-cell RNA sequencing reveals time- and sex-specific responses of mouse spinal cord microglia to peripheral nerve injury and links ApoE to chronic pain. *Nat Commun* (2022) 13:843. doi: 10.1038/s41467-022-28473-8

52. Schomberg D, Olson JK. Immune responses of microglia in the spinal cord: Contribution to pain states. *Exp Neurol* (2012) 234:262–70. doi: 10.1016/j.expneurol.2011.12.021

53. Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, et al. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res* (2021) 49: D605–12. doi: 10.1093/nar/gkaa1074

54. Liu NK, Zhang YP, Han S, Pei J, Xu LY, Lu PH, et al. Annexin A1 reduces inflammatory reaction and tissue damage through inhibition of phospholipase A2 activation in adult rats following spinal cord injury. *J neuropathology Exp Neurol* (2007) 66:932–43. doi: 10.1097/nen.0b013e3181567d59

55. Chalif JI, Martínez-Silva ML, Pagiazitis JG, Murray AJ, Mentis GZ. Control of mammalian locomotion by ventral spinocerebellar tract neurons. *Cell* (2022) 185:328–344.e326. doi: 10.1016/j.cell.2021.12.014

56. Lei Y, Perez MA. Cerebellar contribution to sensorimotor adaptation deficits in humans with spinal cord injury. *Sci Rep* (2021) 11:2507. doi: 10.1038/s41598-020-77543-8

57. Formento E, Minassian K, Wagner F, Mignardot JB, Le Goff-Mignardot CG, Rowald A, et al. Electrical spinal cord stimulation must preserve proprioception to enable locomotion in humans with spinal cord injury. *Nat Neurosci* (2018) 21:1728–41. doi: 10.1038/s41593-018-0262-6

58. Matson KJE, Russ DE, Kathe C, Hua I, Maric D, Ding Y, et al. Single cell atlas of spinal cord injury in mice reveals a pro-regenerative signature in spinocerebellar neurons. *Nat Commun* (2022) 13:5628. doi: 10.1038/s41467-022-33184-1

59. Morris R, Whishaw IQ. A proposal for a rat model of spinal cord injury featuring the rubrospinal tract and its contributions to locomotion and skilled hand movement. *Front Neurosci* (2016) 10:5. doi: 10.3389/fnins.2016.00005

60. van den Brand R, Heutschi J, Barraud Q, DiGiovanna J, Bartholdi K, Huerlimann M, et al. Restoring voluntary control of locomotion after paralyzing spinal cord injury. *Science* (2012) 336:1182–5. doi: 10.1126/science.1217416

61. Borton D, Bonizzato M, Beauparlant J, DiGiovanna J, Moraud EM, Wenger N, et al. Corticospinal neuroprostheses to restore locomotion after spinal cord injury. *Neurosci Res* (2014) 78:21–9. doi: 10.1016/j.neures.2013.10.001

62. Jo HJ, Richardson MSA, Oudega M, Perez MA. Paired corticospinal-motoneuronal stimulation and exercise after spinal cord injury. *J spinal cord Med* (2021) 44:S23–s27. doi: 10.1080/10790268.2021.1970908

63. Jo HJ, Perez MA. Corticospinal-motor neuronal plasticity promotes exercise-mediated recovery in humans with spinal cord injury. *Brain* (2020) 143:1368–82. doi: 10.1093/brain/awaa052

64. Wang C, Zhang L, Ndong JC, Hettinghouse A, Sun G, Chen C, et al. Progranulin deficiency exacerbates spinal cord injury by promoting neuroinflammation and cell apoptosis in mice. *J Neuroinflamm* (2019) 16:238. doi: 10.1186/s12974-019-1630-1

65. Shi Q, Wu Y, Zhang B, Wu S, Wang X, Lin F, et al. Progranulin promotes functional recovery in rats with acute spinal cord injury via autophagy-induced anti-inflammatory microglial polarization. *Mol Neurobiol* (2022) 59:4304–14. doi: 10.1007/s12035-022-02836-0

66. Kuse Y, Tsuruma K, Mizoguchi T, Shimazawa M, Hara H. Progranulin deficiency causes the retinal ganglion cell loss during development. *Sci Rep* (2017) 7:1679. doi: 10.1038/s41598-017-01933-8

67. Menzel L, Kleber L, Friedrich C, Hummel R, Dangel L, Winter J, et al. Progranulin protects against exaggerated axonal injury and astrogliosis following traumatic brain injury. *Glia* (2017) 65:278–92. doi: 10.1002/glia.23091

68. Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, et al. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature* (2017) 541:481–7. doi: 10.1038/nature21029

69. Jha MK, Jo M, Kim J-H, Suk K. Microglia-astrocyte crosstalk: an intimate molecular conversation. (2019) 25:227–40. doi: 10.1177/1073858418783959

70. Brennan FH, Li Y, Wang C, Ma A, Guo Q, Li Y, et al. Microglia coordinate cellular interactions during spinal cord repair in mice. *Nat Commun* (2022) 13:4096. doi: 10.1038/s41467-022-31797-0

71. Lv C, Zhang T, Li K, Gao K. Bone marrow mesenchymal stem cells improve spinal function of spinal cord injury in rats via TGF-beta/Smads signaling pathway. *Exp Ther Med* (2020) 19:3657–63. doi: 10.3892/etm.2020.8640

72. Pan D, Yang F, Zhu S, Li Y, Ning G, Feng S. Inhibition of TGF-beta repairs spinal cord injury by attenuating EphrinB2 expressing through inducing miR-484 from fibroblast. *Cell Death Discovery* (2021) 7:319. doi: 10.1038/s41420-021-00705-8

73. Nakazaki M, Morita T, Lankford KL, Askenase PW, Kocsis JD. Small extracellular vesicles released by infused mesenchymal stromal cells target M2 macrophages and promote TGF-beta upregulation, microvascular stabilization and functional recovery in a rodent model of severe spinal cord injury. *J Extracell Vesicles* (2021) 10:e12137. doi: 10.1002/jev2.12137

74. Tyor WR, Avgeropoulos N, Ohlandt G, Hogan EL. Treatment of spinal cord impact injury in the rat with transforming growth factor-beta. *J Neurol Sci* (2002) 200:33–41. doi: 10.1016/s0022-510x(02)00113-2

75. Xu Y, He X, Wang Y, Jian J, Peng X, Zhou L, et al. 5-Fluorouracil reduces the fibrotic scar via inhibiting matrix metalloproteinase 9 and stabilizing microtubules after spinal cord injury. *CNS Neurosci Ther* (2022) 28:2011–23. doi: 10.1111/cns.13930

76. Li Y, He X, Kawaguchi R, Zhang Y, Wang Q, Monavarfeshani A, et al. Microglia-organized scar-free spinal cord repair in neonatal mice. *Nature* (2020) 587:613–8. doi: 10.1038/s41586-020-2795-6

77. Göritz C, Dias DO, Tomilin N, Barbacid M, Shupliakov O, Frisén J. A pericyte origin of spinal cord scar tissue. *Science* (2011) 333:238–42. doi: 10.1126/science.1203165

78. Dias DO, Kim H, Holl D, Werne Solnestam B, Lundeberg J, Carlén M, et al. Reducing pericyte-derived scarring promotes recovery after spinal cord injury. *Cell* (2018) 173:153–165.e122. doi: 10.1016/j.cell.2018.02.004

79. Narang A, Zheng B. To scar or not to scar. *Trends Mol Med* (2018) 24:522–4. doi: 10.1016/j.molmed.2018.04.007

80. Santini MP, Malide D, Hoffman G, Pandey G, D'Escamard V, Nomura-Kitabayashi A, et al. Tissue-resident PDGFRα(+) progenitor cells contribute to fibrosis versus healing in a context- and spatiotemporally dependent manner. *Cell Rep* (2020) 30:555–570.e557. doi: 10.1016/j.celrep.2019.12.045

81. Moutal A, White KA, Chefdeville A, Laufmann RN, Vitiello PF, Feinstein D, et al. Dysregulation of CRMP2 post-translational modifications drive its pathological functions. *Mol Neurobiol* (2019) 56:6736–55. doi: 10.1007/s12035-019-1568-4

82. Kondo S, Takahashi K, Kinoshita Y, Nagai J, Wakatsuki S, Araki T, et al. Genetic inhibition of CRMP2 phosphorylation at serine 522 promotes axonal regeneration after optic nerve injury. *Sci Rep* (2019) 9:7188. doi: 10.1038/s41598-019-43658-w

83. Liz MA, Mar FM, Santos TE, Pimentel HI, Marques AM, Morgado MM, et al. Neuronal deletion of GSK3beta increases microtubule speed in the growth cone and enhances axon regeneration via CRMP-2 and independently of MAP1B and CLASP2. *BMC Biol* (2014) 12:47. doi: 10.1186/1741-7007-12-47

84. Gogel S, Lange S, Leung KY, Greene ND, Ferretti P. Post-translational regulation of Crmp in developing and regenerating chick spinal cord. *Dev Neurobiol* (2010) 70:456–71. doi: 10.1002/dneu.20789

85. Tsuchida M, Nakamachi T, Sugiyama K, Tsuchikawa D, Watanabe J, Hori M, et al. PACAP stimulates functional recovery after spinal cord injury through axonal regeneration. *J Mol neuroscience: MN* (2014) 54:380–7. doi: 10.1007/s12031-014-0338-z

86. Kase Y, Sato T, Okano Y, Okano H. The GADD45G/p38 MAPK/CDC25B signaling pathway enhances neurite outgrowth by promoting microtubule polymerization. *iScience* (2022) 25:104089. doi: 10.1016/j.isci.2022.104089

frontiers | Frontiers in Immunology

TYPE Review
PUBLISHED 14 March 2024
DOI 10.3389/fimmu.2024.1374796

OPEN ACCESS

EDITED BY
Chun Wai Mai,
UCSI University, Malaysia

REVIEWED BY
Giorgio Mangino,
Sapienza University of Rome, Italy
Sachiko Akashi-Takamura,
Aichi Medical University, Japan

*CORRESPONDENCE
DeLisa Fairweather
Fairweather.DeLisa@mayo.edu

RECEIVED 22 January 2024
ACCEPTED 28 February 2024
PUBLISHED 14 March 2024

CITATION
Di Florio DN, Beetler DJ, McCabe EJ, Sin J, Ikezu T and Fairweather D (2024) Mitochondrial extracellular vesicles, autoimmunity and myocarditis. *Front. Immunol.* 15:1374796. doi: 10.3389/fimmu.2024.1374796

Mitochondrial extracellular vesicles, autoimmunity and myocarditis

Damian N. Di Florio[1,2,3], Danielle J. Beetler[1,2,3], Elizabeth J. McCabe[1], Jon Sin[4], Tsuneya Ikezu[5] and DeLisa Fairweather[1,2,6,7]*

[1]Department of Cardiovascular Medicine, Mayo Clinic, Jacksonville, FL, United States, [2]Center for Clinical and Translational Science, Mayo Clinic, Rochester, MN, United States, [3]Mayo Clinic Graduate School of Biomedical Sciences, Mayo Clinic, Rochester, MN, United States, [4]Department of Biological Sciences, University of Alabama, Tuscaloosa, AL, United States, [5]Department of Neuroscience, Mayo Clinic, Jacksonville, FL, United States, [6]Department of Immunology, Mayo Clinic, Jacksonville, FL, United States, [7]Department of Medicine, Mayo Clinic, Jacksonville, FL, United States

For many decades viral infections have been suspected as 'triggers' of autoimmune disease, but mechanisms for how this could occur have been difficult to establish. Recent studies have shown that viral infections that are commonly associated with viral myocarditis and other autoimmune diseases such as coxsackievirus B3 (CVB3) and SARS-CoV-2 target mitochondria and are released from cells in mitochondrial vesicles that are able to activate the innate immune response. Studies have shown that Toll-like receptor (TLR)4 and the inflammasome pathway are activated by mitochondrial components. Autoreactivity against cardiac myosin and heart-specific immune responses that occur after infection with viruses where the heart is not the primary site of infection (e.g., CVB3, SARS-CoV-2) may occur because the heart has the highest density of mitochondria in the body. Evidence exists for autoantibodies against mitochondrial antigens in patients with myocarditis and dilated cardiomyopathy. Defects in tolerance mechanisms like autoimmune regulator gene (AIRE) may further increase the likelihood of autoreactivity against mitochondrial antigens leading to autoimmune disease. The focus of this review is to summarize current literature regarding the role of viral infection in the production of extracellular vesicles containing mitochondria and virus and the development of myocarditis.

KEYWORDS

autoimmune disease, extracellular vesicles, mitochondria, mitochondrial-derived vesicles, myocarditis, AIRE, coxsackievirus

Highlights

- Mitochondrial extracellular vesicles contain CVB3
- Extracellular vesicles containing mitochondrial components activate TLR4/NLRP3
- Autoantibodies against mitochondria are found in patients with myocarditis
- The autoimmune regulator AIRE may bind few mitochondrial genes

Introduction

The immune system protects the host against infection by specifically recognizing and eliminating foreign pathogens, but in the process must avoid responding to host antigens. During maturation of the immune system, immune cells that react against self-antigens are eliminated providing an immune system that is 'tolerant' to self (1). T cells that escape central tolerance are additionally regulated with peripheral tolerance mechanisms that include the conversion of self-reactive T cells to regulatory T cells. Autoimmunity that progresses to autoimmune disease can occur if this process breaks down (2). Genetic and environmental factors contribute to the development of autoimmune diseases, but twin studies indicate that environmental factors are a significant contributor (3, 4). For many decades viral infections have been suspected as 'triggers' of autoimmune disease, but mechanisms for how this could occur have been difficult to establish (2, 5, 6). Recent findings suggest that subversion of host cellular extracellular vesicle (EV) processing by viral infections may lead not only to activation of the immune response against the virus but also against mitochondrial or other self-antigens thereby contributing to the development of autoimmune disease. In this review, we describe EVs with mitochondrial content, their relationship to viral infections such as coxsackievirus B3 (CVB3), and their potential role in driving autoimmune diseases with a focus on myocarditis.

Extracellular vesicles

In the last decade there has been a major increase in interest in EVs in their role in cell-to-cell communication, as biomarkers and as therapeutics (7, 8). Many terms and definitions are used to describe EVs, and in this review we use the term EVs to refer to all extracellular, lipid bilayer, sub-cellular particles and their functional contents with sizes ranging from several nm to several µm (9, 10). This umbrella term includes the widely recognized major subgroups termed exosomes, microvesicles, and apoptotic bodies, which are currently distinguishable only by their theorized origin and size but not by experimental means (11). EVs are engaged in cellular communication in both health and disease as transporters of molecular signals in the form of nucleic acids (e.g., DNA, mRNA, microRNA/miRs, long-coding RNA/lcRNA and circular RNA/circRNA), proteins and lipids (11).

When tissue environments are perturbed or cells become damaged as occurs during a viral infection, EV content changes based on cellular reprogramming in response to pathological stress (11, 12). EVs can either activate or inhibit innate and adaptive immune cell responses based on their content (13–15). EVs have been demonstrated to express major histocompatibility complex (MHC) class I or II and directly activate innate antigen presenting cells (APCs) or adaptive T and B cells in an antigen/self-antigen-specific manner (16, 17). Tetraspanins like CD9, CD63 and CD81, which are commonly used to characterize EVs, bind factors on innate immune cells like integrins (i.e., CD11b) that are important in activating and modulating immune responses (12, 18).

Viral infection and EVs

Importantly, many viruses use EV cellular machinery (i.e., exosome endosomal sorting complexes required for the transport/ESCRT pathway) for viral transmission such as cytomegalovirus, coxsackievirus, SARS-CoV-2, human immunodeficiency virus 1 (HIV-1), hepatitis viruses B, C and E (HBV, HCV, and HEV), and multiple members of the human herpesvirus (HHV) family (reviewed in (19–22). As a result, EVs can contain infectious virus, viral particles and/or viral proteins following infection that can subvert the immune response to promote viral replication. Important from an autoimmune disease context, a 'mix' of self and foreign antigens in/on EVs that may occur after viral infection may be presented to APCs and drive the immune response to target not only the infectious agent but also host antigens resulting in an autoimmune response.

EVs and autoimmune disease

The role of EVs in the development of autoimmune disease has been studied in patients and animal models. A review article by Tian et al. recently examined the role of EVs in a number of autoimmune diseases including thyroiditis, systemic lupus erythematosus (SLE), multiple sclerosis (MS), rheumatoid arthritis (RA), anti-phospholipid syndrome and type I diabetes (23). Many investigators have reported that the number of circulating EVs are elevated in patients with autoimmune disease compared to controls (24–26); however, the wide variety of methods and procedures for isolating EVs as well as differences in storage conditions makes it difficult to interpret these findings.

Studies examining changes in EV content and function may provide a clearer picture of their effects in patients with autoimmune disease. MicroRNA (miRs) content in blood EVs (exosomes) were identified as biomarkers that distinguished patients with relapsing-remitting MS (i.e., miR-15b-5p, miR-451a, miR-30b-5p, miR-342-3p) from those with progressive MS or healthy controls (27, 28). Eight out of the nine miRs that were identified in the study were confirmed in a separate group of patients indicating that the miRs/EVs could serve as biomarkers

to predict MS type. Similar results have been found for other autoimmune diseases like type I diabetes (29). EVs have also been found to either promote inflammation/remodeling or to inhibit harmful immune responses for a number of autoimmune diseases including RA (30–33), Hashimoto's thyroiditis (34), type I diabetes (35), SLE (36), and myocarditis (15). Additionally, EVs have been found with immunoglobulins (Ig) on their surface including IgG or internally in the form of self-antigen-complement-Ig immune complexes (ICs) (37, 38) suggesting that EVs may initiate and/or promote autoimmune damage and inflammation via ICs (39–42). Understanding the role of EVs in autoimmune disease is an emerging field with many questions still to be answered.

Mitochondrial extracellular vesicles

Another form of EVs that have received recent attention and may play a role in autoimmune disease are those that contain host cellular components such as mitochondria (e.g., primarily mitochondrial proteins or RNA) (43). The earliest evidence of mitochondria and mitochondrial components in vesicles comes from a description by Vishwa Nath in 1932 of work by Koltzoff in 1906 studying sperm cells from *Paratelphusa spinigera* (44). Koltzoff and Nath observed sub-cellular structures in crab spermatocytes undergoing a process that sounds similar to our current understanding of mitochondrial-derived vesicles (MDVs) (intracellular vesicles for mitochondrial transport) (44–46) or mitophagosomes (mitochondria fission products contained in autophagosomes for selective autophagy) (47, 48). Both MDVs and fragmented mitochondria fission products can be sent to autophagosomes for selective autophagy (46, 49) in a specific lysosomal degradation of mitochondria process referred to as 'mitophagy' (49). The formation of the endoplasmic reticulum barrier around fragmented mitochondrial pieces (i.e., the autophagosome), a process that occurs in receptor-mediated mitophagy, is what Nath suspected protected these structures (which he only knew as another membrane around a mitochondrial mass) from rupture and lysis when exposed to acetic acid (44). Another process that has been referred to as 'mitoptosis' involves selective removal of damaged mitochondria from the cell in vesicles (i.e., EVs) that are generally referred to as mitochondrial EVs (50) or mitovesicles (51) that contain whole or pieces of mitochondria (52, 53). See images of mitochondrial EVs budding from cardiac myocytes in Figure 1 (54). Importantly, this process can occur for healthy physiological removal or transfer of mitochondria as well as for damaged mitochondria (55).

EVs that contain mitochondria lack standardized definitions but are known to contain inner and outer mitochondrial membrane components, mitochondrial nucleic acid (i.e., DNA, RNA), and/or cardiolipin - a signature phospholipid that is more concentrated in mitochondrial membranes than cellular membranes (9). The two known major populations of mitochondrial EVs also differ in terms of their size: MDVs are smaller (30-100 nm) and EVs containing larger mitochondrial components or whole mitochondria have a larger size.

Coxsackievirus B3-induced mitochondrial EVs

For decades, small non-enveloped RNA viruses like CVB3 were thought to cause host cell lysis as the primary method of viral dissemination, but recent evidence has demonstrated that infectious CVB3 and viral particles are released in mitochondrial EVs (47, 56). The first evidence that CVB3 infection disrupts cardiac mitochondria was published in 1964 using young Swiss white mice (Webster strain) inoculated with tissue culture-derived virus (57). Investigators utilized microscopy to assess subcellular changes to the myocardium during viral infection. Notably, they identified an increase in mitochondrial fission, disruption of mitochondrial cristae, and smaller mitochondria with additional membranes that enclosed them that likely depict mitophagosomes (57). A later study from 1997 identified CVB3 localization around and *within* cardiac mitochondria during myocarditis in mice (Figure 2) (58).

In 2017, Roberta Gottlieb's laboratory published a study demonstrating CVB3 viral transmission via EVs containing mitochondrial components (47). Using an *in vitro* neural progenitor stem cell model of viral infection, Robinson et al. demonstrated that CVB3 localizes to the mitochondrial compartment of infected cells and is later ejected from cells in vesicles containing virus, inner and outer mitochondrial membrane components, and autophagy machinery (i.e., microtubule-

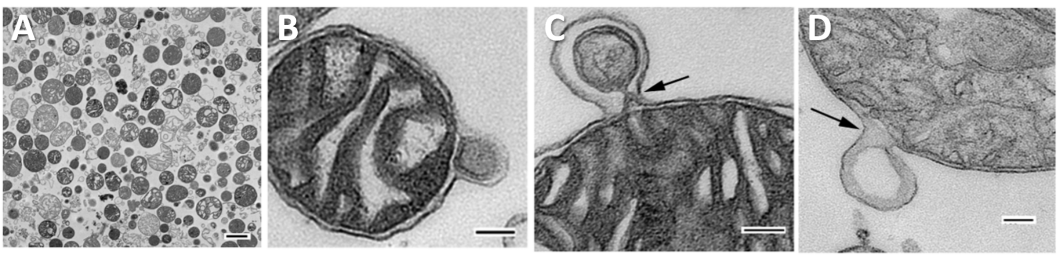


FIGURE 1
Mitochondrial derived vesicles (MDVs) from cardiac mitochondria. Widefield transmission electron microscopic images of mitochondria isolated from bovine heart. **(A)** 60-100 nm vesicles containing mitochondria. Scale bar, 500 nm, **(B)** MDV budding from mitochondria containing inner and outer mitochondrial membrane, **(C)** Protrusion of MDV from mitochondria showing constriction at its base, **(D)** MDV forming with only outer mitochondrial membrane. Panels **(B–D)**, scale bars 100 nm. Reused with permission from (54).

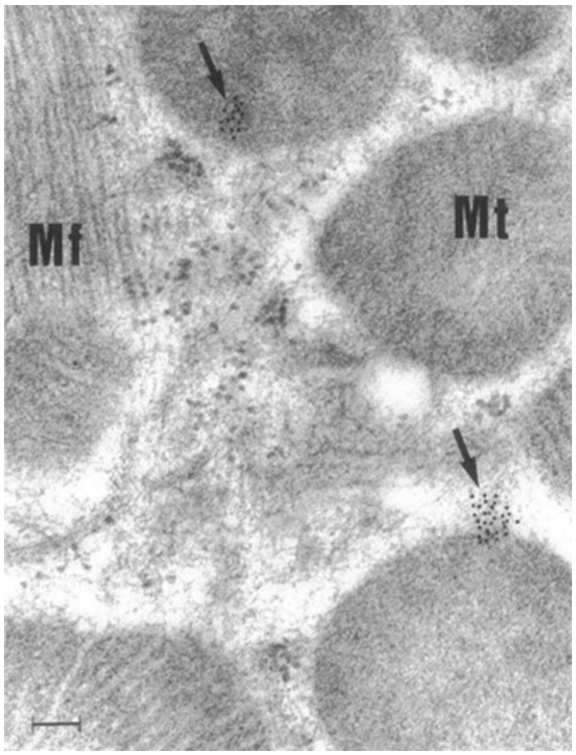


FIGURE 2
CVB3 localizes around and within murine cardiac mitochondria during myocarditis. Immunogold electron micrograph of mouse cardiomyocyte with CVB3 myocarditis on day 8 post infection. Black dots (arrows) are gold staining of CVB3 viral genome localizing around and in cardiomyocyte mitochondria. Scale bar, 100 nm. Mt, mitochondria; Mf, myofibril. Reused with permission from (58).

associated protein light chain 3/LC3-II) (47). Using electron microscopy, they found these particles ranged in size from 100-200 nm in diameter and contained single or multiple virions (Figure 3) (47). They also observed that the ejected particles were infectious to adjacent uninfected host cells.

The protein dynamin-related protein 1 (Drp1) is required for mitochondria to undergo fission. Drs. Gottlieb and Sin showed that CVB3 infection led to Drp1-induced mitochondrial fission resulting in damaged mitochondria being processed into mitophagosomes via mitophagy and released from host HL-1 cardiomyocytes in culture as mitochondrial EVs (48). The role of fission in the production of mitochondrial vesicles was confirmed by inhibition/blocking mitochondrial fission machinery using mitochondrial division inhibitor-1 (Mdivi-1) or direct inhibition of Drp1 with siRNA which resulted in less viral replication and fewer/no virus containing EVs in the culture supernatant (48). Dr. Sin's group additionally showed that Tank binding kinase I (TBK1) increased phosphorylation of GABA type A receptor associated protein-like (GABARAPL) proteins leading to EVs that contain mitochondria being released from the cells (59). CVB3 infection has also been shown to perturb syntaxin-17 facilitated mitophagosome-lysosomal fusion, which may lead to build up and release of formed mitophagosomes from the cell (60). Thus, these studies confirm that CVB3 localizes to mitochondria and is released in mitochondrial vesicles. Further research is needed to better understand the molecular mechanisms of intracellular mitophagosome formation in the context of viral infections to determine how viruses take advantage of mitochondrial compartments and evade intracellular degradation by targeted autophagy. A summary of our current understanding of CVB3-mitochondria interaction and the development of EV populations containing mitochondria and virus is illustrated in Figure 4.

Mitochondrial autoimmunity and myocarditis

It turns out that many viruses are known to localize to mitochondria (61–64), utilize mitochondrial machinery for replication (48, 65), evade immune responses within EVs (66) and modify cellular processes (59, 60, 67). Importantly, most of the viruses that are associated with causing myocarditis [e.g., CVB, influenza, HIV, poliovirus, hepatitis C virus, SARS-CoV-22 (68, 69)] have been found to target mitochondria to gain a replicative advantage (61–64) and are ejected from cells/tissues in EVs (60, 65, 70, 71) suggesting that these mechanisms may provide an explanation for how viruses could cause autoimmune disease.

Mitochondrial autoantibodies in patients with myocarditis

Dr. Peter Schultheiss, a major contributor to the fields of cardiology and myocarditis, began identifying and characterizing autoimmune antibodies in patients with myocarditis in the 1970s. In 1978, Bolte and Schultheiss reported that 76% of 17 patients with viral myocarditis had autoantibodies in sera and 41% of these were anti-nuclear antibodies (72). They went on to show that autoantibodies against the adenine nucleotide transporter (ANT), which is a component of the inner mitochondrial membrane, were elevated in patients with myocarditis and dilated cardiomyopathy (DCM) (73, 74). Myocarditis progresses to DCM in susceptible patients and animal models (75). Patients with suspected or confirmed viral myocarditis or cardiomyopathy/DCM had the highest reactivity to anti-mitochondrial antigen and highest expression of anti-mitochondrial antibodies (74). Further analysis found that the sera had uniquely specific reactivity toward cardiac mitochondrial antigen compared to liver or kidney mitochondrial antigen (76).

Another group independently reported that patients with various cardiomyopathies including myocarditis had autoantibodies reactive against mitochondrial proteins (77). They found that 13% with acute myocarditis, 31% of patients with DCM, and 33% with hypertrophic cardiomyopathy generated antibody responses specifically to the M7 antigen of the mitochondrial enzyme sarcosine dehydrogenase, and 25% of these reacted against the cardiac-specific form of the mitochondrial antigen (77). Another group observed autoantibodies against ANT in patients diagnosed with myocarditis or DCM (78). They also observed cardiac-specific reactivity and suggested mitochondrial autoimmune activity as a potential mechanism for the

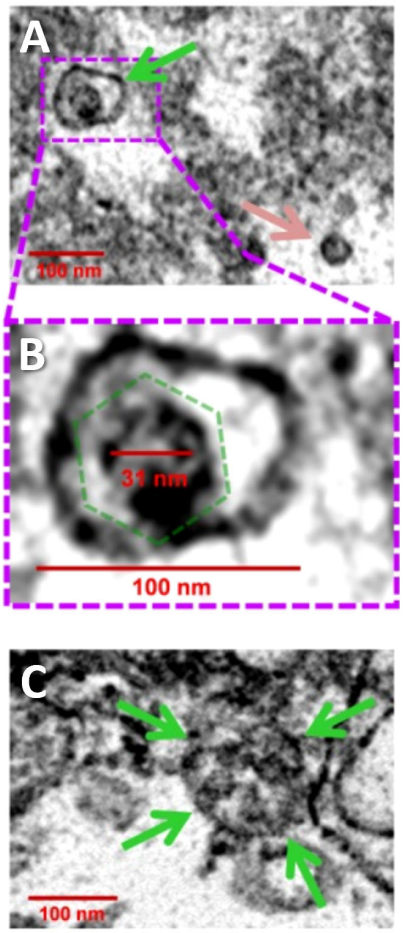


FIGURE 3
CVB3 identified in EVs using transmission electron microscopy. **(A)** Widefield transmission electron microscopic view of single virion (green arrow) in an extracellular EV or free virion (pink arrow) from culture of CVB3 in C2C12 cells. **(B)** Higher digital magnification (dashed purple box) of a virus-like particle revealed an icosahedral shape structure (dashed green polygon) slightly larger than 31 nm in diameter enclosed within a membrane structure. **(C)** Large EV containing multiple virions (green arrows). Scale bars = 100 nm. Reused with permission from (47).

development of DCM following acute myocarditis (78). This agrees with our current understanding of the development of DCM following acute myocarditis (75, 79, 80).

