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The role of microRNAs in self-renewal and differentiation of mesenchymal stem cells

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MicroRNAs (miRNAs) are short non-coding RNAs involved in post-trascriptional regulation of gene expression and diverse biological activities. They are crucial for self-renewal and behavior of embryonic stem cells, but their role in mesenchymal stem cells has been poorly understood. Recently emerging evidence suggests that miRNAs are closely involved in controlling key steps of mesenchymal stem cell differentiation into certain cell lineages. This review focuses on miRNAs identified recently that regulate mesenchymal stem cell differentiation and other activities.

MicroRNAs (miRNAs) represent a class of small noncoding RNAs that uncovered a hidden layer of gene regulation and resulted in a paradigm shift in the way we view the role of RNA in regulating gene expression programs [1,2]. miRNAs act in a complex functional network in which each miRNA probably controls hundreds of distinct target genes and expression of a single coding gene can be regulated by several miRNAs [3,4]. Recent evidence shows their important role in regulation of a multitude of physiological functions, such as stem cell differentiation, neurogenesis, hematopoiesis, immune response, skeletal and cardiac muscle development, and stress [5–11]. To date, >3% of the genes in humans have been found to encode miRNAs, and up to 40% to 90% of the human protein encoding genes are under miRNA-mediated gene regulation [12].

Mesenchymal stem cells (MSCs), which are also referred to as multipotent stromal cells, have been isolated from various adult tissue sources. MSCs are capable of self-renewing and differentiating into multiple cell lineages [13–15]. Despite interest in clinical applications of MSCs, the molecular mechanisms underlying their self-renewal, differentiation, replicative senescence, and wound healing have yet to be fully elucidated. Recent studies suggest that stem cells have discrete miRNA expression profiles that can account for, or contribute to, the intrinsic stem cell properties of self-renewal and pluripotency [16–18]. In particular, miRNA differentiation signatures for MSCs recently identified indicate that progenitors and terminally differentiated cells have distinct miRNA patterns, suggesting that such signatures may be used to define and track rare cell populations [19,20]. Here, we present an overview of miRNAs discovered recently in regulation of MSC properties.

miRNAs in MSC differentiation

During the differentiation of MSCs into specific cell types, corresponding miRNAs are involved (Table 1 and Figure 1).

miRNA in osteogenic differentiation of MSCs

The role of miRNA in osteogenic differentiation of MSCs has been indicated by several studies. With microarray profiling of miRNAs expressed in osteoblast-differentiated mouse MSCs compared to undifferentiated MSCs, Mizuno et al. found that miR-125b was downregulated in the differentiated MSCs [21], implying a role of miR-125b in suppressing osteogenesis of MSCs. Additional data from this group showed that ERBB2 receptor tyrosine kinase was the target gene of miR-125b. MiR-133 and miR-135 functionally inhibited the differentiation of osteoprogenitors by attenuating RUNX2 and SMAD5 pathways that synergistically contribute to bone formation [22]. MiR-206 played an inhibitory role during osteoblast differentiation of

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Table 1. miRNAs in regulation of MSC activities

MSC activities	miRNAs	Target genes	Effects
Osteogenic differentiation	miR-125b	ERBB2	Suppress ostogenesis [21]
	miR-133, -135	RUNX2/SMAD5	Suppress ostogenesis [22]
	miR-206	Connexin 43	Suppress ostogenesis [23]
	miR-204, -211	Runx2	Suppress ostogenesis [24]
	miR-27a, -489	GCA/PEX7/APL	Suppress ostogenesis [25]
	miR-26a	SMAD1	Suppress ostogenesis [26]
	miR-196a	HOXC8	Promote ostogenesis [27]
	miR-210	ACVR1b	Promote ostogenesis [28]
	miR-2861	HDAC5	Promote ostogenesis [29]
	miR-148b	Unknown	Promote ostogenesis [25]
	let-7, miR-24, -125b, -138	Unknown	Promote ostogenesis through PDGF pathway [30]
Chondric differentiation	miR-140	SOX9/COL2A1/DAC4	Promote chondrogenesis [31,32]
	miR-638, -663	Unknown	Unknown [20]
	miR-199a	SMAD1	Suppress chondrogenesis [33]
	miR-145	Unknown	Unknown [20]
Adipogenic differentiation	miR-143	ERK5	Promote adipogenesis [34]
	miR-138	EID-1	Suppress adipogenesis [35]
	miR-31	CEBPA	Suppress adipogenesis [36]
	miR-24	Unknown	Promote adipogenesis [36]
	miR-133	SRF/nPTB	Suppress myogenesis [6,37]
Myogenic differentiation	miR-1	HDAC	Promote myogenesis [6]
	miR-206	Connexin43	Promote myoblast fusion [38]
	miR-24	Unknown	Promote myogenesis [39]
	miR-181	HOX-A11	Promote myoblast differentiation [40]
	miR-143, -208	Unknown	Unknown [41]
	miR-130a,-206	TAC1	Promote neurogenesis [42]
Neuronal differentiation	miR-124	JAG1/DLX2/SOX9	Promote neurogenesis [42]
	miR-9	TLX	Promote neurogenesis [42]
Replicative senescence	miR-371, -369-5p, -29c, -499, let-7f	Unknown	Unknown [43]
	miR-31, -21, -223, -142, -205, -203, -18b, -19a, -130b, -16, -26b, -125b, let-7f	Unknown	Unknown [44]
Wound healing	miR-133a, -181, -30a-3p, -193b, -30a-5p, -204, -200b, -96, -127, -181c, -182, -130a	Unknown	Unknown [44]
	miR-335	Unknown	Probably suppress metastasis [45]
Self-renewal	let-7 family	HNF4A	Maintain self-renewal [46]

MSCs, and connexin 43 was suggested to be one of the target genes [23]. MiR-204/211 acted as negative regulators of osteoblast differentiation and subsequent mineralization of bone marrow-derived MSCs through negative regulation of RUNX2 transcription factor [24]. MiR-489 and -27a were shown to exhibit an inhibitory effect in early osteogenic differentiation of human MSCs, mediated at least in part by repression of grancalcin [25]. Additionally, during the late stages of human adipose tissue-derived stem cell differentiation toward osteogenic lineage, miR-26a negatively regulated the event by targeting the SMAD1 transcription factor, accompanied by downregulation of bone maker genes, such as alkaline phosphatase, type I collagen, osteocalcin, and osteopontin [26].

