Koh, W., 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 Suppl 1: S6.

BACKGROUND: Recent literature has revealed that genetic exchange of microRNA between cells can be essential for cell-cell communication, tissue-specificity and developmental processes. In stem cells, as in other cells, this can be accomplished through microvesicles or exosome mediated transfer. However, molecular profiles and functions of microRNAs within the cells and in their exosomes are poorly studied. Next generation sequencing technologies could provide a broad-spectrum of microRNAs and their expression and identify possible microRNA targets. In this work, we performed deep sequencing of microRNAs to understand the profile and expression of the microRNAs in microvesicles and intracellular environment of human embryonic stem cells derived mesenchymal stem cells (hES-MSC). We outline a workflow pertaining to visualizing, statistical analysis and interpreting deep sequencing data of known intracellular and extracellular microRNAs from hES-MSC). We utilized these results of which directed our attention towards establishing hepatic nuclear factor 4 alpha (HNF4A) as a downstream target of let-7 family of microRNAs. RESULTS: In our study, significant differences in expression profile of microRNAs were found in the intracellular and extracellular environment of hES-MSC. However, a high level of let-7 family of microRNAs is predominant in both intra- and extra- cellular samples of hES-MSC. Further results derived from visualization of our alignment data and network analysis showed that let-7 family microRNAs could affect the downstream target HNF4A, which is a known endodermal differentiation marker. The elevated presence of let-7 microRNA in both intracellular and extra cellular environment further suggests a possible intercellular signalling mechanism through microvesicles transfer. We suggest that let-7 family microRNAs might play a signalling role via such a mechanism amongst populations of stem cells in maintaining self renewal property by suppressing HNF4A expression. This is in line with recent paradigm where microRNAs regulate self-renewal and differentiation pathways of embryonic stem cells by forming an integral biological network with transcription factors. CONCLUSION: In summary, our study using a combination of alignment, statistical and network analysis tools to examine deep sequencing data of microRNAs in hES-MSC has led to a result that (i) identifies intracellular and exosome microRNA expression profiles of hES-MSC with a possible mechanism of miRNA mediated intercellular regulation by these cells and (ii) placed HNF4A within the cross roads of regulation by the let-7 family of microRNAs.

Bauer, N., et al. (2011). "Haematopoietic stem cell differentiation promotes the release of prominin-1/CD133-containing membrane vesicles--a role of the endocytic-exocytic pathway." EMBO Mol Med 3(7): 398-409.

The differentiation of stem cells is a fundamental process in cell biology and understanding its mechanism might open a new avenue for therapeutic strategies. Using an ex vivo co-culture system consisting of human primary haematopoietic stem and progenitor cells growing on multipotent mesenchymal stromal cells as a feeder cell layer, we describe here the exosome-mediated release of small membrane vesicles containing the stem and cancer stem cell marker prominin-1 (CD133) during haematopoietic cell differentiation. Surprisingly, this contrasts with the budding mechanism underlying the release of this cholesterol-binding protein from plasma membrane protrusions of neural progenitors. Nevertheless, in both progenitor cell types, protein-lipid assemblies might be the essential structural determinant in the release process of prominin-1. Collectively, these data support the concept that prominin-1-containing lipid rafts may host key determinants necessary to maintain stem cell properties and their quantitative reduction or loss may result in cellular differentiation.

Xin, H., 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.

Multipotent mesenchymal stromal cells (MSCs) have potential therapeutic benefit for the treatment of neurological diseases and injury. MSCs interact with and alter brain parenchymal cells by direct cell-cell communication and/or by indirect secretion of factors and thereby promote functional recovery. In this study, we found that MSC treatment of rats subjected to middle cerebral artery occlusion (MCAo) significantly increased microRNA 133b (miR-133b) level in the ipsilateral hemisphere. In vitro, miR-133b levels in MSCs and in their exosomes increased after MSCs were exposed to ipsilateral ischemic tissue extracts from rats subjected to MCAo. miR-133b levels were also increased in primary cultured neurons and astrocytes treated with the exosome-enriched fractions released from these MSCs. Knockdown of miR-133b in MSCs confirmed that the increased miR-133b level in astrocytes is attributed to their transfer from MSCs. Further verification of this exosome-mediated intercellular communication was performed using a cel-miR-67 luciferase reporter system and an MSC-astrocyte coculture model. Cel-miR-67 in MSCs was transferred to astrocytes via exosomes between 50 and 100 nm in diameter. Our data suggest that the cel-miR-67 released from MSCs was primarily contained in exosomes. A gap junction intercellular communication inhibitor arrested the exosomal microRNA communication by inhibiting exosome release. Cultured neurons treated with exosome-enriched fractions from MSCs exposed to 72 hours post-MCAo brain extracts significantly increased the neurite branch number and total neurite length. This study provides the first demonstration that MSCs communicate with brain parenchymal cells and may regulate neurite outgrowth by transfer of miR-133b to neural cells via exosomes.

