miRNA Expression in Mesenchymal Stem Cells

Federica Collino · Stefania Bruno · Rafael Soares Lindoso · Giovanni Camussi

Published online: 14 May 2014
© Springer Science+Business Media New York 2014

Abstract Mesenchymal stem cells (MSCs) express defined patterns of miRNAs which have been shown to be involved in stemness and differentiation processes. Modulation of miRNA expression by MSCs has been shown to change their biological properties. Moreover, in light of the paracrine hypothesis of MSC action, increasing evidence indicates that miRNA transfer between MSCs and injured cells in tissues accounts for the healing properties exhibited by MSCs. Extracellular vesicles (EVs) are candidates for the trafficking of encapsulated miRNAs between cells, as they are able to cross easily biological barriers and protect nucleic acids from degradation. Moreover, EVs, by expressing the same surface receptors of MSCs, may be recruited to the site of injury as well as the cell of origin. These properties of EVs may be exploited for the generation of specially engineered EVs carrying specific miRNAs for therapeutic purposes.

Keywords Mesenchymal stem cells · miRNAs · Extracellular vesicles · Tissue regeneration

Introduction

Mesenchymal stem cells (MSCs) are considered to be multipotent progenitor cells, defined according to the International Society for Cellular Therapy, on the basis of

their ability to adhere to plastic, to self-replicate, to differentiate into different mesodermal lineages (osteocytes, chondrocytes, and adipocytes), and by their expression of specific surface markers (CD105, CD73 and CD90) [1]. MSCs were initially isolated and characterized from bone marrow, where they play a primary role in supporting haematopoiesis [2, 3]. Subsequently, MSCs were also obtained and expanded from other tissues, including lung [4], adipose tissue [5], dental pulp [6], placenta [7], and dermis [8].

A large body of evidence, present in the literature, indicates that MSC infusion in different animal models of acute injury induces tissue regeneration [9]. For this reason, there is an increasing interest in the application of MSCs in regenerative medicine. Although MSCs exhibit *in vitro* multilineage differentiation potential and have the capacity to reach injured sites after systemic administration [10], the *in vivo* differentiation of MSCs in cells of injured tissues appears to be a rare event. In addition, MSCs are short-lived after *in vivo* injection, being detectable in various organs and tissues for periods ranging from 24 to 36 h [11, 12]. The current interpretation of these observations is that the biological effects of MSCs mainly depend on paracrine mechanisms that involve the release of bioactive factors. Administration of bioactive soluble factors released by MSCs, in different *in vivo* models of acute tissue injury, induces inhibition of apoptosis and increases proliferation [13–15], leading to tissue regeneration. Moreover, soluble factors are also involved in the immunomodulatory and anti-inflammatory effects of MSCs that contribute to the regeneration of injured tissues [16]. The bioactive factors secreted by MSCs include a variety of growth factors, cytokines and extracellular vesicles (EVs), which are small cellular fragments involved in cell-to-cell communication and capable of altering the behaviour of

F. Collino · R. S. Lindoso · G. Camussi (✉)
Department of Medical Science, MBC, Turin, Italy
e-mail: giovanni.camussi@unito.it

S. Bruno
Molecular Biotechnology and Healthy Sciences, MBC, Turin, Italy

recipient cells [17]. It has been shown that MSCs release EVs that contribute to tissue repair in different animal models of renal, cardiac and lung injury [18–23].

EVs contain proteins and nucleic acids (RNA and small non-coding RNAs) derived from their cell of origin. miRNAs, a group of small non-coding RNAs, have been shown to modulate stemness features and differentiation capacity of MSCs, as well as their biological functions. Moreover, transfer of miRNAs via EVs may modify target cell phenotype and activate regenerative processes.

miRNA Biogenesis and Functions

miRNAs were first discovered in nematodes about 20 years ago where they were shown to play a role in determining cell fate. The presence of miRNAs in humans was described about 10 years ago.

Mature miRNAs are produced by multiple steps in the cell. The first miRNA inactive precursor, known as pri-miRNA, is generated as a stem-loop structure, and is recognized by a complex of different proteins, including DGCR8 and Drosha. These proteins have been shown to process the pri-miRNA into the precursor miRNA (pre-miRNA), a double-stranded hairpin structure of about 70 nucleotides [24, 25]. The pre-miRNA is then exported from the nucleus to the cytoplasm by another protein complex, Exportin-5 bound to Ran [26].