Mitochondrial autoantibodies in models of myocarditis

Although viral-induced myocarditis is often categorized as a distinct condition from autoimmune myocarditis clinically and in animal models, the distinction between the two conditions is not clear-cut because patients with viral myocarditis and mouse models of viral myocarditis have been demonstrated to develop autoantibodies and autoreactive T and B cells against cardiac myosin and other self-antigens including mitochondria (2, 39, 79, 81–83).

Importantly, a study examining autoantibody levels that compared experimental autoimmune myocarditis (EAM) to CVB3-induced myocarditis in mice found that ANT was only produced after viral infection but not in EAM suggesting that viral infection was necessary for the production of mitochondrial autoantibodies whereas both models produced autoantibodies against cardiac myosin (84). Additionally, Lin et al. showed that depletion of Drp1 (required for fission) in mice using the mitochondrial fission inhibitor Mdivi-1 reduced CVB3 myocarditis and restored mitochondrial function in the heart (71) suggesting that mitochondrial EVs containing virus may increase myocarditis, although they did not examine this in the study. Overall, these findings suggest that viral infection may be an important mechanism to produce mitochondrial autoantibodies found in patients with autoimmune diseases.

Anti-mitochondrial antibodies in rheumatic autoimmune diseases

Anti-mitochondrial antibodies (for example, antibodies that target cardiolipin, mitofusin 1, mitochondrial DNA or mitochondrial RNA) are commonly found in patients with rheumatic autoimmune diseases such as RA, SLE, and anti-phospholipid syndrome (85–87). Mobarrez et al. reports most larger EVs (0.7 - 3.0 µm) found in SLE patients contain functional mitochondrial components, as indicated by the presence of the translocase of outer mitochondrial membrane 20 (TOMM20) and hexokinase1 (25). Elevated levels of these type of EVs containing mitochondria are positively associated with increased SLE disease activity, proinflammatory cytokines, and anti-dsDNA antibodies, suggesting that these EVs may be involved in disease pathogenesis (25). Becker et al. recently reviewed the mechanism of immune activation leading to autoimmune disease by mitochondria in these rheumatic conditions but does not discuss the potential role of viral infections in the process or whether the mitochondrial EVs also contain virus or viral components (85). These findings suggest that damage to mitochondria resulting in autoimmune responses may be a common mechanism in the pathogenesis of many autoimmune diseases.

Activation of autoimmunity by mitochondrial EVs

One possible mechanism where myocarditis and other autoimmune diseases could be induced and/or exacerbated by mitochondrial EVs is by activation of Toll-like receptor (TLR)4, interleukin (IL)-1β and leucin-rich repeat (LRR)-containing protein (NLRP)3, which is a pathway that has been demonstrated to increase myocarditis and viral replication in CVB3 models of myocarditis (88, 89). Mitochondria are known to be immunogenic and the TLR4/NLRP3 signaling pathway can be activated by mitochondrial components such as cytochrome c, mitochondrial transcription factor A (TFAM), ATP and cardiolipin, which can all be found in mitochondrial EVs, to initiate a proinflammatory and profibrotic immune response (90–

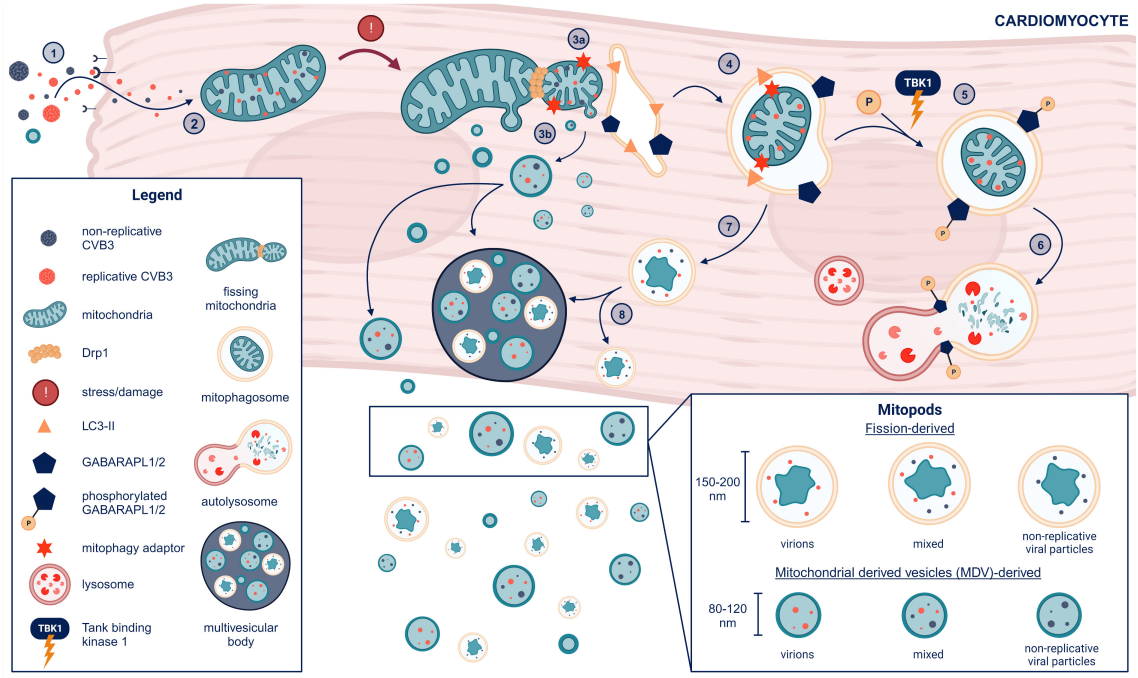


FIGURE 4
Formation of mitochondrial EVs from cardiomyocytes after CVB3 infection (1). CVB3 gains entry to cardiomyocytes via the coxsackievirus adrenoreceptor (CAR) or passive entry from previously formed mitochondrial EVs containing replicative virus (2). CVB3 mitochondrial localization induces mitochondrial stress and damage leading to (3a) mitochondrial-derived vesicle (MDV) formation and Drp1-mediated mitochondrial fission and recruitment of the endoplasmic reticulum (ER) for autophagosome formation alongside LC3 lipidation (LC3-II). (3b) MDVs containing replicative and or non-replicative viral particles may either eject from the cell or join multi-vesicular bodies before release from cardiomyocytes (MDVs can also be slated for receptor mediated mitophagy and potentially escape the cell without GABARAPL phosphorylation, which is not shown in this diagram) (4). LC3-II binds mitophagy adaptors situated on the outer mitochondrial membrane to form a mitophagosome with GABARAPL proteins on the endoplasmic reticulum (ER) facing the cytosol (5). Phosphorylation of the mitophagosome on GABARAPL proteins by tank-binding kinase 1 (TBK1) leads mitophagosomes to subsequent (6) lysosomal fusion and degradation (7). Non-phosphorylated mitophagosomes do not proceed to fusion with the lysosome but either (8) join the multivesicular body for cell release and dissemination or are ejected alone. The resulting EVs containing mitochondrial components and viral particles (replicative and/or non-replicative) we term as "mitopods" or mitochondrial escape-pods for CVB3. The two major sources of mitopods are MDV-derived or fission-derived. Another possible distinguishing feature of fission-derived versus MDV-derived mitopods would be an additional membrane derived from the ER. This figure was created using BioRender.

94). Mitochondrial and viral antigens may be expressed on the cell surface or interior of EVs and activate APCs via MHC class II presentation, TLRs or be processed for presentation after the EV lipid membrane has 'merged' with an APC (23).

However, not all mitochondria found in EVs stimulate the innate immune response. In some cases, healthy mitochondria within EVs are found to fuse with recipient mitochondria in cultured cardiomyocytes and in a mouse model of myocardial infarction where they improve mitochondrial function and disease (95). These investigators showed a similar improvement in doxorubicin-induced toxicity in cultured cardiomyocytes (96). Thus, transfer of healthy intact mitochondria within EVs represents a novel and potentially viable therapy for patients with mitochondrial damage or dysfunction (97, 98).

Tolerance against mitochondrial antigens and myocarditis

As mentioned earlier, a key feature of the immune response that protects against the development of autoimmunity is the generation of tolerance to self-antigens that occurs in the thymus (1). Since the generation of T cell receptors (TCRs) in the thymus is a random process, negative selection of T cells that react too strongly to self-antigen is required to prevent autoimmunity. To determine whether there are too many self-reactive T cells, the thymus utilizes the autoimmune regulator gene (AIRE) and dendritic cells (99, 100).

AIRE and tolerance to self

AIRE is a transcriptional regulator that protects against self-reactivity by inducing the production of tissue-specific antigens normally not expressed in the thymus, a process that occurs in medullary thymic epithelial cells (mTECs) (99). Resident dendritic cells of the thymus take up self-proteins and present them to T cells. If reactivity to self-antigen is too strong, dendritic cells undergo cytokine signalling programs that destroy autoreactive T cells (99). Migratory and peripheral dendritic cell populations further contribute to negative selection of autoreactive T cells by selecting against cells reactive to peripheral antigen from other tissue microenvironments. Migratory dendritic cells take up antigen in their respective resident tissues and travel to the thymus whereas peripheral dendritic cells test autoreactive

10.3389/fimmu.2024.1374796

T cells in the periphery (i.e., their tissue of origin) thereby inducing, depending on the conditions, cell deletion, anergy or polarization toward a regulatory phenotype (100). It is estimated that there are 1,140 murine genes that interact with or localize to mitochondrial compartments (101), so AIRE should protect the host from developing autoimmunity against these mitochondrial antigens. One important question is whether mitochondrial genes represent a gap in the normal negative selection criteria in the thymus.

AIRE and mitochondrial autoimmunity

To our knowledge, no studies examining the function of AIRE describe its ability to produce mitochondrial self-antigen. Two major studies exist with publically available data of AIRE genomic binding and related expression (102, 103). A study by Bansal et al. reported murine transcriptomic data that examined AIRE binding using chromatin immunoprecipitation (ChIP) sequencing (ChIPseq). We examined whether any of the antigens that they reported for AIRE were directed against mitochondrial antigens using their published transcript-level data. They did not have protein/proteomic level data available to assess this question. They estimated AIREs coverage of genes by assessing transcription among AIRE knockout (KO) mice versus wild type (WT) controls in data derived from mouse mTECs (103). A negative log fold-change (LogFC) indicated downregulation of the transcript in the AIRE KO mice, suggesting that in WT conditions, AIRE may be responsible, in part, for regulating the transcription of a respective gene. To determine AIRE regulation of mitochondrial related genes, we performed a keyword search for "mitochondri" (which yielded results for *mitochondrial, mitochondria, and mitochondrion*) in the gene description column of their dataset, which yielded 315 genes. Among these, 20 were significantly downregulated at an FDR $p \leq 0.05$ comparing AIRE WT to KO indicating that AIRE may regulate only 6.3% of the 315 mitochondrial related genes. We also assessed the potential role of AIRE to regulate 85 murine nuclear-encoded mitochondrial respiratory chain genes using keyword searches in the gene name column for "nduf," "sdh," "cox," "uqcc," and "atp," which are the prefixes for gene names among components of respiratory complexes I-V, respectively. Their data showed a significant downregulation of 2 of 85 (2.3%) nuclear encoded mitochondrial respiratory chain genes in AIRE KO vs WT samples. Mitochondrial genes that were regulated by AIRE are listed in Table 1. Thus, only a small percentage of potential mitochondrial genes were regulated by AIRE using this method. More research is needed to better understand whether AIRE contributes to tolerance against mitochondrial antigens. Thus, a lack of mitochondrial tolerance may be one possible explanation for the development of mitochondrial targeted autoimmune responses in myocarditis and other autoimmune diseases.

TABLE 1 Mitochondria related and respiratory complex genes expressed by AIRE in mice from Bansal et al. (103) in order of FDR p value.

Gene Symbol	Nominal p value	FDR p value	LogFC[a]	Category
Mrpl13	0.0000163	0.000559	-1.29492	Mito Related
Mrps30	0.0000776	0.00182	-0.85468	Mito Related
Gls2	0.000243	0.00422	-0.87328	Mito Related
Mtarc1	0.000294	0.00486	-0.74663	Mito Related
Slc25a13	0.000842	0.0107	-0.84998	Mito Related
Cox7a2l	0.000932	0.0116	-0.87238	Resp Chain
Tmem243	0.00111	0.0131	-0.68091	Mito Related
Cox17	0.00149	0.0161	-0.67956	Resp Chain
Immp1l	0.00158	0.0167	-0.58226	Mito Related
Mtrf1l	0.00211	0.0208	-0.5228	Mito Related
Mrpl22	0.00219	0.0215	-0.58552	Mito Related
Mtarc2	0.00239	0.0228	-0.54555	Mito Related
Mterf1	0.00289	0.0260	-0.46945	Mito Related
Mrpl47	0.00307	0.0273	-0.52963	Mito Related
Mto1	0.00388	0.0325	-0.45504	Mito Related
Tomm20	0.00433	0.0349	-0.53508	Mito Related
Micu2	0.0049	0.0381	-0.46659	Mito Related
Diablo	0.00498	0.0385	-0.47024	Mito Related
Tk2	0.00512	0.0394	-0.44682	Mito Related
Bcat2	0.0069	0.0489	-0.53663	Mito Related

[a]Bcat2, branched chain amino acid transaminase 2; Cox7a2l, cytochrome c oxidase subunit 7A2-like; Cox17, cytochrome c oxidase copper chaperone; Diablo, IAP-binding mitochondrial protein; Gls2, glutaminase 2; Immpl1, inner mitochondrial membrane peptidase subunit 1; LogFC, Log Fold-Change; Micu2, mitochondrial calcium uptake 2; Mito Related, mitochondrial related; Mrpl13, mitochondrial ribosomal protein L13; Mrpl22/47, mitochondrial ribosomal protein L22/47; Mrps30, mitochondrial ribosomal protein S30; Mtarc1/2, mitochondrial amidoxime reducing component 1/2; Mterf1, mitochondrial transcription termination factor 1; Mto1, mitochondrial tRNA translations optimization 1; Mtrf1l, mitochondrial translation release factor 1-like; Resp Chain, respiratory chain; Slc25a13, solute carrier family 25 member 13; Tk2, thymidine kinase 2; Tmem243, transmembrane protein 243; Tomm20, translocase of outer mitochondrial membrane 20.

Summary

In summary, we propose the following possible scenario for the role of mitochondrial EVs in the induction of autoimmune diseases like myocarditis. The initial infection with virus will activate antiviral TLRs like TLR3, 7, 8, 9 in the first few minutes/hours after infection. The virus will traffic to the mitochondria at the local site of infection and mitochondria within the virus' favorite cell type/primary tropism to obtain a replicative advantage. The virus will be released from the cell in mitochondrial EVs. Mitochondrial components expressed within or on the surface of the EVs then activate TLR4 on APCs. The presence of virus/viral particles and mitochondrial components together may create a strong 'adjuvant' effect to activate the immune response. During the viremic stage of viral replication, which typically occurs in the first few days after viral infection, the virus within EVs can traffic through the bloodstream or lymphatics to the heart where infection of cardiac tissues can occur in a non-viral receptor specific manner via EVs or also with viral receptors if they are present in cardiac tissue. For example, CVB3 may enter cardiac cells via coxsackievirus-

adenovirus receptor (CAR) which is expressed in the heart. Release of mitochondrial EVs from mitochondrially rich cardiomyocytes may drive a cardiac-specific autoimmune response because the mitochondrial content in/on EVs may contain heart specific mitochondrial antigens. TLR4 signaling has been found to be an important pathway in the pathogenesis of many autoimmune diseases including myocarditis. Autoantibodies against mitochondrial components are found in patients with many different autoimmune diseases including myocarditis and in viral animal models of myocarditis providing evidence of an autoimmune response against mitochondria. Whether mitochondrial EVs that originate from the heart occur at a sufficient level to activate a cardiac-specific autoimmune response may be one reason why myocarditis occurs only rarely. Defects in AIRE may also confer susceptibility to autoimmune responses against mitochondrial antigens in some patients.

Conclusions

For decades the question of whether viruses can cause autoimmune disease has lacked a plausible explanation. Evidence exists that viral infections cause myocarditis that is also associated with autoimmune responses against the heart in patients and animal models, yet how viruses could cause autoimmunity in myocarditis is not clear. Recent evidence substantiates that many viruses, and in particular the viruses that are associated with clinical cases of myocarditis, target mitochondria to promote viral replication and to evade the immune response they are ejected from cells within EVs. Often these EVs also contain mitochondrial components. It is known that EVs contain proteins, receptors and other components that identify them as originating from self-tissue. EVs that contain replicative virus and/or virus particles and mitochondrial components may form powerful danger signals to the immune system activating TLR4- a key pathway in the pathogenesis of myocarditis and DCM. Autoantibodies against mitochondrial components and specifically cardiac mitochondria are found in patients with myocarditis and DCM providing insight that viral infections may promote the release of mitochondrial antigens to activate an autoimmune response. Additionally, defects in AIRE may allow heightened self-reactivity against mitochondrial antigens. These mechanisms provide an explanation for how viral infections may initiate or promote autoimmune diseases like myocarditis.

Author contributions

DD: Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Writing – original draft, Writing – review & editing. DB: Conceptualization, Visualization, Writing – review & editing. EM: Writing – review & editing. JS: Conceptualization, Writing – review & editing. TI: Writing – review & editing. DF: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This research was supported in part by the NIH grant R01 HL164520 to DF and NIH TL1 TR002380 to DD, DB and DF.

Acknowledgments

We utilized previously published images from Robinson et al., 2014 (47) and Soubannier et al., 2012 (54) which are reprinted with copyright permissions via Creative Commons CC-BY-license. The image from Ukimura et al., 1997 (58) was reprinted after obtaining a license from Elsevier. We thank Dr. Heidi McBride for support and input on this review.

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.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

References

1. Sogkas G, Atschekzei F, Adriawan IR, Dubrowinskaja N, Witte T, Schmidt RE. Cellular and molecular mechanisms breaking immune tolerance in inborn errors of immunity. *Cell Mol Immunol.* (2021) 18:1122–40. doi: 10.1038/s41423-020-00626-z

2. Fairweather D, Kaya Z, Shellam GR, Lawson CM, Rose NR. From infection to autoimmunity. *J Autoimmun.* (2001) 16:175–86. doi: 10.1006/jaut.2000.0492

3. Shamim EA, Miller FW. Familial autoimmunity and the idiopathic inflammatory myopathies. *Curr Rheumatol Rep.* (2000) 2:201–11. doi: 10.1007/s11926-000-0080-0

4. Fairweather D, Root-Bernstein R. Autoimmune disease: Mechanism. In: *Encyclopedia of life sciences*. John Wiley & Sons, Ltd, Chichester, UK (2015). p. 1–7. doi: 10.1002/9780470015902.a0020193.pub2

5. Fairweather D, Rose NR. Type 1 diabetes: Virus infection or autoimmune disease? *Nat Immunol.* (2002) 3:338–40. doi: 10.1038/ni0402-338

6. Thomas OG, Olsson T. Mimicking the brain: Epstein-Barr virus and foreign agents as drivers of neuroimmune attack in multiple sclerosis. *Front Immunol.* (2023) 14:1304281. doi: 10.3389/fimmu.2023.1304281

7. Ciferri MC, Quarto R, Tasso R. Extracellular vesicles as biomarkers and therapeutic tools: from pre-clinical to clinical applications. *Biol (Basel)*. (2021) 10:359. doi: 10.3390/biology10050359

8. Beetler DJ, Di Florio DN, Law EW, Groen CM, Windebank AJ, Peterson QP, et al. The evolving regulatory landscape in regenerative medicine. *Mol Aspects Med*. (2023) 91:101138. doi: 10.1016/j.mam.2022.101138

9. Thery C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): A position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *J Extracell Vesicles*. (2018) 7:1535750. doi: 10.1080/20013078.2018.1535750

10. Witwer KW, Goberdhan DC, O'Driscoll L, Thery C, Welsh JA, Blenkiron C, et al. Updating MISEV: Evolving the minimal requirements for studies of extracellular vesicles. *J Extracell Vesicles*. (2021) 10:e12182. doi: 10.1002/jev2.12182

11. Yates AG, Pink RC, Erdbrugger U, Siljander PR, Dellar ER, Pantazi P, et al. In sickness and health: The functional role of extracellular vesicles in physiology and pathology in *in vivo*: Part II: Pathology. *J Extracell Vesicles*. (2022) 11:e12190. doi: 10.1002/jev2.12190

12. Rodrigues M, Fan J, Lyon C, Wan M, Hu Y. Role of extracellular vesicles in viral and bacterial infections: Pathogenesis, diagnostics, and therapeutics. *Theranostics*. (2018) 8:2709–21. doi: 10.7150/thno.20576

13. Admyre C, Bohle B, Johansson SM, Focke-Tejkl M, Valenta R, Scheynius A, et al. B cell-derived exosomes can present allergen peptides and activate allergen-specific T cells to proliferate and produce TH2-like cytokines. *J Allergy Clin Immunol*. (2007) 120:1418–24. doi: 10.1016/j.jaci.2007.06.040

14. Beetler DJ, Di Florio DN, Bruno KA, Ikezu T, March KL, Cooper LT Jr., et al. Extracellular vesicles as personalized medicine. *Mol Aspects Med*. (2023) 91:101155. doi: 10.1016/j.mam.2022.101155

15. Beetler DJ, Bruno KA, Watkins MM, Xu V, Chekuri I, Giresi P, et al. Reconstituted extracellular vesicles from human platelets decrease viral myocarditis in mice. *Small*. (2023) 19:e2303317. doi: 10.1002/smll.202303317

16. Raposo G, Nijman HW, Stoorvogel W, Liejendekker R, Harding CV, Melief CJ, et al. B lymphocytes secrete antigen-presenting vesicles. *J Exp Med*. (1996) 183:1161–72. doi: 10.1084/jem.183.3.1161

17. Hwang I, Shen X, Sprent J. Direct stimulation of naive T cells by membrane vesicles from antigen-presenting cells: distinct roles for CD54 and B7 molecules. *Proc Natl Acad Sci U S A*. (2003) 100:6670–5. doi: 10.1073/pnas.1131852100

18. Thery C, Zitvogel L, Amigorena S. Exosomes: Composition, biogenesis and function. *Nat Rev Immunol*. (2002) 2:569–79. doi: 10.1038/nri855

19. Feng Z, Hensley L, McKnight KL, Hu F, Madden V, Ping L, et al. A pathogenic picornavirus acquires an envelope by hijacking cellular membranes. *Nature*. (2013) 496:367–71. doi: 10.1038/nature12029

20. Alenquer M, Amorim MJ. Exosome biogenesis, regulation, and function in viral infection. *Viruses*. (2015) 7:5066–83. doi: 10.3390/v7092862

21. Schorey JS, Harding CV. Extracellular vesicles and infectious diseases: New complexity to an old story. *J Clin Invest*. (2016) 126:1181–9. doi: 10.1172/JCI81132

22. Hernandez-Rodriguez J, Alba MA, Prieto-Gonzalez S, Cid MC. Diagnosis and classification of polyarteritis nodosa. *J Autoimmun*. (2014) 48–49:84-9. doi: 10.1016/j.jaut.2014.01.029

23. Tian J, Casella G, Zhang Y, Rostami A, Li X. Potential roles of extracellular vesicles in the pathophysiology, diagnosis, and treatment of autoimmune diseases. *Int J Biol Sci*. (2020) 16:620–32. doi: 10.7150/ijbs.39629

24. Sellam J, Proulle V, Jungel A, Ittah M, Miceli Richard C, Gottenberg JE, et al. Increased levels of circulating microparticles in primary Sjögren's syndrome, systemic lupus erythematosus and rheumatoid arthritis and relation with disease activity. *Arthritis Res Ther*. (2009) 11:R156. doi: 10.1186/ar2833

25. Mobarrez F, Fuzzi E, Gunnarsson I, Larsson A, Eketjall S, Pisetsky DS, et al. Microparticles in the blood of patients with SLE: Size, content of mitochondria and role in circulating immune complexes. *J Autoimmun*. (2019) 102:142–9. doi: 10.1016/j.jaut.2019.05.003

26. Saenz-Cuesta M, Irizar H, Castillo-Trivino T, Munoz-Culla M, Osorio-Querejeta I, Prada A, et al. Circulating microparticles reflect treatment effects and clinical status in multiple sclerosis. *Biomark Med*. (2014) 8:653–61. doi: 10.2217/bmm.14.9

27. Ebrahimkhani S, Vafaee F, Young PE, Hur SSJ, Hawke S, Devenney E, et al. Exosomal microRNA signatures in multiple sclerosis reflect disease status. *Sci Rep*. (2017) 7:14293. doi: 10.1038/s41598-017-14301-3

28. Selmaj I, Cichalewska M, Namiecinska M, Galazka G, Horzelski W, Selmaj KW, et al. Global exosome transcriptome profiling reveals biomarkers for multiple sclerosis. *Ann Neurol*. (2017) 81:703–17. doi: 10.1002/ana.24931

29. Guay C, Kruit JK, Rome S, Menoud V, Mulder NL, Jurdzinski A, et al. Lymphocyte-derived exosomal microRNAs promote pancreatic beta cell death and may contribute to type 1 diabetes development. *Cell Metab*. (2019) 29:348–361.e6. doi: 10.1016/j.cmet.2018.09.011

30. Boilard E, Nigrovic PA, Larabee K, Watts GF, Coblyn JS, Weinblatt ME, et al. Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. *Science*. (2010) 327:580–3. doi: 10.1126/science.1181928

31. Vinuela-Berni V, Doniz-Padilla L, Figueroa-Vega N, Portillo-Salazar H, Abud-Mendoza C, Baranda L, et al. Proportions of several types of plasma and urine microparticles are increased in patients with rheumatoid arthritis with active disease. *Clin Exp Immunol*. (2015) 180:442–51. doi: 10.1111/cei.12598

32. Lo Cicero A, Majkowska I, Nagase H, Di Liegro I, Troeberg L. Microvesicles shed by oligodendroglioma cells and rheumatoid synovial fibroblasts contain aggrecanase activity. *Matrix Biol*. (2012) 31:229–33. doi: 10.1016/j.matbio.2012.02.005

33. Headland SE, Jones HR, Norling LV, Kim A, Souza PR, Corsiero E, et al. Neutrophil-derived microvesicles enter cartilage and protect the joint in inflammatory arthritis. *Sci Transl Med*. (2015) 7:315ra190. doi: 10.1126/scitranslmed.aac5608

34. Cui X, Liu Y, Wang S, Zhao N, Qin J, Li Y, et al. Circulating exosomes activate dendritic cells and induce unbalanced CD4+ T cell differentiation in Hashimoto thyroiditis. *J Clin Endocrinol Metab*. (2019) 104:4607–18. doi: 10.1210/jc.2019-00273

35. Shigemoto-Kuroda T, Oh JY, Kim DK, Jeong HJ, Park SY, Lee HJ, et al. MSC-derived extracellular vesicles attenuate immune responses in two autoimmune murine models: Type 1 diabetes and uveoretinitis. *Stem Cell Rep*. (2017) 8:1214–25. doi: 10.1016/j.stemcr.2017.04.008

36. Dieker J, Hilbrands L, Thielen A, Dijkman H, Berden JH, van der Vlag J. Enhanced activation of dendritic cells by autologous apoptotic microvesicles in MRL/lpr mice. *Arthritis Res Ther*. (2015) 17:103. doi: 10.1186/s13075-015-0617-2

37. Cloutier N, Tan S, Boudreau LH, Cramb C, Subbaiah R, Lahey L, et al. The exposure of autoantigens by microparticles underlies the formation of potent inflammatory components: The microparticle-associated immune complexes. *EMBO Mol Med*. (2013) 5:235–49. doi: 10.1002/emmm.201201846

38. Nielsen CT, Ostergaard O, Stener L, Iversen LV, Truedsson L, Gullstrand B, et al. Increased IgG on cell-derived plasma microparticles in systemic lupus erythematosus is associated with autoantibodies and complement activation. *Arthritis Rheumatol*. (2012) 64:1227–36. doi: 10.1002/art.34381

39. Fairweather D, Frisancho-Kiss S, Njoku DB, Nyland JF, Kaya Z, Yusung SA, et al. Complement receptor 1 and 2 deficiency increases coxsackievirus B3-induced myocarditis, dilated cardiomyopathy, and heart failure by increasing macrophages, IL-1beta, and immune complex deposition in the heart. *J Immunol*. (2006) 176:3516–24. doi: 10.4049/jimmunol.176.6.3516

40. Fairweather D, Petri MA, Coronado MJ, Cooper LT. Autoimmune heart disease: Role of sex hormones and autoantibodies in disease pathogenesis. *Expert Rev Clin Immunol*. (2012) 8:269–84. doi: 10.1586/eci.12.10

41. Ullal AJ, Reich CF 3rd, Clowse M, Criscione-Schreiber LG, Tochacek M, Monestier M, et al. Microparticles as antigenic targets of antibodies to DNA and nucleosomes in systemic lupus erythematosus. *J Autoimmun*. (2011) 36:173–80. doi: 10.1016/j.jaut.2011.02.001

42. Rother N, Yanginlar C, Pieterse E, Hilbrands L, van der Vlag J. Microparticles in autoimmunity: Cause or consequence of disease? *Front Immunol*. (2022) 13:822995. doi: 10.3389/fimmu.2022.822995

43. She Z, Xie M, Hun M, Abdirahman AS, Li C, Wu F, et al. Immunoregulatory effects of mitochondria transferred by extracellular vesicles. *Front Immunol*. (2020) 11:628576. doi: 10.3389/fimmu.2020.628576

44. Nath V. The spermatid and the sperm of the crab, Paratelphusa spinigera. *J Cell Sci*. (1932) 75:543–56. doi: 10.1242/jcs.s2-75.299.543

45. Neuspiel M, Schauss AC, Braschi E, Zunino R, Rippstein P, Rachubinski RA, et al. Cargo-selected transport from the mitochondria to peroxisomes is mediated by vesicular carriers. *Curr Biol*. (2008) 18:102–8. doi: 10.1016/j.cub.2007.12.038

46. Sugiura A, McLelland GL, Fon EA, McBride HM. A new pathway for mitochondrial quality control: Mitochondrial-derived vesicles. *EMBO J*. (2014) 33:2142–56. doi: 10.15252/embj.201488104

47. Robinson SM, Tsueng G, Sin J, Mangale V, Rahawi S, McIntyre LL, et al. Coxsackievirus B exits the host cell in shed microvesicles displaying autophagosomal markers. *PloS Pathog*. (2014) 10:e1004045. doi: 10.1371/journal.ppat.1004045

48. Sin J, McIntyre L, Stotland A, Feuer R, Gottlieb RA. Coxsackievirus B escapes the infected cell in ejected mitophagosomes. *J Virol*. (2017) 91:e01347-17. doi: 10.1128/JVI.01347-17

49. Youle RJ, Narendra DP. Mechanisms of mitophagy. *Nat Rev Mol Cell Biol*. (2011) 12:9–14. doi: 10.1038/nrm3028

50. Jang SC, Crescitelli R, Cvjetkovic A, Belgrano V, Olofsson Bagge R, Sundfeldt K, et al. Mitochondrial protein enriched extracellular vesicles discovered in human melanoma tissues can be detected in patient plasma. *J Extracell Vesicles*. (2019) 8:1635420. doi: 10.1080/20013078.2019.1635420

51. D'Acunzo P, Perez-Gonzalez R, Kim Y, Hargash T, Miller C, Alldred MJ, et al. Mitovesicles are a novel population of extracellular vesicles of mitochondrial origin altered in Down syndrome. *Sci Adv*. (2021) 7:eabe5085. doi: 10.1126/sciadv.abe5085

52. Soubannier V, McLelland GL, Zunino R, Braschi E, Rippstein P, Fon EA, et al. A vesicular transport pathway shuttles cargo from mitochondria to lysosomes. *Curr Biol*. (2012) 22:135–41. doi: 10.1016/j.cub.2011.11.057

53. Lyamzaev KG, Nepryakhina OK, Saprunova VB, Bakeeva LE, Pletjushkina OY, Chernyak BV, et al. Novel mechanism of elimination of malfunctioning mitochondria (mitoptosis): Formation of mitoptotic bodies and extrusion of mitochondrial material from the cell. *Biochim Biophys Acta*. (2008) 1777:817–25. doi: 10.1016/j.bbabio.2008.03.027

54. Soubannier V, Rippstein P, Kaufman BA, Shoubridge EA, McBride HM. Reconstitution of mitochondria derived vesicle formation demonstrates selective enrichment of oxidized cargo. *PloS One*. (2012) 7:e52830. doi: 10.1371/journal.pone.0052830

55. Lyamzaev KG, Knorre DA, Chernyak BV. Mitoptosis, twenty years after. *Biochem (Mosc).* (2020) 85:1484–98. doi: 10.1134/S0006297920120020

56. Zhai X, Qin Y, Chen Y, Lin L, Wang T, Zhong X, et al. Coxsackievirus B3 induces the formation of autophagosomes in cardiac fibroblasts both *in vitro* and *in vivo*. *Exp Cell Res*. (2016) 349:255–63. doi: 10.1016/j.yexcr.2016.10.020

57. Rabin ER, Hassan SA, Jenson AB, Melnick JL. Coxsackie virus B3 myocarditis in mice. An electron microscopic, immunofluorescent and virus-assay study. *Am J Pathol*. (1964) 44:775–97.