Chen, T. S. and S. K. Lim (2013). "Measurement of precursor miRNA in exosomes from human ESC-derived mesenchymal stem cells." Methods Mol

Biol 1024: 69-86.

Mesenchymal stem cells (MSCs) derived from human embryonic stem cells (ESCs) have been shown to secrete exosomes that are cardioprotective against myocardial ischemia reperfusion injury in a mouse model. To elucidate this cardioprotective mechanism, we have characterized the protein, nucleic acid, and lipid composition of MSC exosomes. Here we describe the isolation and analysis of RNA in MSC exosome. We have previously reported that RNAs in MSC exosome are primarily small RNA molecules of <300 nt and they include many miRNAs. Many of these miRNAs are in the precursor form suggesting that pre-miRNAs, and not mature miRNAs are preferentially loaded into exosomes. The protocols described here include assays to ascertain the presence of pre-miRNAs, profiling of miRNA and pre-miRNA, and quantitative estimation of mature and pre-miRNA.

Greco, S. J. and P. Rameshwar (2013). "Analysis of the transfer of circulating microRNA between cells mediated by gap junction." Methods Mol Biol 1024: 87-96.

A significant breakthrough in the field of research was the identification of microRNAs (miRNAs), which are small molecule, single-stranded nucleic acids. MiRNAs have diverse roles in cellular biology with the ability for translation in different areas of medicine. The size of miRNAs provides them for passage through gap junction. Recent studies have demonstrated the passage of miRNAs through gap junctional intercellular communication (GJIC) among cancer cells and between breast cancer cells and bone marrow stroma. The transfer of miRNAs has been implicated in the etiology of breast cancer dormancy. To this end, we have developed a miRNA reporter assay to assess the ability of miRNAs to be transferred between stem and breast cancer cells and target a specific recognition sequence.

Katoh, M. (2013). "Therapeutics targeting angiogenesis: genetics and epigenetics, extracellular miRNAs and signaling networks (Review)." Int J Mol Med 32(4): 763-767.

Angiogenesis is a process of neovascular formation from pre-existing blood vessels, which consists of sequential steps for vascular destabilization, angiogenic sprouting, lumen formation and vascular stabilization. Vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), angiopoietin, Notch, transforming growth factor-beta (TGF-beta), Hedgehog and WNT signaling cascades orchestrate angiogenesis through the direct or indirect regulation of quiescence, migration and the proliferation of endothelial cells. Small-molecule compounds and human/humanized monoclonal antibodies interrupting VEGF signaling have been developed as anti-angiogenic therapeutics for cancer and neovascular age-related macular degeneration (AMD). Gene or protein therapy delivering VEGF, FGF2 or FGF4, as well as cell therapy using endothelial progenitor cells (EPCs), mesenchymal stem cells (MSCs) or induced pluripotent stem cells (iPSCs) have been developed as pro-angiogenic therapeutics for ischemic heart disease and peripheral vascular disease. Anti-

angiogenic therapy for cancer and neovascular AMD is more successful than pro-angiogenic therapy for cardiovascular diseases, as VEGF-signal interruption is technically feasible compared with vascular re-construction. Common and rare genetic variants are detected using array-based technology and personal genome sequencing, respectively. Drug and dosage should be determined based on personal genotypes of VEGF and other genes involved in angiogenesis. As epigenetic alterations give rise to human diseases, polymer-based hydrogel film may be utilized for the delivery of drugs targeting epigenetic processes and angiogenesis as treatment modalities for cardiovascular diseases. Circulating microRNAs (miRNAs) in exosomes and microvesicles are applied as functional biomarkers for diagnostics and prognostics, while synthetic miRNAs in polymer-based nanoparticles are applicable for therapeutics. A more profound understanding of the spatio-temporal interactions of regulatory signaling cascades and advances in personal genotyping and miRNA profiling are required for the optimization of targeted therapy.

Katsuda, T., et al. (2013). "The therapeutic potential of mesenchymal stem cell-derived extracellular vesicles." Proteomics 13(10-11): 1637-1653.

Extracellular vesicles (EVs), membrane vesicles that are secreted by a variety of mammalian cell types, have been shown to play an important role in intercellular communication. The contents of EVs, including proteins, microRNAs, and mRNAs, vary according to the cell type that secreted them. Accordingly, researchers have demonstrated that EVs derived from various cell types play different roles in biological phenomena. Considering the ubiquitous presence of mesenchymal stem cells (MSCs) in the body, MSC-derived EVs may take part in a wide range of events. In particular, MSCs have recently attracted much attention due to the therapeutic effects of their secretory factors. MSC-derived EVs may therefore provide novel therapeutic approaches. In this review, we first summarize the wide range of functions of EVs released from different cell types, emphasizing that EVs echo the phenotype of their parent cell. Then, we describe the various therapeutic effects of MSCs and pay particular attention to the significance of their paracrine effect. We then survey recent reports on MSC-derived EVs and consider the therapeutic potential of MSC-derived EVs. Finally, we discuss remaining issues that must be addressed before realizing the practical application of MSC-derived EVs, and we provide some suggestions for enhancing their therapeutic efficiency.