The pre-miRNA processing is continued by the RNase III enzyme, Dicer, generating a dsRNA, which lacks the hairpin portion. The resulting two mature single strands (ss) are called guide miRNA and passenger miRNA/miRNA*. Only one of the ss-miRNAs, which preferentially assemble with the Argonaute proteins in the RNA-induced silencing complex (miRISC), is selected and used to guide sequence-specific silencing of complementary target mRNAs. The miRISC activation induces RNA silencing via endonucleolytic cleavage or translational repression of the target transcript. These two options depend on the specificity of the interaction of the mature miRNA with the 3'-UTR of the mRNA [27]. If the miRNA/mRNA interaction is precise, the mRNA is immediately degraded by Ago2, one of the Argonaute family proteins. Otherwise, in the case of no exact interaction, the mRNA is translationally repressed [27].

miRNAs and MSC Regenerative Activity

miRNA profile studies have demonstrated that miRNAs are selectively expressed in different organs and at different developmental stages. miRNA signatures for MSCs of different origin have shown the expression of defined

patterns of miRNAs involved in the maintenance of stem cell properties such as proliferation, self-renewal, and differentiation capacity [28, 29, 30]. Moreover, several studies have identified miRNAs as being involved in MSC differentiation in mesenchymal-derived tissues such as osteocytes, chondrocytes and adipocytes [31, 32]. MSC-based therapy in different organs could be partially due to the regulatory activity of miRNAs involved in MSC differentiative programs (Table 1), or in the paracrine effects of MSCs [33].

Different miRNAs have been involved in the ability of MSCs or other stem cells to differentiate into cardiomyocytes [33]. MSCs transfected with miR-133a, which targets EGFR, expressed cardiac-specific markers [34]. Moreover, miR-133a, miR-210, and miR-34a have been associated to the potential of cardiac mesenchymal-like stromal cells to differentiate into cardiomyocytes [35].

Recently, transplantation of miR-1-transfected MSCs was shown to repair infarct injury and improve heart function by enhancing survival of transplanted cells and cardiomyogenic differentiation [36]. miR-1, miR-133, miR-208, and miR-499 have been described to modulate positively or negatively important cardiac genes [37]. Interestingly, the potential of MSCs and other stem cells to engraft and differentiate into endothelial cells (EC) or vascular smooth muscle cells (VSMC) has also been associated with modulation by miRNAs [33, 38, 39]. miR-1, miR-126, miR-210, miR-145, and miR-143 have been demonstrated to be involved in the differentiation of different stem cells to EC and VSMC [33, 40].

Bone marrow (BM)-MSCs can differentiate into neuron-like cells under the appropriate culture conditions [41]. miR-9 has been demonstrated to promote MSC neuronal differentiation by enhancing the neural cell-specific marker, microtubule-associated protein 2, via repression of Notch-1 protein [42].

In neuronal cells differentiated from human MSCs, miR-130 and miR-206 have been described as important regulators of production of neurotransmitter substance P [43]. In addition, miR-34a has been shown to modulate negatively genes implicated in cellular motility and in oxidative phosphorylation in human umbilical cord MSC-derived neural precursors [44]. Recently, miR-128, a brain-enriched miRNA, has been found to be necessary for the neural differentiation of BM-MSCs [45]. In particular, Wu et al., demonstrated the ability of miR-128 to attenuate the differentiation of MSCs, through the modulation of Wnt3a, a key component of the Wnt signalling pathway [45].

MSCs can ameliorate tissue regeneration when injected in different models of liver injury [15]. miRNAs have been involved in the capacity of MSCs to trans-differentiate into hepatocytes [46]. Cui et al. [46] recently found that

Table 1 miRNAs involved in inhibition or stimulation of MSC differentiation

Differentiation capacity	miRNAs	Species	Target gene	Effect on differentiation	References
Cardiomyocytes	miR-133a	Human	<i>EGFR</i>	Stimulatory	Lee et al. [34]
	miR-133a, -210, and -34a	Human	–	Stimulatory	Vecellio et al. [35]
	miR-1	Human	–	Stimulatory	Huang et al. [36]
Vascular smooth muscle cells	Signature of 20 miRNAs	Human	–	Stimulatory	Danielson et al. [38]
	miR-143 and -145	Human	<i>Elk-1 (miR-143)</i> Transcription factors (miR-145)	Stimulatory	Cordes et al. [40]
Neurons	miR-9	Mouse	<i>Notch-1</i>	Stimulatory	Jing et al. [42]
	miR-130a, -206	Human	<i>Tac1</i>	Inhibitory	Greco et al. [43]
	miR-34a	Human	<i>136 neuronal progenitor genes (prediction)</i>	Inhibitory	Chang et al. [44]
Hepatocytes	miR-128	Rat	<i>Wnt3a</i>	Inhibitory	Wu et al. [45]
	miR-1246, -1290, -148a, -30a, -424 and -542-5p, and -122	Human	–	Stimulatory	Cui et al. [46, 47•]

miR-1246, miR-1290, miR-148a, miR-30a, miR-424, and miR-542-5p were specifically overexpressed during hepatic differentiation of human umbilical cord MSCs. Interestingly, the concomitant overexpression of these miRNAs can convert MSCs more efficiently into mature, functional hepatocytes influencing hepatic gene expression [47•].

