

Advances in Immunomodulatory Mechanisms of Mesenchymal Stem Cells-Derived Exosome on Immune Cells in Scar Formation

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Abstract: Pathological scars are the result of over-repair and excessive tissue proliferation of the skin injury. It may cause serious dysfunction, resulting in psychological and physiological burdens on the patients. Currently, mesenchymal stem cells-derived exosomes (MSC-Exo) displayed a promising therapeutic effect on wound repair and scar attenuation. But the regulatory mechanisms are opinions vary. In view of inflammation has long been proven as the initial factor of wound healing and scarring, and the unique immunomodulation mechanism of MSC-Exo, the utilization of MSC-Exo may be promising therapeutic for pathological scars. However, different immune cells function differently during wound repair and scar formation. The immunoregulatory mechanism of MSC-Exo would differ among different immune cells and molecules. Herein, this review gave a comprehensive summary of MSC-Exo immunomodulating different immune cells in wound healing and scar formation to provide basic theoretical references and therapeutic exploration of inflammatory wound healing and pathological scars.

Keywords: exosomes, inflammation, immune cells, immunoregulation, scars

Background

Pathological scars, including hypertrophic scar (HS) and keloids, are abnormal scars at the trauma or surgical sites of the skin and are characterized by the over-proliferation of fibroblasts, abundant production of extracellular matrix (ECM) and excessive deposition of collagen, which normally cause burdens for the patients both psychologically and physiologically.¹⁻³ It's always a troublesome problem for clinical doctors due to its unclear pathogenesis and shortage of effective therapies.^{4,5} Therefore, exploring the potential pathogenesis and developing possible therapeutics for pathological scars are urgently needed.

The process of wound healing experiences 4 stages: hemostasis, inflammation, proliferation and remodeling, which we can see that the inflammation is at the beginning of scar formation.⁶ Although the exact pathogenesis of pathological scars remains unclear, the inflammation was proven to largely influence the formation and development of HS and keloids.^{7,8} An excessive response of inflammation is deemed to contribute to scar formation and fibrosis.⁹ Clinical works also showed that infected wounds always need a longer time to heal and will easily develop into more serious scars in most cases than uninfected wounds.¹⁰ However, there are lots of immune cells and molecules that participate in this process, and the specific mechanism of immunomodulation is not fully understood. For example, the polarity of macrophages plays an important role in the formation of pathological scars, especially M2 macrophages could promote the formation and development of HS and keloids.¹¹ In addition, mast cells, T lymphocytes, neutrophils, Langerhans cells, and natural killer T (NKT) cells are also essential in the immunoregulatory process of scar formation.¹²⁻¹⁶

Subsequently, plenty of immune agents were explored for the treatment of HS and keloids, such as triamcinolone acetonide, tacrolimus, imiquimod, fingolimod, et al^{17–20} Hydrogel biomaterials with inherent antimicrobial properties offer an attractive and viable solution to wound bacterial infections, which might address this issue and also attenuate scar formation.²¹ Thus, further studying the mechanism of immunomodulation during scar formation would provide theoretical references for other researchers and develop more effective immune therapeutics for pathological scars.

Exosomes are one kind of nano-sized extracellular vesicle (EVs) with a diameter of 30–150nm derived from various cells, and serve as mediators for cell-to-cell communication.²² Two other subpopulations of EVs are microvesicles and apoptotic bodies. Exosomes derived from adipose-derived mesenchymal stem cells (adMSC), umbilical cord blood-derived mesenchyme stem cells (uMSC), bone marrow-derived mesenchymal stem cells (bmMSC), induced pluripotent stem cells-derived mesenchymal stem cells (iPSCs-MSC), epidermal mesenchymal stem cells (eMSC) have advantages like small size, low immunogenicity, and do not require for extra procedures for culture expansion or distribution, which were widely observed to help promote tissue repair and wound healing by controlling various inflammatory, proliferative and remodeling processes in vivo.^{23–28} Especially, mesenchymal stem cells-derived exosomes (MSC-Exo) have attracted much attention for their immunomodulatory and regenerative functions in the treatment of HS and keloids by promoting wound healing and tissue repair.^{29,30} As cell-free therapeutics, MSC-Exo mainly modulates the recipient cells through their cargo, including proteins, lipids and nucleic acids, but our understanding of how exosomes activate recipient cells and the cargo is responsible for their subsequent functional impact is limited.^{23,31} Our previous study demonstrated that the miRNA-138-5p loaded in MSC-Exo could attenuate pathological scars by targeting silent information regulator 1 (SIRT1) and further inhibit the proliferation, migration and fibrotic protein expression of human scar fibroblasts.³² Recent studies suggest that exosomes play an important role as immune modulators.³³ The research in MSC-Exo-mediated immunoregulatory mechanisms in wound healing and scar formation achieved great progress due to the importance of inflammation in the formation of HS and keloids.^{34–36} Yet, the clinical application of MSC-Exo still faces major challenges such as production and isolation methods, and ideal cell sources.³⁴

Here, we provide an overview of how MSC-Exo promotes wound healing and tissue repair processes to attenuate the formation of pathological scars by regulating the inflammation via affecting different immune cells, and discuss current assessment challenges and fundamental insights leading to future clinically relevant exosome therapy directions.

The Application of MSC-Exo in Scar Attenuation

Biological Characteristics of Exosomes

Exosomes are nanoscale vesicles secreted by cells through exocytosis, which are present in almost all body fluids.³⁷ Extracellular vesicles are nanoscale vesicle particles that are actively released by cells. According to their origin, size and biological characteristics, they can be divided into three categories: exosomes (30–150 nm), microbubbles (100–1000 nm) and apoptotic bodies (500–2000 nm).³⁸ Exosomes are mainly derived from multi-vesicles formed by intussusception of lysosomal granules in cells, and the outer membrane of the multi-vesicles fuses with the cell membrane and is released into the extracellular matrix. The exosome which contains protein, mRNA, microRNA and other biological information is just 30–150 nm in diameter, and its surface is rich in cholesterol, sphingomyelin, ceramide and other lipid substances. Exocrine bodies are secreted by almost all cells and are widely distributed in body fluids such as blood, urine, saliva, cerebrospinal fluid, pleural effusion, and ascites.^{39,40} Mesenchymal stem cells (MSCs), one of the main sources of exosomes, have high differentiation potentials so that are widely concerned by the scientific community.⁴¹

The precise mechanism of exosomes generated from MSCs is still unknown at this time, but it mainly plays its role in the following aspects:⁴² (1) Paracrine effect: It reduces cell apoptosis, encourages cell proliferation, and aids in the regeneration of injured cells by secreting a range of growth hormones and cytokines. (2) Immune regulation: It can inhibit the body's excessive immune response and induce immune tolerance by interacting with immune cells, and inhibit the response of autoreactive effector T cells by promoting the generation and expansion of regulatory T cells. (3) Adhesion and migration: under the signal stimulation of tissue damage or inflammatory reaction, MSCs can migrate and home to the damaged tissue site, exerting the corresponding tissue repair ability.