Tomasoni, S., et al. (2013). "Transfer of growth factor receptor mRNA via exosomes unravels the regenerative effect of mesenchymal stem cells." Stem Cells Dev 22(5): 772-780.

Bone marrow-mesenchymal stem cells (BM-MSC) ameliorate renal dysfunction and repair tubular damage of acute kidney injury by locally releasing growth factors, including the insulin-like growth factor-1 (IGF-1). The restricted homing of BM-MSC at the site of

injury led us to investigate a possible gene-based communication mechanism between BM-MSC and tubular cells. Human BM-MSC (hBM-MSC) released microparticles and exosomes (Exo) enriched in mRNAs. A selected pattern of transcripts was detected in Exo versus parental cells. Exo expressed the IGF-1 receptor (IGF-1R), but not IGF-1 mRNA, while hBM-MSC contained both mRNAs. R- cells lacking IGF-1R exposed to hBM-MSC-derived Exo acquired the human IGF-1R transcript that was translated in the corresponding protein. Transfer of IGF-1R mRNA from Exo to cisplatin-damaged proximal tubular cells (proximal tubular epithelial cell [PTEC]) increased PTEC proliferation. Coincubation of damaged PTEC with Exo and soluble IGF-1 further enhanced cell proliferation. These findings suggest that horizontal transfer of the mRNA for IGF-1R to tubular cells through Exo potentiates tubular cell sensitivity to locally produced IGF-1 providing a new mechanism underlying the powerful renoprotection of few BM-MSC observed in vivo.

Eirin, A., et al. (2014). "MicroRNA and mRNA cargo of extracellular vesicles from porcine adipose tissue-derived mesenchymal stem cells." Gene 551(1): 55-64.

Mesenchymal stromal/stem cells (MSCs) are clinically useful for cell-based therapy, but concerns regarding their ability to replicate limit their human application. MSCs release extracellular vesicles (EVs) that mediate at least in part the paracrine effects of the parental cells. To understand the molecular basis of their biological properties, we characterized the RNA cargo of EVs from porcine adipose-tissue derived MSCs. Comprehensive characterization of mRNA and miRNA gene expression using high-throughput RNA sequencing (RNA-seq) revealed that EVs are selectively enriched for distinct classes of RNAs. For example, EVs preferentially express mRNA for transcription factors (e.g. MDFIC, POU3F1, NRIP1) and genes involved in angiogenesis (e.g. HGF, HES1, TCF4) and adipogenesis (e.g. CEBPA, KLF7). EVs also express Golgi apparatus genes (ARRB1, GOLGA4) and genes involved in TGF-beta signaling. In contrast, mitochondrial, calcium signaling, and cytoskeleton genes are selectively excluded from EVs, possibly because these genes remain sequestered in organelles or intracellular compartments. RNA-seq generated reads for at least 386 annotated miRNAs, but only miR148a, miR532-5p, miR378, and let-7f were enriched in EVs compared to MSCs. Gene ontology analysis indicates that these miRNAs target transcription factors and genes that participate in several cellular pathways, including angiogenesis, cellular transport, apoptosis, and proteolysis. Our data suggest that EVs transport gene regulatory information to modulate angiogenesis, adipogenesis, and other cell pathways in recipient cells. These observations may contribute to development of regenerative strategies using EVs to overcome potential complications of cell-based therapy.

Tian, T., et al. (2014). "Exosome uptake through clathrin-mediated endocytosis and macropinocytosis and mediating miR-21 delivery." J Biol Chem 289(32): 22258-22267.

Exosomes are nanoscale membrane vesicles secreted from many types of cells. Carrying functional molecules, exosomes transfer information between cells and mediate many physiological and pathological processes. In this report, utilizing selective inhibitors, molecular tools, and specific endocytosis markers, the cellular uptake of PC12 cell-derived exosomes was imaged by high-throughput microscopy and statistically analyzed. It was found that the uptake was through clathrin-mediated endocytosis and macropinocytosis. Furthermore, PC12 cell-derived exosomes can enter and deliver microRNAs (miRNAs) into bone marrow-derived mesenchymal stromal cells (BMSCs), and decrease the expression level of transforming growth factor beta receptor II (TGFbetaRII) and tropomyosin-1 (TPM1) through miR-21. These results show the pathway of exosome internalization and demonstrate that tumor cell-derived exosomes regulate target gene expression in normal cells.

Baglio, S. R., et al. (2015). "Human bone marrow- and adipose-mesenchymal stem cells secrete exosomes enriched in distinctive miRNA and tRNA species." Stem Cell Res Ther 6: 127.

