



Review

Contribution of microRNAs to the immunosuppressive function of mesenchymal stem cells



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ABSTRACT

Mesenchymal stem/stromal cells (MSCs) are progenitor cells identified in many adult tissues, in particular in bone marrow or adipose tissue and, in placental tissues. MSCs exert pleiotropic functions that render them attractive for many clinical applications, both in degenerative and inflammatory diseases. Their main mode of action is through the secretion of trophic factors that can be released in the extracellular milieu or packaged within extracellular vesicles. These factors can be proteins, lipids, mRNAs or miRNAs that possess diverse effects, notably pro-angiogenic, anti-fibrotic, anti-apoptotic or anti-inflammatory. The anti-inflammatory role of a number of miRNAs has been demonstrated with different types of immune cells but few have been associated with the immunomodulatory function of MSCs. In this review, we summarize the data on the miRNAs that have been validated as participating to the anti-inflammatory role of MSCs.

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1. Introduction

Multipotent mesenchymal stem/stromal cells (MSCs) are progenitor cells mainly isolated from adult bone marrow (BM), adipose tissue, placental tissue and probably present in most adults [1]. MSCs are defined by their functional abilities of differentiation towards adipocytes, osteoblasts and chondrocytes. They differ from other stem cells by the expression of mesenchymal markers (CD70, CD90, CD105) and absence of expression of hematopoietic markers (CD11b CD14, CD19, CD34, CD45 and the major histocompatibility complex class II (MHC II)) [2]. MSCs exert several functions including support of haematopoiesis, immune tolerance, regeneration, resistance to fibrosis, apoptosis or hypertrophy [3]. Most of these properties may have a positive impact for many therapeutic applications in various diseases. However, MSCs are populations of cells with heterogeneity in their functional properties that might contribute to the variability of cell therapy outcomes [4]. Evidence that subpopulations of MSCs exist depending on the tissue source or culture conditions highlights the need to better identify the cell subtypes in order to improve therapeutic efficacy. Selection of MSCs is therefore not a minor concern because each MSC subpopulation has particular potential for a devoted application. For tissue engineering applications including bone and cartilage repair, MSCs with a high potential of differentiation and regeneration will be of interest while high anti-inflammatory function will be essential in auto-immune or inflammatory diseases [5]. Such properties are primarily associated with the production of soluble mediators and the role of extracellular vesicles (EVs) in the transport of these factors has gained much attention in the last few years [6]. EVs are the key players in cell-to-cell communication pathways and convey a variety of proteins, lipids, messenger RNAs (mRNAs) as well as microRNAs (miRNAs).

Conserved throughout evolution, miRNAs are an abundant class of endogenous, short non-coding single-stranded, regulatory RNA molecules (19–23 nucleotides). They are processed and cleaved from larger hairpin precursor transcripts (pre-miRNAs) during the maturation phase that leads to the production of effective mature miRNAs loaded onto the RNA-induced silencing complex (RISC). MiRNAs control gene expression in a sequence-specific manner by targeting mRNAs for degradation or translational repression [7]. Rarely, miRNAs have also been shown to enhance protein synthesis [8]. By controlling degradation of the target mRNA and as a consequence, the accumulation of the target protein(s) in cells, miRNAs play critical role in genetic regulatory networks. They have been implicated in many physiological processes such as proliferation, apoptosis or differentiation. There are now 2588 annotated miRNAs that have been identified in the human genome, each being predicted to regulate the expression of hundreds of target genes [9]. Thus, miRNAs represent increasingly interesting cellular components for unravelling the complexity of specific genetic pathways in a more integrated manner.

High throughput transcriptomic analyses of MSCs have identified a large panel of miRNAs and many studies have highlighted the importance of miRNAs in stem cell fate and differentiation [10]. Global miRNA disruption through Droscha and Dicer knockdown (both are essential components for miRNA biogenesis) in MSCs resulted in significantly reduced potential of differentiation [11]. These data underline the importance of miRNAs in regulating the properties of MSCs, and suggest that they may play a role in other key functions, including proliferation, adhesion, survival or immunosuppression. Contribution of miRNAs to immune cell development and function has been described but less attention has been paid to their role in driving the immunoregulatory potential of MSCs [12]. In this review, we will provide an overview of the few described miRNAs that are involved in the

immunosuppressive function of MSCs and the role of EVs-derived miRNAs in this function.

2. Modulation of miRNA expression in MSCs by inflammation

The anti-inflammatory and immunosuppressive properties of MSCs are induced by an inflammatory environment and are primarily associated with the secretion of soluble factors, such as prostaglandin E2 (PGE2), interleukin 6 (IL-6), human leukocyte antigen G (HLA-G) or tumor necrosis factor-inducible gene 6 (TSG6) and/or, EVs that contain those factors. Upon activation, the expression of a number of them, including proteins and miRNAs, are modulated leading to repression of the inflammatory response [13]. MiRNAs that are modulated in activated MSCs may act either, directly on the cell itself by downregulating or upregulating the expression of inhibitors or inducers, respectively, of immunosuppressive functions. They may also be directed to EVs that target and release their content within the immune cells (Fig. 1).

Ten years ago, the miRNA signature of human bone marrow (BM)-MSCs has been reported in steady-state conditions and after IL-1 α stimulation reproducing an inflammatory environment [14]. Upon activation, nine miRNAs (miR-15 b, miR-30 d, miR-30e-5p, miR-99 b, miR-130a, miR-140, miR-206, miR-302a and miR-342) were upregulated and seven miRNAs (let-7g, miR-34a, miR-99a, miR-103, miR-107, miR-181a, miR-210) were downregulated. More recently in adipose tissue-derived MSCs (ASCs), Liu and co-authors showed that several miRNAs, including miR-146a, miR-150 and miR-155 were differentially expressed after transient treatment with lipopolysaccharides (LPS) or tumor necrosis factor (TNF)- α [15]. The authors proposed that MSCs behave as sensors and switchers of inflammation and share several features of immune cells such as a short-term memory of danger signals or environmental stimuli. A short-term memory response is characterized by the maintenance of a biological change following a short stimulus and a more intense response upon a second exposure to the same stimulus. Potential mediators of short-term memory response include regulatory factors, namely chromatin modifications and miRNAs. Indeed, the modulation of miRNAs might mediate the short-term memory response of MSCs to environmental signals and participate to their anti-inflammatory function. Although poorly investigated, the identity and the role of the distinct miRNAs that have been described in MSCs from different sources and origins will be reviewed in the following parts.