Notably, several miRNAs have been found to act as positive regulators in osteogenic differentiation of MSCs. MiR-196a was shown to enhance osteogenic differentiation possibly through its target gene HOXC8 [27]. Similarly, miR-210 enhanced osteogenic differentiation by inhibiting ACVR1b in transforming growth factor-β (TGF-β)/activin signaling pathway [28]. In addition, a novel miRNA, miR-2861, promoted bone morphogenetic protein (BMP)2-induced ST2 osteogenic differentiation by inhibiting histone deacetylase 5 expression [29]. MiR-148b was found among a group of miRNAs that were associated with osteogenic differentiation of human MSCs in a special 3D substrate [25]. Moreover, a subset of miRNAs (let-7, miR-24, -125b, -138) was found to promote osteogenic differentiation of MSCs through a platelet-derived growth factor pathway [30].

Taken together, it is conceivable that an osteogenic class of miRNAs can be potent candidates as osteoblast differentiation biomarkers for the development of preventive or therapeutic agents for osteogenic disorders.

miRNA in chondrogenic differentiation of MSCs

Several miRNAs have been identified in regulation of chondrogenesis of MSCs. Five miRNAs (hsa-miR-130b, hsa-miR-152, hsa-miR-28, hsa-miR-26b, and hsa-miR-193b) were found differentially expressed during chondrogenic differentiation of MSCs through microarray analysis, and four of them were

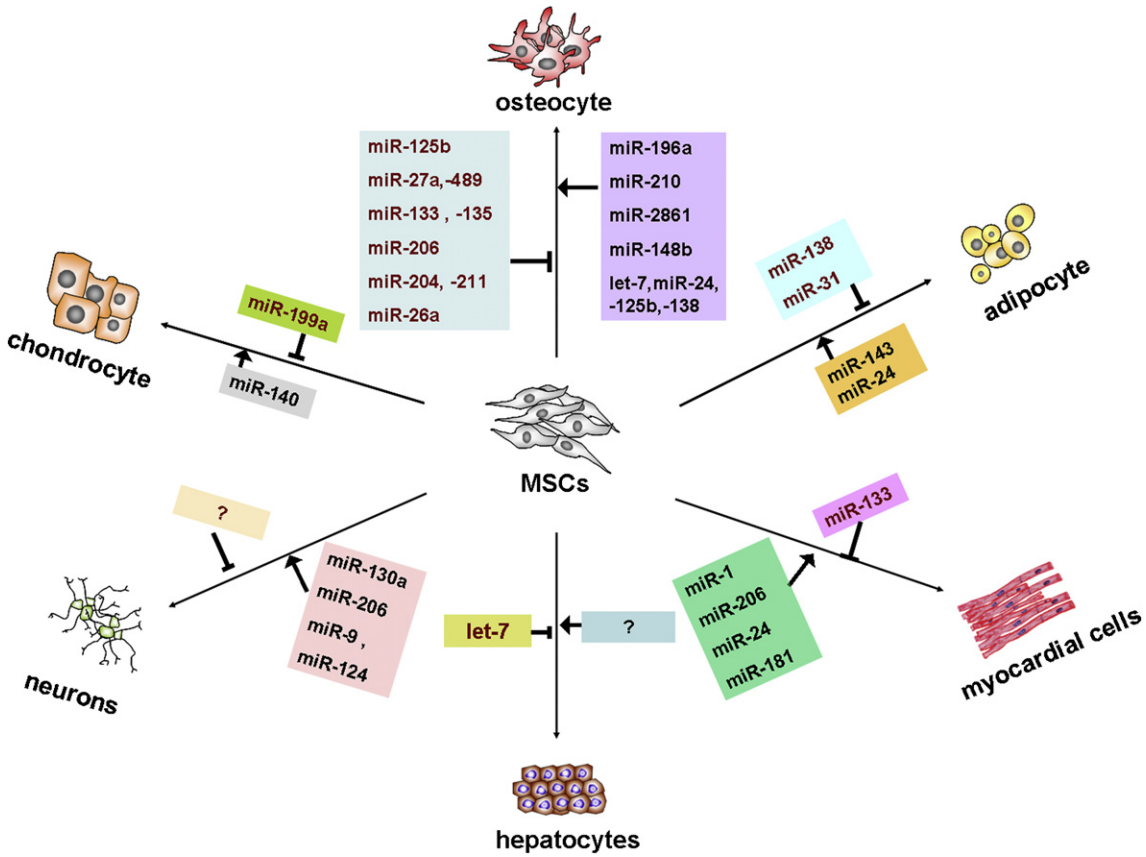

Figure 1. The role of miRNAs in MSC differentiation.

confirmed by real-time polymerase chain reaction analysis [47]. Additional results of this study suggested that the potential targets of hsa-miR-130b, hsa-miR-152, hsa-miR-28, and hsa-miR-26b were genes involved in cartilage formation, such as COL4A1, COL2A1, and COL6A1 [47]. In another microarray analysis, miR-140 was found to have the largest expressional difference between chondrocytes and MSCs [31]. During chondrogenesis of MSCs, miR-140 expression increased in parallel with expression of SOX9 and COL2A1 [31]. Consistent with the finding mentioned previously, another group found that miR-140 was expressed during cartilage development, probably through inhibition of histone deacetylase 4, likely a co-repressor of RUNX2 [32]. Moreover, miR-199a was found to repress early chondrogenesis via direct targeting of the SMAD1 transcription factor [33].