INTRODUCTION: Administration of mesenchymal stem cells (MSCs) represents a promising treatment option for patients suffering from immunological and degenerative disorders. Accumulating evidence indicates that the healing effects of MSCs are mainly related to unique paracrine properties, opening opportunities for secretome-based therapies. Apart from soluble factors, MSCs release functional small RNAs via extracellular vesicles (EVs) that seem to convey essential features of MSCs. Here we set out to characterize the full small RNAome of MSC-produced exosomes. METHODS: We set up a protocol for isolating exosomes released by early passage adipose- (ASC) and bone marrow-MSCs (BMSC) and characterized them via electron microscopy, protein analysis and small RNA-sequencing. We developed a bioinformatics pipeline to define the exosome-enclosed RNA species and performed the first complete small RNA characterization of BMSCs and ASCs and their corresponding exosomes in biological replicates. RESULTS: Our analysis revealed that primary ASCs and BMSCs have highly similar small RNA expression profiles dominated by miRNAs and snoRNAs (together 64-71 %), of which 150-200 miRNAs are present at physiological levels. In contrast, the miRNA pool in MSC exosomes is only 2-5 % of the total small RNAome and is dominated by a minor subset of miRNAs. Nevertheless, the miRNAs in exosomes do not merely reflect the cellular content and a defined set of miRNAs are overrepresented in exosomes compared to the cell of origin. Moreover, multiple highly expressed miRNAs are precluded from exosomal sorting, consistent with the notion that these miRNAs are involved in functional repression of RNA targets. While ASC and BMSC exosomes are similar in RNA class distribution and composition, we observed striking differences in the sorting of evolutionary conserved tRNA species that seems associated with the differentiation status of MSCs, as defined by Sox2, POU5F1A/B and Nanog expression. CONCLUSIONS: We demonstrate that primary MSCs release small RNAs via exosomes, which

are increasingly implicated in intercellular communications. tRNAs species, and in particular tRNA halves, are preferentially released and their specific sorting into exosomes is related to MSC tissue origin and stemness. These findings may help to understand how MSCs impact neighboring or distant cells with possible consequences for their therapeutic usage.

Phinney, D. G., et al. (2015). "Mesenchymal stem cells use extracellular vesicles to outsource mitophagy and shuttle microRNAs." Nat Commun 6: 8472.

Mesenchymal stem cells (MSCs) and macrophages are fundamental components of the stem cell niche and function coordinately to regulate haematopoietic stem cell self-renewal and mobilization. Recent studies indicate that mitophagy and healthy mitochondrial function are critical to the survival of stem cells, but how these processes are regulated in MSCs is unknown. Here we show that MSCs manage intracellular oxidative stress by targeting depolarized mitochondria to the plasma membrane via arrestin domain-containing protein 1-mediated microvesicles. The vesicles are then engulfed and re-utilized via a process involving fusion by macrophages, resulting in enhanced bioenergetics. Furthermore, we show that MSCs simultaneously shed micro RNA-containing exosomes that inhibit macrophage activation by suppressing Toll-like receptor signalling, thereby de-sensitizing macrophages to the ingested mitochondria. Collectively, these studies mechanistically link mitophagy and MSC survival with macrophage function, thereby providing a physiologically relevant context for the innate immunomodulatory activity of MSCs.

Khalkhali-Ellis, Z., et al. (2016). "Lefty Glycoproteins in Human Embryonic Stem Cells: Extracellular Delivery Route and Posttranslational Modification in Differentiation." Stem Cells Dev.

Lefty is a member of transforming growth factor-beta (TGF-beta) superfamily and a potent antagonist of the TGF-beta/Nodal/Activin signaling pathway. Lefty is critical in sustaining self-renewal/pluripotency status, and implicated in the differentiation of embryonic stem cells (ESCs). However, emerging studies depict Lefty as a multifaceted protein involved in myriad cellular events. Lefty proteins (human Lefty A and B) are secreted glycoproteins, but their mode of secretion and the significance of their "glycan" moiety remain mostly unexplored. By employing an in vitro system of human ESCs (hESCs), we observed that Lefty protein(s) are encased in exosomes for extracellular release. The exosomal- and cell-associated Lefty diverge in their proteolytic processing, and possess N-glycan structures of high mannose and complex nature. Differentiation of hESCs to mesenchymal cells (MSCs) or neuronal progenitor cells (NPCs) entails distinct changes in the Lefty A/Lefty B gene(s), and protein expression. Specifically, the proteolytic cleavage and N-glycan composition of the cell-associated and exosomal Lefty differ in the differentiated progenies. These modifications affected Lefty's inhibitory effect on Nodal signaling in aggressive melanoma cells. The

microheterogeneity in the processing and glycosylation of Lefty protein(s) between hESCs, MSCs, and NPCs could present efficient means of diversifying the endogenous functions of Lefty. Whether Lefty's diverse functions in embryonic patterning, as well as its diffusion range in the extracellular environment, are similarly affected remains to be determined. Our studies underscore the potential relevance of Lefty-packaged exosomes for combating debilitating diseases such as cancer.

