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REVIEW PAPER

Molecular signatures of secretomes from mesenchymal stem cells: therapeutic benefits

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Abstract Mesenchymal stem cells (MSCs) have been extensively used in both preclinical and clinical studies for a variety of diseases and injury. Accumulating evidence indicates that paracrine function through their secretomes is considered one of the primary attributes for MSC-mediated repair and regeneration. Secretomes from MSCs include both soluble factors and factors released within extracellular vesicles (EVs). Within EVs there are selective subsets of proteins, lipids, and nucleic acids that can modulate recipient cells and disease microenvironments. In this review, we summarize the current understanding of MSC-derived secretomes at molecular and therapeutic levels, focusing on their potential as novel cell-free therapies.

Keywords: Mesenchymal stem cell, Secretome, Molecular signature, Therapeutic benefit, Cell-free therapy

Introduction

Mesenchymal stem cells (MSCs) were originally identified in bone marrow (BM) as nonhematopoietic stromal cells with multipotent abilities[1-4]. In addition to BM, these cells can be isolated from nearly every organ or tissue in the body including adipose tissue, cord blood, and umbilical cords. In 2006 the International Society for Cellular Therapy (ISCT) defined MSCs as a heterogeneous stem cell population with the following characteristics: (1) adherence to plastic culture dishes with fibroblast-like morphology; (2) positive cell surface expression of CD105, CD73, and CD90, and negative for CD45, CD34, CD14, CD79 and HLA-DR; and (3) capacity for differentiation into osteoblasts, adipocytes, and chondroblasts *in vitro*[5]. Later several studies reported bone and cartilage formation *in vivo*[6] but differentiation into other cell types seems to be rare. In addition to their self-renewal and differentiation stem cell properties, MSCs have been shown to possess tissue protective and immunomodulatory functions[7].

With their great therapeutic potential, MSCs have been tested for their efficacy in numerous animal disease models. Promising results in preclinical studies have led to a rapid expansion of clinical trials with MSCs for treatment of a wide spectrum of pathological indications[8]. Initially, it has been suggested that a direct engraftment and differentiation into appropriate cell types in the damaged region is a major mechanism of action for their beneficial effects[9-13]. More recently, however, paracrine function is considered as one of the primary attributes for MSC-mediated repair and regeneration *in vivo*[14,15]. Consistent with this idea, several studies have demonstrated that administration of MSC-derived conditioned media (CM) had similar therapeutic benefits for myocardial infarction (MI), lung injury, chronic kidney disease, and brain damage[16-19]. MSC can release various growth factors and cytokines such as vascular endothelial growth factor, granulocyte colony-stimulating factor, platelet-derived growth factor, hepatocyte growth factor, insulin growth factor, monocyte chemotactic protein 1, stromal cell-derived factor and transforming growth factor both *in vitro* and *in vivo*[20-24]. Production of paracrine factors potentially modulates cellular processes including cell proliferation, differentiation, immunomodulation, migration, angiogenesis, and survival. However, none of these individual growth

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factors considerably explain the beneficial effects of MSCs[25]. Instead, accumulating evidence suggests that the secretomes from MSCs that includes both soluble factors and factors released within extracellular vesicles (EVs) mainly attributed to their efficacy[26]. Since the cargos are surrounded by lipid bilayer, EV contents could be protected from degradation.

Taken together it is critical to understand the functional molecules and related regulatory networks within MSC secretomes to better understand the paracrine activities of MSCs and ultimately to develop novel secretome-based cell free therapies.

Classes of MSC secretomes

Since its first discovery of secreted microvesicles ranging from 80 nm to 1 μm in size from MSCs[27], it has been known that there are at least three types of EVs in MSCs: exosomes, microvesicles, and apoptotic bodies[28]. They are classified by size, deposited cargo composition, and biogenesis pathway[25,29]. Exosomes are 30 to 120 nm in size and originate from the endosomal compartment, namely multivesicular bodies[30,31]. In contrast, microvesicles which are also known as ectosomes, are larger (with a diameter of 80-1,000 nm) and derived from direct budding of the plasma membrane[32]. Apoptotic bodies are more heterogenous in size, ranging from 50-5,000 nm in diameter and are formed by extensive blebbing during the course of apoptosis[33,34]. In terms of biological contents within EVs, both exosome and microvesicles carry a distinct subsets of proteins, lipids, DNA, mRNA and microRNAs (miRNAs)[35]. In contrast, apototic bodies mainly have fragmented DNA[34]. One of the key steps to understanding secretome function would be to identify the molecular nature of complex cargos and their regulatory networks.

Proteomic signature of MSC-derived secretomes

Principles of secretome proteomics

To further understand mechanisms driving the therapeutic impact of MSCs, significant effort was directed at systemic characterizations of secretome composition to evaluate the secreted factor's role in their therapeutic actions[36]. Proteomics has emerged as a robust technology for profiling high-throughput protein expressions, supplementing global gene expression analysis at RNA level[37-39]. Recent advances in proteomics have enabled molecular profiling of secretome proteins from MSCs, but some crucial steps in secretome analysis still remain challenging due to fundamental technical limitations such as collection of very small quantities of secreted proteins as well as restriction to serum- and protein-free cell culture conditions[40].