miRNA in adipogenic differentiation of MSCs

A number of miRNAs have been shown to regulate adipogenic differentiation of MSCs. Previous studies suggest that modulation of a single miRNA could promote formation of adipocytes from adipogenic precursor cells [34,48]. MiR-103 and miR-107 (virtually indistinguishable in gene sequences) have been predicted to target a large number of genes involved in acetyl-CoA and lipid metabolism. MiR-143 was reported to be involved in adipogenic differentiation by targeting gene ERK5, an intermediate in the LIF signaling pathway [34]. MiR-138 was found to be significantly downregulated during adipogenic differentiation and the expression of E1A-like inhibitor of differentiation 1, a nuclear receptor coregulator, was found inversely correlated with that of miR-138 in adipogenic differentiation of human adipose tissue-derived MSCs (hAD-MSCs) into adipocytes [35]. MiR-24 enhanced BMP2-induced commitment of MSCs to adipocyte lineage and enhanced BMP2-induced G_1 arrest, whereas miR-31 exhibited an opposite effect during adipocytic differentiation by downregulating CCAAT/enhancer-binding protein α (an adipocytic differentiation factor) expression at both transcriptional and translational levels [36].

miRNA in differentiation of MSCs into other cell lineages

MSCs represent a multipotent adult stem cell population. Given appropriate culture conditions, they are able to differentiate into various mesodermal cell lineages, including osteoblasts, chondrocytes, and adipocytes, as discussed here. There is evidence showing that MSCs can also differentiate into myocytes and even into cells of nonmesodermal origin, including hepatocytes and neurons [14,49–52] (Fig. 1). Accumulating evidence supports the role of

miRNAs in the regulation of myogenesis [53]. In a multipotent MSC line, C2C12, miR-1, miR-206, and miR-133 exhibited opposing roles in modulating skeletal muscle proliferation and differentiation. While miR-1 and miR-206 promoted myogenesis, miR-133 inhibited myoblast differentiation and promoted proliferation by repressing serum response factor and a key splicing factor nPTB during myoblast differentiation into myotubes [6,37,54]. Interestingly, miR-133 continued to express well after the cells had entered the differentiation process and was always induced to express concomitantly with miR-1 and miR-206 [37,55]. MiR-181 has been shown to promote myoblast differentiation by downregulating a myogenic inhibitor, the homeobox protein HOX-A11 [39,40]. A recent study has implicated a role of miR-24 in TGF-β mediated myogenic inhibition of MSCs. MiR-24 was upregulated during myoblast differentiation and could be inhibited by TGF-β1, providing a novel mechanism of the genetic regulation of TGF-β signaling during skeletal muscle differentiation [39]. Meanwhile, it appears that miRNA expression in cardiomyocyte differentiation of MSCs is influenced by induction conditions. In human MSC differentiation into cardiomyocyte, the presence of induction reagent 5-azacytidine lead to the expression of primary miRNAs of miR-143 and miR-181, while indirect coculture of human MSCs with neonatal rat myocytes upregulated the expression of primary miRNAs of miR-143, -206, -208, and -181 [41].

Specific miRNAs have been shown to be involved in neurogenesis of MSCs and recently have been implicated in neurotransmitter release by MSC-derived neurons. They also appear to have roles in synaptic plasticity, which may lead to the development of novel MSC-based therapies [19,42,56]. Synergism has been demonstrated in the inhibition of substance-P release from MSC-derived neurons through miR-130a and miR-206 targeting of TAC1 [19]. Another two examples of miRNAs involvement with neurogenesis are miR-9 and miR-124. They are regulated by the transcription factor REST, which is critical in MSC-mediated dopaminergic neurogenesis [57,58]. The significance of these findings is that those miRNAs can potentially influence the effectiveness of MSC therapy for neurological conditions. Their manipulation may be an avenue for future treatments. Little has been known about the involvement of miRNAs in hepatic differentiation of MSCs. One study showed that let-7 family miRNAs could affect expression of HNF4A, which is a known endodermal differentiation marker, suggesting a role of let-7 in suppressing hepatic differentiation of MSCs [46].

While data supporting the role of miRNAs in MSC differentiation have rapidly accumulated in recent years (Table 1, Fig. 1), studies about involvement of miRNAs in self-renewal of MSCs lack. Until very recently, Koh et al. reported let-7 family might play a role in the self-renewal of human embryonic stem cells (ESC)-derived MSCs through a deep sequencing analysis for miRNA expression profile [46], implying potential involvement of this miRNA in the self-renewal of MSCs.

miRNA in replicative senescence and wound healing of MSCs

Ex vivo expansion of MSCs is an indispensable step to acquire sufficient amounts of cells for clinical therapies. However, senescence of MSCs in culture expansion remains an unresolved problem and has caused increasing concerns about the effect of MSC-based therapies. Currently, >100 clinical trials registered at ClinicalTrial.org are underway. MSCs enter senescence after a certain number of cell divisions, which is morphologically characterized by enlarged and irregular cell shapes and ultimately a stop of proliferation [59,60], and inappropriate culture conditions markedly accelerate this process. It has been shown that aged MSCs have reduced multipotent differentiation potential and production of useful cytokines for tissue repair [61–63]. Transplantation of aged MSCs is likely to result in reduced therapeutic effects. In addition, the safety of aged MSCs to patients awaits additional investigation. The molecular mechanisms that underlie senescence in MSCs are still poorly understood. Recent studies have indicated that senescence influences the overall expression of coding genes and miRNAs in MSCs [43,64,65]. miRNA expression profiling of MSCs in replicative senescence revealed an upregulation of hsa-miR-371, hsa-miR-369-5p, hsa-miR-29c, hsa-miR-499, and hsa-let-7f [43]. Interestingly, it has been known that hsa-miR-29c directly targets DNA-methyl transferase 3A (DNMT3A) and 3B (DNMT3B) in lung cancer tissue [66]. In addition, hsa-miR-371 is predicted to target DNMT3A (miRTar), while DNA-methyl transferase 2 (DNMT2) belongs to the predicted targets of hsa-miR-499 (miRTar). Recently, miRNAs have been found to be involved in MSC aging. miRNA expression profiling showed an upregulation of miR-766 and miR-558 and a downregulation of let-7f, miR-125b, miR-222, miR-199-3p, miR-23a, and miR-221 in old monkey MSCs (>12 years) compared to young monkey MSCs (<5 years) [64]. Consistent with the finding mentioned previously, in another study four miRNAs, miR-17, miR-19b, miR-20a, and miR-106a, were found to be downregulated not only in four replicative cell aging models (including endothelial cells, replicated $CD8^+$ T cells, renal proximal tubular epithelial cells, and skin fibroblasts), but also in organismal aging models from old and young donors. Moreover, a decrease in these miRNAs correlated with increased transcript levels of some established target genes, especially the CDK inhibitor p21/CDKN1A [65]. Hence, it is speculated that upregulation of these miRNAs may lead to changes in gene methylation levels, and thereby influence MSC senescence [67,68].