3. Regulatory role of miRNAs involved in the immunosuppressive properties of MSCs

3.1. Role of miR-146

The miR-146 family is composed of 2 members, miR-146a and miR-146 b that are highly conserved across species. Although they have been described in cancer and inflammation, their involvement as negative regulators of inflammation is the most advanced field of miR-146 research [16]. The role of these miRNAs in toll-like receptor (TLR) signaling and the innate immune response has been widely studied [17]. Actually, several ligands of TLR4 signaling such as LPS, TNF- α , interferon (IFN)- γ or IL-1 β are able to increase miR-146a and miR-146 b expression. MiR-146a/b target and repress IL-1 receptor-associated kinase 1 (IRAK1) and TNF receptor associated factor 6 (TRAF6), which are adapter molecules downstream of Toll-like and cytokine receptors (Fig. 2). Indeed, downregulating IRAK1 and TRAF6 decreases the NF- κ B activity leading to downregulation of inflammatory cytokines such as IL-6 and IL-8.

Initially, miR-146a was found to be overexpressed in human BM-MSCs upon activation by TNF- α [18]. This overexpression was

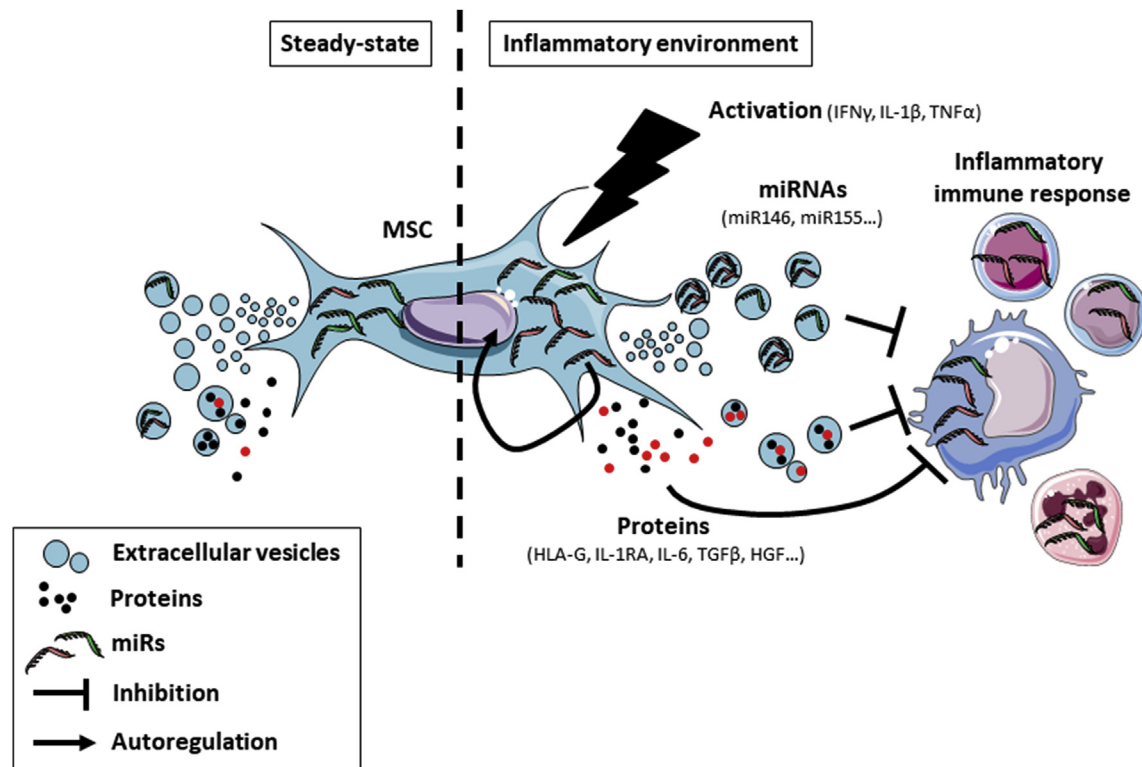


Fig. 1. Mode of action of miRNAs produced by MSCs. In steady state conditions, MSCs constitutively express several hundreds of miRNAs and secrete factors that can be released in the extracellular milieu or within extracellular vesicles. In an inflammatory environment, MSCs can be activated by $\text{IFN}\gamma$, $\text{IL-1}\beta$ or $\text{TNF}\alpha$ and exert an immunosuppressive function. This activation induces or modulates the expression of several proteins and miRNAs that inhibit the inflammatory immune response. MSC-derived factors including miRNAs can either be entrapped within extracellular vesicles and act in a paracrine manner on target immune cells to switch their inflammatory phenotype toward an anti-inflammatory phenotype or, act in an autocrine manner by regulating miRNA biogenesis or signaling pathways involved in the immunosuppressive function of MSCs.

proposed to enhance the production of IL-8 by BM-MSCs. However, the exact function (protective or detrimental) of miR-146a overexpression in BM-MSCs, was not investigated in this study. In a murine model of experimental autoimmune encephalomyelitis (EAE), miR-146a was expressed significantly more in murine BM-MSCs that lacked immunoregulatory function [19]. To confirm the role of miR-146a in the loss of immunoregulatory function, the authors have transfected the murine BM-MSCs with miR-146a antagomir and transplanted the cells in mice with EAE. This resulted in the amelioration of clinical signs confirming the inhibitory effect of miR-146a on the therapeutic effect of murine BM-MSCs. In vitro, overexpression of miR-146a in murine BM-MSCs negatively controlled their immunomodulatory functions by targeting PGE2 synthase (PGES-2), which is a mediator of MSCs known to be involved in immunosuppression. Transfection of BM-MSCs with anti-miR-146a antagomir resulted in inhibition of T cell proliferation. This study was the first demonstrating the role of miR-146 in the immunomodulatory function of BM-MSCs (Table 1).

MSCs can also act on the expression of miR-146 in targeted inflammatory tissues. As an example, one paper suggested that murine BM-MSCs may downregulate the expression of miR-146 in non-healing wounds of diabetic mice [20]. Local treatment with BM-MSCs increased miR-146a expression and decreased the expression of its target genes, IRAK1 and TRAF6 in tissues. Whether the upregulation of miR-146 in tissues is related to the transfer of miR-146 transported within BM-MSC-derived EVs was not determined. Indeed, increasing miR-146a expression by MSC treatment might improve wound healing (Table 2).