58. Ukimura A, Deguchi H, Kitaura Y, Fujioka S, Hirasawa M, Kawamura K, et al. Intracellular viral localization in murine coxsackievirus-B3 myocarditis. Ultrastructural study by electron microscopic *in situ* hybridization. *Am J Pathol*. (1997) 150:2061–74.

59. Sawaged S, Mota T, Piplani H, Thakur R, Lall D, McCabe E, et al. TBK1 and GABARAP family members suppress coxsackievirus B infection by limiting viral production and promoting autophagic degradation of viral extracellular vesicles. *PLoS Pathog*. (2022) 18:e1010350. doi: 10.1371/journal.ppat.1010350

60. Tian L, Yang Y, Li C, Chen J, Li Z, Li X, et al. The cytotoxicity of coxsackievirus B3 is associated with a blockage of autophagic flux mediated by reduced syntaxin 17 expression. *Cell Death Dis*. (2018) 9:242. doi: 10.1038/s41419-018-0271-0

61. Taylor MP, Kirkegaard K. Modification of cellular autophagy protein LC3 by poliovirus. *J Virol*. (2007) 81:12543–53. doi: 10.1128/JVI.00755-07

62. Kyei GB, Dinkins C, Davis AS, Roberts E, Singh SB, Dong C, et al. Autophagy pathway intersects with HIV-1 biosynthesis and regulates viral yields in macrophages. *J Cell Biol*. (2009) 186:255–68. doi: 10.1083/jcb.200903070

63. Gannage M, Dormann D, Albrecht R, Dengjel J, Torossi T, Ramer PC, et al. Matrix protein 2 of influenza A virus blocks autophagosome fusion with lysosomes. *Cell Host Microbe*. (2009) 6:367–80. doi: 10.1016/j.chom.2009.09.005

64. Kim SJ, Syed GH, Khan M, Chiu WW, Sohail MA, Gish RG, et al. Hepatitis C virus triggers mitochondrial fission and attenuates apoptosis to promote viral persistence. *Proc Natl Acad Sci U S A*. (2014) 111:6413–8. doi: 10.1073/pnas.1321114111

65. Oh SJ, Lim BK, Yun J, Shin OS. CVB3-mediated mitophagy plays an important role in viral replication via abrogation of interferon pathways. *Front Cell Infect Microbiol*. (2021) 11:704494. doi: 10.3389/fcimb.2021.704494

66. Blasius AL, Beutler B. Intracellular Toll-like receptors. *Immunity*. (2010) 32:305–15. doi: 10.1016/j.immuni.2010.03.012

67. Xin L, Xiao Z, Ma X, He F, Yao H, Liu Z. Coxsackievirus B3 induces crosstalk between autophagy and apoptosis to benefit its release after replicating in autophagosomes through a mechanism involving caspase cleavage of autophagy-related proteins. *Infect Genet Evol*. (2014) 26:95–102. doi: 10.1016/j.meegid.2014.05.005

68. Tschope C, Ammirati E, Bozkurt B, Caforio ALP, Cooper LT, Felix SB, et al. Myocarditis and inflammatory cardiomyopathy: Current evidence and future directions. *Nat Rev Cardiol*. (2021) 18:169–93. doi: 10.1038/s41569-020-00435-x

69. Fairweather D, Beetler DJ, Di Florio DN, Musigk N, Heidecker B, Cooper LT Jr. COVID-19, myocarditis and pericarditis. *Circ Res*. (2023) 132:1302–19. doi: 10.1161/CIRCRESAHA.123.321878

70. Chen YH, Du W, Hagemeijer MC, Takvorian PM, Pau C, Cali A, et al. Phosphatidylserine vesicles enable efficient en bloc transmission of enteroviruses. *Cell*. (2015) 160:619–30. doi: 10.1016/j.cell.2015.01.032

71. Lin L, Zhang M, Yan R, Shan H, Diao J, Wei J. Inhibition of Drp1 attenuates mitochondrial damage and myocardial injury in coxsackievirus B3 induced myocarditis. *Biochem Biophys Res Commun*. (2017) 484:550–6. doi: 10.1016/j.bbrc.2017.01.116

72. Bolte HD, Schultheiss HP. Immunological results in myocardial diseases. *Postgraduate Med J*. (1978) 54:500–4. doi: 10.1136/pgmj.54.633.500

73. Schultheiss HP, Bolte HD. Immunological analysis of auto-antibodies against the adenine nucleotide translocator in dilated cardiomyopathy. *J Mol Cell Cardiol*. (1985) 17:603–17. doi: 10.1016/s0022-2828(85)80029-8

74. Schultheiss HP, Schwimmbeck P, Bolte HD, Klingenberg M. The antigenic characteristics and the significance of the adenine nucleotide translocator as a major autoantigen to antimitochondrial antibodies in dilated cardiomyopathy. *Adv Myocardiol*. (1985) 6:311–27.

75. Schultheiss HP, Fairweather D, Caforio ALP, Escher F, Hershberger RE, Lipshultz SE, et al. Dilated cardiomyopathy. *Nat Rev Dis Primers*. (2019) 5:32. doi: 10.1038/s41572-019-0084-1

76. Schultheiss HP, Klingenberg M. Immunochemical characterization of the adenine nucleotide translocator. Organ specificity and conformation specificity. *Eur J Biochem*. (1984) 143:599–605. doi: 10.1111/j.1432-1033.1984.tb08412.x

77. Klein R, Maisch B, Kochsiek K, Berg PA. Demonstration of organ specific antibodies against heart mitochondria (anti-M7) in sera from patients with some forms of heart diseases. *Clin Exp Immunol*. (1984) 58:283–92.

78. Ansari AA, Wang YC, Danner DJ, Gravanis MB, Mayne A, Neckelmann N, et al. Abnormal expression of histocompatibility and mitochondrial antigens by cardiac tissue from patients with myocarditis and dilated cardiomyopathy. *Am J Pathol*. (1991) 139:337–54.

79. Myers JM, Cooper LT, Kem DC, Stavrakis S, Kosanke SD, Shevach EM, et al. Cardiac myosin-Th17 responses promote heart failure in human myocarditis. *JCI Insight*. (2016) 1:e85851. doi: 10.1172/jci.insight.85851

80. Fairweather D, Beetler DJ, Musigk N, Heidecker B, Lyle MA, Cooper LT Jr., et al. Sex and gender differences in myocarditis and dilated cardiomyopathy: An update. *Front Cardiovasc Med*. (2023) 10:1129348. doi: 10.3389/fcvm.2023.1129348

81. Caforio AL, Mahon NJ, Tona F, McKenna WJ. Circulating cardiac autoantibodies in dilated cardiomyopathy and myocarditis: Pathogenetic and clinical significance. *Eur J Heart Fail*. (2002) 4:411–7. doi: 10.1016/s1388-9842(02)00010-7

82. Lv H, Havari E, Pinto S, Gottumukkala RV, Cornivelli L, Raddassi K, et al. Impaired thymic tolerance to alpha-myosin directs autoimmunity to the heart in mice and humans. *J Clin Invest*. (2011) 121:1561–73. doi: 10.1172/JCI44583

83. Gil-Cruz C, Perez-Shibayama C, De Martin A, Ronchi F, van der Borght K, Niederer R, et al. Microbiota-derived peptide mimics drive lethal inflammatory cardiomyopathy. *Science*. (2019) 366:881–6. doi: 10.1126/science.aav3487

84. Neumann DA, Rose NR, Ansari AA, Herskowitz A. Induction of multiple heart autoantibodies in mice with coxsackievirus B3- and cardiac myosin-induced autoimmune myocarditis. *J Immunol*. (1994) 152:343–50. doi: 10.4049/jimmunol.152.1.343

85. Becker YLC, Duvvuri B, Fortin PR, Lood C, Boilard E. The role of mitochondria in rheumatic diseases. *Nat Rev Rheumatol*. (2022) 18:621–40. doi: 10.1038/s41584-022-00834-z

86. Burbano C, Villar-Vesga J, Orejuela J, Munoz C, Vanegas A, Vasquez G, et al. Potential involvement of platelet-derived microparticles and microparticles forming immune complexes during monocyte activation in patients with systemic lupus erythematosus. *Front Immunol*. (2018) 9:322. doi: 10.3389/fimmu.2018.00322

87. Chen PM, Tsokos GC. Mitochondria in the pathogenesis of systemic lupus erythematosus. *Curr Rheumatol Rep*. (2022) 24:88–95. doi: 10.1007/s11926-022-01063-9

88. Fairweather D, Yusung S, Frisancho S, Barrett M, Gatewood S, Steele R, et al. IL-12 receptor beta 1 and Toll-like receptor 4 increase IL-1 beta- and IL-18-associated myocarditis and coxsackievirus replication. *J Immunol*. (2003) 170:4731–7. doi: 10.4049/jimmunol.170.9.4731

89. Eriksson U, Kurrer MO, Sonderegger I, Iezzi G, Tafuri A, Hunziker L, et al. Activation of dendritic cells through the interleukin 1 receptor 1 is critical for the induction of autoimmune myocarditis. *J Exp Med*. (2003) 197:323–31. doi: 10.1084/jem.20021788

90. Liu Q, Zhang D, Hu D, Zhou X, Zhou Y. The role of mitochondria in NLRP3 inflammasome activation. *Mol Immunol*. (2018) 103:115–24. doi: 10.1016/j.molimm.2018.09.010

91. Schindler SM, Frank MG, Annis JL, Maier SF, Klegeris A. Pattern recognition receptors mediate pro-inflammatory effects of extracellular mitochondrial transcription factor A (TFAM). *Mol Cell Neurosci*. (2018) 89:71–9. doi: 10.1016/j.mcn.2018.04.005

92. Wenzel TJ, Bajwa E, Klegeris A. Cytochrome c can be released into extracellular space and modulate functions of human astrocytes in a Toll-like receptor 4-dependent manner. *Biochim Biophys Acta Gen Subj*. (2019) 1863:129400. doi: 10.1016/j.bbagen.2019.07.009

93. Bajwa E, Pointer CB, Klegeris A. The role of mitochondrial damage-associated molecular patterns in chronic neuroinflammation. *Mediators Inflamm*. (2019) 2019:4050796. doi: 10.1155/2019/4050796

94. Murray TE, Wenzel TJ, Simtchouk S, Greuel BK, Gibon J, Klegeris A. Extracellular cardiolipin modulates select immune functions of astrocytes in Toll-like receptor (TLR) 4-dependent manner. *Mediators Inflamm*. (2022) 2022:9946439. doi: 10.1155/2022/9946439

95. Ikeda G, Santoso MR, Tada Y, Li AM, Vaskova E, Jung JH, et al. Mitochondria-rich extracellular vesicles from autologous stem cell-derived cardiomyocytes restore energetics of ischemic myocardium. *J Am Coll Cardiol*. (2021) 77:1073–88. doi: 10.1016/j.jacc.2020.12.060

96. O'Brien CG, Ozen MO, Ikeda G, Vaskova E, Jung JH, Bayardo N, et al. Mitochondria-rich extracellular vesicles rescue patient-specific cardiomyocytes from doxorubicin injury: Insights into the SENECA Trial. *JACC CardioOncol*. (2021) 3:428–40. doi: 10.1016/j.jaccao.2021.05.006

97. Liu D, Gao Y, Liu J, Huang Y, Yin J, Feng Y, et al. Intercellular mitochondrial transfer as a means of tissue revitalization. *Signal Transduct Target Ther*. (2021) 6:65. doi: 10.1038/s41392-020-00440-z

98. Clemente-Suarez VJ, Martin-Rodriguez A, Yanez-Sepulveda R, Tornero-Aguilera JF. Mitochondrial transfer as a novel therapeutic approach in disease diagnosis and treatment. *Int J Mol Sci*. (2023) 24:8848. doi: 10.3390/ijms24108848

99. Anderson MS, Su MA. AIRE expands: New roles in immune tolerance and beyond. *Nat Rev Immunol*. (2016) 16:247–58. doi: 10.1038/nri.2016.9

100. Hasegawa H, Matsumoto T. Mechanisms of tolerance induction by dendritic cells *in vivo*. *Front Immunol*. (2018) 9:350. doi: 10.3389/fimmu.2018.00350

101. Rath S, Sharma R, Gupta R, Ast T, Chan C, Durham TJ, et al. MitoCarta3.0: An updated mitochondrial proteome now with sub-organelle localization and pathway annotations. *Nucleic Acids Res*. (2021) 49:D1541–D7. doi: 10.1093/nar/gkaa1011

102. LaFlam TN, Seumois G, Miller CN, Lwin W, Fasano KJ, Waterfield M, et al. Identification of a novel cis-regulatory element essential for immune tolerance. *J Exp Med*. (2015) 212:1993–2002. doi: 10.1084/jem.20151069

103. Bansal K, Yoshida H, Benoist C, Mathis D. The transcriptional regulator AIRE binds to and activates super-enhancers. *Nat Immunol*. (2017) 18:263–73. doi: 10.1038/ni.3675

frontiers | Frontiers in Immunology

TYPE Review
PUBLISHED 24 April 2024
DOI 10.3389/fimmu.2024.1357378

OPEN ACCESS

EDITED BY
Zhiwen Luo,
Fudan University, China

REVIEWED BY
Liang Xu,
Tongji University School of Medicine, China
Jian-Huan Chen,
Jiangnan University, China
Renwen Wan,
Fudan University, China

*CORRESPONDENCE
Ying Gu
13861870460@163.com
Jiaqi Tang
tangjiaqi75@163.com
Na Li
52smrehab@163.com

†These authors have contributed equally to this work and share first authorship

RECEIVED 18 December 2023
ACCEPTED 03 April 2024
PUBLISHED 24 April 2024

CITATION
Li N, Hu L, Li J, Ye Y, Bao Z, Xu Z, Chen D, Tang J and Gu Y (2024) The Immunomodulatory effect of exosomes in diabetes: a novel and attractive therapeutic tool in diabetes therapy.
Front. Immunol. 15:1357378.
doi: 10.3389/fimmu.2024.1357378

The Immunomodulatory effect of exosomes in diabetes: a novel and attractive therapeutic tool in diabetes therapy

Na Li[1*†], Lingli Hu[2†], Jingyang Li[2], Yang Ye[1], Zhengyang Bao[1], Zhice Xu[1], Daozhen Chen[1], Jiaqi Tang[3*] and Ying Gu[1,4*]

[1]Research Institute for Reproductive Health and Genetic Diseases, Wuxi Maternity and Child Health Care Hospital, Wuxi, Jiangsu, China, [2]Graduate School of Nanjing Medical University, Nanjing, Jiangsu, China, [3]Institute for Fetology, First Affiliated Hospital of Soochow University, Suzhou, Jiangsu, China, [4]Department of Obstetrics, Wuxi Maternity and Child Health Care Hospital Affiliated to Nanjing Medical University, Wuxi, Jiangsu, China

Exosomes carry proteins, metabolites, nucleic acids and lipids from their parent cell of origin. They are derived from cells through exocytosis, are ingested by target cells, and can transfer biological signals between local or distant cells. Therefore, exosomes are often modified in reaction to pathological processes, including infection, cancer, cardiovascular diseases and in response to metabolic perturbations such as obesity and diabetes, all of which involve a significant inflammatory aspect. Here, we discuss how immune cell-derived exosomes origin from neutrophils, T lymphocytes, macrophages impact on the immune reprogramming of diabetes and the associated complications. Besides, exosomes derived from stem cells and their immunomodulatory properties and anti-inflammation effect in diabetes are also reviewed. Moreover, As an important addition to previous reviews, we describes promising directions involving engineered exosomes as well as current challenges of clinical applications in diabetic therapy. Further research on exosomes will explore their potential in translational medicine and provide new avenues for the development of effective clinical diagnostics and therapeutic strategies for immunoregulation of diabetes.

KEYWORDS

exosomes, diabetes, anti-inflammation, immune cells, clinical application

1 Introduction

Diabetes mellitus, a group of metabolic disorders characterized by prolonged high blood sugar levels, is a global health issue affecting over 400 million people worldwide (1). This number is expected to surge to approximately 700 million by 2045 (2). The disease occurs either due to insufficient insulin production by the pancreas or the body's inability to effectively utilize the produced insulin (3). The most common symptoms include weight

loss, polydipsia, polyuria, and constant hunger. If not properly managed, diabetes mellitus can lead to severe complications such as kidney failure, unhealed wounds, vision loss, heart attacks, nerve damage, and even increase the risk of cancer (4). There are three main types of diabetes: type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), and gestational diabetes mellitus. T1DM and T2DM account for 7-12% and 85-90% of global diabetes cases respectively. The rapid increase in diabetes mellitus cases worldwide underscores the disease's significance as a public health concern.

Besides traditional treatment with insulin and oral anti-diabetic drugs, clinicians are attempting to enhance patient care through the use of cell therapies involving embryonic stem cells (ESC), induced pluripotent stem cells (iPSC), and adult mesenchymal stem cells (MSC) (5). However, there are unintended safety concerns such as immune rejection, genetic or disease transfer, and ectopic cell differentiation existing in whole-cell therapy. Recently, exosomes have been reported to play a role in multiple diseases and have been shown to be key mediators of various pathogenetic mechanisms. Compared with cell-based therapy, exosomes contain large amounts of bioactive molecules including proteins and nucleic acids. They exhibit high biocompatibility and low immunogenicity (6), and are able to circulate into distant sites and freely pass across the blood-brain barrier duo to their nanoscale size (7).

Recent studies have shown that exosomes play a role in the occurrence, development, and treatment of diabetes and its complications. However, there are few summaries from the perspective of immunity and inflammation regarding the treatment and mechanisms of exosomes from different cell sources in diabetes and its complications. This review summarizes the latest advances concerning the roles of exosomes and immune regulation/inflammation in diabetes.

2 Description of exosomes

Exosomes are small membrane-bound vesicles secreted by cells, usually between 30 and 200 nanometers in diameter. They play an important role in transmitting information between cells, regulating cell function, and participating in the occurrence and development of diseases (8). The biogenesis of exosomes involves three processes: generation, release, and uptake (9). Within the cells, membrane proteins and lipid molecules responsible for membrane synthesis are synthesized and packaged into endoplasmic reticulum vesicles. Subsequently, these vesicles fuse into polyvesicles. Vesicles in polyvesicles can further fuse to form exosomes (9). The release of exosomes is mainly accomplished through the fusion of polyvesicles with cell membranes. When the polyvesicles fuse with the cell membrane, the inner vesicles are released outside the cell to form exosomes (10). Exosomes are taken up by target cells by means of membrane fusion and endocytosis, and then release their cargo into the cytoplasm to exert their effects (11).Therefore, exosomes may manipulate recipient cells and other organs over a long distance (12).

Previous studies have demonstrated that exosomes, functioning as intercellular junctions, transport proteins, lipids, and nucleic acids to target cells. They are involved in a variety of biological processes including nucleic acid regulation, antigen presentation, metabolite transportation, and inflammation management. Furthermore, they hold potential as diagnostic and therapeutic tools for various diseases (13). Significantly, small non-coding RNAs (ncRNAs), which are approximately 19 to 24 nts in length and are a subset of nucleic acids, have garnered considerable interest within the scientific community due to their regulatory function (14). In this review, we have summarized the involvement of exosomes derived from immune cells and non-immune cells (such as stem cells) in the occurrence and intervention mechanisms of diabetes and its complications, many of which involve ncRNAs (Table 1), based on recent reports. Thus, delivery of multiple ncRNAs via exosomes may have promise over a wide range of applications.

3 Immune cell-derived exosomes and diabetes

In 1996, Raposo et al. reported that B lymphocytes secrete antigen-presenting vesicles (36). Since then, more and more studies have found that exosomes secreted by immune cells interact with cells in the immune system to regulate immune responses (37). Therefore, these membranous vesicles are being explored as potential immunotherapeutic reagents. Immune cell-derived exosomes can activate the immune system through various mechanisms (38). Firstly, they can directly activate immune cells such as dendritic cells and T cells through antigen presentation on their surface. Secondly, they can indirectly activate immune cells by releasing immune-stimulating molecules such as cytokines and chemical mediators. In addition, immunogenic exosomes may also regulate the function of immune cells by transferring immune-related nucleic acid molecules such as miRNA and mRNA. Previous studies have shown that immune-derived exosomes played a role in the development and progression of diabetes mellitus, making them a key regulator in the disease (39).

3.1 The roles of neutrophils-derived exosomes in diabetes

Polymorphonuclear neutrophils (PMNs), which make up 40-70% of all white blood cells in humans, are the most prevalent type of granulocytes. Neutrophils act as the first line of defense against invasive pathogens in the host and have a natural ability to phagocytose pathogens (40). Thus, neutrophils serve as important immune and secretory cells and play a crucial role in inflammation and infection processes (41). The status of the parent cell is reflected in the neutrophils-EXOs, which exhibit strong antibacterial ability due to the presence of components like myeloperoxidase, elastase, dermcidin, and lysozyme (42). In a recent research, investigators loaded extracellular matrix (ECM) hydrogel with vascular endothelial growth factor (VEGF)-encapsulated activated neutrophil exosome mimetics (aPMNEM) to develop VEGF-aPMNEM-ECM hybrid hydrogel for treating chronic diabetic

TABLE 1 Changes of exosomal ncRNAs in diabetes.

Source	Models	Contents	Alteration	Functions	References
adipose tissue macrophages	T2DM	miR-210	increase	promoted diabetes pathogenesis by regulating glucose uptake and mitochondrial CIV activity	(15)
adipose tissue macrophage	T2DM	miR-29a	increase	induced insulin resistance	(16)
M1 macrophage	T2DM	miR-212-5p	increase	restricted insulin secretion	(17)
bone marrow-derived macrophages	T2DM	miR-144-5p	increase	impaired bone regeneration	(18)
macrophage	Diabetic vascular disease	miR-150-5p	decrease	promoted resistin expression in macrophages	(19)
M2 macrophages	Diabetic nephropathy	miR-93-5p	increase	attenuated LPS-induced podocyte apoptosis	(20)
EPCs	Diabetic wounds	miRNA-221-3p	increase	downregulated the expression of p27 and p57 proteins in the cell cycle signaling pathway	(21)
EPCs	Diabetic wounds	miR-126-3p	increase	promoted the recovery of tubulogenic function of high-glucose-impaired HUVECs.	(22)
EPCs	Diabetic stroke	miR-126	increase	attenuated acute injury and promoted neurological function recovery	(23)
EPCs	Diabetic wounds	mmu_circ_0000250	increase	enhanced the therapeutic effect of ADSC-exosomes to promote wound healing	(24)
ADSC	Diabetic wounds	miR-132	increase	reduced inflammation, promoting angiogenesis and stimulated M2-macrophages polarization, promote wound healing	(25)
ADSC	Diabetic wounds	miR-21-5p	increase	induced M2 polarization of macrophages and augmented skin wound healing	(26)
HypADSCs	Diabetic wounds	miR-21-3p/miR-126-5p/miR-31-5p	increase	promoted diabetic wounds healing and inhibited inflammation	(27)
HypADSCs	Diabetic wounds	miR-99b/miR-146-a	decrease	promoted diabetic wounds healing and inhibited inflammation	(27)
MSCs	Diabetic kidney disease	miR-424-5p	increase	alleviated high glucose-induced cell apoptosis and EMT	(28)
MSCs	Diabetic kidney disease	miR-22-3p	increase	protected podocytes and reduced inflammation	(29)
MSCs	Diabetic nephropathy	miR-146a-5p	decrease	restored renal function, facilitated M2 macrophage polarization	(30)
MSCs	Retinal inflammation	miR-126	decrease	reduced high glucose-induced HMGB1 expression and the activity of the NLRP3 inflammasome	(31)
MSCs	Diabetic wounds	miR -155	increase	NA	(32)
MSCs	Diabetic foot ulcer	lncRNA H19	decrease	prevented the apoptosis and inflammation of fibroblasts, leading to the stimulated wound-healing process	(33)
MSCs	Diabetic wound	lncRNA KLF3-AS1	increase	down-regulated miR-383, boosted expression of VEGFA	(34)
MSCs	Diabetic stroke	miR-9	decrease	promoted white matter remodeling and anti-inflammatory responses	(35)

EPCs, endothelial progenitor cells; ADSC, adipocyte-derived stem cell; HypADSCs, hypoxia adipose stem cell; MSCs, mesenchymal stem cells; T2DM, type 2 diabetes mellitus; CIV,continuous intravenous infusion; LPS, lipopolysaccharide; HUVECs, human umbilical vein endothelial cells; EMT, epithelial-mesenchymal transition; HMGB1,high mobility group box 1 protein; NLRP3, nod-like receptor thermal protein domain associated protein 3; VEGFA, vascular endothelial growth factor A; NA, not applicable.

wounds (40). Compared to directly using exosomes or using exosomes derived from other cells, this aPMNEM-ECM based biomaterial has the following advantages (1): for wound infection treatment, aPMNEM can play an antibacterial role via bactericidal-associated proteins (2); as a carrier, aPMNEM can deliver cytokines, and protect them from degradation (3); as a hermosensitive material, ECM can function as an *in situ* gel *in vivo* and increase the residence of aPMNEM. The study not only provided a functional biomaterial for the regeneration of chronic diabetic wounds but also created a promising platform for cytokine therapy, which can potentially be used to treat different diseases by loading various available cytokines in aPMNEM-ECM (40).

3.2 The roles of T lymphocytes-derived exosomes in diabetes

Type 1 diabetes mellitus is an autoimmune disorder characterized by infiltration of the islets of Langerhans by immune cells and by selective elimination of the insulin-secreting β cells (43). Regazzi's team reported that miR-142-3p, miR-142-5p and miR-155 are particularly enriched in T lymphocytes of 8 weeks NOD mice with respect to mouse pancreatic islets (44). In type 1 diabetes, T lymphocytes-EXOs carrying specific microRNAs that induce chemokine expression and apoptosis in recipient pancreatic β cells. The inactivation of miR-142-3p/-5p and miR-155 in β cells leads to increased insulin levels, decreased insulitis scores, reduced inflammation, and provides protection against diabetes development in NOD mice (44).

3.3 The roles of macrophages-derived exosomes in diabetes

Macrophage-derived exosomes have been shown to have diverse functions in immune regulation, tissue repair, and communication between cells (45). Based on the functional profiles, macrophages are divided into two sub-populations: type 1 macrophages (M1, pro-inflammation) and type 2 macrophages (M2, anti-inflammation) (46). M1 macrophages play a role in the early phase of inflammation and are linked to tissue damage and pro-inflammatory activities, whereas M2 macrophages release cytokines that suppress inflammation and have anti-inflammatory effects (47). Recent studies have shown that the macrophages-EXOs contribute to the progression of diabetes (48) (Figure 1).