The relevance of miRNAs in regulating the biological properties of MSCs is emphasized by experiments showing that different factors potentiate or reduce the therapeutic properties of MSCs by means of miRNA modulation. Ng et al. [48] demonstrated a reduction in proliferation and diminished locomotion in MSCs treated with nicotine, in concomitance with an alteration in the miRNA profile; this suggested that miRNAs could play an important role in the effect of nicotine on the regenerative potential of MSCs [48]. Conversely, the preconditioning of MSCs with diazoxide led to improve MSC survival and regenerative activity in myocardial infarction, via induction of miR-146a in MSCs [49]. In addition, Nie et al. [50•], showed that up-regulation of miR-21, miR-23a, and miR-210 in MSCs submitted to hypoxia enhanced their survival. Moreover, Mao et al. [51] demonstrated the importance of miR-23a in preventing apoptosis of MSCs infused in a rat model of myocardial infarction; they showed that overexpression of miR-23a in transplanted MSCs, after myocardial infarction, improved cardiac function and reduced infarct size.

miRNAs may also be involved in the paracrine action of MSCs. In fact, miRNAs derived from MSCs may restore an altered miRNA pattern in injured cells. Indeed, increasing evidence has demonstrated a correlation between altered miRNA patterns and the pathogenesis of different organ injuries [33, 52•, 53, 54, 55, 56••, 57]. In particular, the

transfer of miRNAs from MSCs to injured cells has been demonstrated to mainly occur via EVs [29•].

Extracellular Vesicles as Carriers of Extracellular RNA

EVs may contribute to cell-to-cell communication by membrane expression of specific receptors that can interact with ligands on target cells or, after internalization, EVs may transfer cytoplasmic constituents of the cell of origin, influencing the behaviour of recipient cells [58]. EVs are composed of a heterogeneous population produced by cells via distinct processes. Vesicles may be derived from the endosomal membrane compartments that, after fusion with the plasma membrane, are extruded from the cell surface as exosomes [59] or from budding of the plasma membrane, as shedding vesicles [59]. EVs retain the signature of the cell of origin, and contain cytoplasmic constituents (proteins, nucleic acids and lipids). EVs from MSCs express surface molecules that are characteristic of MSCs (CD29, CD73, CD44, CD105) [17] and contain cytoplasmic proteins associated with intracellular vesicle biogenesis and trafficking (RAB protein family), along with proteins associated with MSC self-renewal and differentiation (TGF- β , MAPK, PPAR, etc.) [60•]. The fact that the same adhesion molecules are expressed by EVs and MSCs may favour the recruitment of EVs to the site of injury, and subsequent delivery of their contents to damaged cells.

Analyses of the nucleic acid content shuttled by MSC-derived EVs have demonstrated the presence of nucleic acids, including mRNA, miRNAs, and long noncoding RNAs.

The mRNA present in EVs are representative of the multiple differentiation and functional properties of MSCs, including transcripts related to several different cell functions (e.g. the control of transcription, cell proliferation and immune regulation) [17]. EVs from MSCs also contain mRNA for receptors of specific growth factors, such as the insulin-like growth factor 1 (IGF-1) receptor. EVs have been shown to transfer the IGF-1 receptor mRNA to target renal tubular cells in an in vitro model of renal toxic injury induced by cisplatin, inducing proliferation of damaged proximal tubular cells [61].

EVs derived from human MSCs stimulate in vitro proliferation and apoptosis resistance of renal tubular epithelial cells, and accelerate the functional and morphological recovery in different murine models of kidney injury in vivo [17–20]. These regenerative effects of EVs have been ascribed to their RNA cargo, as inactivation of RNAs reduced both the in vitro and in vivo effects of EVs [18–20]. Moreover, it was found that human MSC-derived mRNA were functionally active, as they were translated into proteins in tubular epithelial cells of mice, both in vitro and in vivo [18, 20].