3.2. Role of miR-155

MiR-155 was initially described as being rapidly increased in response to injury or infection [21]. It is induced by alarmins ($\text{IL-1}\alpha$), inflammatory cytokines ($\text{TNF}\alpha$, $\text{IL-1}\beta$) and pathogen-associated molecular patterns and damage-associated molecular patterns (PAMPs/DAMPs). It regulates both innate and adaptive immunity, including macrophage polarization, dendritic cell maturation, T cell differentiation and controls B lymphocyte proliferation. MiR-155 targets the translation of many proteins playing important functions in immune cells, notably molecules of IL-4/IL-13 family, transforming growth factor ($\text{TGF}\beta$) pathway as well as those of apoptotic histone deacetylase 4 (HDAC4)/B cell lymphoma protein 6 (Bcl6) pathways among others (for detailed review, see Ref. [21]).

Expression of miR-155 has also been reported in murine BM-MSCs and transfection of miR-155 mimics increased the expression levels of IL-6 and IL-8 and decreased inducible nitric oxide synthase (iNOS). In fact, hypoxia was shown to upregulate expression of miR-155 and of hypoxia-inducible factor 1 (HIF1), which in turn downregulated iNOS [22]. In a report investigating the modulation of miRNAs in murine BM-MSCs activated by the inflammatory cytokines $\text{IFN}\gamma$ and $\text{TNF}\alpha$, a number of miRNAs has been identified as being upregulated (miR-155, miR-720, miR-1937a) or downregulated (miR-27a, miR-27 b, miR-148a and miR-762) [23]. In this study, miR-155 was the most significantly upregulated miRNA. Overexpression and downregulation of miR-155 was subsequently shown to decrease and increase, respectively, the proliferation of T cells. Importantly, miR-155 overexpression was associated with a decreased production of iNOS and Tak1-

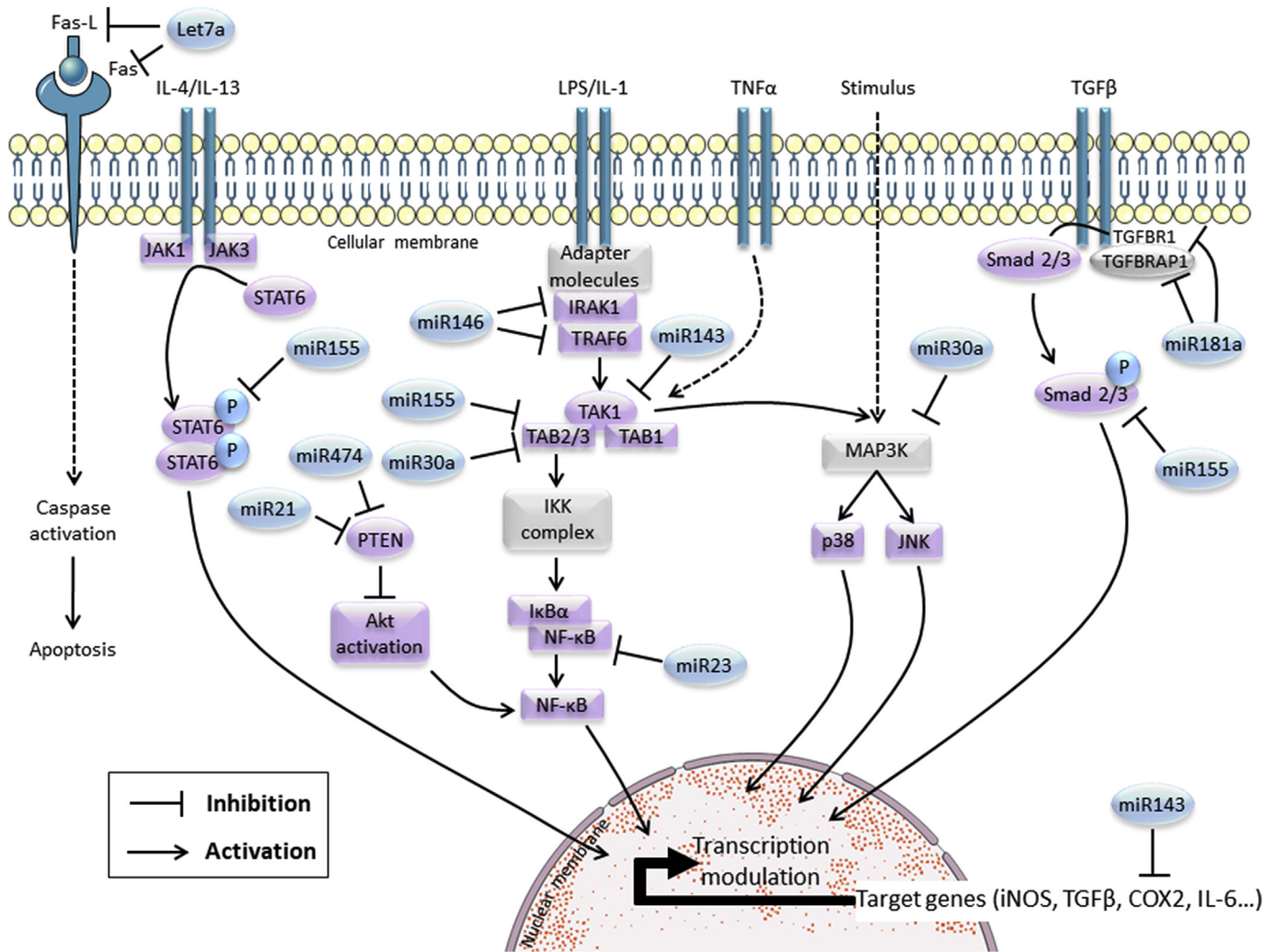


Fig. 2. Main signaling genes and pathways targeted by immunomodulatory MSC-derived miRNAs. List of genes belonging to main immunosignaling pathways that have been demonstrated as direct targets of MSC-derived miRNAs.

binding protein 2 (TAB2) (Fig. 2; Table 1). In fact, miR-155 directly targeted TAB2, which indirectly downregulated iNOS. Production of nitric oxide (NO) by iNOS is one mechanism mediating the immunosuppressive effect of murine MSCs by inducing the death of neighboring immune cells. Indeed, increased expression of miR-155 results in lower secretion of NO and therefore lower immunosuppressive effect of BM-MSCs. This study underscored the role of miR-155 in regulating the immunosuppressive effect of MSCs, both by being produced by pre-activated MSCs in the inflammatory context, as well as by reducing the immunosuppressive effect of MSCs by decreasing iNOS.