Little attention has been paid to the role of miRNAs in the proliferation and differentiation of MSCs in the setting of wound healing [69]. Using a skin excision model, altered

expression in a panel of miRNAs, including upregulated expression of miR-31, -21, -223, -142, -205, -203, -18b, -19a, -130b, -16, -26b, -125b, and let-7f, and downregulated expression of miR-133a, -181, -30a-3p, -193b, -30a-5p, -204, -200b, -96, -127, -181c, -182, and -130a were demonstrated in wounded tissue in the stage of active granulation formation [44]. Furthermore, Zou et al. found that TGF-β, a key growth factor elevated in the wound site, stimulated upregulation of miR-21 in MSCs as well as in multipotential C3H10T1/2 cells, and promoted proliferation and differentiation of these cells in vitro. Consistently, knockdown of miR-21 in the wound bed delayed the healing process. These results suggest that miR-21 regulates gene expression and, subsequently, the behavior of MSCs in wound healing.

miRNA expression in ESCs and MSCs

miRNAs are crucial for self-renewal and differentiation of ESC [17,70–76]. A core network of transcription factors and RNA-binding proteins, including Oct4, Sox2, Nanog, Klf4, c-Myc, Tcf3, and Lin28, cooperate in intricate regulatory circuits to ensure appropriate ESC behavior [71]. Recent investigations have shown that several of these key transcription factors directly regulate miRNA expression in ESCs. By occupying miRNA gene promoter regions, these factors not only activate expression of ESC miRNAs, but also play a role in silencing a subset of miRNAs that are expressed in differentiated cell types. Interestingly, the pattern of miRNA expression in MSC is substantially different from that of ESC [77,78]. Known pluripotent associated markers, such as miR-302a, b, c, and d, and miR-200c, are absent in MSCs [30]. Notably, MSCs in these studies went through considerable successive culture expansion. With culture methods currently used by most research groups, bone marrow-derived MSCs quickly age during culture expansion [79,80], and undergo autonomous differentiation toward osteoblasts [79]. It is unclear whether the difference in miRNA expression between ESCs and MSCs is due to intrinsic difference between these two cell types or alterations of MSCs caused by ex vivo culture.

Let-7 family and miR-125b are involved in the differentiation of both ESCs and MSCs (Fig. 2). Let-7 is expressed late in mammalian embryonic development and plays an evolutionarily conserved role from *caenorhabditis elegans* to drosophila and to mammals [81–84]. The let-7 family of miRNAs is broadly expressed across differentiated tissues and is tightly regulated during ESC differentiation. It has been proposed that let-7 is a prodifferentiation factor with "anti-stemness" properties [85]. Indeed, mature let-7 family miRNAs (let-7-a1, -a2, -a3, -b, -c, -d, -e, -f1, -f2, -g, -i, and miR-98) are undetectable in ESCs and strongly accumulate 10 days after the onset of differentiation, whereas the corresponding pri-let-7 transcripts (from which the mature let-7 miRNAs derive) are readily detectable in both ESCs and differentiated cell types. The developmentally regulated RNA-binding protein Lin28 was recently identified as an inhibitor of let-7 expression [76,84] (Fig. 2A). In MSCs, let-7 family was involved in platelet-derived growth factor signaling pathway-induced osteogenic differentiation [30] (Fig. 2B). MiR-125b is considered a representative miRNA that regulates both cell proliferation and differentiation [21]. In ESC-derived neural progenitor, miR-125b suppressed astrocyte-specific messenger RNAs (mRNAs) during the periods of neural precursor expansion and neuronal differentiation [5,86], while in MSCs, miR-125b inhibited osteogenic differentiation induced by BMP4 by targeting ERBB2 [21], and the effect of miR-125b in osteogenesis of MSCs was likely regulated by platelet-derived growth factor [30].

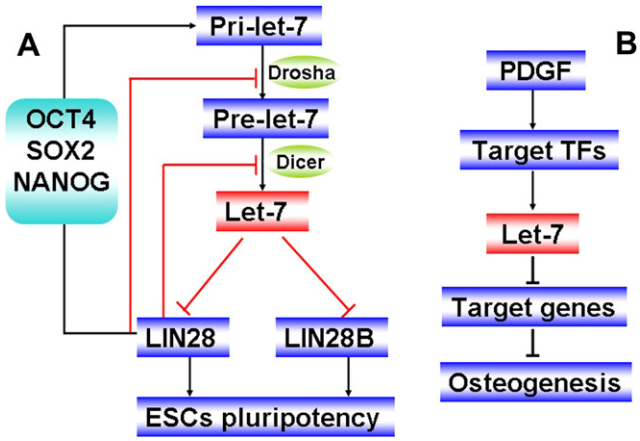

Figure 2. Let-7 in ESCs and MSCs. (**A**) In ESCs, let-7 targets Lin28 and Lin28B are inhibitors of let-7 processing and their expression is restricted to early embryonic development. Proteins Lin28, Lin28B, OCT4, SOX2 and NANOG bind to the loop region of let-7 precursors, resulting in blockade of let-7 processing at either Drosha or Dicer level. (**B**) In MSCs, PDGF signal induces the expression of specific transcription factors (TFs, such as fox and stat3), which upregulate the expression of let-7. Increased let-7 level is predicted to inhibit translation of non-osteogenic target mRNAs to diminish their inhibition of osteogenesis.