EVs released by MSCs, also contain specific pre-miRNAs and miRNAs that can be transferred to target cells [29•, 30•]. Gene ontology analysis of the molecules targeted by the highly expressed miRNAs in MSC-derived EVs revealed genes involved in multi-organ development, cell survival and differentiation [29•].

Paracrine Effects of MSC-Derived miRNAs

EVs may regulate a bi-directional exchange of miRNAs between MSCs and damaged cells [29•, 62]. The possibility of using miRNA-containing EVs for the treatment of different pathologies is now a promising application in regenerative medicine. The direct evidence of a functional role of miRNA-containing EVs in the improvement of a specific injury is supported by the study by Xin et al., who demonstrated that MSCs might communicate with brain parenchymal cells, leading to the regulation of neurite outgrowth by transferring miR-133b to neural cells via EVs [63••]. Moreover, synthetic miR-133b transfected into MSCs was shown to be transferred to astrocytes and neurons via EVs. In these cells, EV-derived miR-133b was shown to regulate gene expression with a consequent benefit for neurite remodelling and recovery following a stroke [64]. Therapy using engineered-MSCs has also been applied in the functional recovery of the ischemic heart. Kim et al. [65] showed that transgenic induction of miR-210 in MSCs, simulates the pro-survival effects of ischemic preconditioning in the ischemic heart. In this study, the engraftment of engineered-MSC supports the functional

recovery, via transfer of miR-210 through gap junction interactions. Recently Yu et al. [66•] demonstrated that EVs released by engineered MSCs overexpressing GATA-4 can enhance the cardio-protective effects of MSCs on neonatal rat cardiomyocytes. This protective effect is mediated, in part, by the transfer of anti-apoptotic miRNAs (miR-221/222) contained within EVs; miR-221/222 function by reducing the expression of the pro-apoptotic gene, *PUMA* [66•].

Xu et al. [67] demonstrated that the therapeutic infusion of MSCs, in a mouse model of diabetic wound healing, improved recovery by induction of miR-146a. Restoration of miR-146a levels in the lesions, following MSC infusion, reduced pro-inflammatory genes and enhanced wound closure [67].

Conclusions

In recent years, the possible application of MSC-based therapies has been extensively studied. The role of miRNAs in the context of MSC biology has been shown to be highly relevant, not only in the regulation of the cell fate of MSCs, but also in their biological activity. The mechanism of action of MSCs via release of bio-agents, such as EVs, has opened new therapeutic perspectives. EVs are able to influence the behaviour of target cells by delivering miRNAs, and this effect could, therefore, be exploited in different diseases. Moreover, the use of EVs to replace cells could represent a novel therapeutic strategy, avoiding the possible adverse effects of cell administration. By utilizing the same adhesion receptors of MSCs, EVs may specifically target the site of injury. Moreover, as miRNAs encapsulated within EVs are protected from degrading enzymes and may easily cross biological barriers, EVs can be envisaged as vehicles for the delivery of synthetic miRNAs. Cells can be engineered to produce EVs enriched with specific miRNAs for therapeutic purposes, in pathological conditions where an evident miRNA dysregulation has been observed.

Compliance with Ethics Guidelines

Conflict of Interest Federica Collino, Stefania Bruno, and Rafael Soares Lindoso declare they have no conflict of interest. Giovanni Camussi reports Grants from Associazione italiana per la Ricerca sul Cancro (AIRC), Fresenius Medical Care, and the NIH, outside the submitted work. In addition, Dr. Camussi has the following pending patents: WO2011107437 (A1), US2011033523 (A1), and US2010233216 (A1).