3.3. Role of miR-27 b

The miR-23/27/24 family contains several members and two paralogs: miR-23a/27a/24-2 (miR-23a cluster) and miR-23 b/27 b/24-1 (miR-23 b cluster). While miR-24-1 and miR-24-2 share the same mature sequences, mature sequences of miR-23a and miR-27a differ by just one nucleotide in comparison to their corresponding paralogs miR-23 b and miR-27 b [24]. Although the mature miRNAs arise from both the 5' and the 3' strands, the guide strand is usually the most prevalent (96–99%) as compared to the passenger strand (for review on biogenesis and nomenclature, see

Ref. [7]). The miR-23 clusters have primarily been associated with tumorigenesis although a role in the control of T cell response is suggested by in silico analysis of predictive targets by the individual miRNAs. The miR-23/27/24 family regulates T cell biology, in particular T helper 2 (Th2) cell immunity. Direct experimental evidence has recently showed that overexpression of the miR-23 cluster or miR-27 alone negatively impacted the differentiation of T helper cell lineages (Th1, Th2, Th17) and induced T regulatory (iTreg) cells. Actually, miR-24 and miR-27 collaboratively control Th2 responses through targeting IL-4 and GATA3, respectively [24]. Indeed, maintaining a low expression level of the family members confers proper effector T cell function at both physiological and pathological settings.

MiR-27 b was first identified as a miRNA upregulated in ASCs isolated from tolerant rats in a model of spontaneous immune tolerance after orthotopic liver transplantation [25]. In this study, miR-27 b knock-down in ASCs had a positive influence on the allosuppressive activity that inhibited T cell proliferation. It also upregulated the expression of stromal cell-derived factor-1α (SDF-1α or CXCL12) (Table 1). However, this study did not specify the exact functions of miR-27 b in modulating the production of cytokines or chemokines as well as inducing a regulatory T response. In another model of liver regeneration after 70% partial hepatectomy

Table 1

List of miRNAs involved in the immunomodulatory function of MSCs. Up- or down-regulation of MSC-derived miRNAs, as indicated by up and down arrows respectively, induces the indicated changes in the function of target cells.

miRNA modulation	Target	MSC-induced effects	MSC source	Reference
↘ let-7a	Fas/FasL	-inhibits T cell proliferation and migration -increases T cell apoptosis	murine BM-MSC	[43] [44],
↘ miR-21	PTEN	-increases TGFβ1, IL-6 expression -promotes Treg induction	murine and human BM-MSC	[37]
↗ miR-23b	NF-κB	- prevents DC maturation - inhibits T cell proliferation	murine BM-MSC	[30]
↗ miR-30a	TAB3	- prevents Treg induction - decreases IL-6, IL-8, COX2 expression	human UC-MSC	[35]
↘ miR-27b	CXCL12	- inhibits T cell proliferation	rat ASC	[25]
↘ miR-143	TAK1 COX2	- increases IL-6, IL-8, IDO, COX2 expression - prevents TNFα, IL-1, MCP-1 production by macrophages	human UC-MSC	[39]
↗ miR-146a	PGES-2	- promotes T cell proliferation	murine BM-MSC	[19]
↗ miR-146a		- stimulates IL-8 secretion	human BM-MSC	[18]
↗ miR-155	iNOS	- stimulates IL-6, IL-8 secretion	human BM-MSC	[22]
↗ miR-155	TAB2	- promotes T cell proliferation - decreases iNOS expression	murine BM-MSC	[23]
↗ miR-181a	TGFBR1 TGFBRAP1	- promotes T cell proliferation - increases IL-6, VEGFA, IDO expression	human UC-MSC	[33]
↗ miR-494	COX2	- increases production of TNFα, IL-6 by macrophages - decreases polarization towards M2 macrophages - decreases IL-10 expression and increases PGE2, IL-6, TNF-α expression	human dMSC	[41]

BM-MSC: bone marrow-derived mesenchymal stem cells; COX2: cyclooxygenase 2, CXCL12: C-X-C motif chemokine 12; dMSC: decidual MSC; iNOS: inducible nitric oxide synthase; IRAK1: interleukin-1 receptor (IL-1R) associated kinase; NF-κB: nuclear factor-kappa B; PGES-2: prostaglandin E2 synthase 2; PTEN: Phosphatase and TENsin homolog; TAB2: TAK1-binding protein 2; TAB3: TAK1-binding protein 3; TAK1: Transforming growth factor beta-activated kinase 1; TGFBR1: Transforming growth factor, beta receptor 1; TGFBRAP1: Transforming Growth Factor Beta Receptor Associated Protein 1; TRAF6: Tumor necrosis factor (TNF) receptor associated factor 6; UC-MSC: umbilical cord-derived MSC.

Table 2

List of miRNAs involved in the immunomodulatory function of MSC-derived extracellular vesicles. Up-regulation of miRNAs in MSC-derived extracellular vesicles induces the indicated changes in the function of target cells.

miRNA modulation	Target	MSC-induced effects	MSC source	Reference
↗ Let-7b	TLR4, NF-κB, STAT3, AKT	- promotes M2 macrophages	human UC-MSC	[49]
↗ miR-146a		- promotes M2 macrophages	human UC-MSC	[51]
↗ miR-181c	TLR4, NF-κB	- increases IL-10 production and decreases TNF-α, IL-1β production by macrophages - decreases macrophage and neutrophil numbers	human UC-MSC	[50]
↗ miR-223	Sema-3A STAT3	- increases M2 polarization - decreases IL-6, IL-1β, TNF-α expression	murine BM-MSC	[52]

BM-MSC: bone marrow-derived mesenchymal stem cells; Sema-3A: Semaphorin-3A; STAT3: Signal transducer and activator of transcription 3; TLR4: Toll-like receptor 4; UC-MSC: umbilical cord-derived MSC.

in rats, ASCs overexpressing miR-27 b displayed significantly higher regenerative capacities than control ASCs [26]. Liver inflammation and fibrosis were reduced and the therapeutic effect was related to heme oxygenase-1 (HO-1)-dependent activity of ASCs. HO-1 is one of the mechanisms involved in the suppressive effect of ASCs. Finally, the same team demonstrated that induction of HO-1 in rat ASCs increased the production of ATP, of protective cytokines/growth factors, and genes involved in mitochondrial biogenesis in a peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC1α)-dependent manner [27]. Greater expression of PGC1α resulted in the induction of several anti-inflammatory cytokines including IL-15, IL-1RA and FGF21. However, discrepancies between these studies were not discussed and the exact genes and pathways targeted by miR-27 b were not investigated.