miRNA expression in MSCs and microvesicles

Recent studies have shown that MSCs secret microvesicles (MVs) [87,88]. MVs are circular fragments of membrane released from the endosomal compartment as exosomes or shed from the surface membranes of most cell types such as cancer cells, blood cells, and endothelial cells [88,89]. Accumulating data suggest that MVs may serve as a means of cell-to-cell communication through which genetic information or gene products are transferred and cell activities are regulated [89,90]. Previous studies show that MVs contain a characteristic composition of proteins [91]. Recently, MSC exosomes have been shown to harbor a variety of mRNAs and miRNAs [88,89,92]. Interestingly, differential miRNA expression profiles in MSCs and MVs derived from MSCs have been observed [88,92] (Table 2). In one study, MSCs were found to preferentially secrete

Table 2. miRNAs expressed in MSCs and their corresponding MVs

MSC types	Expressed only in MSCs	Expressed only in MSC-derived MVs
hESC-derived HuES9.E1	miR-151-3p, miR-424/424*, miR-23a*, miR-152, miR-425, miR-155, miR-15a/b, miR-24-2*, miR-454, miR-25, miR-455-3p, miR-16, miR-483-5p, miR-17, miR-26b, miR-484, let-7g, miR-491-5p, miR-181a*/a-2*/b/c/d, miR-27a/b/b*, miR-503, miR-505*, miR-28-3p/5p, miR-532-5p, miR-106/a/b, miR-185, miR-29a/c, miR-10a, miR-187*,miR-30a/a*/b/c/d/e/e*,miR-584, miR-18a/b, miR-612, miR-625, miR-629, miR-192, miR-193a-5p, miR-195, miR-708, miR-197, miR-31*, miR-744, miR-126, miR-324-5p, miR-766, miR-128, miR-199a-5p/b-5p, miR-328, miR-768-3p/5p, miR-130a/b, miR-330-3p, miR-19b, miR-331-3p, miR-769-5p, miR-20a/b, miR-335, miR-877, miR-137, miR-342-3p, miR-140-3p, miR-345, miR-210, miR-34a/a*, miR-92b, miR-212, miR-93, miR-145*, miR-362-3p/5p, miR-146b-5p, miR-22*, miR-98, miR-148b, miR-365, miR-99a/b, miR-221* miR-374b, miR-421, miR-222*, miR-423-5p [92]	let-7b* let-7d* miR-122 miR-1224-5p miR-1228 miR-1234 miR-1237 miR-1238 miR-124 miR-150* miR-198 miR-296-5p miR-572 miR-765 miR-940 [92]
BM-MSCs	miR-594, miR-654, miR-369-5p, miR-502, miR-376a, miR-362, miR-194 [88]	miR-223 miR-451 miR-564 [88]

BM = bone marrow; hESC-derived HuES9.E1 = human ESC–derived HuES9.E1, MSC line derived from human embryonic stem cells.
*Two arms of a miRNA precursor yield two mature miRNAs respectively, one strand represents the miRNA, the other strand is called miRNA.

miRNA in the precursor instead of the mature form and that these pre-miRNAs were enriched in MVs, which were readily uptaken by neighbor cells, suggesting a potential mechanism in regulation of activities of other cells. Therefore, MSCs can potentially exert miRNA-mediated biological effects on other cells through secretion of pre-miRNA in MVs [92].

It has been recognized that MSCs promote tissue repair/regeneration through release of diverse growth factors, cytokines, and extracellular matrix molecules [93–96]. Beside these conventional substances, transfer of genetic information between MSCs and tissue-injured cells appears to be an important mechanism responsible for MSCs' effect in tissue repair/regeneration. On the one hand, MVs produced by MSCs may reprogram tissue-injured cells by delivering mRNA and/or miRNA that induces cell de-differentiation, modulates soluble paracrine mediator production, and mediates cell-cycle re-entry, thus favoring tissue regeneration. On the other hand, MVs released from tissue-injured cells may mediate phenotypic transfer of MSCs to acquire tissue-specific cell features by delivering mRNAs and/or miRNAs to MSCs [89]. Comparative analysis of the species of miRNAs contained in MVs and cells of origin may reveal information on the mechanism of the therapeutic effects of MSC transplantation.

Conclusions

Although accumulating evidence has suggested an important role of miRNAs in regulating MSC activities, much more remains to be elucidated. Investigations into miRNA-mediated regulation of gene expression in cellular networks must take into account the interplay among various miRNA targets based on the diverse effects of individual miRNAs [19,97]. Likewise, miRNAs themselves could be regulated in a polygenic manner. It is critical to consider gene regulation networks as a whole rather than the effects of individual miRNAs in isolation. Elucidation of miRNA-targeted genes in future studies will definitely be important for better understanding of MSC biology and facilitating development of novel MSC-based therapeutic approaches for various diseases.

Acknowledgments

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Conflict of interest disclosure

No financial interest/relationships with financial interest relating to the topic of this article have been declared.

References

1. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116:281–297.
2. Dykxhoorn DM. MicroRNAs and metastasis: little RNAs go a long way. Cancer Res. 2010;70:6401–6406.
3. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell. 2005;120:15–20.