Contents in Exosomes

Exosomes from various origins have been demonstrated to contain evolutionarily conservative protein components such as cytoskeleton protein, phospholipase D2, heat shock protein (HSP), signal transduction protein, CD55, and CD59, according to numerous research.⁴³

Heat shock proteins like HSP70 and HSP90, membrane transporters and fusion proteins like GTPases, annexins, and flotillin, and members of the four transmembrane protein superfamily called tetraspanins such as CD9, CD63, CD81 and CD82 are the proteins that are most frequently found in exosomes. Exosomes from various sources also have unique protein molecules on their surfaces, like T cell receptors, granzymes, and perforin, which are employed to stimulate the body's immune system.⁴⁴ The exosomes secreted by neurons contain glutamate receptors.⁴⁵ Integrin CD41a was found on the exocrine membrane derived from platelets; The exosomes secreted by antigen-presenting cells usually express costimulatory factors, such as CD54 and CD48.⁴⁶ The exosomes secreted by intestinal epithelial cells contain various metabolic enzymes; Dendritic cells secrete exosomes that contain almost all antigen-presenting molecules and can induce or amplify acquired immunity.⁴⁷

A list of the lipids was found in exosomes, including ceramides (Cer), sphingomyelins (SM), gangliosides GM3 from the class of sphingolipids, phosphatidylserines (PS), phosphatidylethanolamines (PE), phosphatidylcholines (PC), and lysophosphatidylcholine, phosphatidylinositols.⁴⁸ Exosome lipid content is tightly correlated with the cell type from which they are produced. Exosomes of cholesterol, sphingomyelin, saturated phosphatidylcholine, and phosphatidylethanolamine have larger lipid contents than plasma membranes.⁴⁹

The nucleic acid in the exosome is an important symbol to distinguish from vesicles. The nucleic acid contained in the exocrine body mainly includes DNA, mRNA and miRNA.⁵⁰ MiRNA participates in the post-transcriptional regulation of mRNA and is related to disease occurrence and immune regulation. In order to degrade or prevent the expression of target mRNA, miRNA, an endogenous single-stranded non-coding miRNA with a length of 19 to 23 nt, binds to the 3' non-coding region of mRNA.⁵¹ It is reported that exosome miRNA is crucial for intercellular communication. MiR-99a-5p, miR-128, miR-124-3p, miR-22-3p, and miR-99b-5p are the five most prevalent miRNAs.⁵² Due to the protection of exosomes, the inclusion of miRNAs can be resistant to RNA-dependent enzyme degradation and can be transported remotely in body fluids. Different types of cells from different diseases can secrete exosomes containing miRNAs. When the expression of miRNAs in cells changes, the corresponding miRNAs in exosomes will show the same trend.

Function of Exosome

Exosomes as Biomarkers

As the exosomal membranes are homologous to the cell membranes of the source cells, so using exosomes as a biomarker is easy to detect and less damaging. Since urine samples are the easiest to get, the majority of recent studies on biomarkers have concentrated on pee-derived exosomes. Prostate cancer patients had considerably higher urinary exosome expression of ERG, PCA3, PSMA, CK19, and EpCAM compared to healthy males. Additionally discovered to have diagnostic significance in primary prostate cancer are CK19 and EpCAM.⁵³ A prevalent kind of cancer of the head and neck, oral cavity, pharynx, and larynx mucosal epithelium gives rise to head and neck squamous cell carcinoma (HNSCC). To participate in metabolic reprogramming and microenvironmental remodeling, HNSCC cells produce exosomes, which interact and communicate with recipient cells. This results in metabolic alterations. There is a study revealed that exosomes may be a new therapeutic target for HNSCC cure.⁵⁴ In the field of lung cancer, research about exosomes is also popular. An earlier study's findings revealed that non-small cell lung cancer patients had considerably lower amounts of the exosomes tRF-Lys-CTT-049, tRF-Leu-TAA-005, tRF-Asn-GTT-010, tRF-Trp-CCA-057, and tRF-Ala-AGC-036 indicating that these five exosomal tRFs could be promising non-small cell lung cancer diagnostic biomarkers.⁵⁵

Exosomes as Drug Carriers

An ideal drug carrier should be able to bypass the host immune system, be precisely absorbed by target cells, have a sufficient cycle half-life, be non-toxic, and have the capacity to load a range of different medications. Therefore, as a natural liposome, exosomes are considered to have more advantages than synthetic liposomes widely used at present.⁵⁵

It has been reported that therapeutic proteins like catalase or brain-derived neurotrophic factor, as well as low molecular chemotherapeutic drugs like PTX and doxorubicin (DOX), are incorporated into the exosomes through electroporation, saponin penetration, extrusion, freeze-thaw cycles, or sonication.⁵⁶ Gomes et al developed a hybrid nanocarrier of tumor-derived exosomes based on the fusible characteristics of liposomes and exosomes. For the treatment of breast cancer, the exosomes were combined with long-circulation, pH-sensitive liposomes carrying DOX (ExoSphL-DOX).⁵⁷ In kanchanapally's study, MSCs-Exo were exposed to cancer cells by loading with honokiol with the sonication method. And the results showed that these particles had better cytotoxic effects than the free honokiol.⁵⁸

Exosomes Promote Tissue Repair

As a promising therapeutic strategy, exosome has attracted extensive attention for its application in tissue repair. Based on a systematic review of relevant studies in recent years, exosome plays a positive role after the damage of tissues, such as bone, cartilage, skin, spinal cord and tendons, which means it could widely promote tissue repair.^{59–63} Studies have shown that exosome participates in various processes of skin tissue repair. It can promote wound healing and skin tissue regeneration by promoting the proliferation and migration of skin cells, promoting angiogenesis, and regulating the immune response.^{24,64} Interestingly, exosome plays a dynamic role in skin wound healing that it can promote collagen synthesis to accelerate wound repair in the early stage of wound healing, but inhibit collagen synthesis to restrict the scar formation in the later stage.^{24,30,65} Therefore, exosomes can inhibit the proliferation of scars to a great extent by accelerating wound healing.

The Immune Function of Exosomes

It has been known that exosomes are key substances in intercellular communication and innate immunity is an important part of the host's defense mechanism. According to recent findings, exosomes could control immunity in the tumor microenvironment. Through exosomes, CD8+T cells can kill the stromal cells that support mesenchymal tumors and prevent tumor cell invasion and metastasis. CD8+T cell-derived exosomes can increase the expression of matrix metalloproteinase-9 in tumor cells, thereby promoting tumor progression. In addition, exosomes derived from CD8+T cells reduce the effect of anti-tumor response by inhibiting the proliferation of CD8+T cells mediated by dendritic cells.⁵⁸ As innate immune cells, macrophages are divided into M1 macrophages (M1) with proinflammatory phenotype and M2 macrophages with anti-inflammatory phenotype. M1-generated exosomes inhibit tumor growth by polarizing M2 to M1, whereas tumor-derived exosomes enhance M2 polarization. PGE2 and IL1RA were detected in MP and exosomes, which have been proven to be important mediators of MSCs' anti-inflammatory effect in vitro and inflammatory arthritis. Cosenza showed that MSCs-derived MP and exosomes play a similar immunosuppressive function by reducing the proliferation of T and B lymphocytes and inducing Treg cell population.⁶⁶ The exosome is one of the paracrine components and the main contributor to the efficacy of stem cells. Chamberlain found that the exposure of CD14 macrophages to the exosomes derived from MSC leads to a decrease of M1/M2 macrophage ratio. They may improve ligament healing and reduce scar formation.⁶⁷ ADSC-Exo have a profound potential for scar attenuation. By delivering miRNA-125a and miRNA-31 to vascular endothelial cells, they can contribute to the immune response of wound inflammation and stimulate angiogenesis.²⁴ In another study, MSCs secrete secretions containing miR-432-5p to inhibit target gene TRAM2, prevent ECM deposition, and promote stromal regeneration.⁶⁸

The applications of MSC-Exo in scar formation can be summarized in one picture, which was shown in Figure 1.

Clinical Application of MSC-Exo in Pathological Scars and Existing Difficulties

MSC-Exo can be a promising option as an innovative cell-free approach to support wound healing and skin regeneration as well as scar attenuation. It conveys functional cargos (such as growth factor, cytokine, miRNA, etc.) from MSCs to target cells, thereby affecting the recipient skin cells' biological events, such as migration, proliferation, and also secretion of ECM components (mainly collagen).³⁰ Moreover, MSC-Exo could also mediate crosstalk between skin cells and immune cells in cutaneous wound healing, which further accelerates wound healing and alleviates pathological scar formation.⁶⁹

Although lots of studies showed that MSC-Exo plays a positive role in wound healing and tissue regeneration as well as pathological scars attenuation, most of them were just in vitro and animal experiments. Thus, the results of them still need to be verified in clinical trials. But changes from basic to clinical research remain huge challenges.