The step for CM preparation in serum-free media to avoid contamination of serum proteins is a key procedure in secretome analysis. Secretome profiles of CM are differentiated depending on how the CM was prepared from MSCs[41]. These results indicate the importance of optimizing CM preparation step prior to any proteomics[41].

Proteomic identification of MSC secretomes

Since the first analysis of human MSC secretomes via proteomics in 2003[40,42], diverse examinations with molecular profiling have allowed the elucidation of connections between secreted proteins from MSCs and their therapeutic functions. To date, secretomes from a number of different tissues, such as bone marrow, adipose tissue, umbilical cord, dental apical papilla and human brain, have been analyzed via proteomic approaches[43-45].

Choi *et al*. identified 410 secretory protein profiles of bone marrow-derived MSCs (BMSCs) grown in osteogenic medium (OSM) by LC-ESI-MS/MS[46]. Among the identified proteins, 64 of which were selectively secreted by high osteogenic potential BMSCs, SPARC-related modular calcium-binding protein 1 (SMOC1) was prominently expressed and secreted in BMSCs stimulated with OSM. In addition, knockdown of SMOC1 using shRNA remarkably reduced mineralization and the expression of osteoblast differentiation markers, while overexpression of SMOC1 significantly enhanced the expression of osteoblast differentiation-specific genes. Thus, SMOC1 was suggested as a putative regulator of osteoblast differentiation of BMSCs through proteomic tools.

Diverse proteomic examinations in which adipose derived-MSCs (ADSCs) might be associated with beneficial effects of tissue repair, immunomodulation, angiogenesis and regeneration, have been attempted. As reviewed by Kapur and Katz[47], 68 commonly expressed proteins in ADSC secretomes were suggested to be involved in differentiation of ADSCs[48-51]. Interestingly, Serpine 1 (Plasminogen activator inhibitor-1, PAI-1) was the differentially expressed proteins detected in all reports analyzed.

In addition, the secretome of ADSCs significantly inhibited the lipopolysaccharide (LPS)-driven microglia activation via regulation of sphingosine kinase/S1P signaling[52]. This study suggested the potential of ADSC secretomes for cell-free therapeutics for diseases involving excessive microglial activation like neurodegenerative diseases.

MSCs share common features with neural stem cells (NSCs) such as secretion of common growth factors including nerve growth factor and their association

Table 1. Molecular and cellular functions associated with human bone marrow MSC-derived microvesicles[a].

Biological functions	*p*-value	No. of molecules (42 mRNAs total[b])
Cell death and survival	4.45E-02 - 1.76E-04	21
Cell-to-cell signaling and interaction	4.45E-02 - 3.42E-04	10
Cellular growth and proliferation	4.93E-02 - 4.50E-04	16
Cellular assembly and organization	4.83E-02 - 5.09E-04	5
Cellular function and maintenance	4.93E-02 - 5.09E-04	10

[a] Data generated using IPA software.
[b] List of 42 mRNAs enriched in MSC-derived microvesicles from Bruno *et al*.[27].

with bone morphogenetic protein family in their differentiation. In addition, they interact in the neurogenic niches. Thus, MSC secretomes might be utilized in the development of therapeutics for CNS related diseases[53]. Their therapeutic potentials have been reported in animal models of Parkinson's disease, ischemic stroke, and glioblastoma multiforms[53].

The potential of human MSCs from dental apical papilla (SCAPs) secretome for tissue regeneration and therapeutic application was also suggested by Yu *et al*., using isobaric chemical tags and high-performance liquid chromatography with tandem mass spectrometry[54]. 151 secreted proteins in SCAPs include chemokines, angiogenic, immunomodulatory, antiapoptotic, neuroprotective factors, and extracellular matrix (ECM) proteins. Notably, compared to secretomes from BMSCs, the secreted proteins involved in metabolic processes and transcription were higher, whereas those associated with biological adhesion, developmental processes, and immune function were lower. In addition, the SCAPs secretome contained highly elevated levels of chemokines and neurotrophins than BMSCs but fewer ECM proteins and proangiogenic factors.

By using antibody array, a comprehensive cytokine secretion profile of human BMSCs was conducted[55]. With the use of antibody array recognizing 120 cytokines and chemokines, a predominant hybridization signal for IL-6 and moderately elevated signals for IL-8, TIMP-2, MCP-1, VEGF and OPG were obtained. This result also showed distinct features of MSCs with different cell origins, but not with donor individuality.