3.3.1 Exosomes derived from M1 macrophages

3.3.1.1 Impairing insulin sensitivity, secretion and glucose uptake through miRNAs

Chronic tissue inflammation caused by accumulation of M1 macrophages is an important hallmark of insulin resistance. According to prior research, the population of activated M1 macrophages residing within adipose tissue increased in obese mice, resulting in an increased ratio of M1 to M2 macrophages (49). The M1 macrophage is the predominant cell responsible for secreting exosomes containing miR-29a in obese mice (16). MiR-29a targets peroxisome proliferator-activated receptor-δ, leading to impairments of insulin sensitivity both *in vitro* and *in vivo* (16). Moreover, M1 macrophage secreted exosomal miRNA may directly

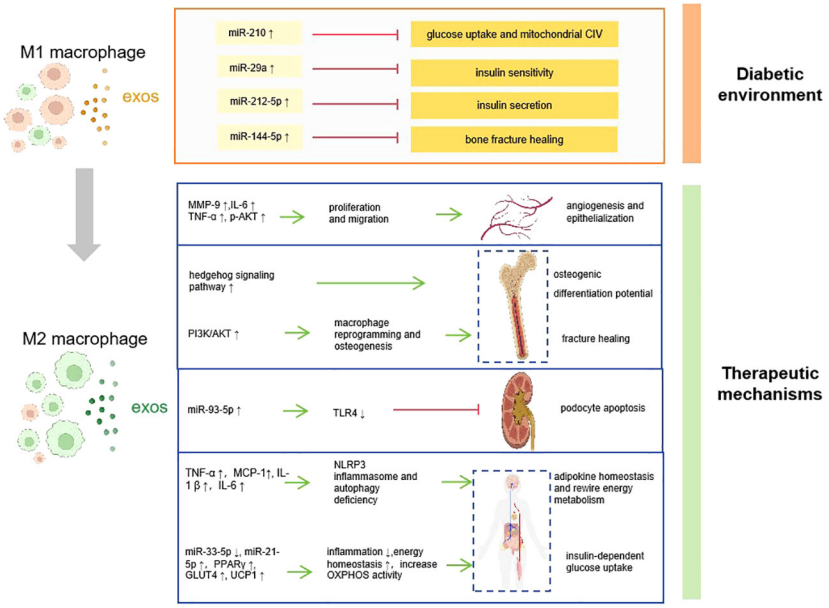


FIGURE 1
How macrophage derived-exosomes contribute to the pathogenesis, complications, and therapy of diabetes. Diabetic environment induce macrophage to M1 polarization, and the M1 macrophage secret exosomes which contains abnormal ncRNAs that promote diabetes and associated complications. Converting the ratio of M1/M2 macrophage polarization is supposed to be a therapeutic application, which accelerates diabetes recovery via various mechanisms. CIV, complex IV; MMP-9, matrix metalloproteinase-9; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; p-AKT, phospho-Akt; PI3K, phosphoinositide 3-kinase; TLR4, toll-like receptor 4; MCP-1, monocyte chemotactic protein-1; interleukin-1β; NLRP3, NOD-like receptor thermal protein domain associated protein 3; PPARγ, peroxisome proliferator-activated receptor γ; GLUT4, glucose transporter type 4; UCP1, uncoupling protein 1; OXPHOX, oxidative phosphorylation ⊣:inhibit →:promote.

give rise to beta cell impairment. Qian et al. reported that the M1 macrophage-EXOs contained miR-212-5p, which regulated the Protein Kinase B (Akt)/Glycogen synthase kinase3β (GSK-3β)/β-catenin pathway in receptor beta cells by targeting the sirtuin 2 gene to restrict insulin secretion (17). Thus, targeting miRNA or inhibiting M1 macrophage-EXOs could be manipulated to inhibit beta cell injury in T2DM.

3.3.1.2 Promoting autophagy deficiency and resistin expression

It was found that high glucose stimulation promoted the polarization of macrophages to the M1-phenotype and produced more exosomes, thereby inducing activation of NOD-like receptor thermal protein domain associated protein 3 (NLRP3) inflammasome and autophagy defects in mesangial cells, promoting development of diabetic nephropathy (50). Besides, exosomal miR-7002-5p are highly expressed in high glucose treated macrophages, which suppress autophagy activity through targeting Atg9b in mouse tubular epithelial cell and C57 mouse kidney (51). In addition to regulate functions of kidney, macrophage-derived exosomes shows impact on diabetic vascular diseases. For example, under high glucose conditions, macrophage-derived exosomal metastasis associated lung adenocarcinoma transcript 1 (MALAT1) is upregulated, inhibiting the expression of miR-150-5p and counteracting its inhibitory effect on macrophage resistance factor expression, and promoting vascular diseases. Thus, macrophage-EXOs containing MALAT1 may serve as a novel target for diabetic vascular diseases (19).

3.3.1.3 Impairing bone fracture healing

Patients with diabetes have an increased risk of nonunion and delayed union of fractures. Exosomes derived from diabetic bone marrow-derived macrophages (dBMDM-EXOs) transfer miR-144-5p to bone marrow stromal cells, inhibiting the expression of Smad1, thereby reducing bone repair and regeneration both *in vivo* and *in vitro* (18). Suppression of miR-144-5p remarkably reversed the adverse effects of dBMDM-EXOs on the osteogenic potential and the ability of fracture repair (18). However, the author didn't test the ratio of M1/M2 or confirm the phenotype of the macrophages that transferred specific miRNAs. Given the function of M1 macrophages, they may be the predominant cell responsible for secreting exosomes containing miR-144-5p, which can lead to bone impairment.

3.3.2 Exosomes derived from M2 macrophages (M2 macrophages-EXOs)

M2 macrophages release cytokines that play a role in anti-inflammatory and tissue repair (47). Previous data validate the association between treatment of diabetic-related diseases and the exosomes secreted by M2 macrophages. For example, the M2 macrophages-EXOs reduced lipopolysaccharides-induced podocyte apoptosis by regulating the miR-93-5p/TLR4 axis, which provided a new perspective for the treatment of diabetic nephropathy patients (20). Tuan et al. Demonstrated (52) that M2 macrophage-EXOs could control chronic inflammatory diseases caused by excessive energy storage. Interleukin 4 (IL-4) stimulated THP-1 macrophage-derived extracellular vesicles can improve the homeostasis of adipose factors, retargeting the energy metabolism of macrophages and adipocytes, thereby controlling the occurrence of cardiac metabolic tissue inflammation in obesity-related diabetes.

In addition to diabetic nephropathy and cardiac diseases, M2 macrophage-EXOs are necessary for accelerating diabetic bone fracture healing. A research has shown that M2 macrophage-EXOs can activate the Hedgehog signaling pathway in BMSCs in a high glucose and high insulin microenvironment, promoting osteogenic differentiation. This suggests that they can serve as a new approach for reshaping the immune homeostasis in diabetic bone (53). Additionally, the research has demonstrated that M2 macrophage-EXOs induced the transformation of M1 macrophages into M2 macrophages by stimulating the phosphoinositide 3-kinase (PI3K)/AKT pathway, significantly reducing the proportion of M1 macrophages and regulating the bone immune microenvironment, thereby accelerating diabetic bone fracture healing (54).

4 Exosomes derived from stem cell and their effect on immune/inflammation in diabetes

In recent years, exosomes-based therapy have gained increasing attention for their comparatively high safety, biocompatibility and low immunogenicity (6). This part reviewed the exosomes from different kinds of stem cells and their main mechanisms underlying regulatory effects on inflammation/immunity in diabetes (Figure 2).

4.1 Cord-blood-derived stem cells

Cord blood-derived stem cells are multipotent stem cells that exhibit a distinct phenotype characterized by both embryonic and hematopoietic markers, distinguishing them from other known stem cell types (55, 56). Phenotypic characterization reveals that CBSCs exhibit embryonic cell markers. Moreover, CBSCs exhibit minimal immunogenicity, as evidenced by their low expression of major histocompatibility complex (MHC) antigens and their inability to stimulate the proliferation of allogeneic lymphocytes (55, 57). Specifically, CBSCs adhere firmly to culture dishes, displaying a large rounded morphology, and are resistant to common detachment methods (trypsin/EDTA), facilitating the collection of suspended lymphocytes after co-culture (55, 57). Based on the unique properties of immune modulation mentioned above and their ability to adhere tightly to the surface of Petri dishes, a new technology called Stem Cell Educator (SCE) therapy was designated for use in clinical trials (58, 59). Stem Cell Educator therapy (Educator therapy) has been utilized with a closed-loop system and open-loop system. During SCE therapy, a

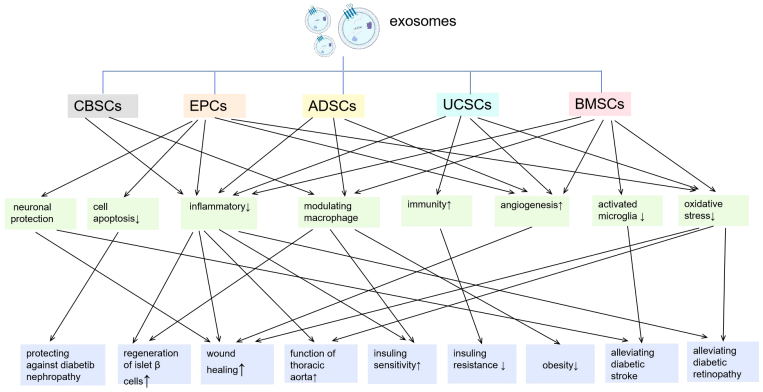


FIGURE 2
Exosomes from different kinds of stem cells and their main mechanisms underlying regulatory effects on inflammation/immunity in diabetes. CBSCs, cord-blood-derived stem cells; EPCs, endothelial progenitor cells; ADSCs, adipose stem cells; UCSCs, umbilical cord mesenchymal stem cells; BMSCs, bone marrow-derived mesenchymal stem cells.

patient's peripheral blood mononuclear cells (PBMCs) are collected and circulated through a cell separator, where they are co-cultured with adherent human CBSCs *in vitro*. The resulting "educated" cells, known as CBSC-treated PBMCs, are then reintroduced into the patient's circulation (60). These "educated" immune cells can educate other immune cells after infusion, thereby reverse the root cause(s) of the autoimmune disease and resulting in the long-lasting clinical efficacy of Educator therapy. Unlike traditional immune therapies, SCE therapy does not destroy the cells responsible for autoimmunity but modifies them (61). The clinical phase 1/2 trials indicate that SCE therapy reverses autoimmunity, promotes regeneration of islet β cells, and improves metabolic control for the treatment of Type 1 diabetes (59, 62, 63) and T2DM (59, 63).

Mechanistic studies revealed that the secretion of CBSC-derived exosomes (CBSC-EXOs) enabled polarization of human blood monocytes/macrophages into M2 macrophages, thereby fundamentally correcting self-immunity and inducing immune tolerance through various molecular and cellular mechanisms (60). CBSC-EXOs preferably and quickly bind to monocytes within 2-3 h. During the coculture of CBSCs with patient's immune cells for clinical treatment during 8-9 h, the SCE-treated monocytes may transport the CBSC-EXOs back into the body, potentially leading to additional M2 differentiation and induction of tolerance (59, 62). Therefore, Educator therapy is the leading immunotherapy to date to safely and efficiently correct autoimmunity through CBSCs mediated immune modulation and anti-inflammatory clinical effects, without the safety and ethical concerns associated with conventional immune and/or stem-cell based approaches.

4.2 Endothelial progenitor cells

Chronic diabetic foot ulceration (DFU) is among the most debilitating long-standing diabetes complications and it is also one of the main causes of physical disability. DFU is partially a result of unregulated foot wound infection caused by neuropathy, hindered angiogenesis, chronic low-grade inflammation, and peripheral vascular/arterial disease (64). Prolonged hyperglycemia intensifies the expression of inflammatory cytokines and reactive oxygen species (ROS), which severely impede angiogenesis (65–67). Thus, wound healing in diabetes always heavily relies on the formation of new blood vessels to restore reperfusion (68). EPCs are the precursors of endothelial cells, which hold great potential in treating chronic non-healing diabetic wounds because of their abilities for vascular and neuronal protection, repair and regenesis (69, 70). Nevertheless, the direct utilization of stem/progenitor cells is constrained by concerns such as potential immunological rejection, chromosomal variation, and emboli formation (71–73). Therefore, it is crucial to devise a new approach that can maximize the therapeutic benefits of stem/progenitor cells while mitigating the risks associated with their direct application.

It has been reported that the exosomes derived from EPCs (EPC-EXOs) can regulate vascular endothelial cells through miRNA. For example (21), EPC-EXOs exhibited a high expression of miRNA-221-3p. Treating skin wounds in diabetic mice with EPC-EXOs demonstrated a similar effect to that seen with miRNA-221-3p administration. MiRNA-221-3p potentially downregulated critical proteins in the AGERAGE signaling pathway, inhibiting reactive oxygen species generation and inactivating nuclear factor-kappa B (NF-kB). This process may reduce inflammatory responses, cell apoptosis, and microvascular diseases. Except for miRNA-221-3p, recent results revealed that treatment with miR-126-3 overexpressing EPC-EXOs accelerated the healing of rat skin wounds and resulted in better tissue repair with slower scar formation. In this process, the expression of caspase-1, NRLP3, interleukin-1b, inteleukin-18, PIK3R2 and SPRED1 was suppressed, promoting diabetic wound repair (22).

Exosomes derived from EPCs were reported to promote angiogenesis and the homing ability of EPCs in diabetic wound healing. Li et al. treated a diabetic rat wound model with EPC-EXOs and found that exosomes enhanced the proliferation, migration and tube formation of vascular endothelial cells *in vitro*. Furthermore,

endothelial cells stimulated with EPC-EXOs showed increase expression of angiogenesis-related molecules such as fibroblast growth factor-1 (FGF-1), VEGFA, VEGFR-2, angiotensin I, E-selectin, Chemokine (C-X-C motif) ligand-16 (CXCR-16), endothelial nitric oxide synthase and IL-8 (74). In addition to promoting angiogenesis in wound healing, microvesicles derived from EPCs were demonstrated to be capable of changing the properties of adipose stem cells (ADSCs), thereby, improving their homing ability to migrate to the wound site. Tu TC et al. transfected exosomes derived from Alde-Low EPCs (EMVs) into human ADSCs. After receiving EMVs, the ADSCs showed a remarkable elevation in the expression of the CXCR4 chemokine receptor *in vitro*, and CD45+ inflammatory cells were successfully recruited to the wound sites *in vivo*, promoting ischemic skin repair (75).

Diabetes mellitus not only increases the risk of ischemia-reperfusion by 3-4 times compared to those without diabetes mellitus, but also exacerbates cerebral damage due to impaired endothelial function and reduced angiogenesis (23). EPCs were demonstrated to hold great potential in the treatment of stoke due to the cerebrovascular protection in the acute phase and promoting neurological recovery in chronic phases (76, 77). Previously experiment in mice indicated that enrichment of miR126 enhanced the therapeutic efficacy of EPC-EXOs on diabetic ischemic stroke by attenuating acute injury and promoting neurological function recovery (23).

Moreover, EPC-EXOs could potentially be a potential therapeutic application for treating Aherosclerosis (AS) resulting from diabetes. AS is a major macrovascular complication of diabetes mellitus characterized by inflammation and endothelial damage (78). The dysfunction of the endothelium is considered an early marker of AS. EPCs are derived from bone marrow and can differentiate into endothelium cells. In cases where ECs are damaged, EPCs may replace them to assist in the recovery from endothelial dysfunction (79). It was demonstrated that EPCs-EXOs had a significant impact on reducing D-AS plaques, lowering the levels of inflammatory factors such as intercellular cell adhesion molecule-1, IL-8, and C-reactive protein, decreasing oxidative stress factors like malondialdehyde and superoxide dismutase, and improving the function of thoracic aorta vasodilation and constriction in a mouse model of diabetic AS (80).

4.3 Mesenchymal stem cell

Mesenchymal stem cells possess various biological characteristics, such as immunomodulation, anti-inflammatory properties, and promotion of angiogenesis, making them widely used in clinical treatment and regenerative medicine (81). MSC-EXOs have been shown to be similar effective as MSCs in the treatment of diabetes and related complications (82–84), but in some contexts, they exert different biological properties (85).

4.3.1 Adipose stem cells

Adipocyte-derived stem cells have been attracting attention as an effective therapeutic tool for tissue regeneration. Exosomes derived from ADSCs (ADSC-EXOs) can ameliorate inflammation by regulating immune cells, thereby promoting the treatment of diabetes and its related complications.

4.3.1.1 ADSC-EXOs modulate macrophage polarization and immune cell activities in diabetes

Zhao et al. demonstrated that treatment with ADSC-EXOs improved metabolic homeostasis in obese mice, including enhanced insulin sensitivity (27.8% improvement), reduced obesity, and alleviated hepatic steatosis. ADSC-EXOs induced M2 macrophage polarization, reduced inflammation, and promoted Beiging in white adipose tissues (WAT) of diet-induced obese mice. Such exosomes carried active signal transducer and activator of transcription 3 (STAT3), which facilitated arginase-1 expression in macrophages, leading to the induction of anti-inflammatory M2 phenotypes. Additionally, the M2 macrophages induced by ADSC-EXOs stimulated ADSC proliferation and lactate production, thereby promoting WAT beiging and maintaining homeostasis in response to high-fat challenge (86). Luo et al. reported that overexpression of hematopoietic prostaglandin D synthase HPGDS in ADSCs accelerated chronic wound healing by improving the anti-inflammatory state and promoting M2 macrophage polarization in type 2 diabetic mice (87). As for M1 macrophages, ADSCs-EXOs play an immunosuppressive role by reducing IFN-α secretion, thus inhibiting activation of T cells, leading to enhanced aggregation capacity of M1 macrophages (88, 89). Besides, ADSC-EXOs promoted T-regulatory cell activation and facilitated wound healing by inhibiting interferon-g production and M1 macrophage accumulation in an EFGR signal-dependent manner (90).

Moreover, recent research found ADSC-EXOs to be a vital source of non-coding RNA to enhance M2 macrophage polarization and promote diabetic wound healing. For example, hypoxic treatment significantly increased circ-Snhg11 contents in ADSC-EXOs and promoted M2 polarization by inhibiting miR-144-3p expression and the STAT3 signaling pathway in skin wounds (91, 92). In another study, the *in vivo* experiment demonstrated that exosomes derived from miR-132-overexpressing ADSC significantly improved the survival of skin flaps and accelerated diabetic wound healing. This was achieved by reducing local inflammation, promoting angiogenesis, and stimulating M2 macrophage polarization through the NF-κB signaling pathway (25). Li et al. found that treating diabetic foot ulcer wounds with ADSC-EXOs increased miR-21-5p levels in macrophages, promoted M2 polarization, and inhibited Keuppel-like factor 6 KLF6, which has been reported to enhance the inflammatory phenotype in macrophages (26).

These findings delineate novel exosome-mediated mechanisms for ADSC-macrophage crosstalk that facilitates immune and metabolic homeostasis, thus providing potential therapy for obesity and diabetes.

4.3.1.2 ADSC-EXOs revers the inflammatory condition in wound healing

Wound healing can be delayed by chronic and excessive inflammation, therefore a well-regulated inflammation guarantees wound healing (88). ADSCs-EXOs contain immunoregulatory

proteins such as tumor necrosis factor-α (TNF-α), macrophage colony-stimulating factor and retinol-binding protein 4 (93). In addition to the local effects, ADSC-EXOs can reverse the systematic inflammatory condition in diabetes models. Qiu et al. demonstrated that high glucose treatment significantly increased inflammatory factors IL-6, IL-1β, and TNF-α levels in EPCs from healthy volunteers. Such elevated levels could be partially and completely reversed by ADSC-EXOs and linc00511-overexpressing ADSCs (94). They found Exosomes from linc00511-overexpressing ADSCs promotes diabetic foot ulcers healing by accelerating angiogenesis via suppressing PAQR3-induced Twist1 ubiquitin degradation as well as suppressed inflammatory. Zhang et al. found that ADSC-EXOs significantly reduced levels of inflammatory cytokines IL-6, TNF-a, and monocyte chemotactic protein-1 (MCP-1) by decreasing ROS production and protecting mitochondrial function via sirtuin-3 (95). Wang et al. found that hypoxic ADSC-EXOs exhibited distinct miRNA expression profiles compared to ADSC-EXOs. Specifically, up-regulation of miR-21-3p, miR-126-5p, and miR-31-5p, and down-regulation of miR-99b and miR-146-a in hypoxic ADSC-EXOs promoted wound healing in diabetic mice and suppressed inflammatory factors through the PI3K/AKT signaling pathway (27). Shi reported that exosomes derived from mmu_circ_0000250-modified ADSCs promoted wound healing in diabetic mice by inducing miR-128-3p/SIRT1-mediated autophagy and improving the hyperglycemic-induced inflammatory microenvironment and recover the function of EPCs (24).

4.3.2 Umbilical cord mesenchymal stem cells

Human umbilical cord tissue (Wharton's jelly) serves as a potent and rich source of MSCs. UCSCs-derived exosomes (UCSC-EXOs) have shown promising results in the treatment of diabetes and may become a successful strategy for treating diabetes and its complications. Injection of UCSC-EXOs significantly ameliorated hyperglycemia in rats with T2DM (96). Besides, UCSC-EXOs also contributes to the therapy of other diabetic complications, such as diabetic nephropathy, retinopathy and wound ulcer.

4.3.2.1 UCSC-EXOs increase insulin sensitivity by suppress inflammatory factors

Chronic inflammation in tissues is typically the primary cause of insulin resistance, which results in the secretion of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) or IL-6 by inflammatory cells. These cytokines then inhibit the activation of the insulin signaling pathway (97, 98). It is found that injection of human UCSC-EXOs significantly ameliorated hyperglycemia in rats with T2DM. UCSC-EXOs could increase insulin sensitivity by increasing the activation of insulin/AKT signaling pathway and inhibiting the secretion of proinflammatory cytokines like TNF-α, which could reverse insulin resistance in T2DM (96).

4.3.2.2 The role of UCSC-EXOs in diabetic nephropathy

It is demonstrated that UCSC-EXOs could be a promising treatment strategy for diabetic nephropathy rats. Xiang et al. reported that UCSC-EXOs apparently reduced the levels of pro-inflammatory cytokines (IL-6, IL-1β, and TNF-α) and pro-fibrotic factor transforming growth factor β (TGF-β) in the kidney and blood of diabetic nephropathy rats. *In vitro* experiments showed that umbilical cord MSC conditioned medium and UCSC-EXOs decreased the production of these cytokines in high glucose injured renal tubular epithelial cells, and renal glomerular endothelial cells (99). Besides, UCSC-EXOs miR-424-5p can inhibit the activation of yes associated protein 1 in HK2 cells, reduce cell apoptosis, and epithelial-to-mesenchymal transition induced by high glucose, thereby attenuating diabetic nephropathy (28). MiR-22-3p, highly expressed in UCSC-EXOs, may play a protective role in podocytes and diabetic mice by regulating the NLRP3 inflammasome. This suggests that MSC-derived exosomes could be a promising cell-free therapeutic strategy for diabetic kidney disease (29). Another study showed that UCSC-EXOs miR-146a-5p enhanced M2 macrophage polarization by inhibiting the TRAF6/STAT1 signaling pathway, thereby protecting against diabetic nephropathy in rats (30).

4.3.2.3 The role of UCSC-EXOs in wound healing and diabetic retinopathy

UCSC-EXOs serve as a novel therapeutic approach to enhance wound healing in diabetes. Studies have shown that UCSC-EXOs can induce anti-inflammatory macrophages (100), leading to a reduction in the expression of inflammatory factors such as IL-1β, IL-6, and TNF-α (101), as well as promoting angiogenesis and collagen deposition. Furthermore, UCSC-EXOs have the potential to inhibit oxidative stress injury, thereby facilitating macro-level angiogenesis and ultimately expediting the healing of diabetic wounds (101).

In addition to diabetic wounds, diabetic retinopathy is another common complication of diabetes. Previous studies have shown the therapeutic effect of UCSC-EXOs in diabetic retinopathy. For example, the administration of miR-126-expressing UCSC-EXOs significantly reduced high glucose-induced high-mobility group box 1 expression and the activity of the NLRP3 inflammasome in human retinal endothelial cells, therefore suppressing suppressed inflammation in diabetic rats (31).

At last, UCSC-EXOs treatment could be beneficial for diabetic rats to recover from the anemia-like symptoms and increase immunity by improving the erythrocytes and hemoglobin levels as well as maintaining the number of white blood cells (102). 1 mg/kg of UCSC-EXOs improved glucose tolerance in T2DM rats and ameliorate insulin resistance. Moreover, there was no significant difference in white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, and basophils between the diabetic rat groups treated with both glibenclamide (one of the traditional hypoglycemic drug) and 1 mg/kg of UCSC-EXOs and the non-diabetic animal group. This finding suggests that the administration of UCSC-EXOs at 1 mg/kg could improve the immune system of diabetic rats, which is essential for reducing infections and increasing survival rates (102).

4.3.3 Bone marrow-derived mesenchymal stem cells

Bone marrow mesenchymal stem cells are multilineage progenitors with self-renewal, multidirectional differentiation, and pleiotropic paracrine functions (103). It is demonstrated that purified

BMSC-derived exosomes (BMSC-EXOs) have more specific distinct benefits in damaged tissue repair than BMSCs themselves, including superior stability, tissue permeability, excellent biocompatibility, and immunomodulatory properties (104).

4.3.3.1 The role of BMSC-EXOs in diabetic wound healing

Accumulative studies have shown that BMSC-EXOs contribute to wound healing through non-coding RNAs. For example, Liu et al. found that miR-155-inhibitor-loaded BMSC-EXOs enhanced keratinocytes migration, FGF-7 recovery, and anti-inflammatory effects *in vitro*. Additionally, they could also be utilized to treat a diabetic wound model by promoting collagen deposition, angiogenesis, and re-epithelization. The functional coordination between miR-155-inhibitor and BMSC-EXOs played a crucial role in enhancing diabetic wound healing (32). Li reported that the injection of BMSC-EXOs overexpressing lncRNA H19 facilitated wound healing in mice with diabetic foot ulcers. Results revealed that BMSC-EXOs overexpressing lncRNA H19 led to higher level of IL-10 and lower levels of IL-1b and TNF-a, and the mechanism by which was associated with promoting fibroblast proliferation and migration, inhibiting cell apoptosis and inflammation (33). In a murine diabetic cutaneous wound model, exosomes from lncRNA KLF3-AS1-expressing BMSCs demonstrated the best effects in promoting cutaneous wound healing in diabetic mice, which were associated with minimal weight loss, increased blood vessel formation, reduced inflammation, decreased miR-383 expression, and up-regulated VEGFA (34). Except for non-coding RNAs, the anti-inflammation effect by BMSC-EXOs could induced by specific pathways that may not directly related to non-coding RNAs. Wang reported that the wounds treated with exosomes showed reduced inflammation, with decreased levels of the inflammatory cytokines TNF-α and IL-1β, and increased levels of the anti-inflammatory cytokines IL-4 and IL-10 (105). Such regenerative and anti-inflammatory effects were eliminated by Lenti-sh-Nrf2 administration, suggesting the participation of the activation of Nrf2 anti-oxidant pathway in wound healing by exosomes. In addition to miRNAs, Liu et al. reported that melatonin-pretreated BMSC-EXOs could promote diabetic wound healing by suppressing the inflammatory response, which was achieved by increasing the ratio of M2 polarization to M1 polarization through activating the phosphatase and tensin homolog/AKT signaling pathway (106).

4.3.3.2 The role of BMSC-EXOs in diabetic stroke

Diabetes increases the risk of stroke by 3-4 fold, and about 30% of stroke patients suffer from diabetes (107). Treating patients with diabetic stroke is challenging because it may cause extensive damage to the cerebral vasculature, exacerbate neurological deficits, enhance inflammatory responses, which are prone to recurrent strokes (108, 109). Therefore, it is crucial to devise therapeutic strategies specifically aimed at enhancing neurological function after stroke in individuals with diabetes. MSCs interact with and alter brain parenchymal cells via the secretion of trophic and growth factors as well as exosomes to exert therapeutic effects (110). Exosome therapy offers several advantages compared to cell therapy, as exosomes do not elicit immune rejection, do not cause vascular obstruction, and have a low risk of triggering tumors or malignant transformation (111). Besides, exosomes are more suitable for clinical use since they are relatively stable, can be obtained in large quantities from a small number of cells, and can be stored until therapeutic needed (112). Therefore, systemic administration of exosomes could be a method of delivering the active components of cell therapy to the central nervous system (113).

Studies (35, 114) have indicated that T2DM stroke was associated with increased inflammatory responses and proinflammatory microglial/macrophage phenotype. The inflammatory factor matrix metalloproteinase-9 (MMP-9) was elevated after stroke and has been implicated in aggravating blood-brain barrier disruption, neuronal death, myelin degradation and white matter injury. In addition, the inflammatory factor MCP-1 was elevated in the serum of both diabetic and stroke patients, and it aids in the accumulation of phagocytic M1 macrophages in the infarct border (115, 116). However, T2DM-BMSC-EXOs treatment has been demonstrated to significantly decrease activated microglia, M1 macrophage, and inflammatory factors MMP-9 and MCP-1 expression in the ischemic brain in T2DM stroke rats (35). Such therapeutic effects in neurological functional recovery were only induced by injection of exosomes derived from BMSCs of T2DM rats but not from BMSCs of non-diabetic animals, which may be partially mediated by decreasing miR-9 and upregulating ABCA1-IGFR1 pathway (35).

4.3.3.3 The role of BMSC-EXOs in diabetic retinopathy

BMSCs-Exos also possess other immunomodulatory properties and can suppress the activation and function of various immune cells involved in islet transplantation and diabetic retinopathy. It is reported that co-delivery of siFas and anti-miR-375 by BMSCs and derived exosomes suppressed early apoptosis of transplanted human islets, while further immune activity could be suppressed by intravenously injection of human BMSC and PBMC co-cultured exosomes. Thus, BMSC and peripheral blood mononuclear cell co-cultured exosomes performed a immunosuppressive effect for improving islet transplantation (117). Besides, BMSC-EXOs improve diabetes-induced retinal damage by inhibiting the Wnt/β-catenin signaling pathway, subsequently reducing oxidative stress, inflammation, and angiogenesis (118). BMSC-EXOs miR-146a regulates the inflammatory response of diabetic retinopathy by mediating the TLR4/MyD88/NF-κB pathway, reducing the levels of TNF-α, IL-1β, and IL-6 (119).