Chen, C., et al. (2017). "Mesenchymal stem cell transplantation in tight-skin mice identifies miR-151-5p as a therapeutic target for systemic sclerosis." Cell Res 27(4): 559-577.

Systemic sclerosis (SSc), an autoimmune disease, may cause significant osteopenia due to activation of the IL4Ralpha/mTOR pathway. Mesenchymal stem cell transplantation (MSCT) can ameliorate immune disorders in SSc via inducing immune tolerance. However, it is unknown whether MSCT rescues osteopenia phenotype in SSc. Here we show that MSCT can effectively ameliorate osteopenia in SSc mice by rescuing impaired lineage differentiation of the recipient bone marrow MSCs. Mechanistically, we show that donor MSCs transfer miR-151-5p to the recipient bone marrow MSCs in SSc mice to inhibit IL4Ralpha expression, thus downregulating mTOR pathway activation to enhance osteogenic differentiation and reduce adipogenic differentiation. Moreover, systemic delivery of miR-151-5p is capable of rescuing osteopenia, impaired bone marrow MSCs, tight skin, and immune disorders in SSc mice, suggesting that miR-151-5p may be a specific target for SSc treatment. Our finding identifies a previously unrecognized role of MSCT in transferring miRNAs to recipient stem cells to ameliorate osteopenia via rescuing a non-coding RNA pathway.

Cheng, Q., et al. (2017). "Multiple Myeloma-Derived Exosomes Regulate the Functions of Mesenchymal Stem Cells Partially via Modulating miR-21 and miR-146a." Stem Cells Int 2017: 9012152.

Exosomes derived from cancer cells can affect various functions of mesenchymal stem cells (MSCs) via conveying microRNAs (miRs). miR-21 and miR-146a have been demonstrated to regulate MSC proliferation and transformation. Interleukin-6 (IL-6) secreted from transformed MSCs in turn favors the survival of multiple myeloma (MM) cells. However, the effects of MM exosomes on MSC functions remain largely unclear. In this study, we investigated the effects of OPM2 (a MM cell line) exosomes (OPM2-exo) on regulating the proliferation, cancer-associated fibroblast (CAF) transformation, and IL-6 secretion of MSCs and determined the role of miR-21 and miR-146a in these effects. We found that OPM2-exo harbored high levels of miR-21 and miR-146a and that OPM2-exo coculture significantly increased MSC proliferation with upregulation of miR-21 and miR-146a. Moreover, OPM2-exo induced CAF transformation of MSCs, which was evidenced by increased fibroblast-activated protein (FAP), alpha-smooth muscle actin (alpha-SMA), and stromal-derived factor 1 (SDF-1) expressions and IL-6 secretion. Inhibition of miR-21 or miR-146a reduced these

effects of OPM2-exo on MSCs. In conclusion, MM could promote the proliferation, CAF transformation, and IL-6 secretion of MSCs partially through regulating miR21 and miR146a.

Davis, C., et al. (2017). "MicroRNA-183-5p Increases with Age in Bone-Derived Extracellular Vesicles, Suppresses Bone Marrow Stromal (Stem) Cell Proliferation, and Induces Stem Cell Senescence." Tissue Eng Part A 23(21-22): 1231-1240.

Microvesicle- and exosome-mediated transport of microRNAs (miRNAs) represents a novel cellular and molecular pathway for cell-cell communication. In this study, we tested the hypothesis that these extracellular vesicles (EVs) and their miRNAs might change with age, contributing to age-related stem cell dysfunction. EVs were isolated from the bone marrow interstitial fluid (supernatant) of young (3-4 months) and aged (24-28 months) mice to determine whether the size, concentration, and miRNA profile of EVs were altered with age in vivo. Results show that EVs isolated from bone marrow are CD63 and CD9 positive, and the concentration and size distribution of bone marrow EVs are similar between the young and aged mice. Bioanalyzer data indicate that EVs from both young and aged mice are highly enriched in miRNAs, and the miRNA profile of bone marrow EVs differs significantly between the young and aged mice. Specifically, the miR-183 cluster (miR-96/-182/-183) is highly expressed in aged EVs. In vitro assays demonstrate that aged EVs are endocytosed by primary bone marrow stromal cells (BMSCs), and these aged EVs inhibit the osteogenic differentiation of young BMSCs. Transfection of BMSCs with miR-183-5p mimic reduces cell proliferation and osteogenic differentiation, increases senescence, and decreases protein levels of the miR-183-5p target heme oxygenase-1 (Hmox1). In vitro assays utilizing H2O2-induced oxidative stress show that H2O2 treatment of BMSCs increases the abundance of miR-183-5p in BMSC-derived EVs, and Amplex Red assays demonstrate that H2O2 is elevated in the bone marrow microenvironment with age. Together, these data indicate that aging and oxidative stress can significantly alter the miRNA cargo of EVs in the bone marrow microenvironment, which may in turn play a role in stem cell senescence and osteogenic differentiation by reducing Hmox1 activity.

