International Immunopharmacology 97 (2021) 107823

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Review

Anti-inflammatory and M2 macrophage polarization-promoting effect of mesenchymal stem cell-derived exosomes

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ARTICLE INFO

Keywords:
Exosomes
Inflammatory diseases
M2 macrophages
Mesenchymal stem cells
Tissue repair

ABSTRACT

Mesenchymal stem cells (MSCs) are multipotent cells beneficial in regenerative medicine and tissue repair. The therapeutic potential of MSCs for inflammatory diseases and conditions is partly due to secreted exosomes. Exosomes are one group of extracellular vesicles with 50–150 nm in diameter. They can carry numerous molecules and introduce them to the recipient cells to produce various biological effects. Macrophages are classified into M1 and M2 subtypes based on their activation states. M1 macrophages release pro-inflammatory factors like tumor necrosis factoralfa (TNF-α), interleukin1alfa (IL-1α), interleukin1beta (IL-1β), interleukin6 (IL-6), C-X-C motif chemokine ligand 9 (CXCL9), and C-X-C motif chemokine ligand 10 (CXCL10), while M2 macrophages secrete anti-inflammatory mediators including interleukin10 (IL-10), transforming growth factor beta (TGF-β), C-C motif chemokine ligand 1 (CCL1), C-C motif chemokine ligand 17 (CCL17), C-C motif chemokine ligand 18 (CCL18), and C-C motif chemokine ligand 22 (CCL22). This review summarizes the effect of MSC-derived exosomes in the polarization of M2 macrophages, which their anti-inflammatory and immunomodulatory properties are potentially effective in inflammation diseases and conditions such as central nervous system (CNS) diseases, autoimmune diseases, inflammatory bowel disease, cardiomyopathy, graftversushost disease, kidney, liver, lung, and skin injuries.

1. Introduction

Mesenchymal stem cells (MSCs) are a class of adult stem cells and can be isolated from various tissues like cord blood, umbilical cord, bone marrow, adipose tissue, dental pulp, placenta, amniotic fluid, brain, liver, kidney, spleen, lung, pancreas, and thymus [1,2]. MSCs produce components that can recover the damages, and they are a proper candidate for regenerative medicine and tissue engineering [1,3]. Also, they have properties that favor cell-therapy for inflammatory/autoimmune diseases and cancer [4]. MSCs affect the immune cells, including macrophages, T lymphocytes, dendritic cells, and natural killer (NK) cells [4]. The definite lack of major histocompatibility complex (MHC) II enables MSCs to inhibit T cell activation [5]. Thus, they have low immunogenicity and high immunosuppressive properties. MSCs can induce immunomodulatory M2 macrophages, which can inhibit the function of T cell and NK cell and induce regulatory T cells (Tregs) [4]. According to the previous investigations, MSCs show their therapeutic features through two main mechanisms: a paracrine factor-mediated mechanism involving cytokines and hormones and an exosome-mediated mechanism engaging RNAs and other molecules [6]. MSCs produce a higher amount of immunoregulatory exosomes compared with human cell lines. Thus, these stem cells can be sources of immunomodulatory components [1]. MSC-derived exosomes can mimic most biological properties of MSCs, such as anti-inflammatory and anti-apoptotic effects (8). These exosomes contain proteins that reflect features of both MSCs and exosomes [3].

Macrophages comprise 20–30% of all leukocytes and have plasticity that refers to macrophage polarization [7]. Tissue-resident macrophages include osteoclasts (bone), alveolar macrophages (lung), microglial cells (CNS), histiocytes (connective tissue), Kupffer cells (liver), and Langerhans cells (skin), but the functions of all macrophages are identical in all tissues [8]. Macrophages in tissues like colon have critical functions

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https://doi.org/10.1016/j.intimp.2021.107823
Received 18 March 2021; Received in revised form 23 May 2021; Accepted 25 May 2021
Available online 5 June 2021

in induction of inflammation. These macrophages enhance secretion of inflammatory cytokines and chemokines such as tumor necrosis factoralpha (TNFα), interleukin 1beta (IL-1β), chemokine (C-C motif) ligand 17 (CCL-17), and C-C motif chemokine ligand 24 (CCL-24) that attract inflammatory M1 macrophages [9]. Inflammation is a part of the pathology of diverse diseases such as obesity, diabetes, myocardial infarction, and CNS disease [10]. Macrophage polarization is important for tissue regeneration and homeostasis maintenance [7]. Numerous studies have demonstrated that MSC-derived exosomes promote M1 to M2 polarization and increase anti-inflammatory cytokines and chemokines, and this process alleviates inflammation. Here, we review exosomes secreted by MSCs and their functions in M2 macrophage polarization, which is beneficial in inflammatory diseases and conditions, wound healing, and graft versus host disease (GvHD).

2. Exosome biogenesis, components, and functions

Extracellular vesicles (EVs) have various subtypes including exosomes with 50–150 nm in diameter; microvesicles with 100–1000 nm in diameter; and apoptotic bodies with 50–4000 nm in diameter [11]. Some markers and proteins of exosomes help distinguish exosomes from microvesicles and apoptotic bodies [11]. These markers and proteins include surface markers such as CD9, CD63, CD81 of the tetraspanin family, heat-shock proteins (HSP60, HSP70, HSP90), multivesicular bodies, biogenic proteins (Alix, and tumor susceptibility gene 101 (TSG101)), lipid-related proteins, and phospholipases [2,6].