5 Exosomes as an innovative therapeutic tools for diabetes: current status and promising directions

5.1 Promising directions

Exosomes exhibit high biocompatibility and low immunogenicity, which makes them have great potential in delivering nucleic acid sequences and chemotherapy drugs (6). However, studies have shown that the natural half-life of most exosomes *in vivo* is relatively short (<6 h) (120), and the contents of natural exosomes

are limited by the secreting cells, resulting in limited therapeutic effects when loaded with drug molecules. To date, increasing researches demonstrated that under certain stress or modified conditions, stem cells can produce more exosomes or exosomes with different compositions compared to basal conditions. Meanwhile, many studies demonstrated the beneficial effects of modified or pretreated stem cell-derived exosomes on preventing comorbidities or microvascular complications in diabetes. These benefits mainly stem from the following three perspectives (Table 2): a. Exosomes from genetically modified stem cells display enhanced effects on diabetic wound healing compared to wild-type exosomes; b. By adding specific drugs to the culture medium, cells may secrete exosomes that are more effective in targeting angiogenesis, anti-inflammation, promoting proliferation and migration, and inhibiting apoptosis; c. Under certain stress conditions, such as hypoxia, cells may secrete exosomes that

TABLE 2 Pre-intervention to improve the function of exosomes in the treatment of diabetes.

Disease and animal	Cell type releasing Exo	Intervention	Pathways	Effect: *in virto*	Effect: *in vivo*	Effect on inflammation /immune system	ref
Diabetic cutaneous wound, Rat	hAMSCs	miR-21-5p overexpressing	Wnt/β-catenin pathways ↑	proliferation and migration of keratinocyte cells ↑	vessel growth and maturing ↑, wound healing process ↑	inflammatory cell infiltration↓	(121)
Diabetic wound, Mice	hAMSCs	hypoxia	PI3K/Akt pathways ↑	fibroblast proliferation and migration ↑	re-epithelialization ↑	CD31↑, TGF-β ↑, COLI ↑ and COLIII ↑, IL-6 ↓	(27)
Diabetic full-thickness excisional wound, Mice	ADSCs	mmu_circ_0000250-overexpressing	miR-128-3p/SIRT1 pathway↑	HG-induced EPC apoptosis ↓, autophagy of EPC ↑	wound closure ↑	SIRT1-mediated anti-inflammatory ↑	(24)
Diabetic foot ulcer, Mice	ADSCs	mmu_circ_0001052 overexpressing	miR-106a-5p ↓, FGF4/p38MAPK pathway ↑	proliferation ↑, migration and angiogenesis of high glucose-induced HUVEC ↑	speed of healing ↑	NA	(122)
Diabetic foot ulcer, Rat	ADSC	Nrf2 overexpression	SMP30 ↑, VEGF ↑, p-VEGFR2 ↑, ROS ↓	increased cell viability ↑, tube formation of EPCs ↑	Ulcerated area ↓, angiogenesis ↑, inflammation ↓, oxidative stress ↓	IL-1β ↓, IL-6 ↓, TNF-α ↓	(123)
Diabetic full-thickness wounds, Rat	BMSC	atorvastatin pretreated	AKT/eNOS pathway ↑	endothelial cell angiogenesis↑	Ascularization ↑ , the wound healing ↑	NA	(124)
Diabetic full thickness dermal dorsal defect, Rat	BMSC	pioglitazone-pretreated	PI3K/AKT/eNOS pathway ↑	migration and tube formation ↑, wound repair ↑, VEGF expression of HUVEC ↑	diabetic wound healing ↑, angiogenesis ↑	NA	(125)
Diabetic full-thickness dermal defect, Rat	BMSC	melatonin-pretreated	PTEN/AKT pathway ↑	ratio of M2 polarization to M1 polarization in RAW264.7 cells ↑	angiogenesis and collagen synthesis ↑	ratio of M2 / M1 polarization ↑,IL-1β ↓, TNF-α ↓, IL-10 ↑, Arg-1 ↑	(106)
Diabetic punch biopsy excisional wound, Mice	BMSC	HOTAIR overexpressing	NA	HOTAIR ↑,VEGF ↑ in endothelial cells	angiogenesis ↑ and wound healin ↑	NA	(125)
Diabetic foot ulcer, mice	BMSC	lncRNA H19 overexpression	miR-152-3p-mediated PTEN inhibition ↓	apoptosis and inflammation of fibroblasts ↓	flammatory cells ↓, granulation tissues thicker around the wound	IL-10 ↑, IL-1b ↓, TNF-a ↓	(33)

(Continued)

TABLE 2 Continued

Disease and animal	Cell type releasing Exo	Intervention	Pathways	Effect: *in virto*	Effect: *in vivo*	Effect on inflammation /immune system	ref
diabetic wounds rat	HEK293	miR-31-5p overexpression	HIF1AN ↓, EMP-1↓	cell proliferation ↑ and migration ↑ in ECs, HFF-1 cells and HaCaT cells; capillary-like construction activity ↑ in ECs	proangiogenesis ↑, profi ↑, brogenesis ↑, reepithelization↑	NA	(126)
Diabetic cutaneous wound, Rat	UC-MSC	Lipopolysaccharide-pretreated	M2 macrophage polarization ↑ through let-7b via TLR4/NF-κB/STAT3/AKT pathway	converted inflammatory THP-1 cells to M2 polarization	inflammatory cell infiltration ↓, new small capillaries and woundhealing ↑	anti-inflammatory cytokines ↑, M2 macrophage activation ↑	(127)

hAMSCs, human adipose-derived mesenchymal stem cells; ADSCs, adipocyte-derived stem cells; ADSC, adipocyte-derived stem cell; BMSC, bone mesenchymal stem cells; HEK293, human embryonic kidney 293T cells; UC-MSC, Umbilical cord-derived mesenchymal stem cells; PI3K, phosphatidyl-inositol 3-kinase; AKT, protein kinase b; SIRT1, silent information regulator 1; FGF4, fibroblast growth factor 4; p38MAPK, P38 mitogen-activated protein kinase; SMP30, senescence marker protein 30; VEGF, vascular endothelial growth factor; VEGFR2 , vascular endothelial growth factor receptor 2; ROS, reactive oxygen species; eNOS, endothelial nitric oxide synthase; NA, ot applicabl; HIF1AN, hypoxia inducible factor 1 subunit alpha inhibitor; EMP-1, EPO mimetic peptide-1; TLR4, toll-like receptor 4; NF-κB,nuclear factor kappa-B; STAT3, Signal transducer and activator of transcription 3; EPC, endothelial progenitor cells; HUVEC, human umbilical vein endothelial cells; VEGF, vascular endothelial growth factor; HOTAIR, HOX transcript antisense RNA; ECs, early career specialists; THP, human monocytic-leukemia cells; CD31, platelet endothelial cell adhesion molecule-1; TGF-β, transforming growth factor β; COLI, Collagen I; IL-6, Interleukin 6; IL-1β, Interleukin-1β; TNF-α,Tumor Necrosis Factor-α; IL-10, Interleukin-10; Arg-1, Arginase 1; IL-1b, Interleukin-1β.

perform better in promoting fibroblast proliferation and migration, and enhancing reepithelialization in chronic wounds. All the above demonstrated that preconditioning or pre-treatment of diabetic MSCs with various agents/stress can be used to optimize/improve cellular function prior to their use in cell therapy.

In addition to modify the donor cells that produce exosomes, direct modification to purified natural exosomes may efficiently and quickly obtain a large number of engineered exosomes, and reduce the uncertainty in the cell culture process, which is of great significance for the mass production of engineered exosomes. For example, taking advantage of natural availability and biocompatibility of exosomes as extracellular miRNA transporting particles (121), Lv et al. reported a human hASC-exos-based miRNA delivery strategy which loaded miRNA into hASC-exos by electroporation. Besides electroporation, other physical methods such as ultrasonic homogenization (128), freeze-thaw cycle (129), may also allow drugs to enter the exosomes more easily, achieving the purpose of engineering exosomes. However, such methods were usually used in treatment of cancers *in vitro* or *in vivo* in animal models, therefore, future research will focus more on the application of these methods in the treatment of diabetes and the associated complications.

Finally, in recent years, due to the high biocompatibility and modifiability, composite hydrogels loaded with exosomes and other nanoparticles have gained increasing attention in managing chronic diabetic wounds. Compared to traditional stem cell therapy, which has been shown to have short survival times, poor stability, and a high risk of immune rejection in diabetic ulcers (130), exosomes-loaded composite hydrogels have been demonstrated to possess superior functions in angiogenesis, anti-inflammatory, antibacterial, and antioxidant properties (Table 3). Since different agents have varying applicability, advantages and disadvantages for wound healing, various therapeutic agents can be incorporated inside the multifunctional hydrogel to create an outstanding drug delivery system (143). Thus, the exosomes-loaded, "all-in-one" composite hydrogels may achieve a controlled drug delivery in diabetic wound healing, prone to better drug applications.

TABLE 3 Functions of composite hydrogels in the treatment of diabetic wound healing (2020 to date).

Publication year	Cell type releasing EXOs	Hydrogels	Anti-inflammatory effect	Antibacterial effect	Angiogenesis	Antioxidant effect	ref
2020	CBSCs	PF-127 hydrogel	inflammatory cell infiltration ↓	unknown	TGFβ-1 ↑, VEGF ↑	unknown	(130)
2022	M2Φ	HA-based hydrogels composed of MnO2 and FGF-2	unknow	+	angiogenic ability ↑	ameliorated ROS damage	(127)
2022	ADSCs	ADSC-exo@MMP-PEG smart	unknow	unknown	CD31 and α-SMA ↑, re-epithelialization and collagen deposition ↑	ROS level ↓	(131)

(Continued)

TABLE 3 Continued

Publication year	Cell type releasing EXOs	Hydrogels	Anti-inflammatory effect	Antibacterial effect	Angiogenesis	Antioxidant effect	ref
2022	HUVECs	GelMA/PEGDA@T+exos MNs patch	unknow	unknown	angiogenesis ↑	unknown	(132)
2022	BMSCs	carboxyethyl chitosan -dialdehyde carboxymethyl cellulose hydrogel	skewing macrophage M1 to M2 phenotype	+	Angiogenesis ↑, VEGF-mediated signaling pathways ↑	unknown	(133)
2022	ESCs	Gel-VH-EVs	unknow	unknown	angiogenesis ↑, HIF-1α-mediated pathway ↑	unknown	(134)
2023	ADSCs	hydrogel loaded with 4-Arm-PEG-Thiol, Ag^{+}, exosomes, CNTs, and metformin hydrochloride	IL-6 ↓, TNF-α ↓, ICAM and VCAM ↓	+	density and quantity of blood vessels ↑	ROS and mtROS production ↓	(135)
2023	M2Φ	hydrogel combined with bioactive M2-Exos and gold nanorods	proinflammatory cytokines ↓	+	CD31+ ↑, vascular network formation ↑	SOD1 ↑, PRDX2 ↑	(136)
2023	ADSCs	extracellular matrix hydrogel	TNF-α ↓, IL-6 ↓	unknow	collagen deposition ↑, skin regeneration ↑, blood vessel numbers ↑	unknown	(136)
2023	PMN	VEGF-aPMNEM-ECM hybrid hydrogel	M1 macrophage transform to M2 macrophage ↑	+	number of blood vessels↑	unknown	(40)
2023	ADSCs	GelMA-Exo hydrogels	unknow	unknow	proliferation, invasion, and tube formation ↑	unknown	(137)
2023	HUVECs	ADM Fe3+@PA-Exos/GelMA	IL-1β ↓	+	proliferation and migration impairment ↓	SOD and GSH-Px activity ↑	(138)
2023	HUVECs	hypoxic exosomes-loaded HGM-QCS hydrogels	IL-6 ↓, TNF-α ↓, ICAM-1↓, SELE ↓, VCAM-1 ↓, M2 polarization ↑	+	collagen deposition ↑, angiogenesis ↑	ROS level ↓	(139)
2024	Umbilical cord blood	UCB-Exos into an ABA-type amphiphilic hydrogel	unknow	unknow	proliferation and tube formation ↑	unknown	(140)
2024	Whole blood	P-Exos-loaded CMC hydrogeL	unknow	unknow	angiogenesis ↑, VEGF mediated signaling pathways ↑	unknown	(141)
2024	hUC-MSCs	hydrogel composed of chitosan nanoparticles, MSC- derived, BG, and TiO2	TGF-β and IL-10 ↑, TNF-α ↓, IL-1β ↓, IL-6 ↓	+	enhanced angiogenesis of ECs by targeting VEGFA and VEGFR2	unknown	(142)

M2Φ, M2 macrophages; ADSCs, adipose-derived stem cells; HUVECs, human umbilical vein endothelial cells; BMSCs, bone marrow mesenchymal stromal cells; ESCs, embryonic stem cell; PMN, polymorphonuclear neutrophils; hUC-MSCs, human umbilical cord mesenchymal stem cells; MnO2, manganese dioxide; FGF-2, fibroblast growth factor-2; MMP, matrix metalloproteinases; PEG, polyethylene glycol; GelMA, gelatin methacryloyl; PEGDA, poly (ethylene glycol) diacrylate; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; ICAM, intercellular cell adhesion molecule; VCAM, vascular cell adhesion molecule; IL-1β, interleukin—1β; ICAM-1, intercellular cell adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; TGF-β, transforming growth factor-β; IL-10, interleukin-10; VEGF, vascular endothelial growth factor; CD31, platelet endothelial cell adhesion molecule-1; α-SMA, α-smooth muscle actin; VEGFA, vascular endothelial growth factor A; VEGFR2, vascular endothelial growth factor receptor 2; ROS, reactive oxygen species; mtROS, mitochondrial reactive oxygen species; SOD1, recombinant superoxide dismutase 1; PRDX2, peroxiredoxin-2; GSH-Px, glutathione peroxidase; SOD, recombinant superoxide dismutase.

5.2 Current challenges of clinical applications

So far, there are mainly three challenges in the clinical translations of exosomes. Firstly, minimize the therapeutic efficacy differences caused by physiological and structural variations between human and animals. Exosomes derived from various stem cell sources have been used in wound healing treatments across animal models including mice (144, 145), rats (123), rabbits (146), consistently demonstrating positive effects such as improved wound closure, reduced healing time, enhanced angiogenesis, and diminished scar formation. However, the outcomes of these preclinical studies do not necessarily translate to human skin due to significant differences in skin structure and physiology, with pig skin being the closest analogue to human skin. Porcine models have emerged as promising models to study wound healing, they possess similar anatomically and physiologically characteristics to humans, including a relatively thick epidermis, distinct rete pegs, dermal papillae, and dense elastic fibers in the dermis (147), porcine collagen (148) et al. In contrast to rodent, rabbit, and canine skin, which exhibits loos adherence to the subcutaneous fascia, porcine skin closely adheres to the underlying structures, resembling human skin (149). The turnover time of pig epidermis is similar to the human epidermis (150). Moreover, the immune cells in pig skin resemble those found in human skin (151). According to research by Sullivan and colleagues, pig models were 78% concordant with human studies. This result exceeded other small-mammal and *in vitro* models, which were only 53% and 57% concordant (152). Therefore, it is crucial to validate the biological effects of exosomes on wound healing using a pig model.

Secondly, the clinical translation of engineered extracellular vesicles is urgently needed. So far, clinical applications of these exosomes are limited to only a few clinical trials exploring the therapeutic effects of stem cell-derived exosomes for diabetes and its complications, such as wound healing. According to data from ClinicalTrials.gov, to date, three completed clinical trials have utilized exosomes derived from plasma (NCT02565264), adipose tissue (NCT05475418), and mesenchymal stem cells (NCT05813379) for wound healing. Another (NCT04134676) has explored the use of stem cell-conditioned medium for chronic ulcer wounds. Apart from wound treatment, very few clinical trials have investigated the use of exosomes for other diabetic conditions [only one for Type 1 diabetes (NCT02138331)].

Thirdly, The scaling-up manufacture of "Good Manufacturing Practice" (GMP)-grade exosomes is the most difficult component in the clinical use of exosomes. Challenges in the further clinical application of exosomes include quality control, such as the cell-culture system, purification, characterization/physicochemical and biological properties of exosomes, as well as the establishment of a "gold standard" for potency assay. Thus, advances in scaling-up technology for GMP-compliant exosomes manufacturing will enhance the clinical applications of these entities for diabetes and the related complications in the near future.

6 Concluding remarks and future perspectives

As a promising candidate for novel cell free therapy, exosomes may be widely used as an alternative to stem cells in management of a variety of immunity-related diseases or inflammation response for maintenance of the microenvironment for tissue homeostasis and tissue regeneration upon injury. In this review article, we describe how immune cell-derived exosomes origin from neutrophils, T lymphocytes and macrophages impact on diabetes and the associated complications. We also discuss the stem cell-derived exosomes and their role in immunomodulatory and inflammation in the progress of diabetic complications. In addition, promising directions involving engineered exosomes as well as current challenges of clinical applications are reviewed. The enhanced properties of engineered exosomes have been verified in lab, which proves that they have great clinical application prospects. However, there is still a long way to go before commercial exosome products are ready for the market, due to the lack of clinical trials and quality control for scaling-up manufacture.

In addition to the above challenges, some questions remain unanswered, which needs more attention to be paid to in the future. For example, how do exosomes transferred specific miRNAs target the genes in recipient cells? Besides, studies about gestational diabetes mellitus (GDM) are still limited. Although researchers have found that some exosomal non-coding RNAs in peripheral blood may be early diagnostic markers for GDM, it is unknown how exosomes interact with the immune system and contribute to the pathophysiology of GDM. Nevertheless, we remain confident that the hurdles facing these innovative approaches will be surmounted and that they will do influence the treatment of diabetes.

Author contributions

NL: Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing. LH: Data curation, Resources, Writing – original draft. JL: Data curation, Investigation, Project administration, Writing – original draft. YY: Data curation, Formal analysis, Methodology, Writing – original draft. ZB: Writing – review & editing. ZX: Conceptualization, Supervision, Writing – review & editing. DC: Supervision, Writing – review & editing. JT: Conceptualization, Funding acquisition, Investigation, Validation, Writing – review & editing. YG: Funding acquisition, Project administration, Resources, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by Top Talent Support Program for young and middle-aged people of Wuxi Health Committee, Youth Project of Wuxi Municipal Health Commission (Q202104), Research Project of Wuxi Municipal Health Commission (M202217) and the Taihu Rencai Project.

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.

The reviewer J-HC declared a shared parent affiliation with the authors NL, YY, ZX, DC, and YG to the handling editor at the time of review.

Publisher's note

References

1. Punthakee Z, Goldenberg R, Katz P. Definition, classification and diagnosis of diabetes, prediabetes and metabolic syndrome. *Can J Diabetes*. (2018) 42 Suppl 1:S10–s5. doi: 10.1016/j.jcjd.2017.10.003

2. Kamal MM, Kassem DH. Therapeutic potential of wharton's jelly mesenchymal stem cells for diabetes: achievements and challenges. *Front Cell Dev Biol*. (2020) 8:16. doi: 10.3389/fcell.2020.00016

3. Jia G, Whaley-Connell A, Sowers JR. Diabetic cardiomyopathy: a hyperglycaemia- and insulin-resistance-induced heart disease. *Diabetologia*. (2018) 61:21–8. doi: 10.1007/s00125-017-4390-4

4. Xiao Y, Zheng L, Zou X, Wang J, Zhong J, Zhong T. Extracellular vesicles in type 2 diabetes mellitus: key roles in pathogenesis, complications, and therapy. *J Extracell Vesicles*. (2019) 8:1625677. doi: 10.1080/20013078.2019.1625677

5. Päth G, Perakakis N, Mantzoros CS, Seufert J. Stem cells in the treatment of diabetes mellitus - Focus on mesenchymal stem cells. *Metabol: Clin Experimental*. (2019) 90:1–15. doi: 10.1016/j.metabol.2018.10.005

6. Zhang Y, Yu M, Tian W. Physiological and pathological impact of exosomes of adipose tissue. *Cell Proliferation*. (2016) 49:3–13. doi: 10.1111/cpr.12233

7. Casado-Díaz A, Quesada-Gómez JM, Dorado G. Extracellular vesicles derived from mesenchymal stem cells (MSC) in regenerative medicine: applications in skin wound healing. *Front Bioeng Biotechnol*. (2020) 8:146. doi: 10.3389/fbioe.2020.00146

8. Pegtel DM, Gould SJ. Exosomes. *Annu Rev Biochem*. (2019) 88:487–514. doi: 10.1146/annurev-biochem-013118-111902

9. Doyle LM, Wang MZ. Overview of extracellular vesicles, their origin, composition, purpose, and methods for exosome isolation and analysis. *Cells*. (2019) 8. doi: 10.3390/cells8070727

10. Hessvik NP, Llorente A. Current knowledge on exosome biogenesis and release. *Cell Mol Life Sci: CMLS*. (2018) 75:193–208. doi: 10.1007/s00018-017-2595-9

11. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. *Sci (New York NY)*. (2020) 367. doi: 10.1126/science.aau6977

12. Wu Y, Deng W, Klinke DJ 2nd. Exosomes: improved methods to characterize their morphology, RNA content, and surface protein biomarkers. *Analyst*. (2015) 140:6631–42. doi: 10.1039/C5AN00688K

13. Luo X, Li Y, Hua Z, Xue X, Wang X, Pang M, et al. Exosomes-mediated tumor metastasis through reshaping tumor microenvironment and distant niche. *J Controlled Release*. (2023) 353:327–36. doi: 10.1016/j.jconrel.2022.11.050

14. Zhang J, Li S, Li L, Li M, Guo C, Yao J, et al. Exosome and exosomal microRNA: trafficking, sorting, and function. *Genomics Proteomics Bioinf*. (2015) 13:17–24. doi: 10.1016/j.gpb.2015.02.001

15. Tian F, Tang P, Sun Z, Zhang R, Zhu D, He J, et al. miR-210 in exosomes derived from macrophages under high glucose promotes mouse diabetic obesity pathogenesis by suppressing NDUFA4 expression. *J Diabetes Res*. (2020) 2020:6894684. doi: 10.1155/2020/6894684

16. Liu T, Sun YC, Cheng P, Shao HG. Adipose tissue macrophage-derived exosomal miR-29a regulates obesity-associated insulin resistance. *Biochem Biophys Res Commun*. (2019) 515:352–8. doi: 10.1016/j.bbrc.2019.05.113

17. Qian B, Yang Y, Tang N, Wang J, Sun P, Yang N, et al. M1 macrophage-derived exosomes impair beta cell insulin secretion via miR-212-5p by targeting SIRT2 and inhibiting Akt/GSK-3β/β-catenin pathway in mice. *Diabetologia*. (2021) 64:2037–51. doi: 10.1007/s00125-021-05489-1

18. Zhang D, Wu Y, Li Z, Chen H, Huang S, Jian C, et al. MiR-144-5p, an exosomal miRNA from bone marrow-derived macrophage in type 2 diabetes, impairs bone fracture healing via targeting Smad1. *J Nanobiotechnol*. (2021) 19:226. doi: 10.1186/s12951-021-00964-8

19. Shyu KG, Wang BW, Fang WJ, Pan CM, Lin CM. Exosomal MALAT1 Derived from High Glucose-Treated Macrophages Up-Regulates Resistin Expression via miR-150-5p Downregulation. *Int J Mol Sci*. (2022) 23. doi: 10.3390/ijms23031095

20. Wang Z, Sun W, Li R, Liu Y. miRNA-93-5p in exosomes derived from M2 macrophages improves lipopolysaccharide-induced podocyte apoptosis by targeting Toll-like receptor 4. *Bioengineered*. (2022) 13:7683–96. doi: 10.1080/21655979.2021.2023794

21. Xu J, Bai S, Cao Y, Liu L, Fang Y, Du J, et al. miRNA-221-3p in endothelial progenitor cell-derived exosomes accelerates skin wound healing in diabetic mice. *Diabetes Metab Syndrome Obesity: Targets Ther*. (2020) 13:1259–70. doi: 10.2147/DMSO.S243549

22. Xiong W, Zhang X, Zhou J, Chen J, Liu Y, Yan Y, et al. Astragaloside IV promotes exosome secretion of endothelial progenitor cells to regulate PI3KR2/SPRED1 signaling and inhibit pyroptosis of diabetic endothelial cells. *Cytotherapy*. (2024) 26:36–50. doi: 10.1016/j.jcyt.2023.08.013

23. Wang J, Chen S, Zhang W, Chen Y, Bihl JC. Exosomes from miRNA-126-modified endothelial progenitor cells alleviate brain injury and promote functional recovery after stroke. *CNS Neurosci Ther*. (2020) 26:1255–65. doi: 10.1111/cns.13455

24. Shi R, Jin Y, Hu W, Lian W, Cao C, Han S, et al. Exosomes derived from mmu_circ_0000250-modified adipose-derived mesenchymal stem cells promote wound healing in diabetic mice by inducing miR-128-3p/SIRT1-mediated autophagy. *Am J Physiol Cell Physiol*. (2020) 318:C848–c56. doi: 10.1152/ajpcell.00041.2020

25. Ge L, Wang K, Lin H, Tao E, Xia W, Wang F, et al. Engineered exosomes derived from miR-132-overexpresssing adipose stem cells promoted diabetic wound healing and skin reconstruction. *Front Bioeng Biotechnol*. (2023) 11:1129538. doi: 10.3389/fbioe.2023.1129538

26. Li J, Wei C, Yang Y, Gao Z, Guo Z, Qi F. Apoptotic bodies extracted from adipose mesenchymal stem cells carry microRNA-21-5p to induce M2 polarization of macrophages and augment skin wound healing by targeting KLF6. *Burns*. (2022) 48:1893–908. doi: 10.1016/j.burns.2021.12.010

27. Wang J, Wu H, Peng Y, Zhao Y, Qin Y, Zhang Y, et al. Hypoxia adipose stem cell-derived exosomes promote high-quality healing of diabetic wound involves activation of PI3K/Akt pathways. *J Nanobiotechnol*. (2021) 19:202. doi: 10.1186/s12951-021-00942-0

28. Cui C, Zang N, Song J, Guo X, He Q, Hu H, et al. Exosomes derived from mesenchymal stem cells attenuate diabetic kidney disease by inhibiting cell apoptosis and epithelial-to-mesenchymal transition via miR-424-5p. *FASEB J*. (2022) 36:e22517. doi: 10.1096/fj.202200488R

29. Wang Y, Liu J, Wang H, Lv S, Liu Q, Li S, et al. Mesenchymal stem cell-derived exosomes ameliorate diabetic kidney disease through the NLRP3 signaling pathway. *Stem Cells (Dayton Ohio)*. (2023) 41:368–83. doi: 10.1093/stmcls/sxad010

30. Zhang Y, Le X, Zheng S, Zhang K, He J, Liu M, et al. MicroRNA-146a-5p-modified human umbilical cord mesenchymal stem cells enhance protection against diabetic nephropathy in rats through facilitating M2 macrophage polarization. *Stem Cell Res Ther*. (2022) 13:171. doi: 10.1186/s13287-022-02855-7

31. Zhang W, Wang Y, Kong Y. Exosomes derived from mesenchymal stem cells modulate miR-126 to ameliorate hyperglycemia-induced retinal inflammation via targeting HMGB1. *Invest Ophthalmol Visual Sci*. (2019) 60:294–303. doi: 10.1167/iovs.18-25617

32. Gondaliya P, Sayyed AA, Bhat P, Mali M, Arya N, Khairnar A, et al. Mesenchymal stem cell-derived exosomes loaded with miR-155 inhibitor ameliorate diabetic wound healing. *Mol Pharmaceutics*. (2022) 19:1294–308. doi: 10.1021/acs.molpharmaceut.1c00669

33. Li B, Luan S, Chen J, Zhou Y, Wang T, Li Z, et al. The MSC-Derived Exosomal lncRNA H19 Promotes Wound Healing in Diabetic Foot Ulcers by Upregulating PTEN via MicroRNA-152-3p. *Mol Ther Nucleic Acids*. (2020) 19:814–26. doi: 10.1016/j.omtn.2019.11.034

34. Han ZF, Cao JH, Liu ZY, Yang Z, Qi RX, Xu HL. Exosomal lncRNA KLF3-AS1 derived from bone marrow mesenchymal stem cells stimulates angiogenesis to promote diabetic cutaneous wound healing. *Diabetes Res Clin Practice*. (2022) 183:109126. doi: 10.1016/j.diabres.2021.109126

35. Venkat P, Zacharek A, Landschoot-Ward J, Wang F, Culmone L, Chen Z, et al. Exosomes derived from bone marrow mesenchymal stem cells harvested from type two

diabetes rats promotes neurorestorative effects after stroke in type two diabetes rats. *Exp Neurol*. (2020) 334:113456. doi: 10.1016/j.expneurol.2020.113456

36. Raposo G, Nijman HW, Stoorvogel W, Liejendekker R, Harding CV, Melief CJ, et al. B lymphocytes secrete antigen-presenting vesicles. *J Exp Med*. (1996) 183:1161–72. doi: 10.1084/jem.183.3.1161

37. Zhao Y, Liu T, Zhou M. Immune-cell-derived exosomes for cancer therapy. *Mol Pharmaceutics*. (2022) 19:3042–56. doi: 10.1021/acs.molpharmaceut.2c00407

38. Veerman RE, Güçlüler Akpinar G, Eldh M, Gabrielsson S. Immune cell-derived extracellular vesicles - functions and therapeutic applications. *Trends Mol Med*. (2019) 25:382–94. doi: 10.1016/j.molmed.2019.02.003