Fatima, F., et al. (2017). "Non-coding RNAs in Mesenchymal Stem Cell-Derived Extracellular Vesicles: Deciphering Regulatory Roles in Stem Cell Potency, Inflammatory Resolve, and Tissue Regeneration." Front Genet 8: 161.

Extracellular vesicles (EVs) are heterogeneous populations of nano- and micro-sized vesicles secreted by various cell types. There is mounting evidence that EVs have widespread roles in transporting proteins, lipids, and nucleic acids between cells and serve as mediators of intercellular communication. EVs secreted from stem cells could function as paracrine factors, and appear to mimic and recapitulate several features of their secreting cells. EV-mediated transport of regulatory RNAs provides a novel source of trans-regulation between cells. As such, stem cells have evolved unique

forms of paracrine mechanisms for recapitulating their potencies with specialized functions by transporting non-coding RNAs (ncRNAs) via EVs. This includes the dissemination of stem cell-derived EV-ncRNAs and their regulatory effects elicited in differentiation, self-renewal, pluripotency, and the induction of reparative programs. Here, we summarize and discuss the therapeutic effects of mesenchymal stem cell-derived EV-ncRNAs in the induction of intrinsic regenerative programs elicited through regulating several mechanisms. Among them, most noticeable are the EV-mediated enrichment of ncRNAs at the injury sites contributing the regulation of matrix remodeling, epithelial mesenchymal transitions, and attraction of fibroblasts. Additionally, we emphasize EV-mediated transmission of anti-inflammatory RNAs from stem cells to injury site that potentially orchestrate the resolution of the inflammatory responses and immune alleviation to better facilitate healing processes. Collectively, this knowledge indicates a high value and potential of EV-mediated RNA-based therapeutic approaches in regenerative medicine.

Hayashi, T., et al. (2017). "Exosomal MicroRNA Transport from Salivary Mesenchyme Regulates Epithelial Progenitor Expansion during Organogenesis." Dev Cell 40(1): 95-103.

Epithelial-mesenchymal interactions involve fundamental communication between tissues during organogenesis and are primarily regulated by growth factors and extracellular matrix. It is unclear whether RNA-containing exosomes are mobile genetic signals regulating epithelial-mesenchymal interactions. Here we identify that exosomes loaded with mesenchyme-specific mature microRNA contribute mobile genetic signals from mesenchyme to epithelium. The mature mesenchymal miR-133b-3p, loaded into exosomes, was transported from mesenchyme to the salivary epithelium, which did not express primary miR-133b-3p. Knockdown of miR-133b-3p in culture decreased endbud morphogenesis, reduced proliferation of epithelial KIT(+) progenitors, and increased expression of a target gene, Disco-interacting protein 2 homolog B (Dip2b). DIP2B, which is involved in DNA methylation, was localized with 5-methylcytosine in the prophase nucleus of a subset of KIT(+) progenitors during mitosis. In summary, exosomal transport of miR-133b-3p from mesenchyme to epithelium decreases DIP2B, which may function as an epigenetic regulator of genes responsible for KIT(+) progenitor expansion during organogenesis.

Lv, J. W., et al. (2017). "Inhibition of microRNA-214 promotes epithelial-mesenchymal transition process and induces interstitial cystitis in postmenopausal women by upregulating Mfn2." Exp Mol Med 49(7): e357.

Our study aims to investigate the roles that microRNA-214 (miR-214) plays in the epithelial mesenchymal transition (EMT) process and the development of interstitial cystitis (IC) in postmenopausal women by targeting Mitofusin 2 (Mfn2). IC bladder tissues and adjacent normal bladder tissues were collected from postmenopausal women. Immunohistochemistry (IHC) staining was conducted. The target

relationship between miR-214 and Mfn2 was determined by a dual luciferase reporter gene assay. Adipose-derived mesenchymal stem cells (ADMSCs) were extracted from postmenopausal rats and assigned to the blank, mimics, miR-214 inhibitors, mimics negative control (NC), inhibitors NC, Mfn2 siRNA, miR-214 inhibitors and Mfn2 siRNA groups. Exosomes secreted by transfected ADMSCs were instilled into the bladders of postmenopausal rats. The expression of miR-214 and Mfn2 mRNA and EMT markers was assessed by qRT-PCR and western blotting. It was confirmed that Mfn2 was the target gene of miR-214 in IC. Compared with the normal bladder tissues, miR-214 decreased, but Mfn2 increased in IC bladder tissues. Compared with the blank group, the expression of miR-214 and the expression levels of N-cadherin, Fibronectin, Twist1, Snail and Vimentin mRNA and protein increased, whereas the expression levels of Mfn2, E-cadherin and ZO-1 mRNA and protein decreased in the miR-214 mimics and Mfn2 groups. The expression of MiR-214 and the expression levels of N-cadherin, Fibronectin, Twist1, Snail and Vimentin mRNA and protein decreased, whereas the expression levels of Mfn2, E-cadherin and ZO-1 mRNA and protein increased in the miR-214 inhibitors group. Our findings indicate that the inhibition of miR-214 promotes the EMT process and contributes to bladder wall fibrosis by up-regulating Mfn2, thus leading to the occurrence of IC in postmenopausal women.