3.4. Role of miR-23 b

As specified above, miR-23 b belongs to the same cluster as miR-27 b. Mature sequences are 21 nucleotides long and differ by 10

nucleotides. In contrast to miR-27 b, which impaired the differentiation of all Th lineages, overexpression of miR-23 b only led to impaired iTreg and Th17 cells without any effect on Th1/Th2 polarization. Indeed, individual miR-23 cluster members can either collaborate or antagonize each other to fine tune the regulation of T cell responses.

MSCs have been shown to regulate dendritic cells (DCs) by inhibiting their differentiation and maturation [28]. Several miRNAs have been reported to participate to the modulation of DCs functions [29]. However, only few studies have investigated the role of miRNAs expressed by BM-MSCs on DCs regulation and miR-23 b is one of these miRNAs. In one study, the authors showed the upregulation of miR-23 b in murine BM-MSCs cocultured with DCs and confirmed the partial inhibition of DCs maturation by BM-MSCs [30]. The overexpression of miR-23 b was responsible of reduced expression of p50 and p65 subunits of the NF-κB pathway (Fig. 2). Moreover, downregulation of miR-23 b in BM-MSCs impaired their potential to inhibit the proliferation of T cells in a lymphocyte proliferative assay. Indeed, miR-23 b overexpression in

BM-MSCs affected the activation of NF- κ B pathway leading to the inhibition of DCs maturation (Table 1). Interestingly, another study reported that DCs incubated with the supernatant of murine BM-MSCs expressed higher levels of miR-23 b while the expression levels of miR-155 did not change [31]. The increased expression of miR-23 b in DCs was associated with the induction of a tolerogenic mechanism. Although not discussed in this paper, we cannot exclude that miR-23 b might have been conveyed within EVs released in the supernatants of BM-MSCs and captured by the targeted DCs.

3.5. Role of miR-181a

The role of miR-181a in inflammation has been widely investigated and proposed to regulate thymic T cell development and macrophage activation. It targets protein tyrosine phosphatase non-receptor type 22 (PTPN22), PTPN11 (or SHP2), dual specificity phosphatase 5/6 (DUSP5/6) and the T cell receptor. In addition, miR-181a directly modulates the expression of IL1 α and is involved in inflamm-aging [32]. It was also proposed to control inflammation during cell aging via the modulation of Bcl2 family members.

MiR-181a has been shown to be a negative regulator of the immunosuppressive properties and proliferation of MSCs. MiR-181a prevented the proliferation of human umbilical cord (UC)-MSCs through the inhibition of TGF β signalling pathway by directly targeting TGF β receptor 1 (TGFBR1) and TGFBR associated protein 1 (TGFBRAP1) (Fig. 2; Table 1) [33]. Furthermore, overexpression of miR-181a in UC-MSCs enhanced the proliferation of T cells in a short-term culture and the secretion of IL-6, vascular endothelial growth factor alpha (VEGFA) and indoleamine 2,3-dioxygenase (IDO). The upregulation of these factors occurred through the activation of p38 and mitogen-activated protein kinase (MAPK) signalling pathway. The authors speculated that miR-181a may regulate MAPK signalling by targeting DUSP8 and DUSP10 that bind JNK and p38 substrates and/or by targeting PTPN members. In vivo, miR-181a-transfected UC-MSCs failed to improve experimental colitis suggesting that miR-181a is a negative endogenous regulator of MSC-mediated immunosuppression.

3.6. Role of miR-30

The miR-30 family contains 6 distinct mature miRNAs: miR-30a, -30b, -30c-1, -30c-2, -30d, -30e) that share the same seed sequence but have different sequences near the 3' end. Consequently, the miR-30 family members target different genes and may have opposite behaviors leading to a variety of physiological or pathological conditions [34]. Their main function is to act as tumor suppressors or as onco-miRNAs. Little is known about their role in inflammation or immunity.

A recent paper found increased expression of miR-30a in human UC-MSCs from preeclamptic women [35]. Actually, overexpression of miR-30a induced the cell cycle arrest of UC-MSCs and suppressed the immunosuppressive effect of IL-1 β -primed UC-MSCs. As compared to control, primed UC-MSCs overexpressing miR-30a produced lower paracrine factors (IL-6 and cyclo-oxygenase 2 (COX2)), which are central cytokines in MSCs-mediated immunomodulation, and less IL-8, which participates in the migration of MSCs to the damage site. In addition, overexpression of miR-30a prevented the induction of CD4⁺CD25⁺Foxp3⁺ Treg cells. Such function occurred through targeting TGF β -activated kinase 1 binding protein 3 (TAB3) and, the NF- κ B and mitogen-activated protein kinases (MAPK) signaling pathways (Fig. 2; Table 1). Overexpression of miR-30a would thus have a negative effect on the immunosuppressive function of MSCs.

3.7. Role of miR-21

MiR-21 is highly expressed in multiple cell types, in particular all types of immune cells, and is upregulated in cancer cells. This highlights miR-21 as a key player in immune pathways, controlling the balance between pro- and anti-inflammatory responses, whose dysregulation contributes to the pathogenesis of inflammatory diseases and cancer [36]. However, identification of miR-21 function has been difficult not only because of the variety of target genes but also the complexity of the responses. MiR-21 can be seen as a "rheostat" regulating the inflammatory switch between inflammatory or immunosuppressive states.

MiR-21 has been shown to be expressed in murine BM-MSCs and be significantly correlated with TGF β 1 expression [37]. Indeed, several miRNAs have been overexpressed in murine BM-MSCs and the secretion of paracrine factors has been quantified in culture supernatants. Together with miR-146, miR-21 was reported to negatively regulate the secretion of TGF β 1 and IL-6, two known immunomodulatory factors of MSCs. In addition, BM-MSCs from miR-21^{-/-} mice displayed higher immunoregulatory properties, which were associated to higher production of TGF β 1 and higher number of CD4⁺Foxp3⁺ Treg cells. The authors demonstrated that miR-21 indirectly inhibited TGF β 1 synthesis by targeting phosphatase and tensin homolog deleted on chromosome 10 (PTEN) and the downstream Akt/NF κ B pathway (Fig. 2; Table 1). Transfer of miR-21^{-/-} murine BM-MSCs in the model of experimental colitis improved colonic inflammation by increasing the production of TGF β 1. Thus, miR-21 negatively regulates the immunosuppressive function of MSCs via the expression of TGF β 1.