Transcriptomic profiles of MSC-derived secretomes

mRNAs enriched in MSC-derived EVs

EVs secreted from MSCs showed improvement of neovascularization in a mouse hindlimb ischemia model[56] and promotion of proliferation and survival of epithelial cells in an acute kidney injury (AKI) mouse[27]. Interestingly, when EVs were treated with RNase, they no longer possessed the protective functions both *in vitro* and *in vivo*, which indicates their RNA-dependent effects[27,57]. To identify RNAs in EVs, Bruno *et al*. performed microarray analysis of human BMSC-derived microvesicles and found that 132 transcripts were selectively expressed in microvesicles[27]. With the list of mRNAs from their study, we performed bioinformatics analysis using the Ingenuity Pathway Analysis (IPA, http://www.ingenuity.com/) software to understand the regulatory functions of EV-enriched mRNAs. Intriguingly, mRNAs showed significant enrichment of their molecular and cellular functions related to cell death and survival, cell-to-cell signaling and interaction, cellular growth and proliferation, and cellular assembly and organization (Table 1). Considering EVs as messengers of intercellular communication, selective enrichment of functional mRNAs in EVs could in part explain the underlying mechanisms of therapeutic effects of MSCs. Furthermore, there is growing evidence suggesting mRNA cargos are selectively loaded. Transcriptomic profiles are different between EVs and parental cells[27,58,59]. It would be interesting to know how mRNAs are selected during EVs formation.

miRNAs enriched in MSC-derived EVs

In addition to mRNAs, EVs often contain miRNAs, small non-coding RNAs that regulate target mRNAs at the post-transcriptional level[60]. It has been known that a single miRNA could repress thousands of genes simultaneously, which suggests miRNAs are key regulators in almost all biological processes[61-64]. Using qRT-PCR, microarray, and next generation sequencing, much effort has been made to identify miRNAs selectively enriched in human MSC-derived EVs compared to parental cells[65-74]. For example, miR-21 and miR-34a were highly expressed in EVs from human BMSCs under serum deprivation conditions[75]. Inhibition of miRNAs using locked nucleic acid (LNA) inhibitors induces cell death, which indicates their tumor supportive functions in cell proliferation and survival[75]. In addition to tumor regulating miRNAs, several EV-derived miRNAs have other functions. For example, miR-24 was shown to have regenerative effects in

AKI[76] and MI[77]. Consistent with these results, miR-24 can inhibit apoptosis[78] and vascular inflammation[79]. Furthermore, developmentally conserved miRNAs, namely the let-7 family, is frequently found in EVs from various species[59,70,74]. Interestingly, the expression of let-7a changes during osteogenic differentiation in MSCs[73] and in some cases, let-7i showed high expression levels in recipient cells pretreated with MSC-derived EVs[80]. Taken together, these results support the idea that miRNAs play an essential role in the diverse functions of EVs both in parental and recipient cells.

Potential functional networks of miRNAs-mRNAs in EVs

The central role of MSC-derived EVs in numerous disease microenvironments is a communication mediator from MSCs to the recipient cells. By secreting bioactive molecules, EVs can modulate cell and organ functions. Distinct subsets of proteins, mRNAs, and miRNAs are well defined in MSC-derived EVs, but a comprehensive understanding of regulatory pathways and/or networks need to be done.

A recent review by Nargesi *et al*. summarized miRNAs consistently enriched in human MSC-derived EVs: miR-34a, miR-302b, miR-451, miR-191, miR-143, miR-22, and miR-21[81]. Since a single miRNA regulates multiple targets, it could be a tool to dissect mechanisms of complex functions of EVs. To this end, we set out to determine regulatory networks of mRNA targets of those 7 miRNAs. A bioinformatics analysis using TargetScan and IPA program identified total of 955 high confidence target mRNAs (Table 2). Disease and functional analysis of these selected target genes revealed that they are related to cancer, organismal injury and abnormalities, tumor morphology, cellular development, cellular growth and proliferation, cellular movement, and cell cycles (Table 3). Furthermore, cellular function and maintenance, molecular transport, and small molecule biochemistry turned out to be a top associated network for these predicted targets (Figure 1). Taken together, the diverse biological and therapeutic functions of secretomes can be revealed in part by understanding the complex miRNA-mRNA networks.

Translation of secretomes

Accumulating promising results from preclinical studies support the idea of developing the secretome-based, cell-free therapies for clinical settings. In contrast to cell-based therapies, secretomes have critical advantages for clinical applications. First, the secretomes have much lower expression of cell surface proteins, which provide low immunogenicity. Indeed, safety evaluation studies showed that intravenous infusion of human MSCs-derived exosomes was well tolerated in different kinds of animal models[82]. Second, secretomes are isolated from cells including MSCs at certain time point during culturing and there is less chance to be affected by genetic instability caused by cellular senescence[83]. Third, secretomes can be stored at a lower temperature, more stable than cellular counterparts.

Table 2. List of miRNAs commonly enriched in human MSC-derived EVs and numbers of their putative targets[a].

miRNAs	Seed sequence	No. of targeted mRNAs
has-miR-143-3p	GAGAUGA	130
has-miR-191-5p	AACGGAA	45
has-miR-21-5p	AGCUUAU	86
has-miR-22-5p	GUUCUUC	105
has-miR-302b-3p	AAGUGCU	159
has-miR-34a-5p	GGCAGUG	422
has-miR-451a	AACCGUU	34

[a] Data generated using IPA software

Table 3. Top diseases and biofunctions associated with putative mRNA targets of human EV-enriched miRNAs[a].