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance

1. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D et al (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 8(4):315–317
2. Prockop DJ (1997) Marrow stromal cells as stem cells for not hematopoietic tissues. *Science* 276:71–74
3. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD et al (1999) Multilineage potential of adult human mesenchymal stem cells. *Science* 284:143–147
4. Collins JJ, Thébaud B (2013) Lung mesenchymal stromal cells in development and disease: to serve and protect? *Antioxid Redox Signal*
5. Tobita M, Mizuno H (2013) Adipose-derived stem cells and periodontal tissue engineering. *Int J Oral Maxillofac Implants* 28:e487–e493
6. Hilkens P, Gervois P, Fanton Y, Vanormelingen J, Martens W, Struys T et al (2013) Effect of isolation methodology on stem cell properties and multilineage differentiation potential of human dental pulp stem cells. *Cell Tissue Res* 353:65–78
7. Indumathi S, Hari Krishnan R, Mishra R, Rajkumar JS, Padmapriya V, Lissa RP et al (2013) Comparison of feto-maternal organ derived stem cells in facets of immunophenotype, proliferation and differentiation. *Tissue Cell* 45:434–442
8. Feisst V, Brooks A, Chen J, Dunbar PR (2013) Characterisation of mesenchymal progenitor cell populations directly derived from human dermis. *Stem Cells Dev*
9. Keating A (2012) Mesenchymal stromal cells: new directions. *Cell Stem Cell* 10:709–716
10. Ji JF, He BP, Dheen ST, Tay SS (2004) Interactions of chemokines and chemokine receptors mediate the migration of mesenchymal stem cells to the impaired site in the brain after hypoglossal nerve injury. *Stem Cells* 22:415–427
11. Tögel F, Hu Z, Weiss K, Isaac J, Lange C, Westenfelder C (2005) Administered mesenchymal stem cells protect against ischemic acute renal failure through differentiation-independent mechanisms. *Am J Physiol Renal Physiol* 289:F31–F42
12. Eggenhofer E, Benseler V, Kroemer A, Popp FC, Geissler EK, Schlitt HJ et al (2012) Mesenchymal stem cells are short-lived and do not migrate beyond the lungs after intravenous infusion. *Front Immunol* 3:297
13. Caplan AI, Dennis JE (2006) Mesenchymal stem cells as trophic mediators. *JCB* 98:1076–1084
14. Bi B, Schmitt R, Israilova M, Nishio H, Cantley LG (2007) Stromal cells protect against acute tubular injury via an endocrine effect. *J Am Soc Nephrol* 18(9):2486–2496
15. van Poll D, Parekkadan B, Cho CH, Berthiaume F, Nahmias Y, Tilles AW et al (2008) Mesenchymal stem cell-derived molecules directly modulate hepatocellular death and regeneration in vitro and in vivo. *Hepatology* 47(5):1634–1643
16. Yagi H, Soto-Gutierrez A, Parekkadan B, Kitagawa Y, Tompkins RG, Kobayashi N et al (2011) Mesenchymal stem cells: mechanism of immunomodulation and homing. *Cell Transplant* 19:667–679
17. Camussi G, Deregibus MC, Bruno S, Cantaluppi V, Biancone L (2010) Exosomes/microvesicles as a mechanism of cell-to-cell communication. *Kidney Int* 78:838–848
18. Bruno S, Grange C, Deregibus MC, Calogero RA, Saviozzi S, Collino F et al (2009) Mesenchymal stem cell-derived microvesicles protect against acute tubular injury. *J Am Soc Nephrol* 20:1053–1067
19. Gatti S, Bruno S, Deregibus MC, Sordi A, Cantaluppi V, Tetta C et al (2011) Microvesicles derived from human adult mesenchymal stem cells protect against ischaemia-reperfusion-induced acute and chronic kidney injury. *Nephrol Dial Transplant* 26:1474–1483
20. Bruno S, Grange C, Collino F, Deregibus MC, Cantaluppi V, Biancone L et al (2012) Microvesicles derived from mesenchymal stem cells enhance survival in a lethal model of acute kidney injury. *PLoS One* 7:e33115
21. He J, Wang Y, Sun S, Yu M, Wang C, Pei X et al (2012) Bone marrow stem cells-derived microvesicles protect against renal injury in the mouse remnant kidney model. *Nephrology* 17:493–500
22. Lai RC, Arslan F, Lee MM, Sze NS, Choo A, Chen TS et al (2010) Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. *Stem Cell Res* 4:214–222
23. Zhu YG, Feng XM, Abbott J, Fang XH, Hao Q, Monsel A et al (2013) Human mesenchymal stem cell microvesicles for treatment of *E. coli* endotoxin-induced acute lung injury in mice. *Stem Cells*. doi:10.1002/stem.1504
24. Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J et al (2003) The nuclear RNase III Drosha initiates microRNA processing. *Nature* 425(6956):415–419
25. Han J, Lee Y, Yeom KH, Kim YK, Jin H, Kim VN (2004) The Drosha-DGCR8 complex in primary microRNA processing. *Genes Dev* 18(24):3016–3027
26. Bohnsack MT, Czapinski K, Gorlich D (2004) Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. *RNA* 10(2):185–191
27. Bartel DP (2009) MicroRNAs: target recognition and regulatory functions. *Cell* 136(2):215–233
28. Valtieri M, Sorrentino A (2008) The mesenchymal stromal cell contribution to homeostasis. *J Cell Physiol* 217(2):296–300
29. • Collino F, Deregibus MC, Bruno S, Sterpone L, Aghemo G, Viltono L et al (2010) Microvesicles derived from adult human bone marrow and tissue specific mesenchymal stem cells shuttle selected pattern of miRNAs. *PLoS One* 5:e11803. *This paper demonstrated that EVs derived from bone marrow and hepatic MSCs contained mature miRNAs. Interestingly, some miRNAs were accumulated within EVs and absent in the cells after EV release. The expressed miRNAs were involved in multi-organ development, cell survival, differentiation and immune system regulation. Moreover, the highly expressed miRNAs in EVs were transferred to target cells after EV incorporation*
30. • Koh W, Sheng CT, Tan B, Lee QY, Kuznetsov V, Kiang LS et al (2010) 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* 11(1):S6. *In this work, the authors performed the deep sequencing of miRNAs in EVs from human embryonic stem cells derived-MSCs (hES-MSC). Significant differences in the expression profile of miRNAs were found in the intracellular and extracellular environment of hES-MSC. In particular, high level of microRNA let-7 family was predominant in both intra- and extra- cellular samples suggesting a possible intercellular signaling through EVs*
31. Fakhry M, Hamade E, Badran B, Buchet R, Magne D (2013) Molecular mechanisms of mesenchymal stem cell differentiation towards osteoblasts. *World J Stem Cells* 5(4):136–148
32. Lin X, Wu L, Zhang Z, Yang R, Guan Q, Hou X et al (2013) MiR-335-5p promotes chondrogenesis in mouse mesenchymal