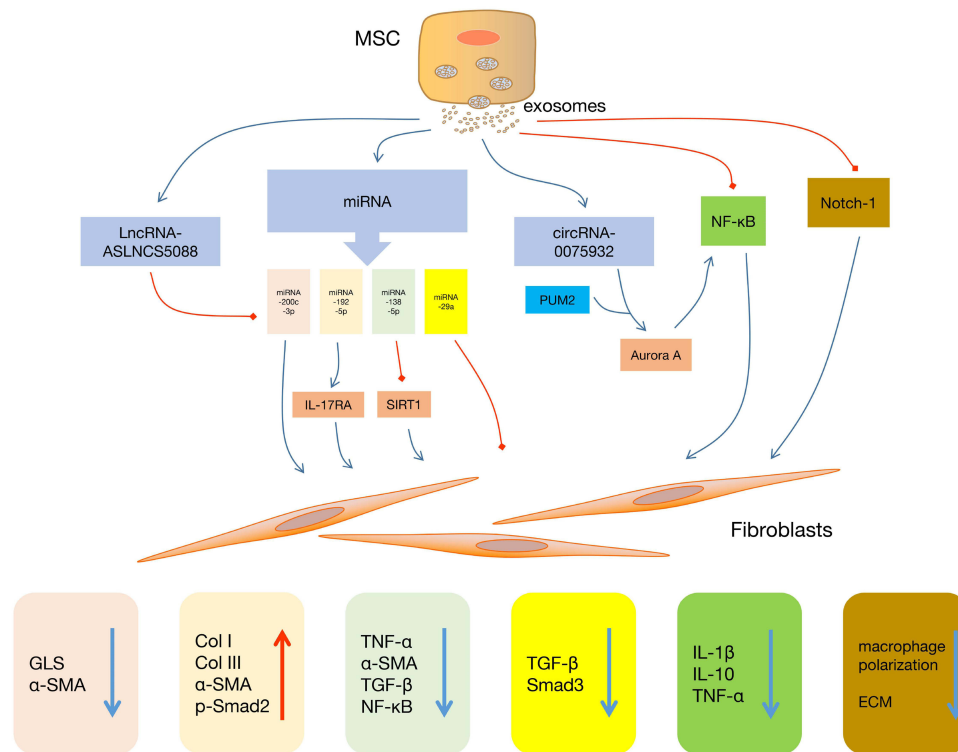


Figure 1 Functions of MSC-Exo in regulating the biological activities of fibroblasts.

Abbreviations: MSC, mesenchymal stem cell; NF-κB, nuclear factor kappa B; PUM2, pumilio RNA binding family member 2; IL-17RA, interleukin-17 Receptor A; SIRT1, silent information regulator 1; GLS, glutaminase; α-SMA, alpha-smooth muscle actin; Col I, collagen I; Col III, collagen III; p-Smad2, phosphorylated-mothers against decapentaplegic homolog 2; TNF-α, tumor necrosis factor-alpha; TGF-β, transforming growth factor-beta; Smad 3, mothers against decapentaplegic homolog 3; IL-1β, interleukin-1 beta; IL-10, interleukin-10; ECM, extracellular matrix.

First, due to technical limitations, it is difficult to obtain high purity and yield MSC-Exo, so further development of technology is needed to improve isolation efficiency and productivity for clinical applications. Second, it is unclear whether the positive effects of MSC-Exo in treatment are directly related to its contents (lipid, protein and nucleic acid). Therefore, the therapeutic efficacy, safety and effective dosage of its contents need to be further studied while using MSC-Exo as a therapeutic agent. Third, non-specific targeted therapy of MSC-Exo may lead to unforeseen side effects. MSC-Exo has been proven to target a variety of cell types, but it is not clear how they choose the priority receptors. Especially in the complex environment of the human body. Therefore, improving the targeting of MSC-Exo could improve its safety and efficiency. Fourth, the use of MSC-Exo as a drug delivery vector is also one of the research hotspots, but drug delivery efficiency is another challenge that needs to be overcome. Therefore, future research needs to develop efficient technologies, which can not only load enough doses of the drug into MSC-Exo but also maintain stable biological activity and physical integrity of MSC-Exo.

Immune Cells in Scar Formation

Macrophages

Overview of macrophages

Macrophage is a kind of immune cell with multiple differentiation sources, which can be self-renewed or supplemented by monocytes. The two primary categories of macrophages are classic activated macrophages (M1, which is also called pro-inflammatory macrophages) and alternative activated macrophages (M2, which is also called anti-inflammatory macrophages).⁷⁰ In addition, M2 macrophages come in four different subtypes: M2a, M2b, M2c, and M2d.⁷⁰ Polarization of macrophages is the phenotypic change of macrophages between different types, which is controlled by a number of signal molecules and pathways.⁷¹ However, the molecular and cytological mechanisms underlying the complicated process of macrophage polarization are not entirely understood.⁷² The polarization of macrophages serves several roles and is strongly associated with the onset and progression of illnesses, depending on the local microenvironment.^{71,73}

Macrophages in Scar Formation

Numerous macrophage infiltration is a remarkable feature of the early stage of wound healing. In the early and middle stages of skin wound healing, dermal macrophage depletion has a beneficial effect on cutaneous scarring, which is characterized by decreased vessel density, decreased fibroblast and myofibroblast proliferation, and reduced post-healing fibrosis and scar formation.⁷⁴ In macrophage phenotypes, M2 macrophages are mainly involved in tissue proliferation and scar formation.⁷⁵ It has been determined that HS tissue contains substantially more M2 macrophages than does typical scar tissue.⁷⁶

Like other immune cells, macrophages play a role in the formation of scar mainly by secreting pro-fibrosis and pro-proliferation cytokines. Macrophages are the primary source of cytokines like transforming growth factor (TGF)-1, fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF)-1, etc., which encourage fibroblast proliferation, migration, and differentiation, promote collagen synthesis and wound contraction, encourage ECM collagen deposition or angiogenesis, and play an important role in scarring (Figure 2a–d).^{76–78} In addition, macrophages can also produce matrix metalloproteinases (MMPs) and play an important role in the remodeling stage of wound healing.⁷⁹ Therefore, reducing the recruitment of macrophages and the release of cytokines or inhibiting the polarization of M2 macrophages may play a role in the prevention and treatment of scarring.^{74,80}