Name	*p*-value	No. of molecules
Diseases and disorders		
Caner	7.19E-04 - 1.00E-18	833
Organismal injury and abnormalities	7.27E-04 - 1.00E-18	848
Tumor morphology	5.48E-04 - 1.52E-08	86
Gastrointestinal disease	5.89E-04 - 3.55E-08	688
Developmental disorder	5.51E-04 - 3.95E-07	111
Molecular and cellular functions		
Cellular development	7.12E-04 - 2.48E-09	292
Cellular growth and proliferation	6.27E-04 - 2.48E-09	253
Cellular movement	7.14E-04 - 3.96E-09	206
Cell cycle	7.12E-04 - 5.85E-09	142
Cellular function and maintenance	5.52E-04 - 7.10E-08	170

[a] Data generated using IPA software.

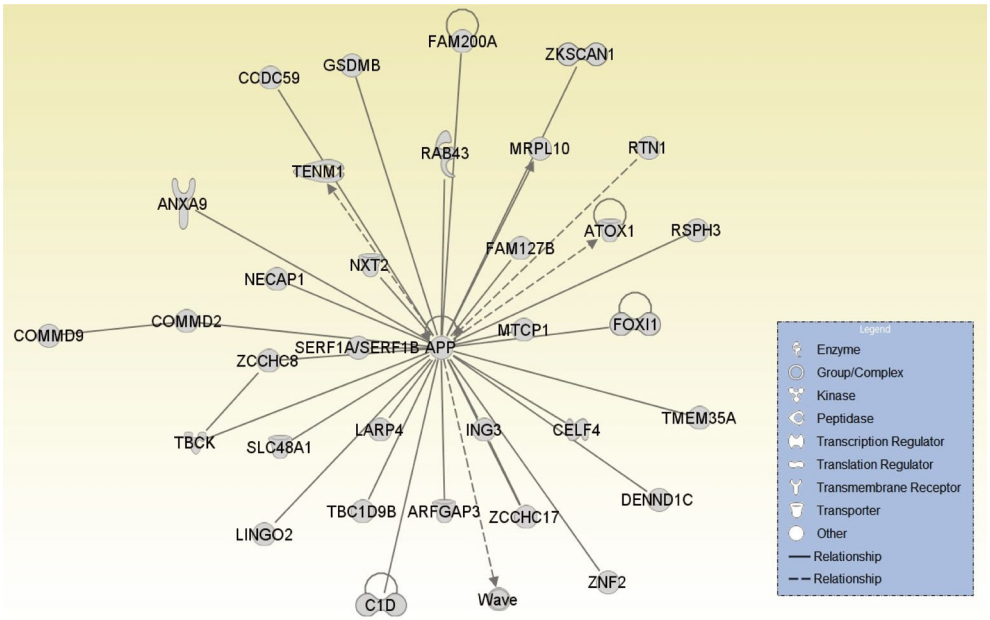

Figure 1. A key network associated with putative mRNA targets of human EV-enriched miRNAs. Using IPA software, a key network that might be regulated by human EV-enriched miRNAs is shown.

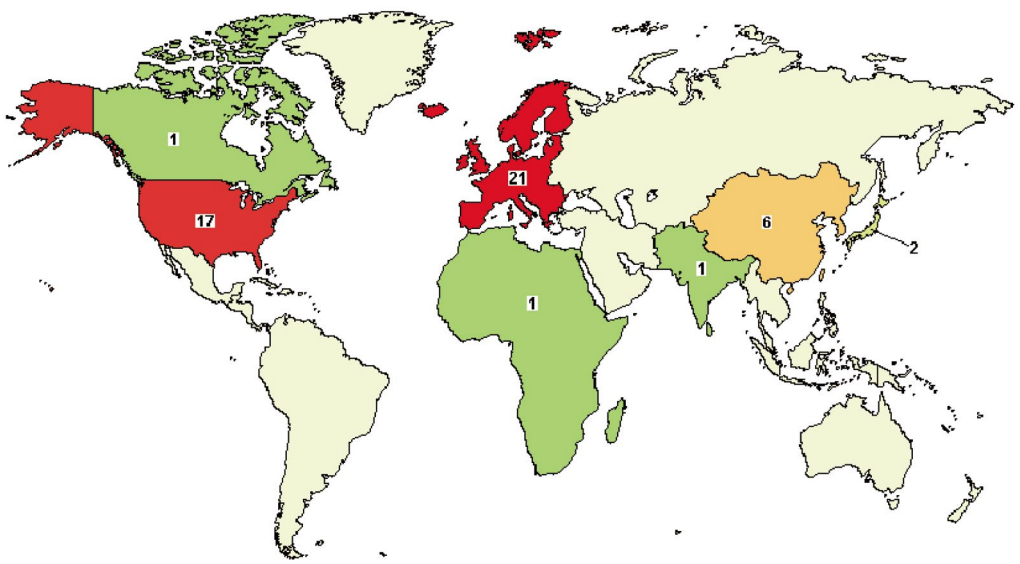

Figure 2. Worldwide clinical trials of exosome therapies. World map showing locations of clinical trials of exosomes (www.clinicaltrials.gov).