- stem cells and is regulated through two positive feedback loops. *J Bone Miner Res*. doi:[10.1002/jbmr.2163](https://doi.org/10.1002/jbmr.2163)
33. Wen Z, Zheng S, Zhou C, Yuan W, Wang J, Wang T (2012) Bone marrow mesenchymal stem cells for post-myocardial infarction cardiac repair: microRNAs as novel regulators. *J Cell Mol Med* 16(4):657–671
 34. Lee SY, Ham O, Cha MJ, Song BW, Choi E, Kim IK, Chang W, Lim S, Lee CY, Park JH, Lee J, Bae Y, Seo HH, Choi E, Jang Y, Hwang KC (2013) The promotion of cardiogenic differentiation of hMSCs by targeting epidermal growth factor receptor using microRNA-133a. *Biomaterials* 34(1):92–99. doi:[10.1016/j.biomaterials.2012.09.069](https://doi.org/10.1016/j.biomaterials.2012.09.069)
 35. Vecellio M, Meraviglia V, Nanni S, Barbuti A, Scavone A, DiFrancesco D, Farsetti A, Pompilio G, Colombo GI, Capogrossi MC, Gaetano C, Rossini A (2012) In vitro epigenetic reprogramming of human cardiac mesenchymal stromal cells into functionally competent cardiovascular precursors. *PLoS One* 7(12):e51694. doi:[10.1371/journal.pone.0051694](https://doi.org/10.1371/journal.pone.0051694)
 36. Huang F, Li ML, Fang ZF, Hu XQ, Liu QM, Liu ZJ, Tang L, Zhao YS, Zhou SH (2013) Overexpression of MicroRNA-1 improves the efficacy of mesenchymal stem cell transplantation after myocardial infarction. *Cardiology* 125(1):18–30. doi:[10.1159/000347081](https://doi.org/10.1159/000347081)
 37. Ohtani K, Dimmeler S (2011) Control of cardiovascular differentiation by microRNAs. *Basic Res Cardiol* 106(1):5–11
 38. Danielson LS, Menendez S, Attolini CS, Guijarro MV, Bisogna M, Wei J et al (2010) A differentiation-based microRNA signature identifies leiomyosarcoma as a mesenchymal stem cell-related malignancy. *Am J Pathol* 177(2):908–917
 39. Kane NM, Meloni M, Spencer HL, Craig MA, Strehl R, Milligan G et al (2010) Derivation of endothelial cells from human embryonic stem cells by directed differentiation: analysis of microRNA and angiogenesis in vitro and in vivo. *Arterioscler Thromb Vasc Biol* 201030(7):1389–1397
 40. Cordes KR, Sheehy NT, White MP et al (2009) miR-145 and miR-143 regulate smooth muscle cell fate and plasticity. *Nature* 460:705–710
 41. Bi Y, Gong M, He Y, Zhang X, Zhou X, Zhang Y et al (2014) AP2 α transcriptional activity is essential for retinoid-induced neuronal differentiation of mesenchymal stem cells. *Int J Biochem Cell Biol* 46:148–160
 42. Jing L, Jia Y, Lu J, Han R, Li J, Wang S et al (2011) MicroRNA-9 promotes differentiation of mouse bone mesenchymal stem cells into neurons by Notch signaling. *NeuroReport* 22(5):206–211
 43. Greco SJ, Rameshwar P (2007) MicroRNAs regulate synthesis of the neurotransmitter substance P in human mesenchymal stem cell-derived neuronal cells. *Proc Natl Acad Sci USA* 104(39):15484–15489
 44. Chang SJ, Weng SL, Hsieh JY, Wang TY, Chang MD, Wang HW (2011) MicroRNA-34a modulates genes involved in cellular motility and oxidative phosphorylation in neural precursors derived from human umbilical cord mesenchymal stem cells. *BMC Med Genomics* 19(4):65
 45. Wu R, Tang Y, Zang W, Wang Y, Li M, Du Y et al (2013) MicroRNA-128 regulates the differentiation of rat bone mesenchymal stem cells into neuron-like cells by Wnt signaling. *Mol Cell Biochem*
 46. Cui L, Zhou X, Li J, Wang L, Wang J, Li Q et al (2012) Dynamic microRNA profiles of hepatic differentiated human umbilical cord lining-derived mesenchymal stem cells. *PLoS One* 7:e44737
 47. • Cui L, Shi Y, Zhou X, Wang X, Wang J, Lan Y, Wang M, Zheng L, Li H, Wu Q, Zhang J, Fan D, Han Y (2013) A set of microRNAs mediate direct conversion of human umbilical cord lining-derived mesenchymal stem cells into hepatocytes. *Cell Death Dis* 14(4):e918. doi:[10.1038/cddis.2013.429](https://doi.org/10.1038/cddis.2013.429). *This study demonstrated that the overexpression of six miRNAs (miR-1246, miR-1290, miR-148a, miR-30a, miR-424 and miR-542-5p) was sufficient to induce differentiation of the MSCs into functional hepatocyte-like cells. Additionally, transplantation of this hepatocyte-like cells into mice with CCL4-induced liver injury, improved liver function and recovery*