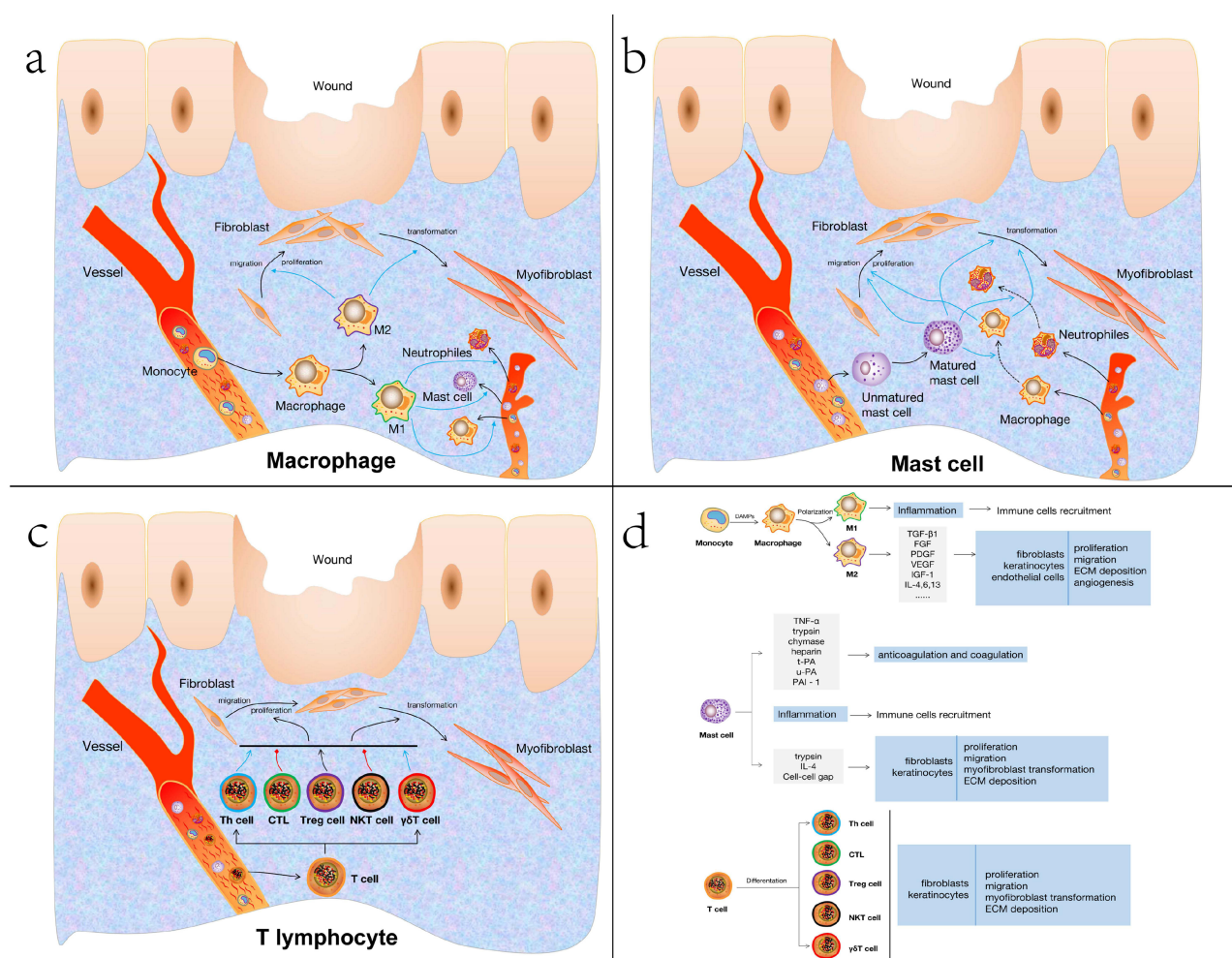


Figure 2 Immunoregulatory mechanisms of different immune cells in scar formation. **a)** Immunoregulatory mechanisms of macrophage in scar formation. **b)** Immunoregulatory mechanisms of mast cell in scar formation. **c)** Immunoregulatory mechanisms of T lymphocyte in scar formation. **d)** Landscape of macrophage, mast cell and T cell result in scar formation.

Abbreviations: Th cell, helper T cell; CTL, cytotoxic T cell; Treg cell, regulatory T lymphocyte; NKT cell, natural killer T lymphocyte; $\gamma\delta$ T cell, gamma delta T lymphocyte; TGF- β 1, transforming growth factor-beta 1; FGF, fibroblast growth factor; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor; IGF-1, insulin-like growth factor; IL-4,6,13, interleukin-4,6,13; TNF- α , tumor necrosis factor-alpha; t-PA, tissue-type plasminogen activator; u-PA, urokinase-type plasminogen activator; PAI-1, plasminogen activator inhibitor 1; IL-4, interleukin-4; ECM, extracellular matrix.

The Notch signal pathway is an important pathway known to participate in inflammatory reactions and fibrosis processes. The expression of Notch is an important condition to promote fibrosis of the liver, kidney, skin and other organs, while inhibiting the Notch pathway of macrophages can effectively improve the fibrosis process.^{81–83} The drug emodin can inhibit the inflammatory reaction and collagen deposition by down-regulating the Notch and TGF- β of macrophages.^{84,85} The expression of the Notch can further inhibit the recruitment and polarization of macrophages in the injured part of the body to achieve the purpose of treating HS.⁸⁴ Therefore, many studies have focused on the control of Notch signal pathway through drug targeting to regulate the polarization of macrophages. For example, astragalus polysaccharide can activate Notch signal pathway to promote the polarization of M1 macrophages, but capsaicin can inhibit Notch signal pathway to induce the polarization of M2 macrophages.^{86,87}

At present, most of the research is focused on the distribution and quantity of macrophages. In view of high plasticity and diversity of macrophages, the further study of the cellular and molecular biological mechanisms of phenotypic transformation is likely to explore new therapeutic targets in the future in reducing excessive injury or proliferation, improving chronic wound healing and scar formation.

Mast Cell

Overview of Mast Cell

Mast cell (MC) was primordially regarded as the main cell involved in IgE-mediated rapid hypersensitivity.⁸⁸ Activated MC plays an important role in the process of host resistance to foreign pathogens at early stage of infection by releasing a variety of special particles (such as histamine, 5-hydroxytryptamine, heparin, trypsin, chymotrypsin, etc.) and recruiting other inflammatory cells, including neutrophils, monocytes, macrophages, etc.^{89–91} Moreover, MC is also involved in adaptive immunity directly or indirectly by secreting a variety of immune molecules. In addition, MC is also an important antigen-presenting cell, which could recognize foreign antigens and present them to T lymphocytes and B lymphocytes. With the development of research, it is found that MC is also closely related to wound healing and scar formation.^{91,92}

Mast Cell in Scar Formation

The role of MC involved in scar formation could be sketched in three aspects: firstly, regulating anticoagulation and coagulation processes in the early stage of wound healing; secondly, triggering a stronger inflammatory reaction by interacting with other immune cells; thirdly, promoting fibroblast proliferation and collagen production.^{12,93,94}

MC migrates to the injury site under the action of stimulation signals, releasing TNF- α to enhance the expression of FXIIIa in dermal dendritic cells.⁹⁵ Additionally, MC-derived trypsin and chymase inhibit the platelet-induced aggregation of fibrin by decomposing the α chain and β chain of fibrinogen.⁹⁵ Meanwhile, MC can create heparin, tissue-type plasminogen activator (t-PA), urokinase-type plasminogen activator (u-PA), and plasminogen activator inhibitor (PAI)-1 to control the coagulation and anticoagulation processes (Figure 2b–d).^{93,96}

The MC in the skin connective tissue is highly expressed in chymotrypsin and trypsin, which are mainly trypsin in the inflammatory process and chymotrypsin in the chronic fibrosis process.⁹⁷ The combination of MC-derived trypsin and protease-activated receptor-2 on the surface of endothelial cells also leads to vasodilation, fluid extravasation, aggravation of local inflammatory reaction, and induction of more inflammatory cells such as monocytes and neutrophils to migrate to the injured site, leading to stronger inflammatory reaction.^{94,98}

Furthermore, MC-derived trypsin could promote the migration and proliferation of keratinocytes and fibroblasts by degrading the basement membranes' components, such as type IV collagen and laminin. It could also promote fibroblasts to transform into myofibroblasts, synthesize ECM and Alpha-smooth smooth actin (α -SMA), and induce collagen deposition, leading to pathological scars and scar contracture. MC-derived IL-4 acts as the second signal, which could amplify the effect of low FGF and PDGF concentrations and further promote the proliferation of fibroblasts.⁹¹ Cell-cell gap junction formed between fibroblasts and MC, the structural basis for the role of MC, enhanced the contact of cells, thus promoting the growth, proliferation, migration, and differentiation of fibroblasts.^{99,100} Therefore, eliminating the gap connection between them may play a role in preventing scarring or reducing the degree of fibrosis.