Song, Y., et al. (2017). "Exosomal miR-146a Contributes to the Enhanced Therapeutic Efficacy of Interleukin-1beta-Primed Mesenchymal Stem Cells Against Sepsis." Stem Cells 35(5): 1208-1221.

Improving the immunomodulatory efficacy of mesenchymal stem cells (MSCs) through pretreatment with pro-inflammatory cytokines is an evolving field of investigation. However, the underlying mechanisms have not been fully clarified. Here, we pretreated human umbilical cord-derived MSCs with interleukin-1beta (IL-1beta) and evaluated their therapeutic effects in a cecal ligation and puncture-induced sepsis model. We found that systemic administration of IL-1beta-pretreated MSCs (betaMSCs) ameliorated the symptoms of murine sepsis more effectively and increased the survival rate compared with naive MSCs. Furthermore, betaMSCs could more effectively induce macrophage polarization toward an anti-inflammatory M2 phenotype through the paracrine activity. Mechanistically, we demonstrated that betaMSC-derived exosomes contributed to the enhanced immunomodulatory properties of betaMSCs both in vitro and in vivo. Importantly, we found that miR-146a, a well-known anti-inflammatory microRNA, was strongly upregulated by IL-1beta stimulation and selectively packaged into exosomes. This exosomal miR-146a was transferred to macrophages, resulted in M2 polarization, and finally led to increased survival in septic mice. In contrast, inhibition of miR-146a through transfection with miR-146a inhibitors partially negated the immunomodulatory properties of betaMSC-derived exosomes. Taken together, IL-1beta pretreatment effectively enhanced the immunomodulatory properties of MSCs partially through exosome-mediated transfer of miR-146a. Therefore, we believe that IL-1beta pretreatment may provide a new

modality for better therapeutic application of MSCs in inflammatory disorders. Stem Cells 2017;35:1208-1221.

Ferguson, S. W., et al. (2018). "The microRNA regulatory landscape of MSC-derived exosomes: a systems view." Sci Rep 8(1): 1419.

Mesenchymal stem cell (MSC)-derived exosomes mediate tissue regeneration in a variety of diseases including ischemic heart injury, liver fibrosis, and cerebrovascular disease. Despite an increasing number of studies reporting the therapeutic effects of MSC exosomes, the underlying molecular mechanisms and their miRNA complement are poorly characterized. Here we microRNA (miRNA)-profiled MSC exosomes and conducted a network analysis to identify the dominant biological processes and pathways modulated by exosomal miRNAs. At a system level, miRNA-targeted genes were enriched for (cardio)vascular and angiogenesis processes in line with observed cardiovascular regenerative effects. Targeted pathways were related to Wnt signaling, pro-fibrotic signaling via TGF-beta and PDGF, proliferation, and apoptosis. When tested, MSC exosomes reduced collagen production by cardiac fibroblasts, protected cardiomyocytes from apoptosis, and increased angiogenesis in HUVECs. The intrinsic beneficial effects were further improved by virus-free enrichment of MSC exosomes with network-informed regenerative miRNAs capable of promoting angiogenesis and cardiomyocyte proliferation. The data presented here help define the miRNA landscape of MSC exosomes, establish their biological functions through network analyses at a system level, and provide a platform for modulating the overall phenotypic effects of exosomes.

Ma, H., et al. (2018). "Analysis of differentially expressed microRNA of TNF-alpha-stimulated mesenchymal stem cells and exosomes from their culture supernatant." Arch Med Sci 14(5): 1102-1111.

Introduction: To analyze the microRNA expression of tumor necrosi factor alpha (TNF-alpha) stimulated mesenchymal stem cells (MSCs) and exosomes from their culture supernatant. Material and methods: TNF-alpha (20 ng/ml) was used to stimulate MSCs, which were then regarded as TNF-alpha cells (TC), while unstimulated cells were the normal control cells (NCC). MSCs and their culture supernatant were harvested after 48 h. Subsequently, exosomes were isolated from culture supernatants with ExoQuick-TC and were divided into two groups, TNF-alpha exosomes (TE) and normal control exosomes (NCE). Then, the microRNAs were measured by high-throughput sequencing and the results were differentially analyzed. Finally, the correlation of the target genes corresponding to differently expressed microRNAs was analyzed by gene ontology (GO) and KEGG pathway analysis. Results: High-throughput sequencing showed that the cellular compartment (TC vs. NCC) had 280 microRNAs. miR-146a-5p was a uniquely up-regulated microRNA ($p < 0.001$) and the most significantly down-regulated microRNA among the 279 microRNAs included was miR-150-5p ($p < 0.001$). There were 180 differentially expressed microRNAs in the exosome compartment (TE vs. NCE), where miR-146-5p ($p < 0.001$) was one of 176 upregulated microRNAs and miR-203b-5p ($p < 0.001$) was one of 4

downregulated microRNAs. Coincidentally, bioinformatics analysis showed that IRAK1 was a critical target gene of miR-146-5p related to the Toll-like receptor (TLR) signaling pathway. Conclusions: In contrast with the control group, there were significantly differentially expressed microRNAs in both MSCs and exosomes. Interestingly, miR-146a-5p was up-regulated in both comparative groups, and its target gene IRAK1 plays a crucial part in the TLR signaling pathway. These investigations demonstrate a new direction for subsequent inflammation mechanistic studies.