The endocytic pathway mediates the formation of exosomes as extracellular vesicles, which then become encapsulated by a lipid bilayer membrane. However, various cellular processes describe to infer other EVs directly derived from the plasma membrane [2,12]. The generation of exosomes is dependent on several cellular mechanisms, including the Endosomal Sorting Complex Required for Transport (ESCRT) that involves ESCRT-dependent and ESCRT-independent transport mechanisms [3]. Formation of intraluminal vesicles (ILVs) occurs through ESCRT-dependent/independent machinery during the maturation of microvesicular bodies (MVBs) from the early endosome [3]. After trafficking and fusion of MVBs with the plasma membrane, ILVs are released as exosomes [3]. ESCRT machinery contains four complexes, including ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III [3]. Studies have shown that exosomes have multiple activities, including anti-inflammatory, anti-immune, anti-coagulant, pro-angiogenic, and pro-tumorigenic activities [10].

Exosome cargo can comprise different molecules such as nucleic acids (DNA, RNA, mRNA, and miRNA), pro-inflammatory and anti-inflammatory cytokines, enzymes, and other various proteins [13]. Exosomes containing different molecules are released into the extracellular space. Consequently, they mediate intercellular communication and impact physiological and pathological conditions of recipient cells [14,15]. Exosomes can enter into the cells through endocytosis, ligand-receptor, or direct binding [2]. Cholesterol, sphingomyelin, ceramide, and lipid raft proteins present in the exosomal membrane help the fusion of exosomes to target cells and cross the biological barriers like the blood-brain barrier through the body [9].

Exosomes can be secreted by various cells, including stem cells, B cells, mast cells, platelets, cancer cells, dendritic cells, T cells, and Schwann cells [6]. The content of exosomes changes according to the source cells and the extracellular environment [2]. Exosomes exist in body fluids like blood, saliva, urine, and amniotic fluid, and they can be easily isolated from patients in a non-invasive manner [12].

3. Characteristics of MSC-derived exosomes

Exosomes as secretory components of MSCs transport cytokines and growth factors of immunoregulation transforming growth factor beta-1 (TGF-β1), IL-6, IL-10, hepatocyte growth factor (HGF), signaling lipids, mRNAs, and regulatory miRNAs which exert biological effects on recipient cells like cell-to-cell communication, tissue regeneration, metabolism, immune modulation, and homing of immune cells [6,9,16,17]. Studies have identified more than 150 miRNAs and 850 unique proteins in the cargo of MSC-derived exosomes that affect target cells through different pathways [17]. Furthermore, MSC-derived exosomes show the expression of vascular endothelial growth factor (VEGF), extracellular matrix metalloproteinase inducer (EMMPRIN), and matrix metallopeptidase 9 (MMP-9), and these factors play important roles in angiogenesis and tissue repair [17]. MSC-derived exosomes express MSC markers such as CD29, CD44, CD90, and CD73 that enable mentioned exosomes to reside in injured and inflamed tissues and express markers of exosomes like CD9, CD63, and CD81 as well [1,9]. MSC-derived exosomes protect their cargo against the degradation that helps them to be uptaken by cells through endocytosis [17]. The phenotype and biological effects of MSC-derived exosomes may change based on the source of MSCs [18].

miRNAs in MSC-derived exosomes are involved in the pathological and physiological processes [19]. For example, miR-155 and miR-146 contribute to organism development, epigenetic regulation, and immunoregulation [19]. Also, miR-23b, miR-451, miR-223, miR-24, miR-125b, miR-31, miR-214, and miR-122 are involved in tumorigenesis and tumor progression [19].

MSC-derived exosomes have some advantages including crossing the barriers like blood-brain barriers and capillaries and the size of these components is small enough to evade the reticuloendothelial system (RES) and are viable in a cell-free space [1,13]. Other features of MSC-derived exosomes include immunosilencing non-oncogenicity high stability cell- and tissue-specific homing [20]. MSC-derived exosomes do not have the major histocompatibility complex (class I and II) responsible for immune rejection and the absence of MHC-I and MHC-II can cause low immunogenicity [1]. The immunosuppressive function of MSC-derived exosomes is related to the immunotolerance of the host [20].

The mononuclear phagocyte system (MPS), also called the macrophage system or reticuloendothelial system, has important roles in the biodistribution of EVs. Studies have demonstrated that clearance of EVs from the circulation is lower in macrophage-depleted mice [9]. Accumulation of MSC-EVs occurs in organs where the MPS is active such as the liver, spleen, and lung [9].

Stem cell-EVs and immune cells exert bidirectional effects on each other through accumulating stem cell-EVs in the injury sites and inhibiting the pro-inflammatory response of immune cells [21]. According to the previous research, downregulation of IL-23 and IL-22 and upregulation of anti-inflammatory prostaglandin E2 (PGE2) occur by stem cell-EVs through repressing the function of T helper type 17 cell (Th17) or inducing conversion of Th17 cells into regulatory T cells (Tregs) [21]. MSC-derived exosomes can inhibit apoptosis, enhance cell and tissue regeneration and cell migration, promote angiogenesis, and regulate the immune and inflammatory response [2].

4. Macrophage polarization

Macrophages can respond to microenvironmental factors within tissues like damaged cells, activated lymphocytes, or products of the microbe to differentiate into two distinct functional phenotypes: classically activated macrophages (M1) and alternatively activated macrophages (M2) [22]. Classically activated macrophages (M1) exert pro-inflammatory activities and are induced through interferon-γ (IFN-γ) alone or in combination with microbial stimuli and/or inflammatory cytokines [23]. Alternatively activated macrophages (M2) are involved in anti-inflammatory response by inducing cytokines like IL-4 and IL-13 [23]. M1/M2 polarity occurs through two antagonistic pathways: 1) M1 macrophages are the iNOS pathway results, which produces citrulline and NO from arginine, 2) M2 macrophages are the results of the arginase pathway, which produces ornithine and polyamines from arginine [7]. NO produced by M1 macrophages is involved in microbial activity and

cell proliferation inhibition, while ornithine produced by M2 macrophages can enhance cell proliferation and tissue repair and remodeling through polyamines [8].