Hence, based on the role of MC in the process of wound healing, inhibiting the activation of MC or targeting the cytokines secreted by MC can reduce the inflammatory reaction and scar formation, which may be a potential method to improve wound healing. In recent years, researchers have had a profound understanding of the role of MC in wound healing, but the research is limited to animal experiments, and the results are still different from human experiments. Therefore, the mechanism of MC in human scarring needs further clinical research.

T Lymphocyte

T lymphocytes are derived from bone marrow pluripotent hematopoietic stem cells or lymphoid cells differentiated from them. A complete T lymphocyte system is necessary to ensure normal wound healing, which plays a major regulatory role in wound healing.¹⁰¹ Over-proliferation or the continuous existence of T lymphocytes in the injury site may be one of the reasons for excessive scarring.^{101,102} However, there are many subtypes of T lymphocytes, and different T lymphocytes play different roles in different microenvironments during wound healing and scar formation.^{101,103} According to the differentiation of functions and cell surface receptors, T lymphocytes were divided into helper T lymphocytes, cytotoxic T lymphocytes, regulatory T lymphocytes and natural killer T lymphocytes (Figure 2c and d).^{104,105}

Helper T Lymphocytes in Scar Formation

In the process of wound healing, a large number of DAMPs-related chemokines in the injury site induce Th1 cells and Th2 cells to reach there.¹⁰³ IFN- γ and IL-12 are mostly secreted by Th1 cells. IFN- γ enhances the gene expression of collagenase, and promotes collagen decomposition and remodeling.¹⁰³ Additionally, cytokines and NOS can be activated by Th1 cells. NOS can enhance the function of collagenase, promote ECM degradation and inhibit fibrosis. Whereas, many other cytokines, including IL-4, IL-5, IL-6, IL-10, IL-13, etc., are secreted by Th2 cells. It was found that IL-4 and IL-13 are the main cytokines leading to skin fibrosis.¹⁰⁶ Overexpression of IL-4 and IL-13 will activate and prolong the fibrosis process of skin tissue, leading to the formation of pathological scars. IL-4 and IL-13 can also induce the expression and secretion of the periosteal protein, which further induces TGF- β through RhoA/ROCK pathway.¹⁰⁷ In turn, TGF- β further promotes the production and secretion of the periosteal protein. Such a positive feedback loop promotes the formation of scarring. Tredget et al confirmed that the serum of patients with HS included considerably higher levels of the cytokines produced by Th2 cells, IL-4, IL-6, and IL-10.¹⁰⁸ On the contrary, IFN- γ and IL-12 produced by Th1 cells were reduced, indicating that Th2 and its cytokines play a significant role in the development of HS after injury.

Regulatory T Lymphocytes in Scar Formation

Skin is rich in Treg cells, which play a role in maintaining skin inflammation and immune homeostasis.¹⁰⁹ Treg cells suppress the immune system by blocking other functional T cells and preserving immunological tolerance in the peripheral body, and then directly affecting collagen deposition, inhibiting wound repair and healing.¹¹⁰ Murao et al found that the collagen synthesis of fibroblasts decreased when co-cultured with T lymphocytes rich in Treg cells compared with lymphocytes lack of Treg cells. However, It was also shown that co-culture of fibroblasts with pure Treg cells can promote the expression and synthesis of collagen without other T lymphocytes.^{111,112} These two seemingly paradoxical results suggested that Treg could directly or indirectly regulate the role of fibroblasts in the process of collagen deposition and subsequent scar formation through their interaction with other T lymphocytes.¹¹³

Natural Killer T Lymphocytes in Scar Formation

Natural Killer T lymphocytes (NKT cells) are natural immune lymphocytes, which simultaneously express TCR and NK cell receptors, and play an important role in infection, autoimmune and tumor.¹¹⁴ It was found that NKT cells infiltration mainly occurred in the first three days of wound repair, and was activated by CD1d (a member of the CD1 family, with a structure similar to MHC I molecule, expressed on the surface of some antigen-presenting cells, mainly presenting glycolipid antigen) related antigen.¹¹⁵ The combination of CD1d-related glycolipid antigen and NKT cell TCR can up-regulate the expression of its surface molecule CD69.¹¹⁶ After intravenous injection of anti-CD1d antibody, the activation of NKT cells is inhibited, and its surface CXCR2 continues to circulate, resulting in the high expression of CXCR2, which promotes NKT cells to migrate to the injury site at the early stage of inflammation, just like neutrophils.¹¹⁷ The

activated NKT cells can inhibit the production of some chemokines that act on the chemotaxis of neutrophils, monocytes and macrophages, and negatively regulate the early inflammatory reactions and fibroproliferative signals.¹¹⁷ Therefore, the lack of NTK cells or the use of anti-CD1d antibodies to inhibit the activation of NKT cells can increase the chemokines and TGF- β of the local inflammation. Therefore, the research on NKT cells and their related molecules is likely to be a new therapeutic target for poor wound healing or scarring in the future.^{117,118} In the view of NKT cells also express NK cell receptors, whether NK cells participate in the process of wound repair and even scar formation requires to be further study.

Natural Killer Cell

Overview of Natural Killer Cell

Natural Killer (NK) cells are the lymphocytes of the innate immune system, which were considered to be an important part of the first defense line of the human body. Human NK cells, or CD3-CD56+, make up about 10% of peripheral blood lymphocytes and are distinguished by CD3 negative and CD56 positive expression. NK cells have two main phenotypes, including CD56bright and CD56dim. CD56bright NK cells can produce higher levels of human immune regulatory cytokines, including IFN- γ , TNF- β , GM-CSF, IL-10 and IL-13, etc., showing that CD56+NK cells have unique natural immune regulation.^{119,120}

NK Cell in Scar Formation

The abnormal infiltration of NKT cells is the characteristic of delayed wound healing, which hinders wound repair and induces over-scarring. Knocking out NK cells with T cell function in vitro during the wound repair and healing process of the mice resulted in significantly accelerated wound healing and less scarring, which confirmed that the continuous inflammatory reaction caused by NK cells can promote the formation of scar.^{117,118} NK cells may also participate in the process of wound repair and even scar formation in consideration of NKT cells also express NK cell receptors. In this regard, our research team has confirmed that NK cells play a key role in wound repair and immune regulation.¹²⁰ However, the study of its immune regulatory mechanism on scar formation has not yet been reported home and abroad.

Other Immune Cells

Neutrophils

Neutrophils are the first immune cells to reach the injury site. It begins to apoptosis only after 24 hours of local survival. They could not only kill the pathogenic microorganisms, but also secrete cytokines and growth factors, such as IL-1, IL-17, VEGF, TNF- α , to induce other immune cells to reach the injury site and promote the proliferation of fibroblasts, keratinocytes and endothelial cells.^{121,122} Therefore, neutrophils may be one of the first cellular signals to activate local cells.

The experimental results of knockout of mouse CXCR2 (the key regulator of neutrophil chemotaxis) gene showed poor wound healing in mice, characterized by reduced migration and proliferation of keratinocytes and fibroblasts, and reduced degree of vascular regeneration and skin regeneration, which suggested the importance of neutrophils to promote wound repair.¹²³ However, some studies have confirmed that neutrophils play a negative regulatory role in the process of wound healing.¹²⁴ Mice with neutrophil deficiency show accelerated wound healing and epithelial regeneration.¹²⁵ This suggests that neutrophils play a complex role in the process of wound healing. Therefore, the interaction between neutrophils and other immune cells may be the reason for the complex role in wound healing and scar formation, which needs to be further studied in the future.

Langerhans Cell

Langerhans cells move to the site of the lesion in order to help with damage repair, which are found in the granular layer of the epidermis. Niessen et al showed that the quantity of Langerhans cells in HS tissue was much larger than that in normal skin tissue at the 3rd and 12th months following the operation, which suggested that Langerhans cells were strongly related to the later formation of HS in the remodeling stage of wound healing.¹⁵ Continuous interaction between epidermal Langerhans cells and keratinocytes in the dermis inhibits the release of IL-1 α and promotes IL-4, which may be related to less degradation of collagen and promote the formation of HS.¹²⁶

Immunomodulation Mechanisms of MSC-Exo in Scar Formation

MSC-Exo Immunomodulates the Activities of Macrophages

MSC-Exo Regulates Macrophage Polarity

Macrophages are in charge of controlling the stages of inflammation, proliferation, and remodeling due to their extremely dynamic flexibility.⁷⁷ In response to the release of cytokines after tissue injury, the recruited macrophage populations reprogram and experience significant phenotypic and functional alterations.¹²⁷ Consequently, as potentially crucial targets, macrophages emit a variety of inflammatory cytokines and regulate tissue repair processes raising the possibility that they are important targets in the formation of HS and keloids.^{128–130} It has been shown that different macrophage activation states play specific and important roles in the various stages of wound healing and scar formation.^{84,131–133}

Recent studies have identified the regulatory effects of MSC-Exo on macrophage polarity, suggesting that MSC-Exo may affect the process of wound healing and scarring through immunomodulating macrophage polarity (Figure 3).^{134–136} Previous research demonstrated that uMSC-Exo promoted angiogenesis and collagen synthesis while triggering M2 macrophage polarization to aid the repair of diabetic wounds.¹³⁷ However, if the period of this process is prolonged and M2 macrophages continue existing, fibroblast over-proliferation, excessive ECM production and collagen deposition will occur, which eventually causes the formation and progress of HS and keloids. Therefore, MSC-Exo could control this process by regulating the polarity of macrophages, and further inhibiting scar formation.

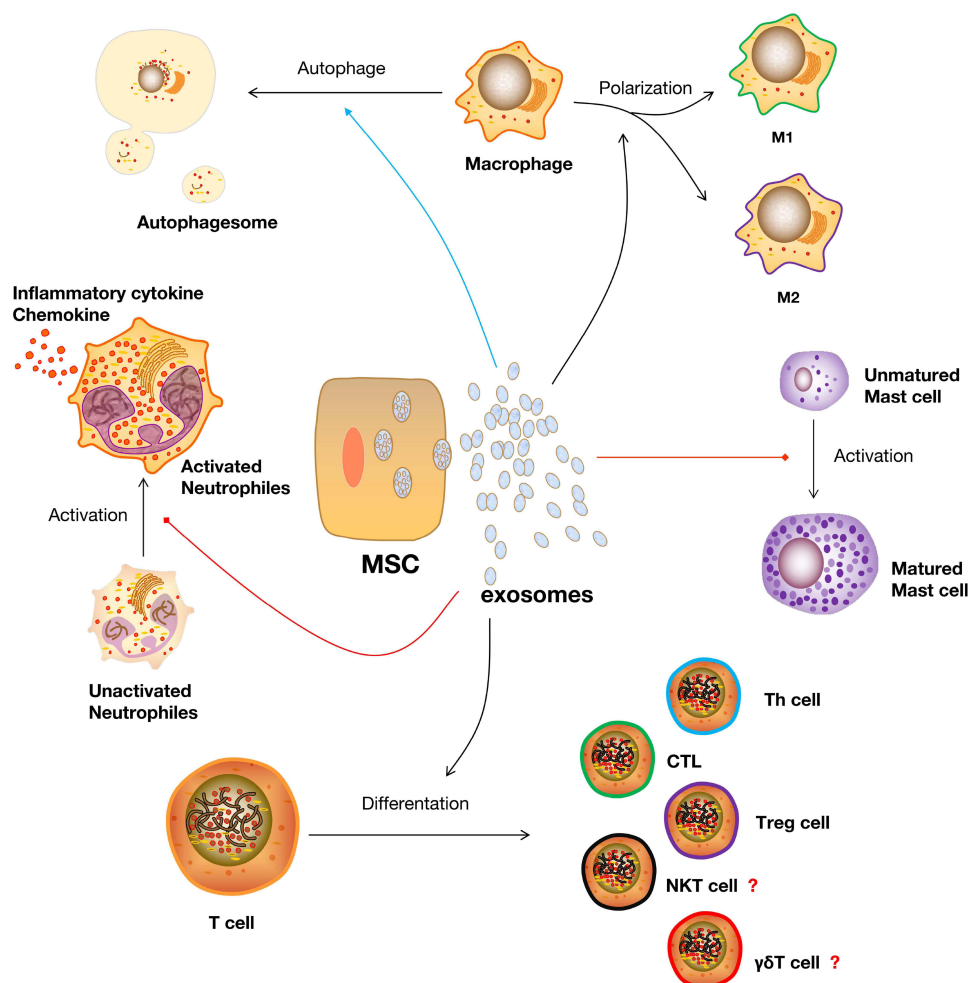


Figure 3 Functions of MSC-Exo in regulating the biological activities of different immune cells in scar formation.

Abbreviations: MSC, mesenchymal stem cell; Th cell, helper T cell; CTL, cytotoxic T cell; Treg cell, regulatory T lymphocyte; NKT cell, natural killer T lymphocyte; γδT cell, gamma delta T lymphocyte.

MSC-Exo Stimulates Macrophage Autophagy

Macrophage autophagy regulates the process of cell proliferation, migration, and neuronal signal transduction, which protects against inflammation- and fibrosis-associated diseases.^{138,139} Macrophage autophagy reduces inflammation by restricting the release of pro-inflammatory cytokines like IL-1 β and inhibiting the activation of the NLRP3-inflammasome via the NF- κ B-p62-mitophagy pathway.¹⁴⁰ Additionally, autophagy controls macrophage polarization via activating the NLRP3 gene and the ROS/ERK signaling pathway.¹⁴¹

Exosomes from human exfoliated deciduous teeth-derived stem cells (SHED-Exo) were previously shown to have potential clinical uses as cell-free therapies for wound healing.¹⁴² Their research showed that miR-1246 in SHED-Exo improved autophagy by controlling macrophage activity via the AKT, ERK1/2, and STAT3 signaling pathways. Lipopolysaccharide (LPS)-induced wounds in a mouse model were used to study inflammatory wound healing. The results demonstrated that SHED-Exo enhances wound healing with less itching in an LPS-induced wound model via promoting macrophage autophagy. Therefore, these results suggest that manipulation of macrophage autophagy by stem cell-derived exosomes, which regulates the macrophage phenotype, may offer clinical options for improved wound healing with reduced pruritus and pathological scar amelioration (Figure 3).

MSC-Exo Inhibits Mast Cell Activation

It has been hypothesized that mast cell activation contributes to each stage of the wound healing and scarring processes.^{12,91,143} Mast cells, which are inflammatory cells that are present in the body, can activate in response to a variety of clinical and environmental factors. Due to their capacity to create a variety of mediators, they facilitate the recruitment of inflammatory cells after skin damage, aid in the prevention of infection, and collaborate with fibroblasts to expedite the creation of scars.^{94,144,145} Activated mast cells undergo degranulation and the cell numbers increase in response to skin injury during scarring.¹⁴⁶ Therefore, blocking mast cell activation would lessen the development of cutaneous scars.⁹²

Recently, Cho et al investigated whether exosomes from tonsil-derived mesenchymal stem cells (tMSC-Exo) have the capacity to control mast cell activation in response to TLR7 stimulation.¹⁴⁷ The result of their study showed that TLR7 agonist-treated HMC-1 cells (a cell line of human mast cells) that contained microRNAs that target inflammatory cytokines greatly decreased the expression of inflammatory cytokines. In addition, tMSC-Exo prevented the development of cutaneous mast cells and CD14-positive cells in TLR7 agonist-treated mice. Another previous study demonstrated that exosomes from iPSCs-MSC could inhibit mast cell activation via the HIF-1 signaling pathway.¹⁴⁸ Both of these studies suggest that MSC-Exo has a negative effect on mast cell activation under inflammatory conditions (Figure 3).

MSC-Exo Regulates T-Cell Differentiation

The role of T cells in wound healing and scar formation is complex due to the variety of cell subsets. For example, IL-10-producing CD4⁺ T lymphocyte subsets, specifically Th1, regulate inflammatory cell cytokine expression to selectively attenuate dermal wound fibrosis.¹⁴⁹ While the NKG2A/CD94 complex (an inhibitory marker of the T cells) was specifically upregulated, it was demonstrated that the downregulation of cytotoxic CD8⁺ T cells is a keloid signature in peripheral blood and keloid lesions.¹⁵⁰ This may explain the significant decrease in CTLs within the scar tissue boundary. In addition, increased infiltration of CD45RO⁺ memory T cells in keloid scars indicated that a disrupted T-cell response may contribute to the progression of keloids.¹⁵¹ Therefore, the balance of T-cell differentiation is crucial for scar alleviation.

A recent study examined how Th17 and regulatory T (Treg) cells differentiate from naïve CD4⁺ T cells in releasing inflammatory factors, Th17, and hypertrophic scar formation to reveal the role of ADSC-Exo.¹⁵² The result of their study showed that naïve CD4⁺ T cells treated with ADSC-Exo in vitro can produce significantly less IL-6, IL-17A, TNF- α , ROR γ t, and more IL-10 and Foxp3 on mRNA and protein levels. Moreover, mice treated with ADSC-Exo showed reduced collagen deposition, reduced levels of IL-17A, TNF- α , and ROR γ t, and elevated levels of IL-10 and Foxp3 production. Furthermore, uMSC-Exo appeared to restore the balance between Th17 and Treg cells, which was accompanied by decreased IL-17 and increased TGF- β and IL-10 levels, indicating that uMSC-Exo serves as a crucial regulator

of the balance between Th1/Th17 and Treg cells during immune and inflammatory responses.^{153,154} Except for Helper T lymphocytes and Treg cells, MSC-Exo could also participate in the differentiation of CTLs. There was a study showed that exosomal PD-L1 suppressed CD8⁺ T cell numbers in the spleen and peripheral lymph nodes and inhibited cytokine production of CD8⁺ T cells, indicating that exosome exerts immune inhibitory effects and promotes tissue repair by suppressing T cells differentiate into CD8⁺ T cells.¹⁵⁵ As is known that NKT cells and $\gamma\delta$ T cells are also involved in wound healing and scar formation. But the existing evidence of MSC-Exo regulating the differentiation of NKT cells and $\gamma\delta$ T cells is limited. Whether MSC-Exo could also work on it still need further research (Figure 3).

MSC-Exo Inhibits Neutrophils Activity

Neutrophils are the first immune cells to reach the injury site to defend against pathogens and recruit other immune cells by releasing various of inflammatory cytokines and chemokines. With the antibacterial property of neutrophils, host damage was also caused by neutrophils resulting from the activity of proteases secreted by these cells. Numerous studies demonstrating a link between elevated neutrophil-derived proteases and persistent non-healing wounds and even severe scarring underline the clinical implications of this issue.¹⁵⁶ Another study demonstrated that neutrophils might control the movement of the preexisting matrix to incorporate fibroblasts that are active or mature into scars that will stay a long time.¹⁴ As such, they could function as a subsequent response to the initial scar-formation process.

The study on the immunomodulatory effects of MSC-Exo on LPS-induced systemic inflammation demonstrates that MSC-Exo reduced neutrophil-to-lymphocyte ratio (NLR) and levels of inflammatory cytokines like IL-6, IL-1, and TNF- α after exosome injection in the mouse model, suggesting that treating mice with MSC-Exo can mitigate the damaging effects of neutrophil-related inflammation brought on by sepsis by reducing inflammatory factors and tissue damage.¹⁵⁷ Neutrophil extracellular trap (NET) is the main property of neutrophil activation in response to inflammatory stimuli and the release of extracellular chromatin structures, which was identified plays a promoting role in the differentiation and function of fibroblasts, and therefore resulting in scar formation.^{158,159} But the C5b-9 assembly-induced production of NETs and IL-17 on neutrophils was suppressed by MSC-Exo, suggesting that MSC-Exo could improve immune dysregulation and break the feed-forward loop between complement and neutrophils to prevent the spread and perpetuation of inflammation.¹⁶⁰ Hence, the eMSC-Exo-inhibited NET formation potentially attenuates inflammation conditions and scarring by reducing neutrophil-related hyperinflammation.

Discussion

HS and keloids are pathological cutaneous scars, which are partially induced by chronic dermal inflammation and result from overwhelming fibroblast production of extracellular matrix.⁸ Their etiology is unclear to a great extent, so they lack effective precautions and treatments. In this study, we reviewed the immunomodulation of MSC-Exo on different immune cells in wound healing and pathological scarring.

Inflammation has long been proven that plays a concrete role in scar formation.⁷ Chemokines, such as C-C chemokine ligand 2 (CCL2), C-C chemokine ligand 4 (CCL4), C-C chemokine ligand 5 (CCL5), C-X3-C Motif Chemokine Ligand 1 (CX3CL1), C-X-C Motif Chemokine Ligand 10 (CXCL10) and C-X-C Motif Chemokine Ligand 12 (CXCL12), at the injury site recruit a variety of immune cells to participate in wound healing and tissue repair and thus result in HS.^{103,161} Additionally, excessive expression of fibrotic cytokines, such as transforming growth factor (TGF) β 1, fibroblast growth factor (FGF), epithelial growth factor (EGF), vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) et al, from immune cells at the injury site is the main reason for the overwhelming tissue repair-caused HS and keloids formation.^{7,76}

cGAS-STING axis, including the synthase for the second messenger cyclic GMP-AMP (cGAS) and the cyclic GMP-AMP receptor stimulator of interferon genes (STING), is a famous inflammation-related pathway and also a research hotspot in inflammatory diseases, cancer immunotherapy, liver diseases, etc.^{162–164} cGAS-STING causes inflammation by identifying pathogenic DNA through igniting an innate immune response that results in a potent type I interferon response or a prodigious release of inflammatory cytokines (IL-1, IL-6, TNF- α , etc.) against microbial infections.¹⁶² The DNA sensor cGAS can detect endogenous DNA as well as microbial DNA (derived from bacteria or viruses), including extranuclear chromatin produced by genotoxic stress and DNA released from mitochondria, which makes cGAS-STING

an important axis in autoimmunity, sterile inflammatory responses, and cellular senescence.^{165,166} Previous studies showed that the cGAS-STING pathway is also an important signal for fibrotic diseases, such as liver, renal, lung or cardiac fibrosis.^{167–170} However, there is not any research putting it together with skin wound healing or scarring. And given the predominant role of cGAS-STING in inflammation, it may be involved in wound healing and scarring (Figure 4).

mesenchymal stem cells produce cytokines in a paracrine or autocrine way to control immune response and tissue regeneration. MSC-Exo, on the other hand, has anti-inflammatory and immunomodulatory qualities that may be useful in inflammatory illnesses and diseases such skin damage and cutaneous scarring.¹⁷¹ Oxidative damage is a critical cause of