Last, the clinical application of secretomes might require more straightforward safety regulations[84].

At present, the exosome is the most characterized among different types of secretomes in both preclinical and clinical studies. According to the United States government-sponsored database (www.clinicaltrials.gov), there are 52 clinical trials with the term "exosome". They are carried out world-wide but mostly in Europe (14 cases) and United States (10 cases) (Figure 2). Targeted conditions include cancer, bacterial and fungal disease, digestive system disease, brain disease, heart and blood disease, respiratory tract disease, and skin disease. However, there is only one study using MSC-derived exosomes in a clinical trial. A phase 1

clinical trial has been done on type I diabetes mellitus (T1DM) using exosomes from human cord-blood derived MSCs (ClinicalTrails.gov identifier: NCT 02138331). This group hypothesized that the anti-inflammatory effect of exosomes from MSCs would reduce the inflammatory state in T1DM. Other studies are currently investigating the immunotherapeutic and vaccination effects of dendritic cell-derived exosomes in non-small cell lung cancer (ClinicalTrails.gov identifier: NCT01159288)[85]. Notably, 15 studies use exosomes as biomarkers in diverse disease conditions, reflecting another important aspect of exosomes[86].

Conclusion

In general, development of novel therapies takes considerable time. Before they are administered to humans, molecular and therapeutic mechanisms must be defined. To date, there is a rapid expansion of both preclinical and clinical studies using MSC-derived secretomes with the advantages of a cell-free system. It appears to be promising therapy for diverse disease or injury but there is still more to be learned about the generation, nature, and modification of MSC-derived secretomes. The potential for therapeutic effects may be optimized by pre-conditioning MSCs with small molecules, biological agents, and biomaterials, and then collect secretomes from them. In addition, secretomes might be directly engineered to selectively load certain factors to treat target diseases more effectively. These strategies will maximize treatment benefits of any kind of secretome from MSCs in the future.

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Conflict of Interest Nayoung Suh declares no conflict of interest. Deepa Subramanyam declares no conflict of interest. Mi-Young Lee declares no conflict of interest.

References

1. Friedenstein, A. J., Piatetzky-Shapiro, I. I. & Petrakova, K. V. Osteogenesis in transplants of bone marrow cells. *J Embryol Exp Morphol* **16**:381-390 (1966).
2. Friedenstein, A. J., Chailakhjan, R. K. & Lalykina, K. S. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. *Cell Tissue Kinet* **3**:393-403 (1970).
3. Friedenstein, A. J., Gorskaja, J. F. & Kulagina, N. N. Fibroblast precursors in normal and irradiated mouse hematopoietic organs. *Exp Hematol* **4**:267-274 (1976).
4. Friedenstein, A. J., Chailakhyan, R. K. & Gerasimov, U. V. Bone marrow osteogenic stem cells: *in vitro* cultivation and transplantation in diffusion chambers. *Cell Tissue Kinet* **20**:263-272 (1987).
5. Dominici, M. *et al*. Minimal criteria for defining multipotent mesenchymal stromal cells. The international society for cellular therapy position statement. *Cytotherapy* **8**:315-317 (2006).
6. Bianco, P., Robey, P. G. & Simmons, P. J. Mesenchymal stem cells: revisiting history, concepts, and assays. *Cell Stem Cell* **2**:313-319 (2008).
7. Li, N. & Hua, J. Interactions between mesenchymal stem cells and the immune system. *Cell Mol Life Sci*, doi:10.1007/s00018-017-2473-5 (2017).
8. Daley, G. Q. The promise and perils of stem cell therapeutics. *Cell Stem Cell* **10**:740-749 (2012).
9. Kopen, G. C., Prockop, D. J. & Phinney, D. G. Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. *Proc Natl Acad Sci U S A* **96**:10711-10716 (1999).
10. Mackenzie, T. C. & Flake, A. W. Human mesenchymal stem cells persist, demonstrate site-specific multipotential differentiation, and are present in sites of wound healing and tissue regeneration after transplantation into fetal sheep. *Blood Cells Mol Dis* **27**: 601-604 (2001).
11. Prockop, D. J. Repair of tissues by adult stem/progenitor cells (MSCs): controversies, myths, and changing paradigms. *Mol Ther* **17**:939-946 (2009).
12. Lee, J. S. *et al*. Transplantation of human mesenchymal stem cells into the cisterna magna and its neuroprotective effects in a parkinsonian animal model. *Mol Cell Toxicol* **11**:373-385 (2015).
13. Kim, K. W. *et al*. Osteogenic differentiation of human mesenchymal stem cells promoted by the crude extracts of the mixture of *Cortex mori* radicis, *Patrinia saniculaefolia*. *Mol Cell Toxicol* **11**:475-482 (2015).
14. Caplan, A. I. Why are MSCs therapeutic? New data: new insight. *J Pathol* **217**:318-324 (2009).
15. Prockop, D. J., Kota, D. J., Bazhanov, N. & Reger, R. L. Evolving paradigms for repair of tissues by adult stem/progenitor cells (MSCs). *J Cell Mol Med* **14**:2190-2199 (2010).
16. Timmers, L. *et al*. Reduction of myocardial infarct size by human mesenchymal stem cell conditioned medium. *Stem Cell Res* **1**:129-137 (2007).
17. Aslam, M. *et al*. Bone marrow stromal cells attenuate lung injury in a murine model of neonatal chronic lung disease. *Am J Respir Crit Care Med* **180**:1122-1130 (2009).