MSCs act as modulators of the inflammatory response and secrete cytokines and factors that can induce switching of pro-inflammatory macrophages toward anti-inflammatory phenotype [23]. Additionally MSCs can reduce pro-inflammatory cytokines produced by macrophages [16]. Besides MSC-derived exosomes induce polarization of M2 macrophages and this group of macrophages affects multiple diseases as illustrated in Fig. 1.

4.1. M1 and M2 macrophages

M1 and M2 macrophages are characterized by the expression of cell surface markers, secreted cytokines and chemokines, transcription, and epigenetic pathways [24]. Based on gene expression profile analysis, M1 macrophages can release pro-inflammatory cytokines such as tumor necrosis factoralfa (TNF-α), C-C motif chemokine ligand 2 (CCL2), IL-6, inducible nitric oxide synthase (iNOS), IL-1α, IL-1β, IL-12, IL-23, IL-18, type I IFNs (-α and -β), CXCL1–3, CXCL5, and CXCL8–10 which apply positive feedback on unpolarized macrophages [25]. Polarization of M1 macrophages takes place following the activation by lipopolysaccharide (LPS) and Th1 cytokines (such as IFN-γ and TNF-α) [7]. M1 macrophages show the expression of toll-like receptor- 2 (TLR-2), TLR-4, CD68, CD80, CD86 (co-stimulatory molecules), and MHC-II [7,11], enabling them to have pro-inflammatory, microbial, and tumoral activities and cause tissue damage [7].

Polarization of M2 macrophages occurs through downstream signals of cytokines like IL-4, IL-13, IL-10, IL-33, and TGF-β and cell exposure to immune complexes, glucocorticoid, or secosteroid (vitamin D_3) [7,26]. In some situations, IL-33 and IL-25 activate M2 macrophages by producing Th2 cytokines (IL-4 and IL-13) [7]. M2 macrophages can express high levels of dectin-1, DCSIGN (CD209), mannose receptor (CD206), scavenger receptor A, scavenger receptor B-1, CD163, CCR2, CXCR1, and CXCR2, Fizz1 (resistin-like-α). They also can produce chitinase 3-like 3 (YM1), macrophage and granulocyte inducer-form 1 (MgI1), arginase-1 (Arg1), IL-1 receptor antagonist (IL-1RA), high levels of IL-10, TGF-β, CCL1, CCL17, CCL18, CCL22, and CCL24, and low levels of IL-12 and IL-23 [7,25]. The functions of M2 macrophages are being anti-inflammatory, tissue regeneration and repair, angiogenesis and immunomodulation, having phagocytosis capacity, and tumor formation and progression [7]. Conversion of M1 to M2 macrophages and vice versa can be observed during responses to infection, wound healing, and cancers [4].

4.2. Subtypes of M2 macrophages

M2 macrophages fall into four subgroups, including M2a, M2b, M2c, and M2d [7]. These macrophages differ from each other in some properties like surface markers, secreted cytokines, and biological functions [7]. Table 1 summarizes different characteristics of M2 subcategories [7].

4.3. The roles of immunoregulatory molecules and signaling pathways in M2 polarization

The regulation of macrophage polarization and functions is dependent on a network of signaling molecules, transcription factors, epigenetic mechanisms, and post-transcriptional regulators [27]. Macrophage polarization involves multiple signaling pathways, such as PI3K/AKT,