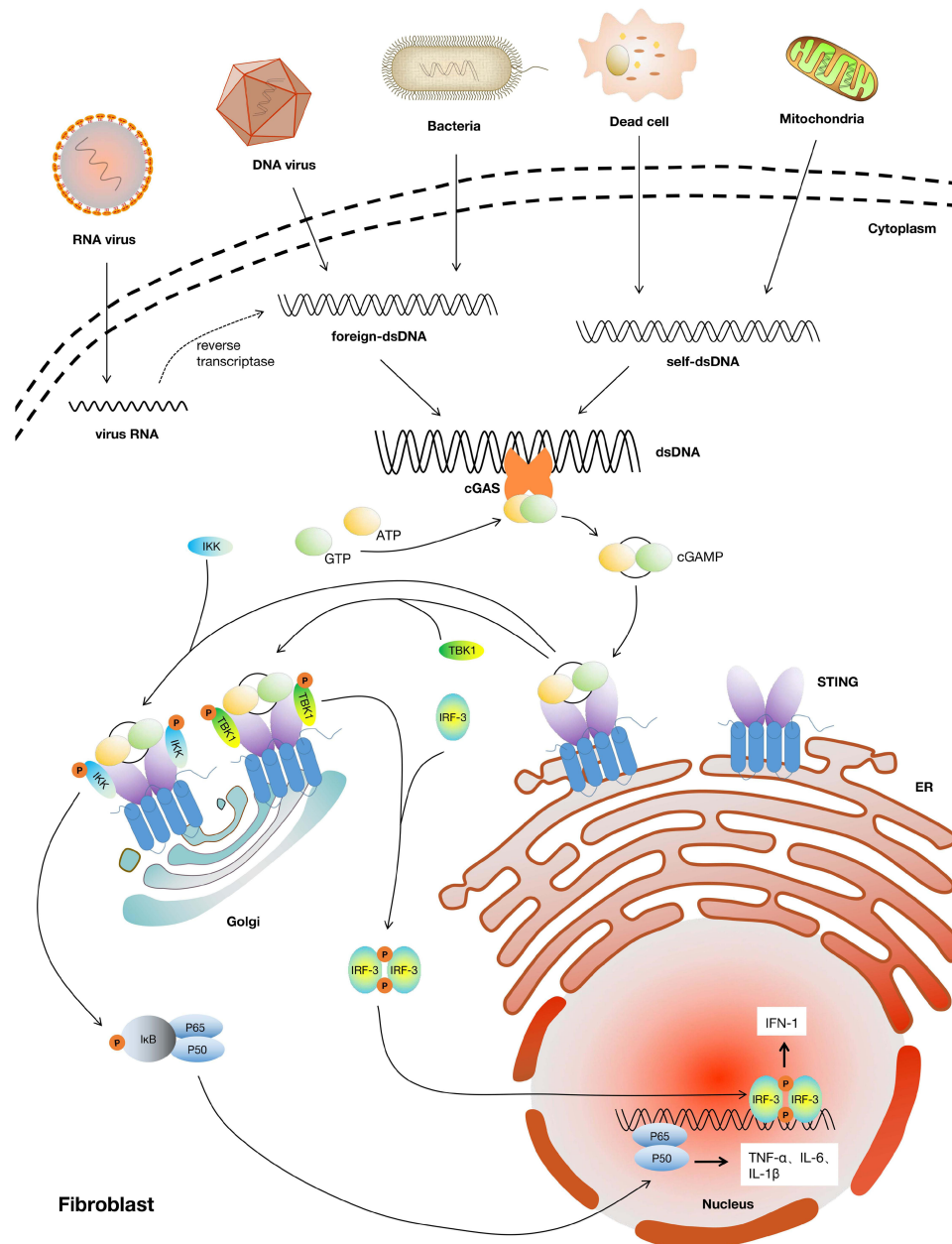


Figure 4 cGAS-STING pathway induced inflammatory effects in fibroblasts.

Abbreviations: cGAS, cyclic guanosine monophosphate-adenosine monophosphate synthase; STING, stimulator of interferon genes; ATP, adenosine triphosphate; GTP, guanosine triphosphate; cGAMP, cyclic guanosine monophosphate-adenosine monophosphate; ER, endoplasmic reticulum; IKK, inhibitor of NF-κB kinase; TBK1, TANK-binding kinase 1; IRF-3, interferon regulatory factor 3; IκB, inhibitor of NF-κB; IFN-1, type I interferon; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6; IL-1β, interleukin-1 beta.

chronic nonhealing wounds. Through the control of oxidative stress, a Chinese research team looked into the likely mechanism by which adMSC-Exo enhances diabetic wound healing. Reactive oxygen species (ROS) levels in keratinocytes, fibroblasts, and endothelial cells were found to be reduced by adMSC-Exo, which also protected these cells from the damaging effects of hypoxia and oxidative stress by activating the exosomal HSP90/LRP1/AKT signaling pathway. One of our previous studies discovered that the cross-talk between human mesenchymal stromal/stem cells and NK cells improved the immune response in both healthy and immunocompromised patients during the process of post-trauma repair.¹²⁰ We also confirmed that this process was driven by MSC-derived CCL2 acting on NK-expressed C-C chemokine receptor 2 (CCR2), indicating that therapeutic application of MSCs or their soluble factors, which was normally secreted by exosomes, might thus improve the NK function after severe injury. However, the mechanism of NK cells as well as its downstream molecules and pathways in pathological scars requires deeper research.

Emerging evidence has shown that MSC-Exo exerts a critical immunomodulatory function in tissue regeneration and scar attenuation.^{172–174} Our recently published study also confirmed that MSC-Exo containing miR-138-5p alleviates pathological scars by downregulating the expression of SIRT1 and inhibiting the biological behaviors of fibroblasts.³² Additionally, the result of our study also demonstrated that SIRT1 could enhance the development of pathological scars by promoting the growth of fibroblasts. But the exact downstream mechanism is yet unclear.³² Furthermore, SIRT1 was proven to participate in innate and adaptive immunes via regulating the maturation of dendritic cells and T-cell differentiation.^{175–178} Taken all together, MSC-Exo may, both directly and indirectly, regulate the immune response during pathological scarring, which still requires further study.

However, the production, isolation, drug loading efficiency, biodistribution, uptake of exosomes and their optimal use remain challenges.¹⁷⁹ Meanwhile, the storage stability, low yield, low purity, and weak targeting of exosomes limit its clinical application.¹⁸⁰ Especially, exosome production is relatively low as a therapy method. Thus, how to improve its yield has become a research hotspot in recent years. With deeper insights into this field, several ways, such as mechanical loading, 3D cultivation conditions and designer exosomes, have been reported to boost its secretion.^{181,182} Recently, designer exosomes have been developed to overcome the limitations of exosomes for the targeted delivery of drugs or functional molecules for the healing of damaged tissue.¹⁸² And exosomes produced from 3D cultures of MSCs by tangential flow filtration also showed higher yield and improved activity.¹⁸¹ Whereas there are still more advanced strategies for the large-scale production of exosomes will be needed for further research and clinical applications. With the development of technologies in the separation, purification, specificity identification, analysis and detection of exosomes, our understanding of the basic biology of exosomes and their application in medical therapy technology will be greatly promoted.

In a word, the MSC-Exo application is a promising therapeutic for both wound healing acceleration and scar attenuation. Particularly, its immunomodulatory effects on immune cells achieve great progress in improving inflammation conditions and diseases, such as wound healing, cutaneous scarring, organ fibrosis and some other inflammation-related diseases. We are devoted to exploring the immunoregulatory mechanism of NK cells during scar formation. The further of our work will reveal the pathogenesis of pathological scars at a deeper level.

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Disclosure

The authors report no conflicts of interest in this work.

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