18. Cheng, K. *et al*. Transplantation of bone marrow-derived MSCs improves cisplatinum-induced renal injury through paracrine mechanisms. *Exp Mol Pathol* **94**:466-473 (2013).
19. van Koppen, A. *et al*. Human embryonic mesenchymal stem cell-derived conditioned medium rescues kidney function in rats with established chronic kidney disease. *PLoS One* **7**:e38746 (2012).
20. Togel, F. *et al*. Administered mesenchymal stem cells protect against ischemic acute renal failure through differentiation-independent mechanisms. *Am J Physiol Renal Physiol* **289**:F31-42 (2005).
21. Kinnaird, T., Stabile, E., Burnett, M. S. & Epstein, S. E. Bone-marrow-derived cells for enhancing collateral development: mechanisms, animal data, and initial clinical experiences. *Circ Res* **95**:354-363 (2004).
22. Nakagami, H. *et al*. Novel autologous cell therapy in ischemic limb disease through growth factor secretion by cultured adipose tissue-derived stromal cells. *Arterioscler Thromb Vasc Biol* **25**:2542-2547 (2005).
23. Van Overstraeten-Schlogel, N., Beguin, Y. & Gothot, A. Role of stromal-derived factor-1 in the hematopoietic-supporting activity of human mesenchymal stem cells. *Eur J Haematol* **76**:488-493 (2006).
24. Lavoie, J. R. & Rosu-Myles, M. Uncovering the secretes of mesenchymal stem cells. *Biochimie* **95**:2212-2221 (2013).
25. Lai, R. C., Yeo, R. W. & Lim, S. K. Mesenchymal stem cell exosomes. *Semin Cell Dev Biol* **40**:82-88 (2015).
26. Konala, V. B. *et al*. The current landscape of the mesenchymal stromal cell secretome: a new paradigm for cell-free regeneration. *Cytotherapy* **18**:13-24 (2016).
27. Bruno, S. *et al*. Mesenchymal stem cell-derived microvesicles protect against acute tubular injury. *J Am Soc Nephrol* **20**:1053-1067 (2009).
28. Lai, R. C. *et al*. MSC secretes at least 3 EV types each with a unique permutation of membrane lipid, protein and RNA. *J Extracell Vesicles* **5**:29828 (2016).
29. Rani, S., Ryan, A. E., Griffin, M. D. & Ritter, T. Mesenchymal stem cell-derived extracellular vesicles: toward cell-free therapeutic applications. *Mol Ther* **23**:812-823 (2015).
30. Mathivanan, S., Ji, H. & Simpson, R. J. Exosomes: extracellular organelles important in intercellular communication. *J Proteomics* **73**:1907-1920 (2010).
31. Gyorgy, B. *et al*. Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles. *Cell Mol Life Sci* **68**:2667-2688 (2011).
32. Cocucci, E., Racchetti, G. & Meldolesi, J. Shedding microvesicles: artefacts no more. *Trends Cell Biol* **19**: 43-51 (2009).
33. Coleman, M. L. *et al*. Membrane blebbing during apoptosis results from caspase-mediated activation of ROCK I. *Nat Cell Biol* **3**:339-345 (2001).
34. Stolzing, A. & Grune, T. Neuronal apoptotic bodies: phagocytosis and degradation by primary microglial cells. *FASEB J* **18**:743-745 (2004).
35. Mathivanan, S., Fahner, C. J., Reid, G. E. & Simpson, R. J. ExoCarta 2012: database of exosomal proteins, RNA and lipids. *Nucleic Acids Res* **40**:D1241-1244 (2012).
36. Teixeira, F. G., Carvalho, M. M., Sousa, N. & Salgado, A. J. Mesenchymal stem cells secretome: a new paradigm for central nervous system regeneration? *Cell Mol Life Sci* **70**:3871-3882 (2013).
37. Kupcova Skalnikova, H. Proteomic techniques for characterisation of mesenchymal stem cell secretome. *Biochimie* **95**:2196-2211 (2013).
38. Sekhon, S. S., Ahn, J. Y., Min, J. & Kim, Y. H. Toxicoproteomic approaches for analysis of microbial community inhabiting Asian dust particles. *Mol Cell Toxicol* **11**:287-294 (2015).
39. Kim, J. H., Ryu, A. R., Kang, M. J. & Lee, M. Y. Berberine-induced changes in protein expression and antioxidant enzymes in melanoma cells. *Mol Cell Toxicol* **12**:53-61 (2016).
40. Tran, C. & Damaser, M. S. Stem cells as drug delivery methods: application of stem cell secretome for regeneration. *Adv Drug Deliv Rev* **82-83**:1-11 (2015).
41. Clabaut, A., Grare, C., Léger, T., Hardouin, P. & Broux, O. Variations of secretome profiles according to conditioned medium preparation: the example of human mesenchymal stem cell-derived adipocytes. *Electrophoresis* **36**:2587-2593 (2015).
42. Potian, J. A., Aviv, H., Ponzio, N. M., Harrison, J. S. & Rameshwar, P. Veto-like activity of mesenchymal stem cells: functional discrimination between cellular responses to alloantigens and recall antigens. *J Immunol* **171**:3426-3434 (2003).
43. Lee, M. J. *et al*. Proteomic analysis of tumor necrosis factor-alpha-induced secretome of human adipose tissue-derived mesenchymal stem cells. *J Proteome Res* **9**:1754-1762 (2010).
44. Skalnikova, H., Motlik, J., Gadher, S. J. & Kovarova, H. Mapping of the secretome of pimary isolates of mammalian cells, stem cells and derived cell lines. *Proteomics* **11**:691-708 (2011).
45. Konala, V. B. *et al*. The current landscape of the mesenchymal stromal cell secretome: a new paradigm for cell-free regeneration. *Cytotherapy* **18**:13-24 (2016).
46. Choi, Y. A. *et al*. Secretome analysis of human BMSCs and identification of SMOC1 as an important ECM protein in osteoblast differentiation. *J Proteome Res* **9**: 2946-2956 (2010).
47. Kapur, S. K. & Katz, A. J. Review of the adipose derived stem cell secretome. *Biochimie* **95**:2222-2228 (2013).
48. Zvonic, S. *et al*. Secretome of primary cultures of human adipose-derived stem cells: modulation of serpins by adipogenesis. *Mol Cell Proteomics* **6**:18-28 (2007)
49. Mutch, D. M., Rouault, C., Keophiphath, M., Lacasa, D. & Clément, K. Using gene expression to predict the secretome of differentiating human preadipocytes. *Int J Obes (Lond)* **33**:354-363 (2009).
50. Kim, J. *et al*. Comparative analysis of the secretory