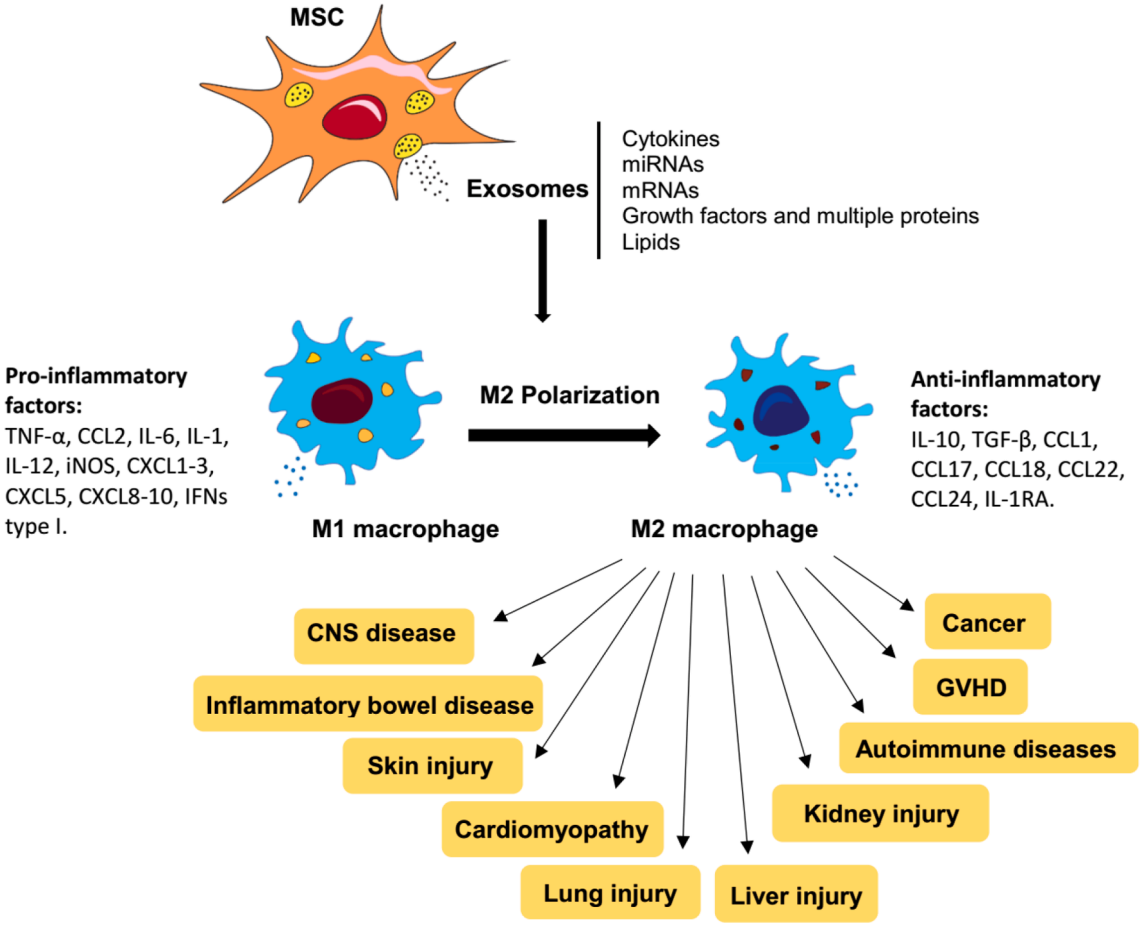

Fig. 1. . Anti-inflammatory and M2 macrophage polarization-promoting effect of MSC-derived exosomes in inflammatory diseases.

Table 1
Characteristics of M2 subgroups.

M2 Subgroup	Activating factors	Surface markers	Secreted cytokines and chemokines	Biological functions
M2a	IL-4, IL-13	IL1R, CD206, Arg-1, FIZZ1, Ym1/2	IL-10, TGF-β, CCL17, CCL18, CCL22	Promote endocytic activity, cell growth, and tissue repair
M2b	Immune complex, TLR ligands, IL-1β	IL-10R, IL-12R, CD86, IL-6R	TNF-α, IL-1β, IL-6, IL-10, CCL1	Promote Th2 differentiation and parasite, bacterial, and fungal infections
M2c	Glucocorticoids, IL-10 and TGF-β	CD163, CD206, TLR-1, TLR-8, Arg-1	IL-10, TGF-β, CCL16, and CCL18, CXCL13	Phagocytosis of apoptotic cells
M2d	TLR antagonists	IL-10R, IL-12R	IL-10, VEGF	Angiogenesis, tumor progression

IL, Interleukin; TGF-β, transforming growth factor-beta; FIZZ, found in the inflammatory zone; Arg-1, arginase-1; CCL, C-C motif chemokine ligand; TNF, tumor necrosis factor; TLR, Toll-like receptor; CXCL, CXC chemokine ligand; VEGF, Vascular endothelial growth factor.

JAK/STAT, NF-κB, Wnt/β-catenin, and notch signaling pathways [15,28].

Regulation of M1 gene expression is done via main transcription factors such as NF-kB p65 subunit, STAT1, STAT5, IRF3, and IRF5 [7]. Regulation of M2 gene expression is accomplished through key transcription factors like STAT3, STAT6, interferon regulatory factor4 (IRF4), lysine demethylase 6B (KDM6B), NF-κB p50 homodimers, peroxisome proliferator-activated receptor-gamma (PPARγ), and PPARδ [7]. Bruton's tyrosine kinase (Btk) has a role in M1 macrophage polarization through responding to LPS stimulation and the absence of Btk skew macrophages towards the M2 phenotype.

Following IFN-γ binding to its receptors, JAK1/2 are phosphorylated which lead to dimerization of STAT1 and then, activate iNOS and IL-12 and induce M1 phenotype [24]. Also, in response to LPS, STAT1-STAT2 make heterodimers and activate TLR4. This process enhances nuclear factor κB (NFκB)–mediated transcription of target inflammatory genes [24]. Also, interaction of STAT1 with IRF5 can induce M1 phenotype [24].

The AKT signaling pathway plays a role in macrophage polarization because knockdown of AKT1 and AKT2 by LPS-primed MSC-EVs abolish macrophage polarization [11]. AKT1 and AKT2 mainly regulate M2 and M1 polarization, respectively [15].

STAT1 and STAT3/STAT6 as members of IRF/STAT signaling pathways are activated by IFNs/TLR pathways. Also, downstream of the IRF/STAT pathways arranges polarized macrophage activation [27]. Krüppel-like factor 4 (KLF4) with STAT6 can induce M2 genes (Arg-1, Mrc1, Fizz1, PPARγ) by suppressing NF-κB/hypoxia-inducible factor 1α (HIF-1α)-dependent transcription [27]. IL-4/IL-13 or IL-10 pathways skew macrophages function toward M1 and M2 phenotypes, respectively [27]. After binding IL-4 or IL-13 to the IL4Rα, IL4Rα cytoplasmic tail becomes phosphorylated, resulting in the activation of JAK1/JAK3 or JAK1/Tyk2, respectively. This, in turn, stimulates STAT6 linked to the regulation of genes involved in M2 polarization like Arg1, CD206, Fizz1, and Ym1 [24].