4. Miranda KC, Huynh T, Tay Y, et al. A pattern-based method for the identification of MicroRNA binding sites and their corresponding heteroduplexes. Cell. 2006;126:1203–1217.
5. Krichevsky AM, Sonntag KC, Isacson O, Kosik KS. Specific microRNAs modulate embryonic stem cell-derived neurogenesis. Stem Cells. 2006;24:857–864.
6. Chen JF, Mandel EM, Thomson JM, et al. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. Nat Genet. 2006;38:228–233.
7. Zhao Y, Samal E, Srivastava D. Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis. Nature. 2005;436:214–220.
8. Pedersen IM, Cheng G, Wieland S, et al. Interferon modulation of cellular microRNAs as an antiviral mechanism. Nature. 2007;449: 919–922.
9. Kloosterman WP, Lagendijk AK, Ketting RF, Moulton JD, Plasterk RH. Targeted inhibition of miRNA maturation with morpholinos reveals a role for miR-375 in pancreatic islet development. PLoS Biol. 2007;5:e203.
10. Tay YM, Tam WL, Ang YS, et al. MicroRNA-134 modulates the differentiation of mouse embryonic stem cells, where it causes post-transcriptional attenuation of Nanog and LRH1. Stem Cells. 2008; 26:17–29.
11. Felli N, Fontana L, Pelosi E, et al. MicroRNAs 221 and 222 inhibit normal erythropoiesis and erythroleukemic cell growth via kit receptor down-modulation. Proc Natl Acad Sci U S A. 2005;102:18081–18086.
12. Hu R, Li H, Liu W, Yang L, Tan YF, Luo XH. Targeting miRNAs in osteoblast differentiation and bone formation. Expert Opin Ther Targets. 2010;14:1109–1120.
13. Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. Science. 1997;276:71–74.
14. Petersen BE, Bowen WC, Patrene KD, et al. Bone marrow as a potential source of hepatic oval cells. Science. 1999;284:1168–1170.
15. Caplan AI. Why are MSCs therapeutic? New data: new insight. J Pathol. 2009;217:318–324.
16. Zhang B, Pan X, Anderson TA. MicroRNA: a new player in stem cells. J Cell Physiol. 2006;209:266–269.
17. Stadler BM, Ruohola-Baker H. Small RNAs: keeping stem cells in line. Cell. 2008;132:563–566.
18. Singh SK, Kagalwala MN, Parker-Thornburg J, Adams H, Majumder S. REST maintains self-renewal and pluripotency of embryonic stem cells. Nature. 2008;453:223–227.
19. Greco SJ, Rameshwar P. MicroRNAs regulate synthesis of the neurotransmitter substance P in human mesenchymal stem cell-derived neuronal cells. Proc Natl Acad Sci U S A. 2007;104:15484–15489.
20. Lakshmipathy U, Hart RP. Concise review: microRNA expression in multipotent mesenchymal stromal cells. Stem Cells. 2008;26:356–363.
21. Mizuno Y, Yagi K, Tokuzawa Y, et al. miR-125b inhibits osteoblastic differentiation by down-regulation of cell proliferation. Biochem Biophys Res Commun. 2008;368:267–272.
22. Li Z, Hassan MQ, Volinia S, et al. A microRNA signature for a BMP2-induced osteoblast lineage commitment program. Proc Natl Acad Sci U S A. 2008;105:13906–13911.
23. Inose H, Ochi H, Kimura A, et al. A microRNA regulatory mechanism of osteoblast differentiation. Proc Natl Acad Sci U S A. 2009;106:20794–20799.
24. Huang J, Zhao L, Xing L, Chen D. MicroRNA-204 regulates Runx2 protein expression and mesenchymal progenitor cell differentiation. Stem Cells. 2010;28:357–364.
25. Schoolmeesters A, Eklund T, Leake D, et al. Functional profiling reveals critical role for miRNA in differentiation of human mesenchymal stem cells. PLoS One. 2009;4:e5605.
26. Luzi E, Marini F, Sala SC, Tognarini I, Galli G, Brandi ML. Osteogenic differentiation of human adipose tissue-derived stem cells is modulated by the miR-26a targeting of the SMAD1 transcription factor. J Bone Miner Res. 2008;23:287–295.
27. Kim YJ, Bae SW, Yu SS, Bae YC, Jung JS. miR-196a regulates proliferation and osteogenic differentiation in mesenchymal stem cells derived from human adipose tissue. J Bone Miner Res. 2009;24: 816–825.
28. Mizuno Y, Tokuzawa Y, Ninomiya Y, et al. miR-210 promotes osteoblastic differentiation through inhibition of AcvR1b. FEBS Lett. 2009; 583:2263–2268.
29. Li H, Xie H, Liu W, et al. A novel microRNA targeting HDAC5 regulates osteoblast differentiation in mice and contributes to primary osteoporosis in humans. J Clin Invest. 2009;119:3666–3677.
30. Goff LA, Boucher S, Ricupero CL, et al. Differentiating human multipotent mesenchymal stromal cells regulate microRNAs: prediction of microRNA regulation by PDGF during osteogenesis. Exp Hematol. 2008;36:1354–1369.
31. Miyaki S, Nakasa T, Otsuki S, et al. MicroRNA-140 is expressed in differentiated human articular chondrocytes and modulates interleukin-1 responses. Arthritis Rheum. 2009;60:2723–2730.
32. Tuddenham L, Wheeler G, Ntounia-Fousara S, et al. The cartilage specific microRNA-140 targets histone deacetylase 4 in mouse cells. FEBS Lett. 2006;580:4214–4217.
33. Lin EA, Kong L, Bai XH, Luan Y, Liu CJ. miR-199a, a bone morphogenic protein 2-responsive MicroRNA, regulates chondrogenesis via direct targeting to Smad1. J Biol Chem. 2009;284:11326–11335.
34. Esau C, Kang X, Peralta E, et al. MicroRNA-143 regulates adipocyte differentiation. J Biol Chem. 2004;279:52361–52365.
35. Yang Z, Bian C, Zhou H, et al. MicroRNA hsa-miR-138 inhibits adipogenic differentiation of human adipose tissue-derived mesenchymal stem cells through adenovirus EID-1. Stem Cells Dev. 2011;20: 259–267.
36. Sun F, Wang J, Pan Q, et al. Characterization of function and regulation of miR-24-1 and miR-31. Biochem Biophys Res Commun. 2009; 380:660–665.
37. Boutz PL, Chawla G, Stoilov P, Black DL. MicroRNAs regulate the expression of the alternative splicing factor nPTB during muscle development. Genes Dev. 2007;21:71–84.
38. Anderson C, Catoe H, Werner R. MIR-206 regulates connexin43 expression during skeletal muscle development. Nucleic Acids Res. 2006;34:5863–5871.
39. Sun Q, Zhang Y, Yang G, et al. Transforming growth factor-beta-regulated miR-24 promotes skeletal muscle differentiation. Nucleic Acids Res. 2008;36:2690–2699.
40. Naguibneva I, Ameyar-Zazoua M, Polesskaya A, et al. The microRNA miR-181 targets the homeobox protein Hox-A11 during mammalian myoblast differentiation. Nat Cell Biol. 2006;8:278–284.
41. Shan ZX, Lin QX, Yu XY, et al. MicroRNAs can be expressed in cardiomyocyte-like cells differentiated from human mesenchymal stem cells]. Nan Fang Yi Ke Da Xue Xue Bao. 2007;27: 1813–1816.
42. Lim PK, Patel SA, Gregory LA, Rameshwar P. Neurogenesis: role for microRNAs and mesenchymal stem cells in pathological states. Curr Med Chem. 2010;17:2159–2167.
43. Wagner W, Horn P, Castoldi M, et al. Replicative senescence of mesenchymal stem cells: a continuous and organized process. PLoS One. 2008;3:e2213.
44. Zou Z, Zhang Y, Hao L, et al. More insight into mesenchymal stem cells and their effects inside the body. Expert Opin Biol Ther. 2010; 10:215–230.
45. Bae S, Ahn JH, Park CW, et al. Gene and microRNA expression signatures of human mesenchymal stromal cells in comparison to fibroblasts. Cell Tissue Res. 2009;335:565–573.
46. Koh W, Sheng CT, Tan B, et al. Analysis of deep sequencing microRNA expression profile from human embryonic stem cells derived mesenchymal stem cells reveals possible role of let-7 microRNA family in downstream targeting of hepatic nuclear factor 4 alpha. BMC Genomics. 2010;11(suppl 1):S6.