39. Ma X, Yao M, Gao Y, Yue Y, Li Y, Zhang T, et al. Functional immune cell-derived exosomes engineered for the trilogy of radiotherapy sensitization. *Adv Sci (Weinheim Baden-Wurttemberg Germany)*. (2022) 9:e2106031. doi: 10.1002/advs.202106031

40. Yu Y, Jin H, Li L, Zhang X, Zheng C, Gao X, et al. An injecta, activated neutrophil-derived exosome mimetics/extracellular matrix hybrid hydrogel with antibacterial activity and wound healing promotion effect for diabetic wound therapy. *J Nanobiotechnol*. (2023) 21:308. doi: 10.1186/s12951-023-02073-0

41. Glennon-Alty L, Hackett AP, Chapman EA, Wright HL. Neutrophils and redox stress in the pathogenesis of autoimmune disease. *Free Radical Biol Med*. (2018) 125:25–35. doi: 10.1016/j.freeradbiomed.2018.03.049

42. Kolonics F, Szeifert V, Timár CI, Ligeti E, Lőrincz ÁM. The functional heterogeneity of neutrophil-derived extracellular vesicles reflects the status of the parent cell. *Cells*. (2020) 9. doi: 10.3390/cells9122718

43. Eizirik DL, Colli ML, Ortis F. The role of inflammation in insulitis and beta-cell loss in type 1 diabetes. *Nat Rev Endocrinol*. (2009) 5:219–26. doi: 10.1038/nrendo.2009.21

44. Guay C, Kruit JK, Rome S, Menoud V, Mulder NL, Jurdzinski A, et al. Lymphocyte-derived exosomal microRNAs promote pancreatic β Cell death and may contribute to type 1 diabetes development. *Cell Metab*. (2019) 29:348–61.e6. doi: 10.1016/j.cmet.2018.09.011

45. Robbins PD, Morelli AE. Regulation of immune responses by extracellular vesicles. *Nat Rev Immunol*. (2014) 14:195–208. doi: 10.1038/nri3622

46. Orecchioni M, Ghosheh Y, Pramod AB, Ley K. Macrophage Polarization: Different Gene Signatures in M1(LPS+) vs. Classically and M2(LPS-) vs. Alternatively Activated Macrophages. *Front Immunol*. (2019) 10:1084. doi: 10.3389/fimmu.2019.01084

47. Gharavi AT, Hanjani NA, Movahed E, Doroudian M. The role of macrophage subtypes and exosomes in immunomodulation. *Cell Mol Biol Lett*. (2022) 27:83. doi: 10.1186/s11658-022-00384-y

48. Shan X, Zhang C, Mai C, Hu X, Cheng N, Chen W, et al. The biogenesis, biological functions, and applications of macrophage-derived exosomes. *Front Mol Biosci*. (2021) 8:715461. doi: 10.3389/fmolb.2021.715461

49. Stefanovic-Racic M, Yang X, Turner MS, Mantell BS, Stolz DB, Sumpter TL, et al. Dendritic cells promote macrophage infiltration and comprise a substantial proportion of obesity-associated increases in CD11c+ cells in adipose tissue and liver. *Diabetes*. (2012) 61:2330–9. doi: 10.2337/db11-1523

50. Liu Y, Li X, Zhao M, Wu Y, Xu Y, Li X, et al. Macrophage-derived exosomes promote activation of NLRP3 inflammasome and autophagy deficiency of mesangial cells in diabetic nephropathy. *Life Sci*. (2023) 330:121991. doi: 10.1016/j.lfs.2023.121991

51. Zhao J, Chen J, Zhu W, Qi XM, Wu YG. Exosomal miR-7002-5p derived from highglucose-induced macrophages suppresses autophagy in tubular epithelial cells by targeting Atg9b. *FASEB J*. (2022) 36:e22501. doi: 10.1096/fj.202200550RR

52. Phu TA, Ng M, Vu NK, Bouchareychas L, Raffai RL. IL-4 polarized human macrophage exosomes control cardiometabolic inflammation and diabetes in obesity. *Mol Ther*. (2022) 30:2274–97. doi: 10.1016/j.ymthe.2022.03.008

53. Zhang C, Bao LR, Yang YT, Wang Z, Li Y. [Role of M2 macrophage exosomes in osteogenic differentiation of mouse bone marrow mesenchymal stem cells under high-glucose and high-insulin]. *Sichuan da xue xue bao Yi xue ban = J Sichuan Univ Med Sci Edition*. (2022) 53:63–70. doi: 10.12182/20220160207

54. Wang Y, Lin Q, Zhang H, Wang S, Cui J, Hu Y, et al. M2 macrophage-derived exosomes promote diabetic fracture healing by acting as an immunomodulator. *Bioactive Mater*. (2023) 28:273–83. doi: 10.1016/j.bioactmat.2023.05.018

55. Zhao Y, Wang H, Mazzone T. Identification of stem cells from human umbilical cord blood with embryonic and hematopoietic characteristics. *Exp Cell Res*. (2006) 312:2454–64. doi: 10.1016/j.yexcr.2006.04.008

56. Zhao Y, Mazzone T. Human cord blood stem cells and the journey to a cure for type 1 diabetes. *Autoimmun Rev*. (2010) 10:103–7. doi: 10.1016/j.autrev.2010.08.011

57. Zhao Y, Huang Z, Qi M, Lazzarini P, Mazzone T. Immune regulation of T lymphocyte by a newly characterized human umbilical cord blood stem cell. *Immunol Lett*. (2007) 108:78–87. doi: 10.1016/j.imlet.2006.10.007

58. Zhao Y, Jiang Z, Zhao T, Ye M, Hu C, Yin Z, et al. Reversal of type 1 diabetes via islet β cell regeneration following immune modulation by cord blood-derived multipotent stem cells. *BMC Med*. (2012) 10:3. doi: 10.1186/1741-7015-10-3

59. Zhao Y, Jiang Z, Zhao T, Ye M, Hu C, Zhou H, et al. Targeting insulin resistance in type 2 diabetes via immune modulation of cord blood-derived multipotent stem cells (CB-SCs) in stem cell educator therapy: phase I/II clinical trial. *BMC Med*. (2013) 11:160. doi: 10.1186/1741-7015-11-160

60. Zhao Y, Knight CM, Jiang Z, Delgado E, Van Hoven AM, Ghanny S, et al. Stem Cell Educator therapy in type 1 diabetes: From the bench to clinical trials. *Autoimmun Rev*. (2022) 21:103058. doi: 10.1016/j.autrev.2022.103058

61. Hu W, Song X, Yu H, Sun J, Zhao Y. Released exosomes contribute to the immune modulation of cord blood-derived stem cells. *Front Immunol*. (2020) 11:165. doi: 10.3389/fimmu.2020.00165

62. Delgado E, Perez-Basterrechea M, Suarez-Alvarez B, Zhou H, Revuelta EM, Garcia-Gala JM, et al. Modulation of autoimmune T-cell memory by stem cell educator therapy: phase 1/2 clinical trial. *EBioMedicine*. (2015) 2:2024–36. doi: 10.1016/j.ebiom.2015.11.003

63. Zhao Y, Jiang Z, Delgado E, Li H, Zhou H, Hu W, et al. Platelet-derived mitochondria display embryonic stem cell markers and improve pancreatic islet β-cell function in humans. *Stem Cells Trans Med*. (2017) 6:1684–97. doi: 10.1002/sctm.17-0078

64. Acosta JB, del Barco DG, Vera DC, Savigne W, Lopez-Saura P, Guillen Nieto G, et al. The pro-inflammatory environment in recalcitrant diabetic foot wounds. *Int Wound J*. (2008) 5:530–9. doi: 10.1111/j.1742-481X.2008.00457.x

65. Bodnár E, Bakondi E, Kovács K, Hegedűs C, Lakatos P, Robaszkiewicz A, et al. Redox profiling reveals clear differences between molecular patterns of wound fluids from acute and chronic wounds. *Oxid Med Cell Longevity*. (2018) 2018:5286785. doi: 10.1155/2018/5286785

66. Rabbani PS, Abdou SA, Sultan DL, Kwong J, Duckworth A, Ceradini DJ. *In vivo* imaging of reactive oxygen species in a murine wound model. *J Visualized Experiments: JoVE*. (2018) 141). doi: 10.3791/58450

67. Rabbani PS, Soares MA, Hameedi SG, Kadle RL, Mubasher A, Kowzun M, et al. Dysregulation of nrf2/keap1 redox pathway in diabetes affects multipotency of stromal cells. *Diabetes*. (2019) 68:141–55. doi: 10.2337/db18-0232

68. Xing Z, Zhao C, Liu H, Fan Y. Endothelial progenitor cell-derived extracellular vesicles: A novel candidate for regenerative medicine and disease treatment. *Adv Healthcare Mater*. (2020) 9:e2000255. doi: 10.1002/adhm.202000255

69. Critser PJ, Yoder MC. Endothelial colony-forming cell role in neoangiogenesis and tissue repair. *Curr Opin Organ Transplantation*. (2010) 15:68–72. doi: 10.1097/MOT.0b013e32833454b5

70. Zhang J, Chen C, Hu B, Niu X, Liu X, Zhang G, et al. Exosomes derived from human endothelial progenitor cells accelerate cutaneous wound healing by promoting angiogenesis through erk1/2 signaling. *Int J Biol Sci*. (2016) 12:1472–87. doi: 10.7150/ijbs.15514

71. Duscher D, Barrera J, Wong VW, Maan ZN, Whittam AJ, Januszyk M, et al. Stem cells in wound healing: the future of regenerative medicine? A mini-review. *Gerontology*. (2016) 62:216–25. doi: 10.1159/000381877

72. Herberts CA, Kwa MS, Hermsen HP. Risk factors in the development of stem cell therapy. *J Trans Med*. (2011) 9:29. doi: 10.1186/1479-5876-9-29

73. Kansu E. Thrombosis in stem cell transplantation. *Hematol (Amsterdam Netherlands)*. (2012) 17 Suppl 1:S159–62. doi: 10.1179/102453312X13336169156735

74. Li X, Jiang C, Zhao J. Human endothelial progenitor cells-derived exosomes accelerate cutaneous wound healing in diabetic rats by promoting endothelial function. *J Diabetes its Complications*. (2016) 30:986–92. doi: 10.1016/j.jdiacomp.2016.05.009

75. Tu TC, Yamashita T, Kato T, Nagano M, Trinh NT, Hamada H, et al. Microvesicles derived from Alde-Low EPCs support the wound healing capacity of AT-MSCs. *Biochem Biophys Res Commun*. (2016) 477:68–75. doi: 10.1016/j.bbrc.2016.06.022

76. Chen J, Chen J, Chen S, Zhang C, Zhang L, Xiao X, et al. Transfusion of CXCR4-primed endothelial progenitor cells reduces cerebral ischemic damage and promotes repair in db/db diabetic mice. *PloS One*. (2012) 7:e50105. doi: 10.1371/journal.pone.0050105

77. Chen J, Xiao X, Chen S, Zhang C, Chen J, Yi D, et al. Angiotensin-converting enzyme 2 priming enhances the function of endothelial progenitor cells and their therapeutic efficacy. *Hypertension (Dallas Tex: 1979)*. (2013) 61:681–9. doi: 10.1161/HYPERTENSIONAHA.111.00202

78. Tang N, Jiang S, Yang Y, Liu S, Ponnusamy M, Xin H, et al. Noncoding RNAs as therapeutic targets in atherosclerosis with diabetes mellitus. *Cardiovasc Ther*. (2018) 36:e12436. doi: 10.1111/1755-5922.12436

79. Zhang Z, Dong J, Lobe CG, Gong P, Liu J, Liao L. CCR5 facilitates endothelial progenitor cell recruitment and promotes the stabilization of atherosclerotic plaques in ApoE-/- mice. *Stem Cell Res Ther*. (2015) 6:36. doi: 10.1186/s13287-015-0026-0

80. Bai S, Yin Q, Dong T, Dai F, Qin Y, Ye L, et al. Endothelial progenitor cell-derived exosomes ameliorate endothelial dysfunction in a mouse model of diabetes. *Biomed Pharmacother = Biomed Pharmacother*. (2020) 131:110756. doi: 10.1016/j.biopha.2020.110756

81. Liang X, Ding Y, Zhang Y, Tse HF, Lian Q. Paracrine mechanisms of mesenchymal stem cell-based therapy: current status and perspectives. *Cell Transplantation*. (2014) 23:1045–59. doi: 10.3727/096368913X667709

82. Mathew B, Ravindran S, Liu X, Torres L, Chennakesavalu M, Huang CC, et al. Mesenchymal stem cell-derived extracellular vesicles and retinal ischemia-reperfusion. *Biomaterials*. (2019) 197:146–60. doi: 10.1016/j.biomaterials.2019.01.016

83. Shabbir A, Cox A, Rodriguez-Menocal L, Salgado M, Van Badiavas E. Mesenchymal stem cell exosomes induce proliferation and migration of normal and chronic wound fibroblasts, and enhance angiogenesis in vitro. *Stem Cells Dev*. (2015) 24:1635–47. doi: 10.1089/scd.2014.0316

84. Nakano M, Kubota K, Kobayashi E, Chikenji TS, Saito Y, Konari N, et al. Bone marrow-derived mesenchymal stem cells improve cognitive impairment in an Alzheimer's disease model by increasing the expression of microRNA-146a in hippocampus. *Sci Rep*. (2020) 10:10772. doi: 10.1038/s41598-020-67460-1

85. Pomatto M, Gai C, Negro F, Cedrino M, Grange C, Ceccotti E, et al. Differential therapeutic effect of extracellular vesicles derived by bone marrow and adipose mesenchymal stem cells on wound healing of diabetic ulcers and correlation to their cargoes. *Int J Mol Sci*. (2021) 22. doi: 10.3390/ijms22083851

86. Zhao H, Shang Q, Pan Z, Bai Y, Li Z, Zhang H, et al. Exosomes from adipose-derived stem cells attenuate adipose inflammation and obesity through polarizing M2 macrophages and beiging in white adipose tissue. *Diabetes*. (2018) 67:235–47. doi: 10.2337/db17-0356

87. Ouyang L, Qiu D, Fu X, Wu A, Yang P, Yang Z, et al. Overexpressing HPGDS in adipose-derived mesenchymal stem cells reduces inflammatory state and improves wound healing in type 2 diabetic mice. *Stem Cell Res Ther*. (2022) 13:395. doi: 10.1186/s13287-022-03082-w

88. Sugimoto MA, Sousa LP, Pinho V, Perretti M, Teixeira MM. Resolution of inflammation: what controls its onset? *Front Immunol*. (2016) 7:160. doi: 10.3389/fimmu.2016.00160

89. Lipsky PE. Systemic lupus erythematosus: an autoimmune disease of B cell hyperactivity. *Nat Immunol*. (2001) 2:764–6. doi: 10.1038/ni0901-764

90. Nosbaum A, Prevel N, Truong HA, Mehta P, Ettinger M, Scharschmidt TC, et al. Cutting edge: regulatory T cells facilitate cutaneous wound healing. *J Immunol (Baltimore Md: 1950)*. (2016) 196:2010–4. doi: 10.4049/jimmunol.1502139

91. Lin CW, Hung CM, Chen WJ, Chen JC, Huang WY, Lu CS, et al. New horizons of macrophage immunomodulation in the healing of diabetic foot ulcers. *Pharmaceutics*. (2022) 14. doi: 10.3390/pharmaceutics14102065

92. Shi R, Jin Y, Zhao S, Yuan H, Shi J, Zhao H. Hypoxic ADSC-derived exosomes enhance wound healing in diabetic mice via delivery of circ-Snhg11 and induction of M2-like macrophage polarization. *Biomed Pharmacother = Biomed Pharmacother*. (2022) 153:113463. doi: 10.1016/j.biopha.2022.113463

93. Blazquez R, Sanchez-Margallo FM, de la Rosa O, Dalemans W, Alvarez V, Tarazona R, et al. Immunomodulatory potential of human adipose mesenchymal stem cells derived exosomes on in *vitro* stimulated T cells. *Front Immunol*. (2014) 5:556. doi: 10.3389/fimmu.2014.00556

94. Qiu J, Shu C, Li X, Ye C, Zhang WC. Exosomes from linc00511-overexpressing ADSCs accelerates angiogenesis in diabetic foot ulcers healing by suppressing PAQR3-induced Twist1 degradation. *Diabetes Res Clin Practice*. (2021) 180:109032. doi: 10.1016/j.diabres.2021.109032

95. Zhang Y, Bai X, Shen K, Luo L, Zhao M, Xu C, et al. Exosomes derived from adipose mesenchymal stem cells promote diabetic chronic wound healing through SIRT3/SOD2. *Cells*. (2022) 11. doi: 10.3390/cells11162568

96. Sun Y, Shi H, Yin S, Ji C, Zhang X, Zhang B, et al. Human mesenchymal stem cell derived exosomes alleviate type 2 diabetes mellitus by reversing peripheral insulin resistance and relieving β-cell destruction. *ACS Nano*. (2018) 12:7613–28. doi: 10.1021/acsnano.7b07643

97. De Taeye BM, Novitskaya T, McGuinness OP, Gleaves L, Medda M, Covington JW, et al. Macrophage TNF-alpha contributes to insulin resistance and hepatic steatosis in diet-induced obesity. *Am J Physiol Endocrinol Metab*. (2007) 293:E713–25. doi: 10.1152/ajpendo.00194.2007

98. Ying W, Riopel M, Bandyopadhyay G, Dong Y, Birmingham A, Seo JB, et al. Adipose tissue macrophage-derived exosomal miRNAs can modulate in Vivo and in Vitro insulin sensitivity. *Cell*. (2017) 171:372–84.e12. doi: 10.1016/j.cell.2017.08.035

99. Xiang E, Han B, Zhang Q, Rao W, Wang Z, Chang C, et al. Human umbilical cord-derived mesenchymal stem cells prevent the progression of early diabetic nephropathy through inhibiting inflammation and fibrosis. *Stem Cell Res Ther*. (2020) 11:336. doi: 10.1186/s13287-020-01852-y

100. Teng L, Maqsood M, Zhu M, Zhou Y, Kang M, Zhou J, et al. Exosomes derived from human umbilical cord mesenchymal stem cells accelerate diabetic wound healing via promoting M2 macrophage polarization, angiogenesis, and collagen deposition. *Int J Mol Sci*. (2022) 23. doi: 10.3390/ijms231810421

101. Yan C, Xv Y, Lin Z, Endo Y, Xue H, Hu Y, et al. Human umbilical cord mesenchymal stem cell-derived exosomes accelerate diabetic wound healing via ameliorating oxidative stress and promoting angiogenesis. *Front Bioeng Biotechnol*. (2022) 10:829868. doi: 10.3389/fbioe.2022.829868

102. Yap SK, Tan KL, Abd Rahaman NY, Saulol Hamid NF, Ooi J, Tor YS, et al. Human umbilical cord mesenchymal stem cell-derived small extracellular vesicles ameliorated insulin resistance in type 2 diabetes mellitus rats. *Pharmaceutics*. (2022) 14. doi: 10.3390/pharmaceutics14030649

103. Mohamed-Ahmed S, Fristad I, Lie SA, Suliman S, Mustafa K, Vindenes H, et al. Adipose-derived and bone marrow mesenchymal stem cells: a donor-matched comparison. *Stem Cell Res Ther*. (2018) 9:168. doi: 10.1186/s13287-018-0914-1

104. Zhou C, Zhang B, Yang Y, Jiang Q, Li T, Gong J, et al. Stem cell-derived exosomes: emerging therapeutic opportunities for wound healing. *Stem Cell Res Ther*. (2023) 14:107. doi: 10.1186/s13287-023-03345-0

105. Wang L, Cai Y, Zhang Q, Zhang Y. Pharmaceutical activation of nrf2 accelerates diabetic wound healing by exosomes from bone marrow mesenchymal stem cells. *Int J Stem Cells*. (2022) 15:164–72. doi: 10.15283/ijsc21067

106. Liu W, Yu M, Xie D, Wang L, Ye C, Zhu Q, et al. Melatonin-stimulated MSC-derived exosomes improve diabetic wound healing through regulating macrophage M1 and M2 polarization by targeting the PTEN/AKT pathway. *Stem Cell Res Ther*. (2020) 11:259. doi: 10.1186/s13287-020-01756-x

107. Ergul A, Hafez S, Fouda A, Fagan SC. Impact of comorbidities on acute injury and recovery in preclinical stroke research: focus on hypertension and diabetes. *Trans Stroke Res*. (2016) 7:248–60. doi: 10.1007/s12975-016-0464-8

108. Callahan A, Amarenco P, Goldstein LB, Sillesen H, Messig M, Samsa GP, et al. Risk of stroke and cardiovascular events after ischemic stroke or transient ischemic attack in patients with type 2 diabetes or metabolic syndrome: secondary analysis of the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) trial. *Arch Neurol*. (2011) 68:1245–51. doi: 10.1001/archneurol.2011.146

109. Chen J, Cui X, Zacharek A, Cui Y, Roberts C, Chopp M. White matter damage and the effect of matrix metalloproteinases in type 2 diabetic mice after stroke. *Stroke*. (2011) 42:445–52. doi: 10.1161/STROKEAHA.110.596486

110. Otero-Ortega L, Laso-García F, Gómez-de Frutos M, Fuentes B, Diekhorst L, Díez-Tejedor E, et al. Role of exosomes as a treatment and potential biomarker for stroke. *Trans Stroke Res*. (2019) 10:241–9. doi: 10.1007/s12975-018-0654-7

111. Xin H, Li Y, Chopp M. Exosomes/miRNAs as mediating cell-based therapy of stroke. *Front Cell Neurosci*. (2014) 8:377. doi: 10.3389/fncel.2014.00377

112. Codispoti B, Marrelli M, Paduano F, Tatullo M. NANOmetric BIO-banked MSC-derived exosome (NANOBIOME) as a novel approach to regenerative medicine. *J Clin Med*. (2018) 7. doi: 10.3390/jcm7100357

113. Moon GJ, Sung JH, Kim DH, Kim EH, Cho YH, Son JP, et al. Application of mesenchymal stem cell-derived extracellular vesicles for stroke: biodistribution and microRNA study. *Trans Stroke Res*. (2019) 10:509–21. doi: 10.1007/s12975-018-0668-1

114. Ma S, Wang J, Wang Y, Dai X, Xu F, Gao X, et al. Diabetes mellitus impairs white matter repair and long-term functional deficits after cerebral ischemia. *Stroke*. (2018) 49:2453–63. doi: 10.1161/STROKEAHA.118.021452

115. Bose S, Cho J. Role of chemokine CCL2 and its receptor CCR2 in neurodegenerative diseases. *Arch Pharmacal Res*. (2013) 36:1039–50. doi: 10.1007/s12272-013-0161-z

116. Hughes PM, Allegrini PR, Rudin M, Perry VH, Mir AK, Wiessner C. Monocyte chemoattractant protein-1 deficiency is protective in a murine stroke model. *J Cereb Blood Flow Metab*. (2002) 22:308–17. doi: 10.1097/00004647-200203000-00008

117. Wen D, Peng Y, Liu D, Weizmann Y, Mahato RI. Mesenchymal stem cell and derived exosome as small RNA carrier and Immunomodulator to improve islet transplantation. *J Controlled Release*. (2016) 238:166–75. doi: 10.1016/j.jconrel.2016.07.044

118. Ebrahim N, El-Halim HEA, Helal OK, El-Azab NE, Badr OAM, Hassouna A, et al. Effect of bone marrow mesenchymal stem cells-derived exosomes on diabetes-induced retinal injury: Implication of Wnt/b-catenin signaling pathway. *Biomed Pharmacother = Biomed Pharmacother*. (2022) 154:113554. doi: 10.1016/j.biopha.2022.113554

119. Methods In Medicine CAM. Retracted: Mesenchymal Stem Cell Exosomal miR-146a Mediates the Regulation of the TLR4/MyD88/NF-κB Signaling Pathway in Inflammation due to Diabetic Retinopathy. *Comput Math Methods Med*. (2023) 2023:9826235. doi: 10.1155/2023/9826235

120. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. *J Cell Biol*. (2013) 200:373–83. doi: 10.1083/jcb.201211138

121. Lv Q, Deng J, Chen Y, Wang Y, Liu B, Liu J. Engineered human adipose stem-cell-derived exosomes loaded with miR-21-5p to promote diabetic cutaneous wound healing. *Mol Pharmaceutics*. (2020) 17:1723–33. doi: 10.1021/acs.molpharmaceut.0c00177

122. Liang ZH, Pan NF, Lin SS, Qiu ZY, Liang P, Wang J, et al. Exosomes from mmu_circ_0001052-modified adipose-derived stem cells promote angiogenesis of DFU via miR-106a-5p and FGF4/p38MAPK pathway. *Stem Cell Res Ther*. (2022) 13:336. doi: 10.1186/s13287-022-03015-7

123. Li X, Xie X, Lian W, Shi R, Han S, Zhang H, et al. Exosomes from adipose-derived stem cells overexpressing Nrf2 accelerate cutaneous wound healing by promoting vascularization in a diabetic foot ulcer rat model. *Exp Mol Med*. (2018) 50:1–14. doi: 10.1038/s12276-018-0058-5

124. Yu M, Liu W, Li J, Lu J, Lu H, Jia W, et al. Exosomes derived from atorvastatin-pretreated MSC accelerate diabetic wound repair by enhancing angiogenesis via AKT/eNOS pathway. *Stem Cell Res Ther*. (2020) 11:350. doi: 10.1186/s13287-020-01824-2

125. Born LJ, Chang KH, Shoureshi P, Lay F, Bengali S, Hsu ATW, et al. HOTAIR-loaded mesenchymal stem/stromal cell extracellular vesicles enhance angiogenesis and wound healing. *Adv Healthcare Mater*. (2022) 11:e2002070. doi: 10.1002/adhm.202002070

126. Huang J, Yu M, Yin W, Liang B, Li A, Li J, et al. Development of a novel RNAi therapy: Engineered miR-31 exosomes promoted the healing of diabetic wounds. *Bioactive Mater*. (2021) 6:2841–53. doi: 10.1016/j.bioactmat.2021.02.007

127. Xiong Y, Chen L, Liu P, Yu T, Lin C, Yan C, et al. All-in-one: multifunctional hydrogel accelerates oxidative diabetic wound healing through timed-release of exosome and fibroblast growth factor. *Small (Weinheim an der Bergstrasse Germany)*. (2022) 18:e2104229. doi: 10.1002/smll.202104229

128. Du J, Wan Z, Wang C, Lu F, Wei M, Wang D, et al. Designer exosomes for targeted and efficient ferroptosis induction in cancer via chemo-photodynamic therapy. *Theranostics*. (2021) 11:8185–96. doi: 10.7150/thno.59121

129. Dumontel B, Susa F, Limongi T, Vighetto V, Debellis D, Canta M, et al. Nanotechnological engineering of extracellular vesicles for the development of actively targeted hybrid nanodevices. *Cell Biosci*. (2022) 12:61. doi: 10.1186/s13578-022-00784-9

130. Yang J, Chen Z, Pan D, Li H, Shen J. Umbilical cord-derived mesenchymal stem cell-derived exosomes combined pluronic F127 hydrogel promote chronic diabetic wound healing and complete skin regeneration. *Int J Nanomed*. (2020) 15:5911–26. doi: 10.2147/IJN.S249129

131. Jiang T, Liu S, Wu Z, Li Q, Ren S, Chen J, et al. ADSC-exo@MMP-PEG smart hydrogel promotes diabetic wound healing by optimizing cellular functions and relieving oxidative stress. *Mater Today Bio*. (2022) 16:100365. doi: 10.1016/j.mtbio.2022.100365

132. Yuan M, Liu K, Jiang T, Li S, Chen J, Wu Z, et al. GelMA/PEGDA microneedles patch loaded with HUVECs-derived exosomes and Tazarotene promote diabetic wound healing. *J Nanobiotechnol*. (2022) 20:147. doi: 10.1186/s12951-022-01354-4

133. Geng X, Qi Y, Liu X, Shi Y, Li H, Zhao L. A multifunctional antibacterial and self-healing hydrogel laden with bone marrow mesenchymal stem cell-derived exosomes for accelerating diabetic wound healing. *Biomater Adv*. (2022) 133:112613. doi: 10.1016/j.msec.2021.112613

134. Wang Y, Cao Z, Wei Q, Ma K, Hu W, Huang Q, et al. VH298-loaded extracellular vesicles released from gelatin methacryloyl hydrogel facilitate diabetic wound healing by HIF-1α-mediated enhancement of angiogenesis. *Acta Biomater*. (2022) 147:342–55. doi: 10.1016/j.actbio.2022.05.018

135. Zhang Y, Li M, Wang Y, Han F, Shen K, Luo L, et al. Exosome/metformin-loaded self-healing conductive hydrogel rescues microvascular dysfunction and promotes chronic diabetic wound healing by inhibiting mitochondrial fission. *Bioactive Mater*. (2023) 26:323–36. doi: 10.1016/j.bioactmat.2023.01.020

136. Song Y, You Y, Xu X, Lu J, Huang X, Zhang J, et al. Adipose-derived mesenchymal stem cell-derived exosomes biopotentiated extracellular matrix hydrogels accelerate diabetic wound healing and skin regeneration. *Adv Sci (Weinheim Baden-Wurttemberg Germany)*. (2023) 10:e2304023. doi: 10.1002/advs.202304023

137. Hu N, Cai Z, Jiang X, Wang C, Tang T, Xu T, et al. Hypoxia-pretreated ADSC-derived exosome-embedded hydrogels promote angiogenesis and accelerate diabetic wound healing. *Acta Biomater*. (2023) 157:175–86. doi: 10.1016/j.actbio.2022.11.057

138. Xiang K, Chen J, Guo J, Li G, Kang Y, Wang C, et al. Multifunctional ADM hydrogel containing endothelial cell-exosomes for diabetic wound healing. *Mater Today Bio*. (2023) 23:100863. doi: 10.1016/j.mtbio.2023.100863

139. Cheng P, Xie X, Hu L, Zhou W, Mi B, Xiong Y, et al. Hypoxia endothelial cells-derived exosomes facilitate diabetic wound healing through improving endothelial cell function and promoting M2 macrophages polarization. *Bioactive Mater*. (2024) 33:157–73. doi: 10.1016/j.bioactmat.2023.10.020