IL-10 can induce M2 polarization through p50 NF-κB homodimer, c-Maf, and STAT3 activities [27]. The binding of IL-10 to the IL-10 receptor complex results in JAK1 phosphorylation and STAT3 activation, leading to the suppression of pro-inflammatory cytokines such as TNFα, IL-1β, IL-12, and IFNγ [24]. miR-27a can suppress IL-10 that decreases STAT3 phosphorylation and elevates the inflammatory response [29]. IRF4 inhibits TLR signaling in a MyD88 independent manner to induce M2 activation [30]. Also, BMP7 promotes M2 polarization *in vitro* through PI3K/AKT/mTOR pathway activation [31]. The HIF has a dual role in macrophage polarization: 1) HIF-1α increases nitric oxide synthase and M1 phenotype, 2) HIF-2α enhances arginase-1 expression and M2 phenotype [11].

According to the previous research, epigenetic mechanisms have important functions in macrophage polarization [25]. One of the epigenetic changes in macrophage polarization is the upregulation of the histone demethylase JMJD3 in mouse macrophages that changes chromatin modifications to induce M2 gene expression [27]. Inhibition of DNA methyltransferases (DNMTs) can increase M2 polarization through the inhibition of PPAR-γ promoter and then prevent inflammation and inflammatory diseases [25]. The expression levels of DNMT3a and DNMT3al were significantly higher in M2 compared to M1 macrophages, but the DNMT3b was significantly lower in M2 compared to M1 macrophages [25]. Acetylation of histones H3 and H4 on suppressors of cytokine signaling 3 (SOCS-3) promoter and activation of STAT3 and MAPK can suppress inflammatory responses and promote M2 polarization [25].

Treatment of MSCs with interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) can cause MSC-EVs to release and enable inducing the M2 macrophage polarization [11]. Heo *et al.* reported that exosomes from adipose-derived MSCs (AdMSCs) induce M2 phenotype in human peripheral blood monocytes (PBMCs) through upregulation of M2 markers such as CD163, Arg1, and CD206 and activating MafB and STAT6 [28].

Exosomes are the third kind of signal transduction pathways and can regulate cellular functions [22]. Exosomes contain a large number of functional RNAs, and the most frequent functional RNAs in exosomes are miRNAs that are involved in the regulation of M1 and M2 macrophage polarization at post-transcriptional levels through targeting various transcription factors [11,22]. Literature reports indicated that hypoxia conditioning MSCs secrete high numbers of extracellular vesicle (EV) containing proteins and miRNAs (such as miR-223, miR-146b, miR126, and miR-199a) involved in M2 macrophage polarization and different phases of the healing process [23].

miR-223 as a novel regulator of macrophage polarization, suppresses classic pro-inflammatory pathways and enhances polarization of macrophages toward alternative M2 macrophages, and decreases inflammatory response and insulin resistance through inhibiting Pknox1 [11,23]. Additionally, the expression of miR-146b can reduce the production of anti-inflammatory cytokines and chemokines like IL-6 [23]. Moreover, the expression of miR-126 and miR-199a have functions in the tissue repair process [23]. Other studies revealed that some of the miRNAs in exosomes derived from stem cells have immunomodulatory properties like miR-21, miR-146a, and miR-181b [16]. miR-146a suppresses IRF5, reduces inducible nitric oxide synthase and finally causes M2 polarization [11]. miR-21 and miR-98 can control IL-10 levels and downregulate inflammatory genes' expression in monocytes and macrophages [25]. Moreover, M1 macrophages display upregulated expression of miR-181a, miR-155, miR-204, and miR-451, along with a downregulated expression of miR-146a, miR-143, and miR-145 compared with M2 macrophages [11]. Also, the expression of let-7c is lower in M1 macrophages than in M2 macrophages and upregulation of this miRNA promotes M2 polarization targeting CCAAT/enhancer-binding protein β (C/EBP-δ) [25]. C/EBP-δ is a transcription factor involved in the inflammatory response [11].

5. Immunomodulatory effects of M2 macrophages promoted by MSC-derived exosomes

5.1. Inflammatory diseases

Extracellular vesicles/exosomes contribute to the therapeutic effects

of MSCs in several animal models such as ischemic stroke, acute kidney injury, and traumatic brain injury [32]. MSC-derived exosomes have shown immunomodulatory effects in skeletal muscle injury, cardiovascular diseases, autoimmune diseases, central nervous system (CNS) diseases, inflammatory bowel diseases and colon injury, liver and lung diseases, kidney injury, and obesity.

5.1.1. Skeletal muscle injury

Administration of EVs in cardiotoxin (CTX)-induced skeletal muscle injury influences macrophage polarization from M1 to M2 phenotype and reduces inflammatory response at *in vivo* level [23]. CCL2 plays a role in macrophages recruitment and activation, and deficiency of this molecule in the skeletal muscle of mice appears to impair muscle regeneration [23].

5.1.2. Cardiovascular diseases

The use of MSC-derived exosomes rather than intra-arterial administration of MSCs can prevent the risks of myocardial micro-infarction and pulmonary embolism caused by MSCs [33]. When packed in MSCs-derived exosomes, miR-182 targets TLR4, inhibits TLR4/NF-κB pathway, and activates PI3K/AKT pathway in myocardial ischemia/reperfusion (I/R) injury and thus, negatively polarizes M1 macrophages and promotes M2 polarization in the heart [16]. Also, the polarization of M2 macrophages can improve myocardial damage of diabetic cardiomyopathy [33]. Transfer of MSC-EVs containing miR-223 to cardiomyocytes downregulates pro-inflammatory genes in a mouse model of sepsis [11]. Macrophages have different properties in the early and late myocardial infarction (MI) stages [15]. In the early stage of MI, M1 macrophages play a role in infarct myocardium, while in the late stage, M2 macrophages increase and are responsible for heart healing [15]. miR-101a-loaded MSC-extracellular nanovesicles (eNVs) enhances heart function, reduces fibrosis after myocardial infarction (MI) through anti-fibrotic and immunoregulatory effects [34]. Xu *et al.* have proved that exosomes from bone marrow-derived mesenchymal stem cells (BMSCs) enhance M2 polarization under LPS stimulation in ischemic heart disease via activation of AKT1/AKT2 signaling pathway and inhibition of LPS-dependent NF-κB signaling pathway [15].