3.8. Role of miR-143

MiR-143 and miR-145 are co-expressed miRNAs that have been associated with tumor suppression. They are primarily expressed in mesenchymal cells where they play a major role in injury response and differentiation through the down-regulation of pluripotency factors (OCT4, SOX2, KLF4) [38]. They have not been associated with inflammation although miR-143 was reported to target TAK1.

A pro-inflammatory role of miR-143 was reported in a study investigating the effect of TLR on the immunosuppressive function of human UC-MSCs [39]. Activation of TLR3 by poly (I:C) upregulated the expression of COX2, IDO and IL-6 and the immunosuppressive function of UC-MSCs. In parallel, the expression of miR-143 was decreased significantly. Up- and down-regulating the expression of miR-143 respectively decreased and increased the secretion of immunomodulatory factors (IL-6, IL-8, COX2 or IDO) by UC-MSCs. Upon coculture with macrophages, overexpressing miR-143 in poly (I:C)-treated UC-MSCs resulted in less efficient down-regulation of TNF α , IL1 β and monocyte chemoattractant protein 1 (MCP1) by LPS-activated macrophages. They identified TAK1 and COX2 as direct targets of miR-143 (Fig. 2; Table 1). Finally, using a murine model of acute bacterial infection, the authors showed that pre-stimulating UC-MSCs by the TLR3 ligand poly (I:C) induced a higher survival rate of infected mice indicating a protective immunomodulatory effect. In addition, premiR-143-expressing UC-MSCs exerted lower immunomodulatory function on septic mice and were less effective to decrease the systemic inflammatory response. Indeed, stimulation of UC-MSCs by the TLR3 ligand improved their therapeutic effect in the model of septic inflammation through the down-regulation of miR-143 while overexpressing miR-143 reversed this protective function.

3.9. Role of miR-494

MiR-494 has been shown to be dysregulated in various

pathological conditions and to regulate cell proliferation, apoptosis and extracellular matrix degradation. The expression of miR-494 was also proposed to contribute to the accumulation of myeloid-derived suppressor cells (MDSC) and the suppressive activity of these cells [40]. TGF β 1 is an inducer of miR-494 that targets PTEN and activates the downstream Akt/NF κ B pathway. Other targets of miR-494 are SOX9, SIRT1, c-myc.

The role of miR-494 in the immunosuppressive effect of decidual MSCs (dMSCs) was recently reported [41]. An aberrant expression of cytokines by dMSCs was observed in preeclampsia patients suggesting that a dysfunction of dMSCs might be involved in abnormal macrophage polarization observed in these patients. Interestingly, over-expression of miR-494 was shown in dMSCs from these preeclampsia patients suggesting a possible role in abnormal immunoregulatory function. Indeed, dMSCs over-expressing miR-494 were less efficient to switch the polarization of macrophages toward M2 type as shown by lower percentages of CD14⁺CD206⁺ macrophages, as well as down-regulation of IL-10 and up-regulation of IL-6 and TNF α secretion [41]. This effect was mediated by the direct inhibition of COX2 expression and reduced PGE2 secretion (Table 1). It was also showed that miR-494 expression levels were up-regulated by TGF β 3. The authors suggest that high expression of TGF β 3 in preeclampsia decidua stimulates the expression of miR-494 in dMSCs leading to the down-regulation of macrophage polarization toward the M2 phenotype that contributes to the immune imbalance at maternal-fetal interface.

3.10. Role of *Let-7a*

The *Let-7* miRNA family includes 12 members (*Let-7a* to *-7k* and miR-98) that are well conserved in various animal species [42]. They can be encoded individually or as clusters with other family members or unrelated miRNAs. Two major roles have been elucidated so far for *Let-7* miRNAs: they are regulators of stem cell differentiation and function as tumor suppressors.

With the idea to improve the efficacy of transplanted MSCs in immunotherapy-based clinical trials, a recent paper identified the Fas ligand (FasL)/Fas system as being crucial for MSC therapy of inflammatory diseases [43]. They showed that transient BM-MSCs-induced T cell apoptosis could ameliorate systemic sclerosis and experimental colitis in mice. Actually, Fas-regulated MCP1 secretion by murine BM-MSCs recruited T lymphocytes for FasL apoptosis. Apoptotic T cells subsequently induced the production of TGF β by macrophages, which led to the upregulation of Treg cells and immune tolerance. In another study based on *in silico* analysis of miRNAs predicted to target FasL and Fas, the authors identified several miRNAs but the *Let-7* family members, and in particular *Let-7a*, were the only conservative miRNAs predicted in all databases [44]. Using gain- and loss-of-function approaches, they confirmed that *Let-7a* modulated Fas and FasL expression by direct targeting of both factors (Fig. 2; Table 1). Importantly, knock-down of *Let-7a* in murine BM-MSCs inhibited T cell proliferation and migration through the increase of apoptosis. Moreover, using the experimental colitis and the graft-versus-host disease mouse models, they demonstrated that BM-MSCs transfected with a *Let-7a* inhibitor were more potent than control BM-MSCs to prevent the body-weight loss, inflammatory reaction and mortality of mice with colitis. Indeed, knockdown of *Let-7a* is a possible therapeutic approach to improve MSC-based therapies for inflammatory diseases in the clinics.

A schematic figure recapitulates the miRNAs that have been identified to date as playing a role in the immunosuppressive function of MSCs and the immune cell subtypes that are targeted by these miRNAs as reviewed here (Fig. 3).

4. miRNAs contained within MSC-derived extracellular vesicles contribute to immunosuppression

Some of those miRNAs or others have been identified in extracellular vesicles released by MSCs. The exact role of the miRNAs packaged within MSC-derived EVs (MSC-EVs) in the modulation of the host immune response is still under evaluation. Nevertheless, current studies have demonstrated that they can act on different cells of the immune response.

Extracellular vesicles (EVs) are released by all cell types and are involved in cell-to-cell communication pathways. EVs subtypes differ by their size and biogenesis and, are divided into 3 main types: exosomes, microparticles or microvesicles and apoptotic bodies. They contain bioactive molecules, including proteins, lipids, mRNAs and miRNAs. Many of the documented functions of EVs are related with immune regulation [45]. There is extensive evidence of the stimulation of immune responses by antigen-containing EVs, derived from both immune and nonimmune cells, that are internalized by antigen-presenting cells after binding to the cell surface. Indeed, endogenous EVs likely modulate immunity, by stimulating immune responses to foreign antigens, and suppressing the response to self-antigens. Moreover, EVs from different sources of MSCs also contribute to immune cell regulation by reducing inflammation and facilitating tissue regeneration, through the release of immunosuppressive factors that are, at least in part, conveyed within EVs. Indeed, the immunosuppressive role of MSC-EVs has been shown in many inflammation-associated diseases [46–48]. However, few studies have identified miRNAs as the effector immunosuppressive factor in MSC-EVs.

The first study on the anti-inflammatory effect of a miRNA shuttled via MSC-EVs reported the identification of *let-7b* as the most up-regulated miRNA in LPS-stimulated human UC-MSCs (Table 2) [49]. LPS-stimulated UC-MSC-EVs were taken up by THP-1 macrophages and regulated the polarization of THP-1 cells towards the M2 phenotype expressing CD163 and producing IL-10 and TGF β . After LPS-activated UC-MSC-EVs treatment, THP1 macrophages expressed lower levels of TLR4 and p-P65 NF- κ B but higher levels of phospho Signal transducer and activator of transcription 3 (p-STAT3) and p-AKT indicating that *let-7b* enhanced the M2 polarization of macrophages via TLR4/NF- κ B/STAT3/AKT regulatory signaling. In addition, local administration of UC-MSC-EVs in a cutaneous wound model in diabetic rats resulted in lower inflammatory cell infiltration and higher number of M2 macrophages in the wound site.

Another study has identified the presence of miR-181c in human UC-MSC-EVs [50]. Addition of UC-MSC-EVs on LPS-activated macrophages downregulated the expression of TLR4, which is a direct target of miR-181c and, of p-P65 NF- κ B. In addition, the levels of TNF α and IL-1 β were downregulated while IL-10 was upregulated, indicating suppression of macrophage activation. *In vivo*, systemic administration of UC-MSC-EVs and more significantly of miR-181c overexpressing UC-MSC-EVs inhibited the inflammatory reaction in a model of burn injury confirming that miR-181c containing UC-MSC-EVs were involved in the anti-inflammatory function of UC-MSCs.

As previously discussed, expression of miR-146a in human BM-MSCs was shown to inhibit PGE2 secretion and the immunosuppressive function of the cells. However, a recent study revealed that miR-146a was upregulated in IL-1 β -stimulated human UC-MSCs and packaged in exosomes, which were taken up by macrophages to induce an anti-inflammatory M2 phenotype [51]. Importantly, miR-146a was expressed at a higher level in IL-1 β -stimulated UC-MSCs-EVs (40-fold higher as compared to unstimulated MSC-EVs). Systemic injection of IL1 β -stimulated UC-MSC-EVs protected mice from sepsis more efficiently than unstimulated UC-

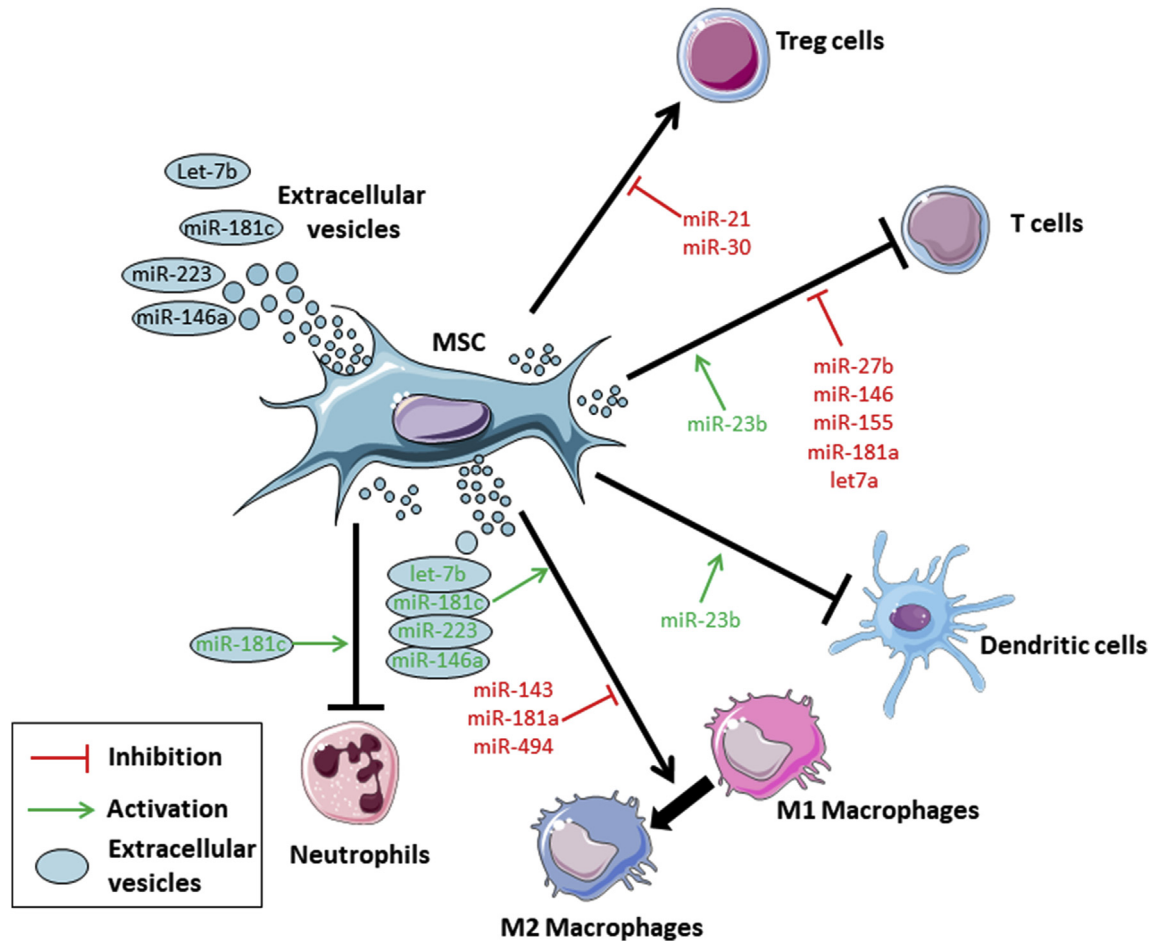


Fig. 3. Regulation of immune cell function by MSC-derived miRNAs. MSC-derived miRNAs can be positive regulators of the immunosuppressive function of MSCs (green arrow) while others are negative regulators (red arrow). Name of MSC-derived miRNAs that have been demonstrated as positive or negative regulators of the proliferation and/or function of one or several immune cell subtypes are indicated in green or in red, respectively. Immune cell function is indirectly regulated through the release of miRNA-responsive immunosuppressive mediators or directly after interaction with miRNA-containing extracellular vesicles (blue bubbles).

MSC-EVs. In contrast, EVs isolated from UC-MSCs transfected with a miR-146a inhibitor were less efficient to improve mice survival. These observations suggested that miR-146a plays an essential role in the therapeutic role of UC-MSC-EVs against sepsis.

An interesting study has evaluated the role of miR-223 in the cardioprotective effect of murine BM-MSCs in polymicrobial sepsis [52]. The authors used murine BM-MSCs knock-out (KO) for miR-223 and demonstrated in vivo that systemic injection of miR-223-KO BM-MSC-EVs did not exert a therapeutic effect and even exaggerated sepsis-induced injury. In vitro, by contrast to wild type BM-MSCs, miR-223-KO BM-MSCs were not able to reduce the levels of IL-1 β , IL-6 and TNF α secreted by activated macrophages. They identified higher levels of miR-223 target proteins, namely Semaphorin 3 A (Sema3A) and STAT3, in miR-223-KO BM-MSC-EVs and in targeted myocardium. These two target proteins are upstream activators of inflammatory signaling pathways during sepsis. Indeed, miR-223 seems mandatory for BM-MSC immunosuppression by counteracting inflammatory macrophage response occurring in sepsis. A more recent study used lentivirus-mediated overexpression of pre-miR-223 or miR-223 inhibitor in murine BM-MSCs to investigate the role of miR-223 in experimental autoimmune hepatitis [53]. After intraperitoneal administration, both wild type BM-MSC-EVs and more significantly pre-miR-223 expressing BM-MSC-EVs were able to reverse liver injury. This effect was

associated with the downregulation of NOD-like receptor family, pyrin domain containing 3 (NLRP3) and caspase-1 mRNA and protein levels. By contrast, miR-223 inhibitor expressing BM-MSC-EVs could not protect liver from injury. The major role of miR-223 in BM-MSC-EVs is therefore to control inflammation as observed in a model of sepsis and of autoimmune diseases.

Interestingly, not only mature miRNAs are present in EVs but also pre-miRNA transcripts along with key components of the miRNA biogenesis machinery, i.e. DICER, TAR RNA binding protein (TRBP), and argonaute 2 (AGO2) have been identified within EVs from tumour cells [54]. In EVs, DICER and TRBP were functional and the cell-independent miRNA maturation was associated with gene-silencing activity in recipient cells. AGO2 has also been described in exosomes from MSCs. Transfection of siRNA against AGO2 in MSCs nearly abolished AGO2 in released exosomes [55]. Remarkably in a model of axonal regeneration, application of siRNA-AGO2 transfected exosomes was not able to promote axonal growth while scramble siRNA transfected exosomes did. The data therefore suggest that AGO2 in exosomes from MSCs is required for exosome function, at least in this in vitro model. However, only scarce literature is found about the functionality of miRNA biogenesis machinery in EVs from MSCs and further investigation is needed to confirm that the process commonly occurs in MSC-EVs. In addition, there is evidence that factors involved in miRNA biogenesis

machinery can be influenced by inflammation. For instance, adenosine deaminase is a RNA-modifying enzyme that is up-regulated during inflammation and can introduce mutations in double-stranded miRNA precursors, thereby modifying the specificity of miRNA targeting [56]. Indeed, whether the miRNA biogenesis machinery is regulated by inflammation in MSCs and whether miRNA biogenesis in MSC-EVs is a process involved in their immunosuppressive function has not been considered but would require to be explored.

5. Conclusion and perspectives

The therapeutic interest of MSCs is widely investigated in several hundreds of clinical trials for many applications worldwide. One of the most promising aspect is their anti-inflammatory function, which has been demonstrated in pre-clinical trials and is being explored in the clinics. Many soluble factors have been identified as playing a major role in this effect but the impact of miRNAs is still at its infancy. The present review discussed the role of the few miRNAs that have been demonstrated as involved in the immunosuppressive effect of MSCs. Importantly, these miRNAs target directly or indirectly the main effector signaling pathways (NF- κ B, TGF β , ...) that have been identified as major in the suppressive function of MSCs. In addition, upon stimulation by inflammatory triggers these miRNAs act either by stimulating or repressing those pathways, indicating that they likely act together for a tightly regulated control of the suppressive function of MSCs (Fig. 3).

In parallel, MSC-EVs, especially exosomes, have gained interest with regard to their use in regenerative medicine. They can be easily isolated from MSCs of various origins and convey bioactive molecules, which are transferred to target cells to exert their therapeutic effect similar to that of the parental MSCs. Of interest, MSC-EVs possess anti-inflammatory functions and modulate the immune system towards a regulatory phenotype to exert many beneficial effects in inflammatory and related diseases. However, before being evaluated in the clinics, standard operating procedures under Good Manufacturing Procedures (GMP) for isolation, characterization and administration of MSC-EVs have to be defined. In addition, a more comprehensive view of their mode of action has to be provided to convince the regulatory authorities that MSC-EV therapy can be an effective and safe therapeutic approach in cell-free regenerative medicine. In this context, the role of miRNAs in the functional mechanisms of MSC-EVs is likely to be of importance, at least in the modulation of the immune response (Fig. 3). This needs further investigation to take full advantage of MSC-EVs in regenerative therapy.

Beside their importance in the mechanism of actions of MSCs, miRNAs might be seen as biomarkers of MSC sub-populations. Identification of miRNA signatures of sub-populations of MSCs that might be more efficient for a dedicated clinical application would be of interest for MSC-based therapies. This will contribute to the development of potency assays, which can help the selection of allogenic MSCs with better predictive efficiency. Future perspectives might also envision the design of novel treatments targeting miRNAs. This includes the injection of selected miRNAs as mimics or antisense-RNA inhibitors in patients to slow down the inflammatory response. MiRNAs of interest (mimics or inhibitors) might be uploaded into engineered EVs to allow better survival in the bloodstream and enhanced delivery to recipient organs. Another possibility might be to use drugs that can directly or indirectly stimulate or inhibit the production of miRNAs or relevant target genes. While the functions and clinical value of miRNAs are still under investigation, the optimal design of therapeutic molecules as well as the drug delivery systems are major challenges to face in the future.

Conflicts of interest

The authors disclose any financial or personal conflict of interest.

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