Qiu, G., et al. (2018). "Mesenchymal stem cell-derived extracellular vesicles affect disease outcomes via transfer of microRNAs." Stem Cell Res Ther 9(1): 320.

Mesenchymal stem cells (MSCs) are adult stromal cells with the capacity to differentiate into multiple types of cells. MSCs represent an attractive option in regenerative medicine due to their multifaceted abilities for tissue repair, immunosuppression, and anti-inflammation. Recent studies demonstrate that MSCs exert their effects via paracrine activity, which is at least partially mediated by extracellular vesicles (EVs). MSC-derived EVs (MSC-EVs) could mimic the function of parental MSCs by transferring their components such as DNA, proteins/peptides, mRNA, microRNA (miRNA), lipids, and organelles to recipient cells. In this review, we aim to summarize the mechanism and role of miRNA transfer in mediating the effects of MSC-EVs in the models of human diseases. The first three sections of the review discuss the sorting of miRNAs into EVs, uptake of EVs by target cells, and functional transfer of miRNAs via EVs. Then, we describe the composition of miRNAs in MSC-EVs. Next, we provide the existing evidence that MSC-EVs affect the outcomes of renal, liver, heart, and brain diseases by transferring their miRNA contents. In conclusion, EV-mediated miRNA transfer plays an important role in disease-modulating capacity of MSCs.

Zhang, H., et al. (2018). "MicroRNA-21 Overexpression Promotes the Neuroprotective Efficacy of Mesenchymal Stem Cells for Treatment of Intracerebral Hemorrhage." Front Neurol 9: 931.

Intracerebral hemorrhage (ICH) has high morbidity and mortality, with no effective treatment at present. One possible therapeutic strategy involves the use of mesenchymal stem cells (MSCs), which have shown promise in experimental models and have great potential for treating nervous illnesses in humans. However, many deficiencies in MSC treatment still need to be addressed, including their poor survival rate post-transplantation. Previously, we reported that the microRNA-21 (miR-21) is downregulated in ICH patients' blood and brain tissue. In this study, we aimed to examine its role and therapeutic efficacy in ICH using miR-21-overexpressing MSCs. We found that this microRNA can enhance MSC survival and recovery of neurological function in ICH rats. Its mechanism of action involves reduced neuronal apoptosis. In addition, we demonstrated that miR-21 can be transported to neurons through exosomes derived from MSCs and

that it can target transient receptor potential melastatin 7 (TRPM7) to alleviate neuronal injury following ICH. We also observed that the NF-kappaB pathway is involved in the regulation of miR-21 in neural cells. In conclusion, miR-21 significantly enhances the survival of MSCs in ICH, and miR-21-overexpressing MSCs clearly improved neurological function in ICH rats. Transplantation of miR-21-overexpressing MSCs may, therefore, provide an effective strategy for neuroprotection and treatment of cerebrovascular diseases.

Baranova, A., et al. (2019). "Adipose may actively delay progression of NAFLD by releasing tumor-suppressing, anti-fibrotic miR-122 into circulation." Obes Rev 20(1): 108-118.

Nonalcoholic fatty liver disease (NAFLD) is the most common liver pathology. Here we propose tissue-cooperative, homeostatic model of NAFLD. During early stages of NAFLD the intrahepatic production of miR-122 falls, while the secretion of miRNA-containing exosomes by adipose increases. Bloodstream carries exosome to the liver, where their miRNA cargo is released to regulate their intrahepatic targets. When the deterioration of adipose catches up with the failing hepatic parenchyma, the external supply of liver-supporting miRNAs gradually tapers off, leading to the fibrotic decompensation of the liver and an increase in hepatic carcinogenesis. This model may explain paradoxical observations of the disease-associated decrease in intrahepatic production of certain miRNAs with an increase in their levels in serum. Infusions of miR-122 and, possibly, some other miRNAs may be efficient for preventing NAFLD-associated hepatocellular carcinoma. The best candidates for exosome-wrapped miRNA producer are adipose tissue-derived mesenchymal stem cells (MSCs), known for their capacity to shed large amounts of exosomes into the media. Notably, MSC-derived exosomes with no specific loading are already tested in patients with liver fibrosis. Carrier exosomes may be co-manufactured along with their cargo. Exosome-delivered miRNA cocktails may augment functioning of human organs suffering from a variety of chronic diseases.