MSCs derived exosomes promote M2 macrophage polarization and suppress macrophage infiltration in atherosclerotic plaque of $ApoE^{-}$ mice through miR-let7 HMGA2 NF-kB pathway and miR-let7 IGF2BP1 PTEN pathway, respectively, to ameliorate atherosclerosis [22]. Atherosclerosis is a chronic inflammatory disease and the main pathological basis of coronary artery diseases [22]. TGF-β secreted by M2 macrophages suppresses the recruitment of inflammatory cells and protects them against atherosclerosis [35].

5.1.3. Autoimmune diseases

There have been reports of immunomodulatory effects of MSC-derived exosomes in several autoimmune diseases like multiple sclerosis (MS), rheumatoid arthritis, type 1 diabetes mellitus (insulin-dependent diabetes mellitus), and uveitis [1]. In the experimental autoimmune encephalomyelitis (EAE) mouse model of multiple sclerosis, microglia cells' polarization into the M2 phenotype by MSC-derived exosomes could alleviate disease phenotypes [1]. Hosseini Shamili *et al.* have shown that the aptamer-exosome bioconjugate could suppress the inflammatory response and decrease demyelination lesion region in CNS of EAE mouse model of MS [36]. The use of MSC-derived exosomes to treat autoimmune diseases is still in its infancy [1].

5.1.4. Central nervous system (CNS) diseases

MSC EVs induce polarization of microglia toward an M2 phenotype in Alzheimer's disease (AD) [9]. In the brain of MSC-Exosomes-treated AβPP/PS1 mice M2 microglia cells express YM1 arginase-1 and mannose receptor C type 1 (MRC1) [9]. Also M2 microglia cells produce Aβ-degrading enzymes (neprilysin (NEP) and insulin-degrading enzyme (IDE)) and anti-inflammatory cytokines (IL-10 and TGF-β) that alleviate inflammation [9].

MSC exosomes inhibit the synthesis of inflammatory cytokines (TNF-α and IL-1β) and promote the production of anti-inflammatory cytokines (IL-10 and TGF-β) in microglia cells and alleviate inflammation in neural injury [9]. MSC-exosomes containing vascular endothelial growth factor C (VEGFC), angiopoietin-2, and fibroblast growth factor-2, lead to neoangiogenesis as a beneficial effect of MSC-EVs [9]. Adipose-derived stem cells (ADSCs) release exosomes with miR-30a-5p overexpression, which can suppress ischemia-induced, autophagy-mediated brain injury by the polarization of M1 to M2 phenotype in acute ischemic stroke (AIS) [37]. MSC-EVs downregulate pro-inflammatory cytokines such as TNF-α, IL-6, and IFN-γ in spinal cord injury and induce M2 polarization and reduce neuroinflammation [11]. Overexpression of CCR2 that inhibits CCL2 promotes the polarization of microglia into M2 macrophages [11]. Wang *et al.* suggested that both MSCs and MSC-derived exosomes have suppressive and anti-inflammatory effects on A1 astrocytes following spinal cord injury [32]. Reduction of A1 astrocyte proportion is mediated by inhibiting NF-κB p65 activation [32]. MSC and MSC-exosomes inhibit pro-inflammatory microglia in spinal cord injury and may impact A1 astrocytes through various inflammatory cells [32]. Li *et al.* have proved that bone marrow mesenchymal stem cell (BMMSC)-derived exosomal miR-124-3p attenuates nerve injury through negative regulation of Ern1 and promotion of M2 polarization in spinal cord ischemia-reperfusion injury (SCIRI) [38].

5.1.5. Inflammatory bowel diseases and colon injury

Administration of immunosuppressive medications suppresses antimicrobial and anti-tumor immunity in autoimmune and inflammatory diseases and may lead to malignancy and infections [9]. Thus, new therapeutic agents without harmful effects of immunosuppressive drugs are necessary for autoimmune and inflammatory diseases. Recent studies have indicated that MSC-derived EVs could alleviate colitis by suppression of colon macrophages and suppression of inflammatory cytokines production and promote polarization of M2 phenotype [9]. Inhibition of colon macrophages induced by MSC-EVs depends on the suppression of NF-κB and iNOS-driven signaling and reduction of TNFα, IL-1β, and IL-6 production, and decreases colon injury and inflammation [9]. Also, MSC-EVs can inhibit IL-7 signaling in colon macrophages through miR-17, which impairs IL-7:IL-7 receptor signaling [9]. Reduction of IL-7 and iNOS-signaling pathways activation attenuates production of TNFα, IL-1β, IL-6 and increases the production of IL-10 that alleviates colitis [9]. MSC-EVs downregulate JAK1/STAT1/STAT6 signaling pathway and increase M2 macrophages and the expression of IL-10 and TGF-β in the colon tissues [11]. Another study revealed that murine bone marrow-MSCs secrete chemokine ligand 2 (CCL2) and C-X-C motif chemokine 12 (CXCL12) to upregulate IL-10 expression in a CCR2-dependant manner and polarize macrophage into IL-10 M2 phenotype with an anti-inflammatory response in toxic colitis [39]. Liu *et al.* reported that human bone marrow-derived MSC-exosomes regulate inflammatory response and polarize M2b macrophages in various models of inflammatory bowel disease (IBD)-related colitis. The metallothionein-2 in MSC-exosomes is essential for suppression of inflammatory response [20].