140. Liu K, Gong B, Li T, Lei H, Li J, Tang J, et al. Bioactive self-healing umbilical cord blood exosomes hydrogel for promoting chronic diabetic wound healing. *Biochem Biophys Res Commun*. (2024) 690:149241. doi: 10.1016/j.bbrc.2023.149241

141. Huang L, Shi Y, Li M, Wang T, Zhao L. Plasma exosomes loaded pH-responsive carboxymethylcellulose hydrogel promotes wound repair by activating the vascular endothelial growth factor signaling pathway in type 1 diabetic mice. *J Biomed Nanotechnol*. (2021) 17:2021–33. doi: 10.1166/jbn.2021.3165

142. Shang S, Zhuang K, Chen J, Zhang M, Jiang S, Li W. A bioactive composite hydrogel dressing that promotes healing of both acute and chronic diabetic skin wounds. *Bioactive Mater*. (2024) 34:298–310. doi: 10.1016/j.bioactmat.2023.12.026

143. Lin Y, Liu X, Liu Z, Xu Y. Visible-light-driven photocatalysis-enhanced nanozyme of tiO(2) nanotubes@MoS(2) nanoflowers for efficient wound healing infected with multidrug-resistant bacteria. *Small (Weinheim an der Bergstrasse Germany)*. (2021) 17:e2103348. doi: 10.1002/smll.202103348

144. Qiu X, Liu J, Zheng C, Su Y, Bao L, Zhu B, et al. Exosomes released from educated mesenchymal stem cells accelerate cutaneous wound healing via promoting angiogenesis. *Cell Proliferation*. (2020) 53:e12830. doi: 10.1111/cpr.12830

145. Zhao G, Liu F, Liu Z, Zuo K, Wang B, Zhang Y, et al. MSC-derived exosomes attenuate cell death through suppressing AIF nucleus translocation and enhance cutaneous wound healing. *Stem Cell Res Ther*. (2020) 11:174. doi: 10.1186/s13287-020-01616-8

146. Zhu YZ, Hu X, Zhang J, Wang ZH, Wu S, Yi YY. Extracellular vesicles derived from human adipose-derived stem cell prevent the formation of hypertrophic scar in a rabbit model. *Ann Plast Surgery*. (2020) 84:602–7. doi: 10.1097/SAP.0000000000002357

147. Lindblad WJ. Considerations for selecting the correct animal model for dermal wound-healing studies. *J Biomater Sci Polymer Edition*. (2008) 19:1087–96. doi: 10.1163/156856208784909390

148. Heinrich W, Lange PM, Stirtz T, Iancu C, Heidemann E. Isolation and characterization of the large cyanogen bromide peptides from the alpha1- and alpha2-chains of pig skin collagen. *FEBS Lett*. (1971) 16:63–7. doi: 10.1016/0014-5793(71)80687-7

149. Davidson JM. Animal models for wound repair. *Arch Dermatol Res*. (1998) 290 Suppl:S1–11. doi: 10.1007/PL00007448

150. Hoekstra MJ, Hupkens P, Dutrieux RP, Bosch MM, Brans TA, Kreis RW. A comparative burn wound model in the New Yorkshire pig for the histopathological evaluation of local therapeutic regimens: silver sulfadiazine cream as a standard. *Br J Plast Surgery*. (1993) 46:585–9. doi: 10.1016/0007-1226(93)90111-N

151. Summerfield A, Meurens F, Ricklin ME. The immunology of the porcine skin and its value as a model for human skin. *Mol Immunol*. (2015) 66:14–21. doi: 10.1016/j.molimm.2014.10.023

152. Sullivan TP, Eaglstein WH, Davis SC, Mertz P. The pig as a model for human wound healing. *Wound Repair Regeneration*. (2001) 9:66–76. doi: 10.1046/j.1524-475x.2001.00066.x

frontiers | Frontiers in Immunology

TYPE Review
PUBLISHED 17 May 2024
DOI 10.3389/fimmu.2024.1360618

OPEN ACCESS

EDITED BY
Chun Wai Mai,
UCSI University, Malaysia

REVIEWED BY
Shareni Jeyamogan,
Northwestern University, United States
Somayeh Keshtkar,
Shiraz University of Medical Sciences, Iran

*CORRESPONDENCE
Giovanna Romeo
giovanna.romeo@uniroma1.it

†These authors have contributed equally to this work and share senior authorship

RECEIVED 23 December 2023
ACCEPTED 07 May 2024
PUBLISHED 17 May 2024

CITATION
Iuliano M, Grimaldi L, Rosa P, Scibetta S, Bernardini N, Proietti I, Tolino E, Skroza N, Potenza C, Mangino G and Romeo G (2024) Extracellular vescicles in psoriasis: from pathogenesis to possible roles in therapy. *Front. Immunol.* 15:1360618. doi: 10.3389/fimmu.2024.1360618

Extracellular vescicles in psoriasis: from pathogenesis to possible roles in therapy

Marco Iuliano[1], Lorenzo Grimaldi[1], Paolo Rosa[1,2], Sofia Scibetta[1], Nicoletta Bernardini[3], Ilaria Proietti[3], Ersilia Tolino[3], Nevena Skroza[1,3], Concetta Potenza[1,3], Giorgio Mangino[1†] and Giovanna Romeo[1*†]

[1]Department of Medico-Surgical Sciences and Biotechnologies, Sapienza University of Rome – Polo Pontino, Latina, Italy, [2]ICOT, Istituto Chirurgico Ortopedico Traumatologico, Latina, Italy, [3]Dermatology Unit "Daniele Innocenzi", "A. Fiorini" Hospital, Terracina, Italy

Psoriasis is a chronic inflammatory disease affecting skin and joints characterized by a chronically altered immune and inflammatory response. Several factors occur from the onset to the development of this disease due to different types of cells spatially and temporally localized in the affected area, such as, keratinocytes, macrophages, neutrophils and T helper lymphocytes. This scenario leads to the chronic release of high levels of inflammatory mediators (*i.e.*, IL-17, IL-23, IL-22, TNF-α, S100 proteins, Defensins) and lastly parakeratosis and thickening of the stratum spinosum. Extracellular vesicles (EVs) are small double membraned biological nanoparticles that are secreted by all cell types and classified, based on dimension and biogenesis, into exosomes, microvesicles and apoptotic bodies. Their role as vessels for long range molecular signals renders them key elements in the pathogenesis of psoriasis, as well as innovative platforms for potential biomarker discovery and delivery of fine-tuned anti-inflammatory therapies. In this review, the role of EVs in the pathogenesis of psoriasis and the modulation of cellular microenvironment has been summarized. The biotechnological implementation of EVs for therapy and research for new biomarkers has been also discussed.

KEYWORDS

psoriasis pathogenesis, extracellular vesicles, exosomes, inflammatory microenvironment, microRNA

1 Introduction

Psoriasis is a chronic inflammatory-mediated disease that affects the skin and has an incidence of 2–3% of the world's population (1). Despite the direct cause behind psoriasis development is still unknown, many risk factors and molecular components have been described. In the beginning, blood vessels enlarge and become tortuous. In this stage the

dermis remains unaltered. Soon after, keratinocyte (KC) hyperproliferation can be observed, accompanied by parakeratosis (*i.e.*, retention of nuclei by corneocytes). Lastly, during the late stages, acanthosis becomes prevalent. This phenomenon is a result of the KCs invading the higher layers of the skin and the thickening of the *stratum spinosum*, producing a darker tone and a scaly texture (2). A molecular trigger for psoriasis has also been identified. In response to a physical trauma of the skin, KCs release the cationic antimicrobial peptide (AMP) LL-37. DNA or RNA fragments released from the lesion are bound to LL-37 and form LL-37/self-DNA/RNA complexes found in psoriasis lesions, which in turn activate TLR7/9-bearing dendritic cells (DCs). This results in type I interferon (IFN) production and initiation of inflammation. One of the main features of psoriasis is the dysregulated and chronic immune response, stemming from a vicious cycle between damaged KCs, DCs and local T cells. Myeloid DCs in the skin are activated by pro-inflammatory cytokines produced by damaged KCs such as Tumor Necrosis Factor-α (TNF-α) Interleukin- (IL)-1β and IL-6 as well as the LL-37/self-DNA/RNA complex. DCs, then, activate γδ-T cells polarization through the release of TNF-α, IL-23, IL-1β and IL-6. The cycle is closed by matured γδ-T cells who induce keratinocyte aberrant differentiation program through IL-17 and the recruitment of neutrophils and other immune cells. Plaque formation and maintenance is enabled by the DC-macrophage IL-23/IL-17 axis which promotes type 17 T helper (Th) and cytotoxic T lymphocyte effector polarization (3). The causes of psoriasis development can be both genetic and environmental (4). It is important to note that, rather than being direct causes, they represent risk factors for psoriasis and likely play roles with different weights in disease emergence. It is, therefore, clear that psoriasis is a disease that occurs when there is an interplay between environment, genetic predisposition factors, an overactive immune system and altered cell-to-cell intercommunication (2, 5).

Extracellular vesicles (EVs) are a class of small double membraned nanoparticles that are secreted by all cell types and mainly act as vessels for delivering cargo and molecular signals (6). Based on their biogenesis and/or size, EVs can be categorized in three subsets: exosomes or small EVs (s-EVs, 30–150 nm in diameter, originating from the endosomal complex); microvesicles or large EVs (l-EVs 100–1000 nm in diameter, secreted from the cell membrane); apoptotic bodies (APs 50–5000 nm in diameter, originated from plasma membrane blebbing during apoptosis) (6–8). Nucleic acids (*i.e.*, several type of RNAs) and proteins are carried by EVs from a donor to an acceptor cell where modulation of expression is carried out. The range of EV signaling can be restricted to adjacent cells localized in the same tissue as the donor cell but can also be extended to a systemic level. While long range cell-to-cell communication is also possible with cell-free RNAs and proteins, the protection provided by EVs from RNAses and proteases represents a major advantage in long distance signaling (8–10). EVs play a crucial role in the regulation of many patho-physiological processes and the definition of the extracellular environment. During disease development EVs are employed both by the host and the pathogen or cancerous cells: the former to regulate the immune response and induce the polarization of macrophages, neutrophils and of other effector cells; the latter to prime the environment for further colonization. Regarding a typical chronic inflammatory disease, as psoriasis, EVs have roles in the regulation of the immune response but also as platforms for therapeutic applications and delivery of innovative drugs. Furthermore, the use of EVs has also had ramifications in disease diagnosis. In recent times, RNA and proteins carried by EVs through the blood stream have been profiled to find molecular biomarkers positively correlated with many diseases. Indeed, such biomarkers have become useful in keeping track of disease progression and amelioration (11–14). The latter aspect is even more important in the case of psoriasis where the patient evaluation and follow-up is still based on clinical criteria, mainly the Psoriasis Area and Severity Index (PASI) and the Dermatology Life Quality Index (DLQI). Hence, the push to molecular characterization of EVs in subjects affected by psoriasis to identify markers for patient management, possibly through a liquid biopsy approach.

Here a selection of the studies about the influence and the potential role of EVs both in the development of psoriasis and in therapeutic intervention to contrast this disease, has been presented. In particular, the review highlights the role of EVs in the composition of psoriasis-associated secretome and microenvironment also suggesting the EV involvement in the spreading of the disease mediators and in the development of associated comorbidities.

2 EV release and composition

While it is not clear if EV production is influenced by psoriasis and by its severity (15), *in vitro* experiments with HaCaT cells performed by Mangino et al. have reported that IL-17A treatment significantly alters the rate of EVs production (16). Moreover, the size of the produced EVs was also subjected to modulation since an enrichment in EVs below 450 nm in diameter was observed. Besides IL-17A, other cytokines have been proven to have an effect in EV release, both in size and cargo. To this end, Capriotti et al. (17) carried out experiments by stimulating HaCaT KCs with recombinant chemokines (*i.e.*, IFN-γ, TNF-α, IL-17A, IL-22 and IL-23). EVs were subsequently isolated and analyzed through Nanoparticle Tracking Analysis (NTA). The results revealed that only IL-17A and INF-γ modulated EV production, increasing the quantity of EVs below 200 nm produced. Recently, EVs have proven to be valuable elements to keep track of disease progression and development. In this case psoriasis is not an exception and there are many examples in which EVs have been used to monitor both amelioration after treatment and disease development. In fact, it has been proposed that the concentration of EVs in psoriatic patients' sera is indicative of the chronicity of the disease (18–20).

The molecular cargo of the EVs is the result of an active process orchestrated by specific pathways for exosomes and microvesicles (21). The evaluation of the EVs content can be useful for the discovery of new biomarkers and for determining the possible role of EVs' cargo in transferring competencies to the acceptor cell.

A study based on proteomic analysis on exosome from KCs stimulated by psoriasis related inflammatory cytokines

demonstrated that 72 (10.9%) proteins were up-regulated and 96 (14.5%) down-regulated. The Gene Ontology (GO) analysis showed that the enrichment was focused on proteins linked to the immune effector process, inflammatory response, endocytosis and in molecules involved in serine hydrolase activity, serine-type peptidase activity and heparin binding. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis highlighted an increase of proteins related to Wingless-related integration site (Wnt), Nuclear Factor kappa B (NF-κB), cytokine-cytokine receptor and Toll Like Receptor (TLR) pathways (22). The microenvironmental stress can lead to a change in exosome composition even if the abundance of Heat Shock Proteins (HSPs) and S100 proteins remains univariate and there is not a specific molecular inducer of neutrophils activation. This scenario reinforces the hypothesis that the exosomes are subjected to different methods of spreading messages from the producer cells (22). One of the most important mediators of the psoriatic disease is the IL-17, a cytokine produced by Th17 lymphocytes and KCs. The composition of psoriasis-derived exosomes is dependent on the severity of the disease. It has been discovered that IL-17A expression increases 5 times from mild to severe psoriasis (15). The altered regulation of the inflammation can be due to different mechanisms. The control of the oxidative status is fundamental to reduce the increase of reactive oxygen species (ROS) that can trigger the induction of inflammation (23). It has been observed that while the levels of iron and TIBC (Total iron-binding capacity) were significantly lower in psoriasis patients compared to healthy donors, Soluble transferrin receptor (sTfR) and Heme Oxygenase-1 (HO-1) were over-expressed. HO-1 is an inducible enzyme which converts heme into iron, carbon monoxide and biliverdin/bilirubin (24). HO-1 plays fundamental roles in cytoprotection, membrane cholesterol and oxysterols metabolism (25), and in the development of the psoriatic lesion by negatively regulating Stat3 signaling (26). The function of HO-1 is mainly protective against oxidative stress and inflammation (23). However, in presence of chronic stress situations, the strong induction of HO-1 may result to be cytotoxic due to extreme iron accumulation (27). In exosomes, the increased HO-1 levels reflected the cytosolic situation and could be a part of the non-specific defense against inflammation and ROS increment into psoriatic KCs. In another study iron levels measured through heme-oxygenase activity as other acute phase reactants present in EVs were used to track disease progression and quantify acuteness (28).

The mRNA cargo carried by EVs from IL-17A treated HaCaT cells was enriched by neutrophil and lymphocyte chemoattractant C-X-C motif ligand (CXCL)1, CXCL3, CXCL5, CXCL6 and C-C motif ligand (CCL)20 and AMP Defensin-β2 (hBD2). The aforementioned results were confirmed through neutralization of rIL-17A by using anti-17-A antibody (*i.e.*, Secukinumab). When administered to the treated cells, Secukinumab reversed the CXCL1, CXCL3, CXCL5, CXCL6, CCL20 and hBD2 mRNAs to levels similar to those of the untreated controls. Interestingly, EVs collected from IL-17A treated KCs also induced endogenous expression of Defensin-β2 mRNAs in acceptor cell (16). Furthermore, IL-17A and IL-23 increase the transcription levels of hBD2 and hS100A12 in cells, and hBD2 in EVs. Conversely, IL-17A, TNF-α, IL-22 and IL-23 upregulated the levels of S100 calcium binding protein A12 (S100A12) in the secreted EVs (17).

A massive study of miRNA exosome content from plasma samples of psoriatic patients discovered 246 miRNAs differentially expressed compared to exosomes from plasma of healthy donors. In particular, 166 miRNAs were upregulated whereas 80 were downregulated compared to healthy donors. Among those analyzed, let-7d-3p, miR-125a-5p, -134–5p, -142–3p, -155–5p, -375–3p, -485–5p, -941 and -1228–5p were the most deregulated. Subsequently, a GO enrichment analysis allowed to define the principal biological processes involved in relation to the deregulated miRNAs. KEGG pathway enrichment analysis was directed against miRNAs targets to find the associated pathways. It has been found that cellular metabolic process, cellular process, signal-organism cellular process, metabolic pathways, endocytosis, apoptosis and spliceosome were the most affected among the others (29). The characterization of exosome content of various cell types concurring to the development of the psoriasis, highlights specific miRNA profile for every cell type. Treg derived exosomes are enriched with miR-146a-5p, -150–5p and -21–5p. Th1/Th17 derived exosomes contain high levels of miR-106a-5p, -155–5p and -19a-3p. The possibility to create an *in vitro* model of the psoriasis has been applied to study the miRNA profile from keratinocyte treated derived exosomes: 28 miRNAs are enriched while 114 result downmodulated compared to untreated cells. By evaluating the circulant exosomes into psoriatic patient, it was an interesting highlight as let-7b-5p and miR-30e-5p could be discriminant for the development of cutaneous-only psoriasis with respect to psoriatic arthritis, while miR-1305 dampen could be a master regulator of psoriasis pathophysiology by modulating Wnt pathways (30). Changes in serum miRNA population between patients with plaque psoriasis, psoriatic arthritis and control patients have been found by Lattekivi et al. (31), suggesting that EV mediated communication could be crucial in the pathophysiological development of these diseases. In this study, instead of an overall change in EV bound miRNAs, major shifts in enrichment profiles were discovered. These insights also usefully correlate with other inflammatory diseases such as osteoarthritis (OA). A deregulated miRNA that was previously found to be downregulated in psoriatic skin biopsies, namely hsa-miR-99b-5p, was found to be deregulated in sera collected from patients with plaque psoriasis. This data fits also with the observation that hsa-miR-99b-5p has been positively correlated with keratinocyte hyperproliferation. Hsa-miR-671–3p was found to be down-modulated in patients with arthritic psoriasis compared to the control groups. This miRNA is also deregulated in OA and its role is to regulate the expression of OA correlated genes in chondrocytes and osteoblasts suggesting a possible connection between psoriasis and other inflammatory diseases (32).

Exosome cargo could be defined also by circulating long non-coding RNAs (lncRNAs). Such type of non-coding RNAs (ncRNAs) are characterized by a sequence of more than 200 nucleotides, a secondary structural conformation and regulatory of gene expression function. The lncRNA PRINS (Psoriasis-susceptibility-Related RNA Gene Induced by Stress) can contribute to the pathogenesis of psoriasis by increasing the expression of the anti-

apoptotic G1P3 gene but until now the presence of lncRNA PRINS into KCs-derived or immune cells associated to psoriasis-derived exosome is not well understood (33).

Besides nucleic acids and proteins, lipids also have a role in EV mediated communication in normal physiology as in psoriasis. The alteration of EV membranes and cargo in phospholipid composition benefits the uptake in acceptor cells thus facilitating effective cell-to-cell communication. Starting from the observation that plasma lipidic profiles in psoriatic patients are altered, Paolino et al. studied the phospholipid composition of plasma microvesicles and exosomes (34). In psoriatic patients undergoing treatment with Secukinumab, Ustekinumab, Adalimumab, an increased production of microvesicles and exosomes in plasma was recorded, with altered membrane phospholipid composition. Membrane phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol and lysophosphatidylcholine were altered in plasma exosomes from psoriatic patients in comparison with those from healthy subjects. Moreover, in plasma microvesicles from psoriatic patients, changes in sphingomyelin and phosphatidylinositol levels were recorded. Interestingly, treatments with the aforementioned drugs seemed to revert the observed lipidic phenotypes. Ustekinumab reverted the phosphatidylethanolamine and phosphatidylcholine levels in exosomes back to levels comparable to those in healthy subjects. Furthermore, a variation in microvesicle and exosome origin was recorded by Takeshita et al. as an increased level of monocyte and endothelial-derived microvesicles in psoriatic patients (32). These data suggest that lipid profiles of the sera of patients could be a potential tool for a quantitative diagnosis and management of psoriasis. A resume about the molecular composition of EVs related to psoriatic disease is shown on Table 1.

3 EV cellular trafficking and microenvironment

Jiang et al. demonstrated the influence of EVs originating from KCs treated with cytokines during psoriatic development, specifically in Th1 and Th17 polarization (36). EVs derived from cytokine-stimulated KCs have been shown to influence T cell response to the point of over proliferation and activation, thus leading to psoriasis. Small RNAs such as miRNAs have a major role in regulating $CD4^+$ T cell polarization into Th1 and Th17 subsets. Indeed, the sequencing of RNA extracted from these EVs showed that 28 miRNAs were upregulated while 114 were downmodulated. Among these modulated miRNAs, miR-381–3p expression increased in EVs from cytokine-treated KCs and in $CD4^+$ T-cells from psoriatic patients. In the receiving cells, IFN-γ, IL-17A, IL-17F, T-box expressed in T cells (T-bet), and RAR-related orphan receptor gamma (RORγt) transcript levels enhancement and IFN-γ and IL-17A protein levels increase were observed. Since miR-381–3p has been positively correlated with the Psoriasis Area Severity Index (PASI) score, a clinical-quantitative scale used to determine the severity of psoriasis cases, such evidence suggests the important role of EV cargo in psoriasis development and management. During the development of psoriasis, the Th cell-keratinocyte axis has a crucial role. Although the underlying mechanism is not entirely clear, it is understood that psoriatic KCs communicate with $CD4^+$ cells and induce Th1 and Th17 polarization. This mechanism may also modulate the hyper immune response that is associated with psoriasis. Jiang et al. determined that miR-381–3p is carried from psoriatic cells to $CD4^+$ cells through vesicle trafficking, thus polarizing T helper cells towards the Th1 and Th17 phenotype (36). These findings can be considered both as an interesting development in scientific understanding of psoriatic pathogenesis and as an innovative platform to contrast this disease since these KC-EVs could be implemented in immunomodulatory therapies.

Neutrophils are able to amplify the psoriatic inflammatory deregulation by building the Neutrophils Extracellular Trap (NETs), a structure composed by proteins and DNA that promotes hBD2 expression in KCs and the induction of type 17 T helper cells from peripheral blood mononuclear cells. It has been observed that cytokine-treated keratinocyte exosomes are able to activate NF-κB and p38 pathways on neutrophils leading to the production of IL-6, IL-8, and TNF-α, and so promoting the induction of NETosis. The precise mechanism behind this process has been not defined yet but it seems to be fundamental that the inflammatory message from KC has been conveyed through exosomes. The NET exploits its role when the structure is complete and psoriatic KC-derived exosomes could also be a part of this structure (22). The ability of the EVs derived from KCs stimulated with psoriatic cytokines to induce NETs was investigated also by Capriotti et al. (17). This was carried out by exposing primary neutrophils to supernatant derived from HaCatT cells treated with IFN-γ, TNF-α, IL-17A, IL-22 and IL-23. All cytokines, apart from IFNγ, were able to induce the formation of the neutrophil traps (17). Psoriatic lesions are known to be characterized by a heightened migration and motility of KCs. To further investigate this evidence, HaCat cells were incubated in transwells with EVs from untreated or treated with IL-17A and IFN-γ HaCaT cell. While the cells exposed to the IL-17A treated EVs showed no relevant change in motility compared to the untreated controls, those treated with the IFN-γ derived EVs were able to migrate more than the controls. Behind these differences between IL-17A and IFN-γ, the authors speculate that the hypercellularity seen in psoriatic lesions could be connected to the effect of IL-17A of halting cell migration. Interestingly, IFN-γ has an opposite effect on NETosis, modulating the phenomenon more than IL-17A (17).

The miRNAs activity can be modulated by the action of the circular RNAs that are able to capture miRNAs due to their structure. Circ_0024028 is a circular RNA highly expressed into psoriasis lesions and IL-22 stimulated HaCaT cells. Moreover, it has been demonstrated that in HaCaT cells Circ_0024028 upregulation was associated with cell proliferation and migration and its expression is dependent to IL-22 stimulus in a concentration-dependent manner. With an elegant demonstration Zhang et al. have found that circ_0024028 can be accumulated into exosome in a specific manner and that exosomes are able to spread circ_0024028 into surrounding cells. Probably the action of circ_0024028 is related to the sponge of miR-486–3p that is an inhibitor of retinoblastoma (pRB) and AKT serine/threonine kinase

TABLE 1 Schematic table summarizing the cargo carried by EVs or exosomes, the quality of modulation in the target cell and the resulting effects.

Component	Mediator	Type of regulation	Effects	Origin	References
mRNA	hBD2 CXCL3 CCL20	Upregulated	Pro-inflammatory	HaCaT	(16)
	S100A12 hBD2	Upregulated	Pro-inflammatory	pso-KC	(17)
ncRNA	miR-146a-5p miR-150–5p miR-21–5p	Upregulated	Pro-inflammatory Onco-regulatory	Treg	(30)
	miR-106a-5p miR-155–5p miR-19a-3p	Upregulated	Pro-inflammatory Onco-regulatory	Th17	(30)
	miR-30e-5p let-7b-5p	Downregulated (in PsA)	Discriminates between cutaneous and arthritic psoriasis	Blood	(35) (30)
	miR-1305	Downregulated	Amelioration of psoriasis development	Blood	(30)
	miR-381–3p miR-365–5p miR-4488 miR-619–5p	Upregulated	Role in Th1/Th17 polarization.	pso-KC	(36)
	miR-4505	Upregulated	Induction of M1 macrophage differentiation.	pso-KC	(37)
	ASO-210	Delivery	Anti-inflammatory	MSC-EVs	(38)
	circ_0024028	Expression	Sponge activity for miR-486–3p.	pso-KC	(39)
Protein	OLFM4	Expression	Positively correlated with severity of GPP Pro-inflammatory	pso-Neutrophils	(40)
	JPH203	Expression	LAT-1 inhibitor.	pso-KC	(41)
	Heme-oxygenase sTfR	Upregulation	Anti-inflammatory	Blood	(28)
Lipids	Pristimerin	Expression	Anti-inflammatory	Melanoma cells	(42)
	Phosphatidylcholine Phosphatidylethanolamine Phosphatidylglycerol Lysophosphatidylcholine	Upregulation	Enrichment of EV plasma membrane in psoriasis patients	pso-KC	(34)

RNAs, proteins or lipids that are expressed ex novo in EVs or exosomes and are delivered in innovative therapy strategies have been labeled as "Expression".

3 (AKT3) genes and an activator of extracellular matrix protein 1 (ECM1) level (39).

The ability of neutrophil-derived vesicles to interact with immune cells for the orchestration of the adaptive immune response is well documented. Shao et al. determined that, Olfactomedin 4 protein (OLFM4), was expressed in exosomes from neutrophils collected from generalized pustular psoriasis (GPP) (40). This protein belongs to the olfactomedin family and is known to be an anti-apoptotic and tumor promoting factor. In their work, the authors approached exosome characterization from a proteomic standpoint analyzing EV cargo from healthy and GPP subjects. OLFM4 was not only found in mRNA form in psoriatic neutrophils but also as a protein in circulating exosomes (determined through Western Blot), shedding interesting information on cell-to-cell communication between neutrophils and psoriatic KCs. Interestingly, when recombinant OLFM4 was introduced in KCs, a spike in CXCL1, CXCL2, CXCL8, and CCL20-containing exosomes was registered. This expression phenotype is typically associated with a psoriatic microenvironment, which leads to increased proliferation and migration of neutrophils and other immune cells to the inflamed area. Thus, the role of OLFM4 has been correlated with the exosome-assisted pathogenesis of GPP.

Mast cells derived exosomes contain phosphatidylcholine 2-acylhydrolase (PLA2) and are able to bind CD1a on T lymphocytes, thus stimulating an inflammatory response. This inflammatory response was determined by Enzyme-Linked immuno-SPOT (ELISPOT) experiments on T cells from healthy and psoriatic subjects stimulated with mast cells derived exosomes. T cells from psoriasis patients had a greater IFN-γ, IL-17 and IL-22 production due to an increased CD1a response compared to healthy individuals. Such CD1a high T cells were preferentially localized near the lesional skin but could also be found into the non-lesional skin and peripheral blood. PLA2 was produced by endogenous cytosolic phospholipase A2 group IV D (PLA2G4D) that was

expressed in mast cells and KCs within psoriatic lesions, loaded into exosomes and transferred to CD1a-expressing target cells in a clathrin-dependent manner (43).

Psoriasis is characterized by the increase of the asymmetric division of the basal stem cells. Such situation is caused by the hyperactivation of the Par3/mInsc/LGN signaling pathway. Moreover, proteinase-activated receptor (PAR) proteins cooperate with atypical protein kinase C (aPKC) λ to induce skin tumor and to modulate inflammatory signaling. It has been observed that, during psoriasis, macrophages show high levels of Par3 expression. Exosomes derived from psoriatic macrophage containing Par3 are able to induce asymmetric division of the basal stem cells and inflammation when inoculated in mice skin (44).

Psoriatic KCs show a low expression of the vitamin D receptor (VDR). It has been demonstrated that it is possible to induce M1 polarization and inhibition of apoptosis by stimulating macrophages with HaCaT cells derived-VDR deficient exosomes. Moreover, starting from the knowledge that miR-4505 was highly express in psoriatic skin it has been observed that VDR deficient HaCaT cells showed miR-4505 overexpression and the exosomes produced by these cells exerted their M1 polarization and anti-apoptotic activity through the delivery of miR-4505. The M1 polarization of the macrophages, then, seems central in maintaining the inflammation (37). A table resuming all the interaction mediated by EVs between KCs and other immune cells located into psoriatic microenvironment has been represented in Figure 1.