proteome of human adipose stromal vascular fraction cells during adipogenesis. *Proteomics* **10**:394-405 (2010).
51. Zhong, J. *et al*. Temporal profiling of the secretome during adipogenesis in humans. *J Proteome Res* **9**: 5228-5238 (2010).
52. Marfia, G. *et al*. The adipose mesenchymal stem cell secretome inhibits inflammatory responses of microglia: evidence for an involvement of sphingosine-1-phosphate signalling. *Stem Cells Dev* **25**:1095-1107 (2016).
53. Antonio J. S. *et al*. Mesenchymal stem cells secretome as a modulator of the neurogenic niche: basic insights and therapeutic opportunities. *Front Cell Neurosci* **9**: 249 (2015).
54. Yu, S., Zhao, Y., Ma, Y. & Ge, L. Profiling the secretome of human stem cells from dental apical papilla. *Stem Cells Dev* **25**:499-508 (2016).
55. Park, C. W. *et al*. Cytokine secretion profiling of human mesenchymal stem cells by antibody array. *Int J Stem Cells* **2**:59-68 (2009).
56. Ranghino, A. *et al*. Endothelial progenitor cell-derived microvesicles improve neovascularization in a murine model of hindlimb ischemia. *Int J Immunopathol Pharmacol* **25**:75-85 (2012).
57. Deregibus, M. C. *et al*. Endothelial progenitor cell derived microvesicles activate an angiogenic program in endothelial cells by a horizontal transfer of mRNA. *Blood* **110**:2440-2448 (2007).
58. Tomasoni, S. *et al*. Transfer of growth factor receptor mRNA via exosomes unravels the regenerative effect of mesenchymal stem cells. *Stem Cells Dev* **22**:772-780 (2013).
59. Eirin, A. *et al*. MicroRNA and mRNA cargo of extracellular vesicles from porcine adipose tissue-derived mesenchymal stem cells. *Gene* **551**:55-64 (2014).
60. Fabian, M. R. & Sonenberg, N. The mechanics of miRNA-mediated gene silencing: a look under the hood of miRISC. *Nat Struct Mol Biol* **19**:586-593 (2012).
61. Bartel, D. P. MicroRNAs: target recognition and regulatory functions. *Cell* **136**:215-233 (2009).
62. Cha, H. J. *et al*. MicroRNA expression profiling of p-phenylenediamine treatment in human keratinocyte cell line. *Mol Cell Toxicol* **11**:19-28 (2015).
63. Son, G. W. *et al*. Analysis of miRNA expression profiling in melatonin-exposured endothelial cells. *Mol Cell Toxicol* **12**:73-81 (2016).
64. Lee, W. *et al*. Expression profiling of microRNAs in lipopolysaccharide induced acute lung injury after hypothermia treatment. *Mol Cell Toxicol* **12**:243-253 (2016).
65. Ono, M. *et al*. Exosomes from bone marrow mesenchymal stem cells contain a microRNA that promotes dormancy in metastatic breast cancer cells. *Sci Signal* **7**:ra63 (2014).
66. Nakamura, Y. *et al*. Mesenchymal-stem-cell-derived exosomes accelerate skeletal muscle regeneration. *FEBS Lett* **589**:1257-1265 (2015).
67. Muntion, S. *et al*. Microvesicles from mesenchymal stromal cells are involved in HPC-microenvironment crosstalk in myelodysplastic patients. *PLoS One* **11**: e0146722 (2016).
68. Qin, Y., Wang, L., Gao, Z., Chen, G. & Zhang, C. Bone marrow stromal/stem cell-derived extracellular vesicles regulate osteoblast activity and differentiation *in vitro* and promote bone regeneration *in vivo*. *Sci Rep* **6**:21961 (2016).