5.1.6. Liver diseases

MSC EVs protect hepatocyte in chronic liver inflammation and fibrosis through prevention of TGF-β1/Smad2-induced EMT in hepatocytes and suppressing the production of inflammatory cytokines (TNF-α IL-1β and IL-6) and pro-fibrotic TGF-β1 in liver macrophages (Kupffer cells) [9]. MSC-EVs ameliorate liver injury and reduce the production of pro-inflammatory cytokines (TNF-α IL-1β IL-6) in macrophages [11]. MSC-EVs with miR-223-3p which targets STAT3 reduce the inflammatory response and attenuate liver injury [11]. Exosomes derived from human umbilical cord MSCs (hucMSCs) inhibit both epithelial-mesenchymal transition of hepatocytes and collagen production resulting in alleviation of liver fibrosis [19].

5.1.7. Lung diseases

MSC EVs can effectively treat acute lung injury and transfer miR-27a-3p to macrophages and induce M2 polarization of alveolar macrophages *in vitro* and *in vivo* [40]. MSC-EVs have different effects on alveolar macrophages based on anti-microbial inflammatory response [9]. During the onset of inflammation, MSC-EVs increase alveolar macrophages' phagocyte activity and eliminate bacterial pathogens from the lungs [9]. However, during the resolution of inflammation, MSC-EVs can promote alternatively activated M2 macrophages involved in tissue repair and regeneration [9]. The alveolar macrophages promote chronic inflammation and MSC-EVs suppress chronic and macrophage-driven inflammation of lung disease through alveolar macrophages' polarization [9]. MSC-exosomes reduce the expression of iNOS mRNA and enhance the expression of Arginase-1 mRNA that leads to the switching of inflammatory M1 towards M2 macrophages [9]. Administration of MSC-exosomes in mice with ischemia-reperfusion (I/R)-induced lung injury decreases M1-related inflammatory cytokines (IL-8 IL-1β IL-6 and TNF-α) while increases the M2-related anti-inflammatory cytokines (IL-10 TGF-β) [9]. Intratracheal administration of MSC-EVs containing miR-21-5p decreases M1 polarization of alveolar macrophages and alleviates lung ischemia/reperfusion injury [11]. Intratracheal administration of MSC-EVs containing miR-27a-3p alleviates acute lung injury and elevates M2 macrophage polarization [11]. Young and aging MSC-EVs have displayed similar markers (CD63 CD81 CD105 and CD44) but their ability to polarize the alveolar macrophages is different [9]. Aging MSC-EVs have a lower capacity to inhibit the production of inflammatory M1-related cytokines and induce polarization of M2 macrophages compared to the young MSC-EVs [9]. Young MSC-EVs have indicated higher expression of miR-223-5p which induces M2 polarization in alveolar macrophages and lower expression of miR-127-3p and miR-125b-5p that are responsible for M1 polarization [9]. Also, another study revealed that macrophages are the main targets of MSC-exosomes in alleviation of pulmonary fibrosis [9].

5.1.8. Kidney injury

Let-7b in MSC-EVs is responsible for generating M2 phenotype in renal macrophages, and mediates immunosuppressive effects. Also, mice that received let-7b containing MSC-EVs show a lower concentration of M1-derived inflammatory cytokines in their I/R-injured kidneys [9].

5.1.9. Obesity

Zhao *et al.* demonstrated that exosomes from adipose-derived MSCs alleviate inflammation and obesity through M2 macrophage polarization. These MSCs express Arg-1 and IL-10 depending on activation of STAT3 transferred by exosomes [20]. Obesity causes low-grade chronic inflammation in white adipose tissues (WAT), which results in insulin resistance and metabolic syndromes and consequently, leading to type 2 diabetes, cardiovascular disease, and cancers [14]. PPARγ is an important molecule for M2 polarization in obesity and diabetes. Studies have demonstrated that PPARγ inhibitors can reduce M2 polarization and enhance insulin sensitivity in mouse adipose tissue [35].

5.1.10. Spleen disease

Pacienza *et al.* showed that exosomes derived from MSCs suppress IL-1β and IL-6 in the spleen of mice subjected to LPS-induced systemic inflammation and inhibit M1 phenotype in LPS-stimulated macrophages [10]. Secretion of IL-1β and IL-6 is mediated by Toll-like receptor 4 (TLR4)-expressing monocytes and macrophages [10].

5.2. Wound healing

Multiple studies have investigated the effects of MSC-derived EVs in skin disorders and wound healing [33]. Four wound healing stages include hemostasis, inflammation, hyperplasia, and remodeling [33]. Diverse cell types are involved in the different phases of chronic wound healing like neutrophils, macrophages, mast cells, dendritic cells, T cells, and fibroblasts [41]. M2 macrophages secrete anti-inflammatory cytokines and diverse growth factors and play important roles in wound healing [16]. Furthermore, regulation of M2 polarization through exosomes derived from MSCs can enhance skin wound healing. The amount of IL-1β increases in diabetic wounds, and inhibition of IL-1β improves the wounds through polarization of macrophages to repair phenotype [33]. Liu *et al.* demonstrated that melatonin (MT)-pretreated MSCs-derived exosomes (MT-Exo) notably suppress the pro-inflammatory factors IL-1β and TNF-α and promote the anti-inflammatory factors IL-10 [33]. MT-Exo lead to the upregulation of PTEN expression, and inhibition of the phosphorylation of AKT increases the ratio of M2 polarization to M1 polarization *in vitro* [33]. At *in vivo* level, MT-Exo inhibit inflammation, facilitate angiogenesis and collagen synthesis, and increase diabetic wound healing [33]. miR-146a negatively regulates wound healing in a diabetic murine wound healing model [42]. Based on the studies done before, LPS-preconditioned MSC-derived exosomes (LPS pre-Exo) elevate diabetic cutaneous wound healing via M2 macrophages activation [15]. Ti *et al.* demonstrated that let-7b in LPS pre-Exo contributes to macrophage plasticity regulation to attenuate chronic inflammation and improves cutaneous wound healing [43].