 48. Ng TK, Carballosa CM, Pelaez D, Wong HK, Choy KW, Pang CP et al (2013) Nicotine alters MicroRNA expression and hinders human adult stem cell regenerative potential. *Stem Cells Dev* 22(5):781–790
 49. Suzuki Y, Kim HW, Ashraf M, Haider HKh (2010) Diazoxide potentiates mesenchymal stem cell survival via NF-kappaB-dependent miR-146a expression by targeting Fas. *Am J Physiol Heart Circ Physiol* 299(4):H1077–H1082
 50. • Nie Y, Han BM, Liu XB, Yang JJ, Wang F, Cong XF et al (2011) Identification of MicroRNAs involved in hypoxia- and serum deprivation-induced apoptosis in mesenchymal stem cells. *Int J Biol Sci* 7(6):762–768. *In this paper it is reported that overexpression of miR-21, miR-23a and miR-210 promotes the survival of MSCs exposed to hypoxia and serum deprivation. Conversely, down-regulation of these miRNAs induces apoptosis of MSCs*
 51. Mao J, Lv Z, Zhuang Y (2013) MicroRNA-23a is involved in tumor necrosis factor- α induced apoptosis in mesenchymal stem cells and myocardial infarction. *Exp Mol Pathol*. doi:[10.1016/j.yexmp.2013.11.005](https://doi.org/10.1016/j.yexmp.2013.11.005)
 52. • Garza-Manero S, Pichardo-Casas I, Arias C, Vaca L, Zepeda A (2013) Selective distribution and dynamic modulation of miRNAs in the synapse and its possible role in Alzheimer's Disease. *Brain Res*. doi:[10.1016/j.brainres.2013.12.009](https://doi.org/10.1016/j.brainres.2013.12.009). *Synaptic loss is an early event in the pathogenesis of Alzheimer's Disease (AD). MiRNA-mediated gene silencing represents a candidate event for the repression of specific mRNAs and protein synthesis that could account for synaptic dysfunction. In this paper, the authors evaluated the participation of miRNAs in synaptic function and considered their possible role in synaptic alterations in AD*
 53. Parisi C, Arisi I, D'Ambrosi N, Storti AE, Brandi R, D'Onofrio M et al (2013) Dysregulated microRNAs in amyotrophic lateral sclerosis microglia modulate genes linked to neuroinflammation. *Cell Death Dis* 12(4):e959. doi:[10.1038/cddis.2013.491](https://doi.org/10.1038/cddis.2013.491)
 54. Wei Q, Bhatt K, He HZ, Mi QS, Haase VH, Dong Z (2010) Targeted deletion of Dicer from proximal tubules protects against renal ischemia-reperfusion injury. *J Am Soc Nephrol* 21(5):756–761. doi:[10.1681/ASN.2009070718](https://doi.org/10.1681/ASN.2009070718)
 55. Godwin JG, Ge X, Stephan K, Jurisch A, Tullius SG, Iacomini J (2010) Identification of a microRNA signature of renal ischemia reperfusion injury. *Proc Natl Acad Sci USA* 107(32):14339–14344
 56. • Xu X, Kriegl AJ, Liu Y, Usa K, Mladinov D, Liu H, Fang Y, Ding X, Liang M (2012) Delayed ischemic preconditioning contributes to renal protection by upregulation of miR-21. *Kidney Int* 82(11):1167–1175. doi:[10.1038/ki.2012.241](https://doi.org/10.1038/ki.2012.241). *In this article, the authors examined the in vivo role of miR-21 in the renal protection conferred by delayed ischemic preconditioning in mice. Up-regulation of miR-21 contributed to the protective effect of delayed ischemic preconditioning against subsequent renal ischemia-reperfusion injury*
 57. Kaucsár T, Révész C, Godó M, Krenács T, Albert M, Szalay CI et al (2013) Activation of the miR-17 family and miR-21 during murine kidney ischemia-reperfusion injury. *Nucleic Acid Ther* 23(5):344–354
 58. György B, Szabó TG, Pásztói M, Pál Z, Misják P, Aradi B et al (2011) Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles. *Cell Mol Life Sci* 68:2667–2688
 59. Akers JC, Gonda D, Kim R, Carter BS, Chen CC (2013) Biogenesis of extracellular vesicles (EV): exosomes, microvesicles,