47. Han J, Yang T, Gao J, et al. Specific microRNA expression during chondrogenesis of human mesenchymal stem cells. Int J Mol Med. 2010;25:377–384.
48. Zhang D, Fan GC, Zhou X, et al. Over-expression of CXCR4 on mesenchymal stem cells augments myoangiogenesis in the infarcted myocardium. J Mol Cell Cardiol. 2008;44:281–292.
49. Schwartz RE, Reyes M, Koodie L, et al. Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. J Clin Invest. 2002;109:1291–1302.
50. Reyes M, Lund T, Lenvik T, Aguiar D, Koodie L, Verfaillie CM. Purification and ex vivo expansion of postnatal human marrow mesodermal progenitor cells. Blood. 2001;98:2615–2625.
51. Jiang Y, Jahagirdar BN, Reinhardt RL, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature. 2002;418:41–49.
52. Greco SJ, Liu K, Rameshwar P. Functional similarities among genes regulated by OCT4 in human mesenchymal and embryonic stem cells. Stem Cells. 2007;25:3143–3154.
53. Zhao Y, Srivastava D. A developmental view of microRNA function. Trends Biochem Sci. 2007;32:189–197.
54. Kim HK, Lee YS, Sivaprasad U, Malhotra A, Dutta A. Muscle-specific microRNA miR-206 promotes muscle differentiation. J Cell Biol. 2006;174:677–687.
55. Rao PK, Kumar RM, Farkhondeh M, Baskerville S, Lodish HF. Myogenic factors that regulate expression of muscle-specific microRNAs. Proc Natl Acad Sci U S A. 2006;103:8721–8726.
56. Hebert SS, Horre K, Nicolai L, et al. MicroRNA regulation of Alzheimer's amyloid precursor protein expression. Neurobiol Dis. 2009; 33:422–428.
57. Yoo AS, Staahl BT, Chen L, Crabtree GR. MicroRNA-mediated switching of chromatin-remodelling complexes in neural development. Nature. 2009;460:642–646.
58. Trzaska KA, Reddy BY, Munoz JL, Li KY, Ye JH, Rameshwar P. Loss of RE-1 silencing factor in mesenchymal stem cell-derived dopamine progenitors induces functional maturity. Mol Cell Neurosci. 2008;39:285–290.
59. Hayflick L. The limited in vitro lifetime of human diploid cell strains. Exp Cell Res. 1965;37:614–636.
60. Ho AD, Wagner W, Mahlknecht U. Stem cells and ageing. The potential of stem cells to overcome age-related deteriorations of the body in regenerative medicine. EMBO Rep. 2005;6(spec no): S35–S38.
61. Galderisi U, Helmbold H, Squillaro T, et al. In vitro senescence of rat mesenchymal stem cells is accompanied by downregulation of stemness-related and DNA damage repair genes. Stem Cells Dev. 2009;18:1033–1042.
62. Kretlow JD, Jin YQ, Liu W, et al. Donor age and cell passage affects differentiation potential of murine bone marrow-derived stem cells. BMC Cell Biol. 2008;9:60.
63. Crisostomo PR, Wang M, Wairiuko GM, et al. High passage number of stem cells adversely affects stem cell activation and myocardial protection. Shock. 2006;26:575–580.
64. Yu JM, Wu X, Gimble JM, Guan X, Freitas MA, Bunnell BA. Age-related changes in mesenchymal stem cells derived from rhesus macaque bone marrow. Aging Cell. 2011;10:66–79.
65. Hackl M, Brunner S, Fortschegger K, et al. miR-17, miR-19b, miR-20a, and miR-106a are down-regulated in human aging. Aging Cell. 2010;9:291–296.
66. Fabbri M, Garzon R, Cimmino A, et al. MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. Proc Natl Acad Sci U S A. 2007;104:15805–15810.
67. Noer A, Boquest AC, Collas P. Dynamics of adipogenic promoter DNA methylation during clonal culture of human adipose stem cells to senescence. BMC Cell Biol. 2007;8:18.
68. Chambers SM, Shaw CA, Gatza C, Fisk CJ, Donehower LA, Goodell MA. Aging hematopoietic stem cells decline in function and exhibit epigenetic dysregulation. PLoS Biol. 2007;5:e201.
69. Shilo S, Roy S, Khanna S, Sen CK. MicroRNA in cutaneous wound healing: a new paradigm. DNA Cell Biol. 2007;26:227–237.
70. Houbaviy HB, Murray MF, Sharp PA. Embryonic stem cell-specific MicroRNAs. Dev Cell. 2003;5:351–358.
71. Marson A, Levine SS, Cole MF, et al. Connecting microRNA genes to the core transcriptional regulatory circuitry of embryonic stem cells. Cell. 2008;134:521–533.
72. Gunaratne PH. Embryonic stem cell microRNAs: defining factors in induced pluripotent (iPS) and cancer (CSC) stem cells? Curr Stem Cell Res Ther. 2009;4:168–177.
73. Judson RL, Babiarz JE, Venere M, Blelloch R. Embryonic stem cell-specific microRNAs promote induced pluripotency. Nat Biotechnol. 2009;27:459–461.
74. Malchenko S, Galat V, Seftor EA, et al. Cancer hallmarks in induced pluripotent cells: new insights. J Cell Physiol. 2010;225: 390–393.
75. Martinez NJ, Gregory RI. MicroRNA gene regulatory pathways in the establishment and maintenance of ESC identity. Cell Stem Cell. 2010; 7:31–35.
76. Melton C, Judson RL, Blelloch R. Opposing microRNA families regulate self-renewal in mouse embryonic stem cells. Nature. 2010;463: 621–626.
77. Lakshmipathy U, Love B, Goff LA, et al. MicroRNA expression pattern of undifferentiated and differentiated human embryonic stem cells. Stem Cells Dev. 2007;16:1003–1016.
78. Josephson R, Ording CJ, Liu Y, et al. Qualification of embryonal carcinoma 2102Ep as a reference for human embryonic stem cell research. Stem Cells. 2007;25:437–446.
79. Krampera M, Pasini A, Rigo A, et al. HB-EGF/HER-1 signaling in bone marrow mesenchymal stem cells: inducing cell expansion and reversibly preventing multilineage differentiation. Blood. 2005;106: 59–66.
80. Baxter MA, Wynn RF, Jowitt SN, Wraith JE, Fairbairn LJ, Bellantuono I. Study of telomere length reveals rapid aging of human marrow stromal cells following in vitro expansion. Stem Cells. 2004;22: 675–682.
81. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel genes coding for small expressed RNAs. Science. 2001; 294:853–858.
82. Schulman BR, Esquela-Kerscher A, Slack FJ. Reciprocal expression of lin-41 and the microRNAs let-7 and mir-125 during mouse embryogenesis. Dev Dyn. 2005;234:1046–1054.
83. Caygill EE, Johnston LA. Temporal regulation of metamorphic processes in Drosophila by the let-7 and miR-125 heterochronic microRNAs. Curr Biol. 2008;18:943–950.
84. Peter ME. Let-7 and miR-200 microRNAs: guardians against pluripotency and cancer progression. Cell Cycle. 2009;8:843–852.
85. Viswanathan SR, Daley GQ, Gregory RI. Selective blockade of microRNA processing by Lin28. Science. 2008;320:97–100.
86. Foshay KM, Gallicano GI. Small RNAs, big potential: the role of MicroRNAs in stem cell function. Curr Stem Cell Res Ther. 2007;2:264–271.
87. Bruno S, Grange C, Deregibus MC, et al. Mesenchymal stem cell-derived microvesicles protect against acute tubular injury. J Am Soc Nephrol. 2009;20:1053–1067.
88. Collino F, Deregibus MC, Bruno S, et al. Microvesicles derived from adult human bone marrow and tissue specific mesenchymal stem cells shuttle selected pattern of miRNAs. PLoS One. 2010;5: e11803.
89. Camussi G, Deregibus MC, Bruno S, Cantaluppi V, Biancone L. Exosomes/microvesicles as a mechanism of cell-to-cell communication. Kidney Int. 2010;78:838–848.
90. Lotvall J, Valadi H. Cell to cell signalling via exosomes through esRNA. Cell Adh Migr. 2007;1:156–158.
91. Smalheiser NR. Exosomal transfer of proteins and RNAs at synapses in the nervous system. Biol Direct. 2007;2:35.

92. Chen TS, Lai RC, Lee MM, Choo AB, Lee CN, Lim SK. Mesenchymal stem cell secretes microparticles enriched in pre-microRNAs. Nucleic Acids Res. 2010;38:215–224.
93. Chen L, Tredget EE, Wu PY, Wu Y. Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. PLoS One. 2008;3:e1886.
94. Wu Y, Chen L, Scott PG, Tredget EE. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. Stem Cells. 2007;25:2648–2659.
95. Wu Y, Zhao RC, Tredget EE. Concise review: bone marrow-derived stem/progenitor cells in cutaneous repair and regeneration. Stem Cells. 2010;28:905–915.
96. Gnecchi M, He H, Liang OD, et al. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. Nat Med. 2005;11:367–368.
97. Tran DH, Satou K, Ho TB. Finding microRNA regulatory modules in human genome using rule induction. BMC Bioinformatics. 2008; 9(suppl 12):S5.