5.3. SARS-CoV-2 infection

Macrophage polarization is involved in viral infections. M2 macrophages can suppress inflammation, while their apoptotic functions enhance viral clearance. In severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, M2 macrophages can protect patients from progression of their disease to fibrotic lung disease via the STAT pathway [35]. MSC-derived exosomes might be effective in SARS-CoV-2 infection by suppressing the pro-inflammatory immune response, promoting M2 macrophage polarization via delivering PGE2, secretion of anti-inflammatory cytokines like IL-10, and repairing tissue injury by producing keratinocyte growth factor (KGF), VEGF, and HGF [44]. These MSC-derived exosomes' actions can reduce the damage of alveolar epithelial cells and capillary endothelial cells [44].

5.4. Graft versus host disease (GvHD)

GvHD is a problem associated with high morbidity and mortality after allogeneic hematopoietic stem cell transplantation (allo-HSCT) [45]. GvHD includes chronic and acute types [45]. Antigen-presenting cells (APCs) like macrophages and dendritic cells play a role in the pathogenesis of aGvHD and cGvHD [45]. The most common treatment for acute and chronic GvHD is immunosuppression with corticosteroids, but the response rate is 40–50%, and more therapeutic approaches are required [13]. Multiple studies have demonstrated the successful application of exosomes secreted by MSCs in GvHD [6]. Studies have illustrated that MSC-derived exosomes' administration alleviates symptoms of steroid-resistant acute GvHD patients without side effects [46]. Also, these exosomes diminish pro-inflammatory cytokines like TNF-α, IL-1β, and IFN-γ and increase anti-inflammatory cytokines like IL-10 both *in vivo* and *ex vivo* [46]. BM-MSC-derived exosomes containing CD73 can modulate GvHD by mediating the conversion of ATP to adenosine and inhibition of Th1 cells function [46].

5.5. Cancer

Tumors have tumor-infiltrating inflammatory cells like macrophages, which can attain a definite phenotype defining as tumor-associating macrophages (TAMs) [25]. These cells attenuate anti-tumor activity, induce tumor-supporting functions and show M2-like features [25]. Moreover, TAMs can promote tumor progression through different mechanisms and pathways [25].

Biswas *et al.* revealed that exosomes derived from tumor-associated MSCs contribute to the differentiation of monocytic myeloid-derived

suppressor cells (M-MDSCs) toward immunosuppressive M2 macrophages and accelerate breast cancer progression [47]. Moreover, EVs secreted by hypoxia-pre-challenge MSC induce M2 polarization through PTEN downregulation by miR-21-5p delivery and promote cell growth and mobility of non-small cell lung cancer [48].

Human bone marrow mesenchymal stem cells (hBMSCs)-derived exosomes overexpress several miRNAs. These miRNAs including miR-101-3p [49], miR-205 [50], miR-16-5p [51], and miR-34a [52] suppress proliferation, invasion, and migration of oral cancer, prostate cancer, colorectal cancer, and glioblastoma by inhibiting their targets, respectively.

6. Therapeutic potential

Previous studies have found that MSCs are a safe and effective strategy for tissue repair and regeneration in various diseases [22]. Some beneficial properties of MSCs for regenerative medicine are ease of isolation, self-renewal, ability to grow *in vitro*, low immunogenicity, multilineage differentiation potential, and secretion of components for promotion of tissue renovation [2]. The capacity of MSCs for immunosuppression and tissue regeneration is age-dependent, and aging harms immunosuppressive features of MSCs and weakens their therapeutic potential [9]. The therapeutic effects of MSCs might be related to exosomes with the potential for cell-free therapy [32]. Also, injection of MSCs may lead to malignant transformation, but MSC-derived exosomes do not have this complication [2]. Some features make the MSC-derived exosomes an ideal delivery system for small molecules and gene therapy for cancer and regenerative medicine [18]. These features help the transportation of genetic materials, their protection from extracellular degradation, and selectively delivering them to the recipient cells [18].

7. Conclusion

The alternatively activated macrophages secrete immunomodulatory mediators involved in tissue repair and regeneration and regulate the immune response in many inflammatory diseases through signaling pathways such as PI3K/AKT/mTOR, JAK/STAT, NF-κB, Wnt/β-catenin, and notch signaling pathways. MSCs can induce macrophage polarization towards the M2 phenotype, but this cell-based therapy has several clinical risks and problems. A new therapeutic strategy is the usage of MSC-derived exosomes that carry most of the therapeutic effects of MSCs, and this cell-free therapy overcomes the problems of MSCs and has some advantages. The inflammatory diseases like autoimmune disease and conditions affecting the heart, kidney, lung, liver, brain, muscle, and skin can be efficiently and safely managed through this therapeutic approach and it can increase patients' survival, as well. Effects of MSC-derived exosomes in treating disease are related to M2 polarization with anti-inflammatory activity. Further studies are required to investigate more about this strategy in clinical use and potential issues about it.

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