- retrovirus-like vesicles and apoptotic bodies. *J Neurooncol* 113:1–11
60. • Kim HS, Choi DY, Yun SJ, Choi SM, Kang JW, Jung JW et al (2012) Proteomic analysis of microvesicles derived from human mesenchymal stem cells. *J Proteome Res* 11:839–849. *This paper provided the characterization of protein content of EVs produced by MSCs. 730 proteins were identified, including surface receptors (PDGFRB, EGFR, and PLAUR), signaling molecules controlling self-renewal, proliferation and differentiation of MSCs and exosome/EV production*
 61. Tomasoni S, Longaretti L, Rota C, Morigi M, Conti S, Gotti E et al (2013) Transfer of growth factor receptor mRNA via exosomes unravels the regenerative effect of mesenchymal stem cells. *Stem Cells Dev* 22:772–780
 62. Aliotta JM, Sanchez-Guijo FM, Dooner GJ, Johnson KW, Dooner MS, Greer KA et al (2007) Alteration of marrow cell gene expression, protein production, and engraftment into lung by lung-derived microvesicles: a novel mechanism for phenotype modulation. *Stem Cells* 25(9):2245–2256
 63. •• Xin H, Li Y, Buller B, Katakowski M, Zhang Y, Wang X et al (2012) Exosome-mediated transfer of miR-133b from multipotent mesenchymal stromal cells to neural cells contributes to neurite outgrowth. *Stem Cells* 30(7):1556–1564. *This study provided the first in vitro and in vivo demonstration that MSCs communicate with brain parenchymal cells and regulate neurite outgrowth by transfer of miR-133b via exosomes*
 64. Xin H, Li Y, Liu Z, Wang X, Shang X, Cui Y et al (2013) Mir-133b promotes neural plasticity and functional recovery after treatment of stroke with multipotent mesenchymal stromal cells in rats via transfer of exosome-enriched extracellular particles. *Stem Cells*. doi:10.1002/stem.1409
 65. Kim HW, Jiang S, Ashraf M, Haider KH (2012) Stem cell-based delivery of Hypoxamir-210 to the infarcted heart: implications on stem cell survival and preservation of infarcted heart function. *J Mol Med (Berl)* 90(9):997–1010
 66. • Yu B, Gong M, Wang Y, Millard RW, Pasha Z, Yang Y et al (2013) Cardiomyocyte protection by GATA-4 gene engineered mesenchymal stem cells is partially mediated by translocation of miR-221 in microvesicles. *PLoS One* 8(8):e73304. *In this study, the authors found that miR-221 translocation by EVs played an important role in cardio-protection mediated by GATA-4 over-expressed MSCs*
 67. Xu J, Wu W, Zhang L, Dorset-Martin W, Morris MW, Mitchell ME et al (2012) The role of microRNA-146a in the pathogenesis of the diabetic wound-healing impairment: correction with mesenchymal stem cell treatment. *Diabetes* 61(11):2906–2912