69. Li, X. *et al*. Exosome derived from human umbilical cord mesenchymal stem cell mediates miR-181c attenuating burn-induced excessive inflammation. *EBioMedicine* **8**:72-82 (2016).
70. Garcia-Contreras, M., Vera-Donoso, C. D., Hernandez-Andreu, J. M., Garcia-Verdugo, J. M. & Oltra, E. Therapeutic potential of human adipose-derived stem cells (ADSCs) from cancer patients: a pilot study. *PLoS One* **9**:e113288 (2014).
71. Phinney, D. G. *et al*. Mesenchymal stem cells use extracellular vesicles to outsource mitophagy and shuttle microRNAs. *Nat Commun* **6**:8472 (2015).
72. Baglio, S. R. *et al*. Human bone marrow- and adipose-mesenchymal stem cells secrete exosomes enriched in distinctive miRNA and tRNA species. *Stem Cell Res Ther* **6**:127 (2015).
73. Xu, J. F. *et al*. Altered microRNA expression profile in exosomes during osteogenic differentiation of human bone marrow-derived mesenchymal stem cells. *PLoS One* **9**:e114627 (2014).
74. Ti, D. *et al*. LPS-preconditioned mesenchymal stromal cells modify macrophage polarization for resolution of chronic inflammation via exosome-shuttled let-7b. *J Transl Med* **13**:308 (2015).
75. Vallabhaneni, K. C. *et al*. Extracellular vesicles from bone marrow mesenchymal stem/stromal cells transport tumor regulatory microRNA, proteins, and metabolites. *Oncotarget* **6**:4953-4967 (2015).
76. Collino, F. *et al*. AKI recovery induced by mesenchymal stromal cell-derived extracellular vesicles carrying microRNAs. *J Am Soc Nephrol* **26**:2349-2360 (2015).
77. Shao, L. *et al*. miRNA-sequence indicates that mesenchymal stem cells and exosomes have similar mechanism to enhance cardiac repair. *Biomed Res Int* **2017**:4150705 (2017).
78. Qian, L. *et al*. miR-24 inhibits apoptosis and represses Bim in mouse cardiomyocytes. *J Exp Med* **208**:549-560 (2011).
79. Maegdefessel, L. *et al*. miR-24 limits aortic vascular inflammation and murine abdominal aneurysm development. *Nat Commun* **5**:5214 (2014).
80. Zhang, Z. *et al*. Pretreatment of cardiac stem cells with exosomes derived from mesenchymal stem cells enhances myocardial repair. *J Am Heart Assoc* **5**: e002856 (2016).
81. Nargesi, A. A., Lerman, L. O. & Eirin, A. Mesenchymal stem cell-derived extracellular vesicles for renal repair. *Curr Gene Ther* doi:10.2174/1566523217666170412110724 (2017).
82. Sun, L. *et al*. Safety evaluation of exosomes derived

from human umbilical cord mesenchymal stromal cell. *Cytotherapy* **18**:413-422 (2016).
83. Binato, R. *et al*. Stability of human mesenchymal stem cells during *in vitro* culture: considerations for cell therapy. *Cell Prolif* **46**:10-22 (2013).
84. Kordelas, L. *et al*. MSC-derived exosomes: a novel tool to treat therapy-refractory graft-versus-host disease. *Leukemia* **28**:970-973 (2014).
85. Viaud, S. *et al*. Dendritic cell-derived exosomes for cancer immunotherapy: what's next? *Cancer Res* **70**: 1281-1285 (2010).
86. Kourembanas, S. Exosomes: vehicles of intercellular signaling, biomarkers, and vectors of cell therapy. *Annu Rev Physiol* **77**:13-27 (2015).