4 EVs and therapeutic applications

Zhang et al. demonstrated that EVs derived from IFN-γ treated mesenchymal stem cells (MSC-EVs) have the ability to effectively modulate the proliferation of peripheral monocellular cells and T cells in a psoriatic setting (38). MSC-EVs decreased the intensity and presence of hallmark psoriatic symptoms such as skin thickness, scaling and erythema, but also decreased the production of pro-inflammatory cytokines such as IL-6, IL-17A, IFN-γ and TNF-α. Interestingly, Th cell subsets were also modulated with less exhausted Th17 cells and more Th2 cells. Furthermore, these types of cells were shown to be potentially effective tools in disease management. Indeed, antisense nucleotides, in this case ASO-210, were delivered with more efficacy by using MSC-EVs. Nucleotide stability was also heightened, resulting in a successful control of immune imbalance which is at the core of psoriatic development. The ability of EVs to efficiently carry and protect bioactive cargo has been exploited in experiments using innovative compounds. In one case Jiang et al. used ultraviolet induced EVs from cultured KCs to transport an inhibitor of LAT1 (JPH203) with the purpose of blocking the mTOR pathway through leucine sequestration (41). Moreover, the exposure of KCs to UVB radiation caused an accumulation of IL-1 receptor antagonists that, when released in an *in vitro* model, effectively decreased IL-1 mediated inflammation. Positive results of the *in vivo* treatment were also the blocking of NF-κB pathway. In successive *in vivo* psoriasis imiquimod (IMQ)-induced murine experiments, the EVs significantly reduced the typical psoriatic symptoms like acanthosis as well as suppressing the over-active immune reaction, pointed out by the IL-17 release and Th17 expansion (41).

MSCs-exosomes are known to dampen chronic inflammation associated with bowel disease, atopic dermatitis and chronic graft versus host disease (GVHD). On the other hand, EVs derived from umbilical cord blood mononuclear cell are able to downmodulate the expression of inflammatory mediators namely IL-6, IL-8, CXCL10, Cyclooxygenase 2 (COX2), S100A7, and hBD4 in a 3D model of psoriatic skin. When the umbilical cord blood mononuclear cell derived EVs had been administered to IMQ-treated mice the inflammatory dampening was incomplete suggesting a use in combination with standard therapies (45). MSC derived exosomes are able to reduce the expression of IL-17, IL-23 and C5b-9 in IMQ treated mouse skin. Zhang et al. have been tested for topical application demonstrating that they remain along the stratum corneum. Here they can regulate complements components, one of the most induced molecules by NETosis. Since neutrophils are the major producer of IL-17 during psoriasis it could be reasonable to hypothesize that the MSC exosome activity into the *stratum corneum* can finally damp the inflammatory mediators into epidermis (46). A study confirmed the ability of umbilical cord blood mononuclear cell derived exosome to reduce inflammation. Specifically, they reduced the expression of CCL20, IL-17 and IL-23 in IMQ-induced mice and treated HaCaT cells. The entire inflammatory process of psoriasis needs the orchestration of various factors. Mature DCs participate to the development of the disease through the continue production of IL-23 that activating Th17 cells, finally, producing an abnormal production of IL-17, IL-21 and IL-22 and KCs deregulation. The use of umbilical cord blood mononuclear cell derived exosome can reduce the secretion of IL-23 by DCs. Moreover, the phosphorylation of STAT3 is a central mechanism of the IL-17/-23 axis and hucMSCs-Exo were able to inhibit this process in the epidermis of IMQ-induced psoriatic mice and in HaCaT cells (47).

The therapeutical uses of exosomes derived and modified from tumor cells, immune cells or mesenchymal stem cells could be promising. A characteristic of tumoral exosomes, as is the case of melanoma derived exosomes, is the high presence of programmed death-ligand 1 (PD-L1) expressed to achieve immune escape. Jia et al. engineered exosome derived from melanoma cells by introducing a natural anti-inflammatory triterpenoid substance called Pristimerin. The combinatory activity of PD-1/PD-L1 interaction and Pristimerin allowed the engineered melanoma-derived exosomes to dampen inflammation when administrated to psoriatic skin more than the sum of each treatment. The activity of the immune infiltrate in the psoriatic skin is fundamental for the development of the disease. Macrophages are one of the most important immune cells that collaborate into psoriatic inflammation promotion. Engineered exosomes derived from melanoma cells are able to reduce the inflammatory macrophages infiltrate and to drive their polarization into M2 subset. In addition, the interaction between PD-L1 on the surface of the exosomes and PD-1 on the surface of immunosuppressive T cells produced the

10.3389/fimmu.2024.1360618

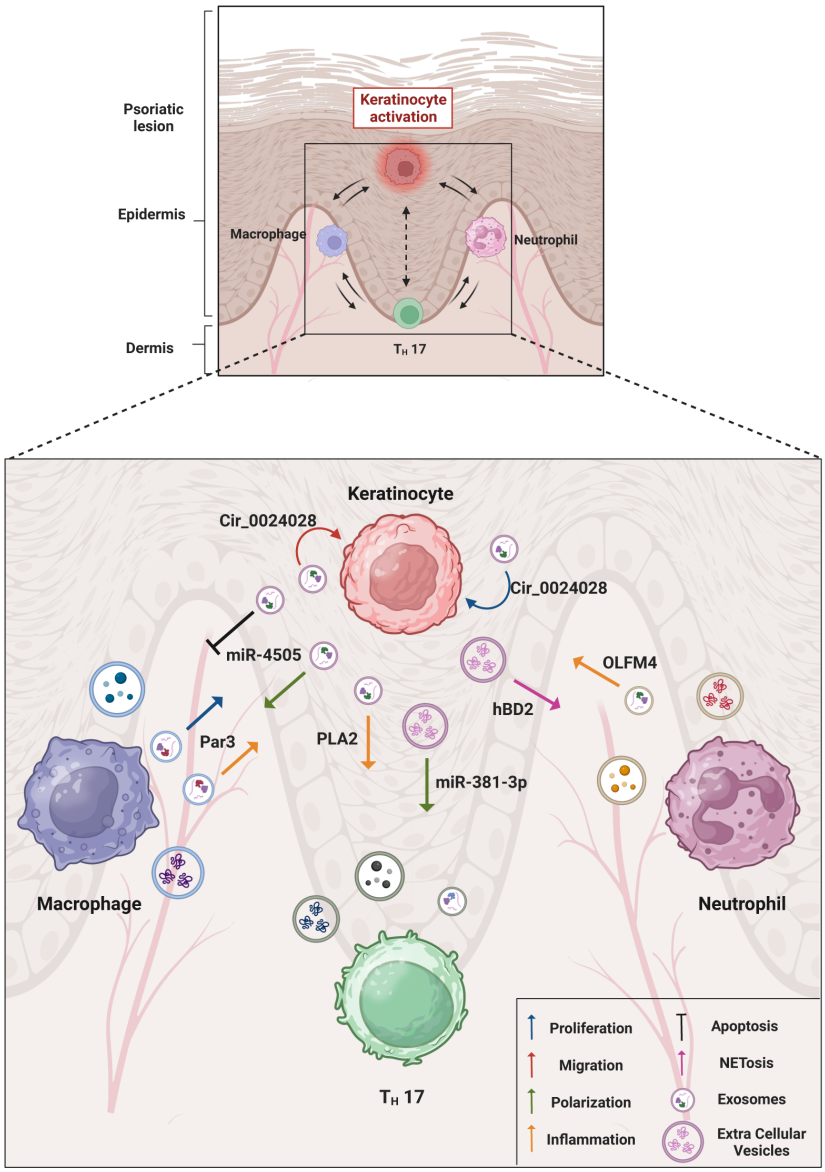


FIGURE 1
Overview of keratinocytes and immune cells interactions in psoriatic microenvironment. The cartoon summarizes the main molecular mediators found within the EVs and elucidates the resulting inflammatory effects obtained through EV trafficking. An emphasis has been given on the cell-to-cell communication between psoriatic keratinocytes and immune cells (macrophages, T helper lymphocytes and neutrophils) localized in the lesion area. The role of EVs in the psoriatic disease, in particular in the composition of psoriasis-associated secretome and microenvironment indicates the EV involvement in the spreading of disease mediators and in the possible associated comorbidities. Created with BioRender.com.

exhaustion of this kind of T cell so improving the inflammatory status of the affected skin (42).

Correlation between psoriasis and other inflammatory diseases and microbiota diversity has been a subject of scientific interest as of recent years. In order to study microbial skin heterogeneity, α and β diversity is usually employed. The former represents the diversity within a sample of an ecological community, while the latter is used to measure how much two distinct communities differ. In this setting, interesting developments have also arisen in the relationship between host and commensal bacteria of the skin, intestinal mucosa and other tissues. Indeed, communication between the host and non-pathogenic bacteria are crucial for certain mechanisms such as tissue healthiness, functionality and defense against pathogens and can be carried out through EVs. Chang et al. expanded on this subject by performing metanalyses of skin and intestine microbiota by analyzing the origin and diversity of serum EVs (48). By searching EV contents for Microbial Associated Molecular patterns (MicroAMPS), their findings pointed out a lower richness and microbial diversity in intestinal and skin microbiota in psoriatic patients. This is in line with previous studies in which a decreased alpha-diversity was measured in the intestinal microbiota of psoriasis patients, thus speculating an

interesting correlation between dysbiosis, psoriasis and Inflammatory Bowel Disease (IBS) related diseases. Furthermore, the authors found that the presence of *Staphylococcaceae*, most importantly *S. aureus*, took up a higher percentage of the entire microbiota in psoriatic patients compared to healthy controls. This Gram-positive bacterium is responsible for the inflammation of skin mucosa and KCs through allergic reaction and release of alpha-toxins. *S. aureus* infection has also been highly correlated with psoriasis severity (49). In conclusion, analyzing EV content for Metabolism Associated Molecular Patterns (MAMPS) or deviation from biochemical homeostasis could represent a viable option for psoriasis diagnosis and management. Commensal bacteria play an active role in skin health by promoting regeneration after injury and by impeding pathogen colonization. Among the many species that inhabit the skin microbiota, *S. epidermidis* is one of the most active in protection against pathogens and immunomodulation during inflammation. Chang et al. reported that in psoriatic skin *S. epidermidis* and *Propionibacterium acnes* were under-represented, suggesting a possible role of these species in psoriasis contrast. Since the mechanism through which the immunomodulation occurs is unknown, it is possible to hypothesize that EVs could play a role in signal transduction (48). Gomez-Chavez et al. presented a study in which EVs extracted from two *S. epidermidis* strains, one commensal (ATC12228) and a second one of clinical origin (983), were used to test the effect on a psoriatic skin platform, namely the *in vivo* IMQ-induced murine model (50). In an *in vitro* experiment with the keratinocyte cell line HaCaT, both the ATC12228 and the 983 derived EVs were capable of inducing proinflammatory IL-6 expression, although EVs from the clinical strain induced a higher level of other inflammatory mediators like Vascular endothelial growth factor-A (VEGF-A), LL-37, IL-8, and IL-17F. In the *in vivo* experiment using the IMQ-induced murine model the ATC12228 EVs actually reversed typical psoriatic symptoms like acanthosis and cell infiltration as well as VEGF-A, IL-6, IL-23, IL-17F and IL-36 mRNA transcription. Conversely FoxP3 expression had no significant change in expression and IL-36 receptor antagonist was found to be increased. With these findings the authors evaluated the therapeutic potential of *S. epidermidis* EVs in regulating the immune response in psoriatic skin (51).

5 Discussion

This is a state of the art update on the importance and future perspectives of EVs, the emergent regulatory biological structures in virology, immunology and pathology, as explored by researchers from both inside and outside the EV community. This work focused on the role of EVs in the pathogenesis of the chronic inflammatory disease, psoriasis.

EVs are heterogeneous, membrane-enclosed nanostructures that are evolutionally conserved and released by cells of living organisms. EVs are identified as an alternative secretory mechanism for cytokines/chemokines and the regulatory role of specific cytokines in vesicle release, trafficking and/or content is almost recognized. Their role as important mediators of cell-to-cell communication in physiologic and pathologic conditions has emerged in the last two decades. The composition of the EV cargo is diverse and critical for intercellular communication. EV cargo is defined by the lineage of the parental cells and their state of activation. EVs protect their cargo, *e.g.* miRNAs secreted into the microenvironment are preserved from serum RNAse degradation as a result of being encapsulated inside a double membrane structure; once transferred to recipient cells, EVs could promote inflammation by regulating gene expression leading to multiple physiological changes in cell proliferation, migration, intercellular communication and/or stromal modification. The inflammatory microenvironment is characterized by the presence and activity of specific combinations of molecular, cellular and sub-cellular mediators derived from both immune and non-immune cells that collectively contribute to inflammation. Among the sub-cellular mediators are EVs that are derived through budding processes from cellular membranes and are secreted into the extracellular space by many cell types. Many inflammatory-associated pathological disorders, ranging from autoimmune diseases to cancer (52–55), are mainly characterized by a microenvironment with specific inflammatory elements (*i.e.*, immune cells infiltrate, cytokines, chemokines, AMPs and Damage-associated molecular patterns). The role of EVs in the psoriatic disease, in particular in the composition of psoriasis-associated secretome and microenvironment indicates the EV involvement in the spreading of disease mediators and in the possible associated comorbidities.

However, the studies are still at their infancy in the dermatological field. The sections on different topics of recent EV studies, from EV specificity, production, cargo and extracellular functions, as well as some pilot therapeutic applications, aim to address the emerging challenges up to date limiting the broader translational use of EVs. The molecular cargo and the origin of EVs related to psoriatic disease is outlined in Table 1. The main interactions mediated by EVs between KCs and other immune cells located in the psoriatic microenvironment are represented in Figure 1.

Likewise, the highlighted new strategies and more comprehensive studies appear to be in progress to identify EV subpopulations with high accuracy and selectivity. Notably, new technologies have flourished in recent years allowing future applications to benefit from EVs' identification and profiling, with the aim to detect and treat inflammatory skin diseases.

Author contributions

MI: Conceptualization, Writing – original draft, Writing – review & editing. LG: Writing – original draft, Writing – review & editing. PR: Writing – original draft, Writing – review & editing. SS: Writing – original draft, Writing – review & editing. NB: Supervision, Writing – review & editing. IP: Supervision, Writing – review & editing. ET: Supervision, Writing – review & editing. NS: Supervision, Writing – review & editing. CP: Supervision, Writing – review & editing. GM: Conceptualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing. GR: Conceptualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was financially supported by the "Bando di Ateneo Sapienza-2020", Grant no. RM120172B8B7B997 and "Bando di Ateneo Sapienza-2021", Grant no. RM12117A8778D221.

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.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

References

1. Damiani G, Bragazzi NL, Karimkhani Aksut C, Wu D, Alicandro G, McGonagle D, et al. The global, regional, and national burden of psoriasis: results and insights from the global burden of disease 2019 study. *Front Med (Lausanne)*. (2021) 8:743180. doi: 10.3389/fmed.2021.743180

2. Di Meglio P, Villanova F, Nestle FO. Psoriasis. *Cold Spring Harb Perspect Med*. (2014) 4:a015354. doi: 10.1101/cshperspect.a015354

3. Di Cesare A, Di Meglio P, Nestle FO. The IL-23/Th17 axis in the immunopathogenesis of psoriasis. *J Invest Dermatol*. (2009) 129:1339–50. doi: 10.1038/jid.2009.59

4. Enamandram M, Kimball AB. Psoriasis epidemiology: the interplay of genes and the environment. *J Invest Dermatol*. (2013) 133:287–9. doi: 10.1038/jid.2012.434

5. Griffiths CE, Barker JN. Pathogenesis and clinical features of psoriasis. *Lancet*. (2007) 370:263–71. doi: 10.1016/S0140-6736(07)61128-3

6. van der Pol E, Böing AN, Harrison P, Sturk A, Nieuwland R. Classification, functions, and clinical relevance of extracellular vesicles. *Pharmacol Rev*. (2012) 64:676–705. doi: 10.1124/pr.112.005983

7. Kakarla R, Hur J, Kim YJ, Kim J, Chwae YJ. Apoptotic cell-derived exosomes: messages from dying cells. *Exp Mol Med*. (2020) 52:1–6. doi: 10.1038/s12276-019-0362-8

8. Lo Cicero A, Stahl PD, Raposo G. Extracellular vesicles shuffling intercellular messages: for good or for bad. *Curr Opin Cell Biol*. (2015) 35:69–77. doi: 10.1016/j.ceb.2015.04.013

9. Kim KM, Abdelmohsen K, Mustapic M, Kapogiannis D, Gorospe M. RNA in extracellular vesicles. *Wiley Interdiscip Rev RNA*. (2017) 8:10.1002/wrna.1413. doi: 10.1002/wrna.1413

10. Théry C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. *Nat Rev Immunol*. (2002) 2:569–79. doi: 10.1038/nri855

11. Medhin LB, Beasley AB, Warburton L, Amanuel B, Gray ES. Extracellular vesicles as a liquid biopsy for melanoma: Are we there yet? *Semin Cancer Biol*. (2023) 89:92–8. doi: 10.1016/j.semcancer.2023.01.008

12. Sabato C, Noviello TMR, Covre A, Coral S, Caruso FP, Besharat ZM, et al. A novel microRNA signature for the detection of melanoma by liquid biopsy. *J Transl Med*. (2022) 20:469. doi: 10.1186/s12967-022-03668-1

13. Shao S, Fang H, Li Q, Wang G. Extracellular vesicles in inflammatory skin disorders: from pathophysiology to treatment. *Theranostics*. (2020) 10:9937–55. doi: 10.7150/thno.45488

14. Rosa P, De Falco E, Pacini L, Piazza A, Ciracì P, Ricciardi L, et al. Next-generation sequencing comparative analysis of DNA mutations between blood-derived extracellular vesicles and matched cancer tissue in patients with grade 4 glioblastoma. *Biomedicines*. (2022) 10:2590. doi: 10.3390/biomedicines10102590

15. Jacquin-Porretaz C, Cordonnier M, Nardin C, Boullerot L, Chanteloup G, Vautrot V, et al. Increased levels of interleukin-17A exosomes in psoriasis. *Acta Derm Venereol*. (2019) 99:1143–7. doi: 10.2340/00015555-3300

16. Mangino G, Iuliano M, Carlomagno S, Bernardini N, Rosa P, Chiantore MV, et al. Interleukin-17A affects extracellular vesicles release and cargo in human keratinocytes. *Exp Dermatol*. (2019) 28:1066–73. doi: 10.1111/exd.14015

17. Capriotti L, Iuliano M, Lande R, Frasca L, Falchi M, Rosa P, et al. Potential pathogenetic role of antimicrobial peptides carried by extracellular vesicles in an in *vitro* psoriatic model. *J Inflammation Res*. (2022) 15:5387–99. doi: 10.2147/JIR.S373150

18. Tamagawa-Mineoka R, Katoh N, Kishimoto S. Platelet activation in patients with psoriasis: increased plasma levels of platelet-derived microparticles and soluble P-selectin. *J Am Acad Dermatol*. (2010) 62:621–6. doi: 10.1016/j.jaad.2009.06.053

19. Longobardi A, Benussi L, Nicsanu R, Bellini S, Ferrari C, Saraceno C, et al. Plasma extracellular vesicle size and concentration are altered in Alzheimer's disease, dementia with lewy bodies, and frontotemporal dementia. *Front Cell Dev Biol*. (2021) 9:667369. doi: 10.3389/fcell.2021.667369

20. Freeman DW, Noren Hooten N, Eitan E, Green J, Mode NA, Bodogai M, et al. Altered extracellular vesicle concentration, cargo, and function in diabetes. *Diabetes*. (2018) 67:2377–88. doi: 10.2337/db17-1308

21. Anand S, Samuel M, Kumar S, Mathivanan S. Ticket to a bubble ride: Cargo sorting into exosomes and extracellular vesicles. *Biochim Biophys Acta Proteins Proteom*. (2019) 1867:140203. doi: 10.1016/j.bbapap.2019.02.005

22. Jiang M, Fang H, Shao S, Dang E, Zhang J, Qiao P, et al. Keratinocyte exosomes activate neutrophils and enhance skin inflammation in psoriasis. *FASEB J*. (2019) 33:13241–53. doi: 10.1096/fj.201900642R

23. Campbell NK, Fitzgerald HK, Dunne A. Regulation of inflammation by the antioxidant haem oxygenase 1. *Nat Rev Immunol*. (2021) 21:411–25. doi: 10.1038/s41577-020-00491-x

24. Wojas-Pelc A, Marcinkiewicz J. What is a role of haeme oxygenase-1 in psoriasis? Current concepts of pathogenesis. *Int J Exp Pathol*. (2007) 88:95–102. doi: 10.1111/j.1365-2613.2006.00505.x

25. Rosa P, Zerbinati C, Crestini A, Canudas AM, Ragona G, Confaloni A, et al. Heme oxygenase-1 and brain oxysterols metabolism are linked to egr-1 expression in aged mice cortex, but not in hippocampus. *Front Aging Neurosci*. (2018) 10:363. doi: 10.3389/fnagi.2018.00363

26. Zhang B, Xie S, Su Z, Song S, Xu H, Chen G, et al. Heme oxygenase-1 induction attenuates imiquimod-induced psoriasiform inflammation by negative regulation of Stat3 signaling. *Sci Rep*. (2016) 6:21132. doi: 10.1038/srep21132

27. Liu R, Yang J, Li Y, Xie J, Wang J. Heme oxygenase-1: The roles of both good and evil in neurodegenerative diseases. *J Neurochem*. (2023) 167:347–61. doi: 10.1111/jnc.15969

28. El-Rifaie AA, Sabry D, Doss RW, Kamal MA, Abd El Hassib DM. Heme oxygenase and iron status in exosomes of psoriasis patients. *Arch Dermatol Res*. (2018) 310:651–6. doi: 10.1007/s00403-018-1852-6

29. Chen XM, Yao DN, Wang MJ, Wu XD, Deng JW, Deng H, et al. Deep sequencing of plasma exosomal microRNA level in psoriasis vulgaris patients. *Front Med (Lausanne)*. (2022) 9:895564. doi: 10.3389/fmed.2022.895564

30. Mirzaei R, Zamani F, Hajibaba M, Rasouli-Saravani A, Noroozbeygi M, Gorgani M, et al. The pathogenic, therapeutic and diagnostic role of exosomal microRNA in the autoimmune diseases. *J Neuroimmunol*. (2021) 358:577640. doi: 10.1016/j.jneuroim.2021.577640

31. Lättekivi F, Guljavina I, Midekessa G, Viil J, Heath PR, Bæk R, et al. Profiling blood serum extracellular vesicles in plaque psoriasis and psoriatic arthritis patients reveals potential disease biomarkers. *Int J Mol Sci*. (2022) 23:4005. doi: 10.3390/ijms23074005

32. Takeshita J, Mohler ER, Krishnamoorthy P, Moore J, Rogers WT, Zhang L, et al. Endothelial cell-, platelet-, and monocyte/macrophage-derived microparticles are elevated in psoriasis beyond cardiometabolic risk factors. *J Am Heart Assoc*. (2014) 3:e000507. doi: 10.1161/JAHA.113.000507

33. Karimi B, Dehghani Firoozabadi A, Peymani M, Ghaedi K. Circulating long noncoding RNAs as novel bio-tools: Focus on autoimmune diseases. *Hum Immunol*. (2022) 83:618–27. doi: 10.1016/j.humimm.2022.06.001

34. Paolino G, Buratta S, Mercuri SR, Pellegrino RM, Urbanelli L, Emiliani C, et al. Lipidic profile changes in exosomes and microvesicles derived from plasma of

monoclonal antibody-treated psoriatic patients. *Front Cell Dev Biol.* (2022) 10:923769. doi: 10.3389/fcell.2022.923769

35. Pasquali L, Svedbom A, Srivastava A, Rosén E, Lindqvist U, Ståhle M, et al. Circulating microRNAs in extracellular vesicles as potential biomarkers for psoriatic arthritis in patients with psoriasis. *J Eur Acad Dermatol Venereol.* (2020) 34:1248–56. doi: 10.1111/jdv.16203

36. Jiang M, Fang H, Dang E, Zhang J, Qiao P, Yu C, et al. Small extracellular vesicles containing miR-381–3p from keratinocytes promote T helper type 1 and T helper type 17 polarization in psoriasis. *J Invest Dermatol.* (2021) 141:563–74. doi: 10.1016/j.jid.2020.07.009

37. Sun W, Chen J, Li J, She X, Ma H, Wang S, et al. Vitamin D receptor-deficient keratinocytes-derived exosomal miR-4505 promotes the macrophage polarization towards the M1 phenotype. *PeerJ.* (2023) 11:e15798. doi: 10.7717/peerj.15798

38. Zhang W, Lin J, Shi P, Su D, Cheng X, Yi W, et al. Small extracellular vesicles derived from MSCs have immunomodulatory effects to enhance delivery of ASO-210 for psoriasis treatment. *Front Cell Dev Biol.* (2022) 10:842813. doi: 10.3389/fcell.2022.842813

39. Zhang B, Wu S. Downregulation of circ_0024028 inhibits IL-22-induced keratinocyte proliferation and migration by miR-486–3p/AKT3 axis. *Arch Dermatol Res.* (2023) 315:2079–90. doi: 10.1007/s00403-023-02597-3

40. Shao S, Fang H, Zhang J, Jiang M, Xue K, Ma J, et al. Neutrophil exosomes enhance the skin autoinflammation in generalized pustular psoriasis. *FASEB J.* (2019) 33:6813–28. doi: 10.1096/fj.201802090RR

41. Jiang X, Jiang Z, Huang S, Mao P, Zhang L, Wang M, et al. Ultraviolet B radiation-induced JPH203-loaded keratinocyte extracellular vesicles exert etiological interventions for psoriasis therapy. *J Control Release.* (2023) 362:468–78. doi: 10.1016/j.jconrel.2023.08.059

42. Jia H, Liu T, Yang Q, Zheng H, Fu S, Hong J, et al. Tumor-derived PD-L1. *Bioconjug Chem.* [Online ahead of print] (2023). doi: 10.1021/acs.bioconjchem.3c00129

43. Cheung KL, Jarrett R, Subramaniam S, Salimi M, Gutowska-Owsiak D, Chen YL, et al. Psoriatic T cells recognize neolipid antigens generated by mast cell phospholipase delivered by exosomes and presented by CD1a. *J Exp Med.* (2016) 213:2399–412. doi: 10.1084/jem.20160258

44. Yan J, Zhang K, Guo T, Yang S, Jia H. Par3 regulates the asymmetric division of basal stem cells in psoriasis via the Par3/mInsc/LGN signaling axis. *Cell Immunol.* (2022) 373:104496. doi: 10.1016/j.cellimm.2022.104496

45. Rodrigues SC, Cardoso RMS, Freire PC, Gomes CF, Duarte FV, Neves RPD, et al. Immunomodulatory properties of umbilical cord blood-derived small extracellular vesicles and their therapeutic potential for inflammatory skin disorders. *Int J Mol Sci.* (2021) 22:9797. doi: 10.3390/ijms22189797

46. Zhang B, Lai RC, Sim WK, Choo ABH, Lane EB, Lim SK. Topical application of mesenchymal stem cell exosomes alleviates the imiquimod induced psoriasis-like inflammation. *Int J Mol Sci.* (2021) 22:720. doi: 10.3390/ijms22020720

47. Zhang Y, Yan J, Li Z, Zheng J, Sun Q. Exosomes derived from human umbilical cord mesenchymal stem cells alleviate psoriasis-like skin inflammation. *J Interferon Cytokine Res.* (2022) 42:8–18. doi: 10.1089/jir.2021.0146

48. Chang CJ, Zhang J, Tsai YL, Chen CB, Lu CW, Huo YP, et al. Compositional features of distinct microbiota base on serum extracellular vesicle metagenomics analysis in moderate to severe psoriasis patients. *Cells.* (2021) 10:2349. doi: 10.3390/cells10092349

49. Crusz SA, Yates C, Holden S, Kearns A, Boswell T. Prolonged outbreak of Staphylococcus aureus surgical site infection traced to a healthcare worker with psoriasis. *J Hosp Infect.* (2014) 86:42–6. doi: 10.1016/j.jhin.2013.10.006

50. Gómez-Chávez F, Cedillo-Peláez C, Zapi-Colín LA, Gutiérrez-González G, Martínez-Torres I, Peralta H, et al. The extracellular vesicles from the commensal staphylococcus epidermidis ATCC12228 strain regulate skin inflammation in the imiquimod-induced psoriasis murine model. *Int J Mol Sci.* (2021) 22:13029. doi: 10.3390/ijms222313029

51. Chang HW, Yan D, Singh R, Liu J, Lu X, Ucmak D, et al. Alteration of the cutaneous microbiome in psoriasis and potential role in Th17 polarization. *Microbiome.* (2018) 6:154. doi: 10.1186/s40168-018-0533-1

52. Chiantore MV, Mangino G, Zangrillo MS, Iuliano M, Affabris E, Fiorucci G, et al. Role of the microenvironment in tumourigenesis: focus on virus-induced tumors. *Curr Med Chem.* (2015) 22:958–74. doi: 10.2174/0929867322666141212121751

53. Ferrari SM, Fallahi P, Elia G, Ragusa F, Ruffilli I, Paparo SR, et al. Thyroid autoimmune disorders and cancer. *Semin Cancer Biol.* (2020) 64:135–46. doi: 10.1016/j.semcancer.2019.05.019

54. Wu MY, Li CJ, Hou MF, Chu PY. New insights into the role of inflammation in the pathogenesis of atherosclerosis. *Int J Mol Sci.* (2017) 18:2034. doi: 10.3390/ijms18102034

55. Colgan SP, Curtis VF, Campbell EL. The inflammatory tissue microenvironment in IBD. *Inflammation Bowel Dis.* (2013) 19:2238–44. doi: 10.1097/MIB.0b013e31828dcaaf

Frontiers in Immunology

Explores novel approaches and diagnoses to treat immune disorders.

The official journal of the International Union of Immunological Societies (IUIS) and the most cited in its field, leading the way for research across basic, translational and clinical immunology.

Discover the latest Research Topics

See more →

Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact