CARDIAC

Lai, R. C., et al. (2010). "Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury." Stem Cell Res 4(3): 214-222.

Human ESC-derived mesenchymal stem cell (MSC)-conditioned medium (CM) was previously shown to mediate cardioprotection during myocardial ischemia/reperfusion injury through large complexes of 50-100 nm. Here we show that these MSCs secreted 50- to 100-nm particles. These particles could be visualized by electron microscopy and were shown to be phospholipid vesicles consisting of cholesterol, sphingomyelin, and phosphatidylcholine. They contained coimmunoprecipitating exosome-associated proteins, e.g., CD81, CD9, and Alix. These particles were purified as a homogeneous population of particles with a hydrodynamic radius of 55-65 nm by size-exclusion fractionation on a HPLC. Together these observations indicated that these particles are exosomes. These purified exosomes reduced infarct size in a mouse model of myocardial ischemia/reperfusion injury. Therefore, MSC mediated its cardioprotective paracrine effect by secreting exosomes. This novel role of exosomes highlights a new perspective into intercellular mediation of tissue injury and repair, and engenders novel approaches to the development of biologics for tissue repair.

Lai, R. C., et al. (2011). "Mesenchymal stem cell exosome: a novel stem cell-based therapy for cardiovascular disease." Regen Med 6(4): 481-492.

Cardiovascular disease is a major target for many experimental stem cell-based therapies and mesenchymal stem cells (MSCs) are widely used in these therapies. Transplantation of MSCs to treat cardiac disease has always been predicated on the hypothesis that these cells would engraft, differentiate and replace damaged cardiac tissues. However, experimental or clinical observations so far have failed to demonstrate a therapeutically relevant level of transplanted MSC engraftment or differentiation. Instead, they indicate that transplanted MSCs secrete factors to reduce tissue injury and/or enhance tissue repair. Here we review the evidences supporting this hypothesis including the recent identification of exosome as a therapeutic agent in MSC secretion. In particular, we will discuss the potential and practicality of using this relatively novel entity as a therapeutic modality for the treatment of cardiac disease, particularly acute myocardial infarction.

Barile, L., et al. (2012). "Ultrastructural evidence of exosome secretion by progenitor cells in adult mouse myocardium and adult human cardiospheres." J Biomed Biotechnol 2012: 354605.

The demonstration of beneficial effects of cell therapy despite the persistence of only few transplanted cells in vivo suggests secreted factors may be the active component of this treatment. This so-called paracrine hypothesis is supported by observations that culture media conditioned by progenitor cells contain growth factors that mediate proangiogenic and cytoprotective effects. Cardiac progenitor cells in semi-suspension culture form

spherical clusters (cardiospheres) that deliver paracrine signals to neighboring cells. A key component of paracrine secretion is exosomes, membrane vesicles that are stored intracellularly in endosomal compartments and are secreted when these structures fuse with the cell plasma membrane. Exosomes have been identified as the active component of proangiogenic effects of bone marrow CD34(+) stem cells in mice and the regenerative effects of embryonic mesenchymal stem cells in infarcted hearts in pigs and mice. Here, we provide electron microscopic evidence of exosome secretion by progenitor cells in mouse myocardium and human cardiospheres. Exosomes are emerging as an attractive vector of paracrine signals delivered by progenitor cells. They can be stored as an "off-the-shelf" product. As such, exosomes have the potential for circumventing many of the limitations of viable cells for therapeutic applications in regenerative medicine.

Arslan, F., et al. (2013). "Mesenchymal stem cell-derived exosomes increase ATP levels, decrease oxidative stress and activate PI3K/Akt pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury." Stem Cell Res 10(3): 301-312.

We have previously identified exosomes as the paracrine factor secreted by mesenchymal stem cells. Recently, we found that the key features of reperfusion injury, namely loss of ATP/NADH, increased oxidative stress and cell death were underpinned by proteomic deficiencies in ischemic/reperfused myocardium, and could be ameliorated by proteins in exosomes. To test this hypothesis in vivo, mice (C57Bl6/J) underwent 30 min ischemia, followed by reperfusion (I/R injury). Purified exosomes or saline was administered 5 min before reperfusion. Exosomes reduced infarct size by 45% compared to saline treatment. Langendorff experiments revealed that intact but not lysed exosomes enhanced viability of the ischemic/reperfused myocardium. Exosome treated animals exhibited significant preservation of left ventricular geometry and contractile performance during 28 days follow-up. Within an hour after reperfusion, exosome treatment increased levels of ATP and NADH, decreased oxidative stress, increased phosphorylated-Akt and phosphorylated-GSK-3beta, and reduced phosphorylated-c-JNK in ischemic/reperfused hearts. Subsequently, both local and systemic inflammation were significantly reduced 24h after reperfusion. In conclusion, our study shows that intact exosomes restore bioenergetics, reduce oxidative stress and activate pro-survival signaling, thereby enhancing cardiac function and geometry after myocardial I/R injury. Hence, mesenchymal stem cell-derived exosomes are a potential adjuvant to reperfusion therapy for myocardial infarction.

Askar, S. F., et al. (2013). "Engraftment patterns of human adult mesenchymal stem cells expose electrotonic and paracrine proarrhythmic mechanisms in myocardial cell cultures." Circ Arrhythm Electrophysiol 6(2): 380-391.

BACKGROUND: After intramyocardial injection, mesenchymal stem

cells (MSCs) may engraft and influence host myocardium. However, engraftment rate and pattern of distribution are difficult to control in vivo, hampering assessment of potential adverse effects. In this study, the role of the engraftment patterns of MSCs on arrhythmicity in controllable in vitro models is investigated. METHODS AND RESULTS: Cocultures of 4x10(5) neonatal rat cardiomyocytes and 7% or 28% adult human MSCs (hMSCs) in diffuse or clustered distribution patterns were prepared. Electrophysiological effects were studied by optical mapping and patch-clamping. In diffuse cocultures, hMSCs dose-dependently decreased neonatal rat cardiomyocyte excitability, slowed conduction, and prolonged action potential duration until 90% repolarization (APD90). Triggered activity (14% versus 0% in controls) and increased inducibility of re-entry (53% versus 6% in controls) were observed in 28% hMSC cocultures. MSC clusters increased APD90, slowed conduction locally, and increased re-entry inducibility (23%), without increasing triggered activity. Pharmacological heterocellular electric uncoupling increased excitability and conduction velocity to 133% in 28% hMSC cocultures, but did not alter APD90. Transwell experiments showed that hMSCs dose-dependently increased APD90, APD dispersion, inducibility of re-entry and affected specific ion channel protein levels, whereas excitability was unaltered. Incubation with hMSC-derived exosomes did not increase APD in neonatal rat cardiomyocyte cultures. CONCLUSIONS: Adult hMSCs affect arrhythmicity of neonatal rat cardiomyocyte cultures by heterocellular coupling leading to depolarization-induced conduction slowing and by direct release of paracrine factors that negatively affect repolarization rate. The extent of these detrimental effects depends on the number and distribution pattern of hMSCs. These results suggest that caution should be urged against potential adverse effects of myocardial hMSC engraftment.

Bian, S., et al. (2014). "Extracellular vesicles derived from human bone marrow mesenchymal stem cells promote angiogenesis in a rat myocardial infarction model." J Mol Med (Berl) 92(4): 387-397.

UNLABELLED: Mesenchymal stem cells (MSCs) have been increasingly tested experimentally and clinically for cardiac repair. However, the underlying mechanisms remain controversial due to the poor viability and considerable death of the engrafted cells in the infracted myocardium. Recent reports have suggested that extracellular vesicles (EVs) released by MSCs have angiogenesis-promoting activity; however, the therapeutic effect of MSC-EVs on an ischemic heart is unclear. In the present study, we reported that MSCs could release a large quantity of EVs around 100 nm in diameter upon hypoxia stimulation though the majority of the cells had not experienced apoptosis. MSC-EVs could be promptly uptaken by human umbilical vein endothelial cells, and the internalization resulted in dose-dependent enhancement of in vitro proliferation, migration, and tube formation of endothelial cells. Using an acute myocardial infarction rat model, we found that intramyocardial injection of MSC-EVs markedly enhanced blood flow recovery, in accordance with reduced infarct size and preserved cardiac systolic and diastolic performance compared to those

treated with PBS. These data suggest that like MSCs, MSC-EVs could also protect cardiac tissue from ischemic injury at least by means of promoting blood vessel formation, though further detailed investigations should be performed to define the functionality of MSC-EVs. KEY MESSAGES: MSCs released extracellular vesicles (EVs) upon hypoxia stimulation. MSC-EVs were a mixture of microvesicles and exosomes. MSC-EVs could be promptly uptaken by human umbilical vein endothelial cells. MSC-EVs promoted neoangiogenesis in vitro and in vivo. MSC-EVs preserved cardiac performance in an AMI model.

Tsao, C. R., et al. (2014). "Mesenchymal Stem Cell Derived Exosomes: A New Hope for the Treatment of Cardiovascular Disease?" Acta Cardiol Sin 30(5): 395-400.

UNLABELLED: Cardiovascular disease is a major target for numerous experimental stem (progenitor) cell-based therapies. Mesenchymal stem cells (MSCs) from different sources confer regenerative effects in animal models of cardiovascular disease. Some of these investigations have proceeded into phase I and II clinical trials for limb ischemia, heart failure, and acute myocardial infarction. The rationale for MSC therapy is increasingly recognized on a secretion (paracrine) rather than differentiation mechanism. Recently, several groups have demonstrated that the "exosome" is a secreted agent mediating MSC therapeutic efficacy. Unlike cell therapy, exosomes have no risk of aneuploidy, and a lower rate of immune rejection following allogeneic administration. In this short review, we will focus on the potential of using this novel therapeutic modality for the treatment of cardiovascular disease, particularly acute myocardial infarction. KEY WORDS: Cardiovascular disease; Exosome; Mesenchymal.

Huang, L., et al. (2015). "Exosomes in mesenchymal stem cells, a new therapeutic strategy for cardiovascular diseases?" Int J Biol Sci 11(2): 238-245.

Cardiovascular diseases (CVDs) are still a major cause of people deaths worldwide, and mesenchymal stem cells (MSCs) transplantation holds great promise due to its capacity to differentiate into cardiovascular cells and secrete protective cytokines, which presents an important mechanism of MSCs therapy for CVDs. Although the capability of MSCs to differentiate into cardiomyocytes (CMCs), endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) has been well recognized in massive previous experiments both in vitro and in vivo, low survival rate of transplanted MSCs in recipient hearts suggests that therapeutic effects of MSCs transplantation might be also correlated with other underlying mechanisms. Notably, recent studies uncovered that MSCs were able to secret cholesterol-rich, phospholipid exosomes which were enriched with microRNAs (miRNAs). The released exosomes from MSCs acted on hearts and vessels, and then exerted anti-apoptosis, cardiac regeneration, anti-cardiac remodeling, anti-inflammatory effects, neovascularization and anti-vascular remodeling, which are considered

as novel molecular mechanisms of therapeutic potential of MSCs transplantation. Here we summarized recent advances about the role of exosomes in MSCs therapy for CVDs, and discussed exosomes as a novel approach in the treatment of CVDs in the future.

Kang, K., et al. (2015). "Exosomes Secreted from CXCR4 Overexpressing Mesenchymal Stem Cells Promote Cardioprotection via Akt Signaling Pathway following Myocardial Infarction." Stem Cells Int 2015: 659890.

Background and Objective. Exosomes secreted from mesenchymal stem cells (MSC) have demonstrated cardioprotective effects. This study examined the role of exosomes derived from MSC overexpressing CXCR4 for recovery of cardiac functions after myocardial infarction (MI). Methods. In vitro, exosomes from MSC transduced with lentiviral CXCR4 (Exo(CR4)) encoding a silencing sequence or null vector were isolated and characterized by transmission electron microscopy and dynamic light scattering. Gene expression was then analyzed by qPCR and Western blotting. Cytoprotective effects on cardiomyocytes were evaluated and effects of exosomes on angiogenesis analyzed. In vivo, an exosome-pretreated MSC-sheet was implanted into a region of scarred myocardium in a rat MI model. Angiogenesis, infarct size, and cardiac functions were then analyzed. Results. In vitro, Exo(CR4) significantly upregulated IGF-1alpha and pAkt levels and downregulated active caspase 3 level in cardiomyocytes. Exo(CR4) also enhanced VEGF expression and vessel formation. However, effects of Exo(CR4) were abolished by an Akt inhibitor or CXCR4 knockdown. In vivo, Exo(CR4) treated MSC-sheet implantation promoted cardiac functional restoration by increasing angiogenesis, reducing infarct size, and improving cardiac remodeling. Conclusions. This study reveals a novel role of exosomes derived from MSC(CR4) and highlights a new mechanism of intercellular mediation of stem cells for MI treatment.

Teng, X., et al. (2015). "Mesenchymal Stem Cell-Derived Exosomes Improve the Microenvironment of Infarcted Myocardium Contributing to Angiogenesis and Anti-Inflammation." Cell Physiol Biochem 37(6): 2415-2424.

BACKGROUND/AIMS: Bone marrow mesenchymal stem cells (MSCs) widely applied for treating myocardial infarction face survival challenges in the inflammatory and ischemia microenvironment of acute myocardial infarction. The study hypothesized that MSC-derived exosomes play a significant role in improving microenvironment after acute myocardial infarction and aimed to investigate the paracrine effects of exosomes on angiogenesis and anti-inflammatory activity. METHODS: MSCs were cultured in DMEM/F12 supplemented with 10% exosome-depleted fetal bovine serum and 1% penicillin-streptomycin for 48 h. MSC-derived exosomes were isolated using ExoQuick-TC. Tube formation and T-cell proliferation assays were performed to assess the angiogenic potency of MSC-derived exosomes. Acute myocardial infarction was induced in Sprague-Dawley rats, and myocardium bordering the infarcted zone was injected at four different sites with phosphate-buffered saline (PBS, control), MSC-derived exosomes, and

exosome-depleted MSC culture medium. RESULTS: MSC-derived exosomes significantly enhanced the tube formation of human umbilical vein endothelial cells, impaired T-cell function by inhibiting cell proliferation in vitro, reduced infarct size, and preserved cardiac systolic and diastolic performance compared with PBS markedly enhancing the density of new functional capillary and hence blood flow recovery in rat myocardial infarction model. CONCLUSIONS: Exosomes stimulate neovascularization and restrain the inflammation response, thus improving heart function after ischemic injury.

Wang, X., et al. (2015). "Exosomal miR-223 Contributes to Mesenchymal Stem Cell-Elicited Cardioprotection in Polymicrobial Sepsis." Sci Rep 5: 13721.

Mesenchymal stem cells (MSCs) have been shown to elicit cardio-protective effects in sepsis. However, the underlying mechanism remains obscure. While recent studies have indicated that miR-223 is highly enriched in MSC-derived exosomes, whether exosomal miR-223 contributes to MSC-mediated cardio-protection in sepsis is unknown. In this study, loss-of-function approach was utilized, and sepsis was induced by cecal ligation and puncture (CLP). We observed that injection of miR-223-KO MSCs at 1 h post-CLP did not confer protection against CLP-triggered cardiac dysfunction, apoptosis and inflammatory response. However, WT-MSCs were able to provide protection which was associated with exosome release. Next, treatment of CLP mice with exosomes released from miR-223-KO MSCs significantly exaggerated sepsis-induced injury. Conversely, WT-MSC-derived-exosomes displayed protective effects. Mechanistically, we identified that miR-223-KO exosomes contained higher levels of Sema3A and Stat3, two known targets of miR-223 (5p &3p), than WT-exosomes. Accordingly, these exosomal proteins were transferred to cardiomyocytes, leading to increased inflammation and cell death. By contrast, WT-exosomes encased higher levels of miR-223, which could be delivered to cardiomyocytes, resulting in down-regulation of Sema3A and Stat3. These data for the first time indicate that exosomal miR-223 plays an essential role for MSC-induced cardio-protection in sepsis.

Yu, B., et al. (2015). "Exosomes secreted from GATA-4 overexpressing mesenchymal stem cells serve as a reservoir of anti-apoptotic microRNAs for cardioprotection." Int J Cardiol 182: 349-360.

BACKGROUND: Exosomes play an important role in intercellular signaling and exert regulatory function by carrying bioactive molecules. This study investigated (1) the cardioprotective capabilities of exosomes derived from mesenchymal stem cells (MSCs) overexpressing GATA-4 (MSC(GATA-4)) and (2) its underlying regulatory mechanisms for expression of target proteins in recipient cells. METHODS AND RESULTS: Exosomes were isolated and purified from MSC(GATA-4) (Exo(GATA-4)) and control MSCs (Exo(Null)). Cell injury was investigated in primary cultured rat neonatal cardiomyocytes (CM) and in the rat heart. Exosomes contributed to increased CM survival, reduced CM apoptosis, and preserved mitochondrial membrane potential

in CM cultured under a hypoxic environment. Direct intramyocardial transplantation of exosomes at the border of an ischemic region following ligation of the left anterior descending coronary artery significantly restored cardiac contractile function and reduced infarct size. Real-time PCR revealed that several anti-apoptotic miRs were highly expressed in Exo(GATA-4). Rapid internalization of Exo(GATA-4) by CM was documented using time-lapse imaging. Subsequent expression of these miRs, particularly miR-19a was higher in CM and in the myocardium treated with Exo(GATA-4) compared to those treated with Exo(Null). The enhanced protective effects observed in CM were diminished by the inhibition of miR-19a. The expression level of PTEN, a predicted target of miR-19a, was reduced in CM treated with Exo(GATA-4), which resulted in the activation of the Akt and ERK signaling pathways. CONCLUSIONS: Exo(GATA-4) upon transplantation in the damaged tissue mediate protection by releasing multiple miRs responsible for activation of the cell survival signaling pathway.

Zhao, Y., et al. (2015). "Exosomes Derived from Human Umbilical Cord Mesenchymal Stem Cells Relieve Acute Myocardial Ischemic Injury." Stem Cells Int 2015: 761643.

This study is aimed at investigating whether human umbilical cord mesenchymal stem cell- (hucMSC-) derived exosomes (hucMSC-exosomes) have a protective effect on acute myocardial infarction (AMI). Exosomes were characterized under transmission electron microscopy and the particles of exosomes were further examined through nanoparticle tracking analysis. Exosomes (400 mug protein) were intravenously administrated immediately following ligation of the left anterior descending (LAD) coronary artery in rats. Cardiac function was evaluated by echocardiography and apoptotic cells were counted using TUNEL staining. The cardiac fibrosis was assessed using Masson's trichrome staining. The Ki67 positive cells in ischemic myocardium were determined using immunohistochemistry. The effect of hucMSC-exosomes on blood vessel formation was evaluated through tube formation and migration of human umbilical vein endothelial cells (EA.hy926 cells). The results indicated that ligation of the LAD coronary artery reduced cardiac function and induced cardiomyocyte apoptosis. Administration of hucMSC-exosomes significantly improved cardiac systolic function and reduced cardiac fibrosis. Moreover, hucMSC-exosomes protected myocardial cells from apoptosis and promoted the tube formation and migration of EA.hy926 cells. It is concluded that hucMSC-exosomes improved cardiac systolic function by protecting myocardial cells from apoptosis and promoting angiogenesis. These effects of hucMSC-exosomes might be associated with regulating the expression of Bcl-2 family.

Nguyen, P. K., et al. (2016). "Potential Strategies to Address the Major Clinical Barriers Facing Stem Cell Regenerative Therapy for Cardiovascular Disease: A Review." JAMA Cardiol 1(8): 953-962.

Importance: Although progress continues to be made in the field of stem cell regenerative medicine for the treatment of

cardiovascular disease, significant barriers to clinical implementation still exist. Objectives: To summarize the current barriers to the clinical implementation of stem cell therapy in patients with cardiovascular disease and to discuss potential strategies to overcome them. Evidence Review: Information for this review was obtained through a search of PubMed and the Cochrane database for English-language studies published between January 1, 2000, and July 25, 2016. Ten randomized clinical trials and 8 systematic reviews were included. Findings: One of the major clinical barriers facing the routine implementation of stem cell therapy in patients with cardiovascular disease is the limited and inconsistent benefit observed thus far. Reasons for this finding are unclear but may be owing to poor cell retention and survival, as suggested by numerous preclinical studies and a small number of human studies incorporating imaging to determine cell fate. Additional studies in humans using imaging to determine cell fate are needed to understand how these factors contribute to the limited efficacy of stem cell therapy. Treatment strategies to address poor cell retention and survival are under investigation and include the following: coadministration of immunosuppressive and prosurvival agents, delivery of cardioprotective factors packaged in exosomes rather than the cells themselves, and use of tissue-engineering strategies to provide structural support for cells. If larger grafts are achieved using these strategies, it will be imperative to carefully monitor for the potential risks of tumorigenicity, immunogenicity, and arrhythmogenicity. Conclusions and Relevance: Despite important achievements to date, stem cell therapy is not yet ready for routine clinical implementation. Significant research is still needed to address the clinical barriers outlined herein before the next wave of large clinical trials is under way.

Safari, S., et al. (2016). "Mesenchymal stem cell-derived exosomes: A novel potential therapeutic avenue for cardiac regeneration." Cell Mol Biol (Noisy-le-grand) 62(7): 66-73.

Coronary artery diseases (CADs) represent a significant cause of death worldwide. During recent decades the rate of cardiovascular mortality has been declined as a result of modern medicine and surgery. However, despite the fact that cardiac cells, including cardiomyocytes (CMCs), vascular smooth muscle cells (VSMC) and vascular endothelial cells (VEC), can be regenerated by cardiac adult stem cell, the regenerative capacity of these cells are limited and inadequate to functionally regenerate heart damaged tissue. Thus, growth reserve of the heart fails to restore the structural integrity of the myocardium after infarction and healing is associated with scar formation. An explanation for this is that cardiac reside stem cells are present throughout the infarction site but die rapidly by apoptosis. Furthermore, microenvironment surrounding the damage site is not promising for the cells survival and renewal. Hence, recent advances in the stem cell therapy have emerged as an attractive approach to replace the lost cells. In this context, mesenchymal stem

cells (MSCs) has considered as one of the most promising candidates for regeneration of cardiac cells, lost upon injury. The regenerative capacity of MSCs has primarily been centered on the hypothesis that these cells would engraft, differentiate and replace damaged cardiac cells. However, experimental and clinical observations so far have failed to establish if this differentiated is considerably relevant to MSCs cardiac regenerative properties. Recent reports have suggested that these therapeutic properties, at least in part, are mediated by paracrine factors released from MSCs. This review provides a concise summary of current evidences supporting the paracrine hypothesis of MSCs. In particular, the scope of this review focuses on the role of MSC-derived exosome (MSC-EXs) as a therapeutic modality for the treatment of CADs, particularly ischemic myocardial dysfunctions.

Suzuki, E., et al. (2016). "Stem cell-derived exosomes as a therapeutic tool for cardiovascular disease." World J Stem Cells 8(9): 297-305.

Mesenchymal stem cells (MSCs) have been used to treat patients suffering from acute myocardial infarction (AMI) and subsequent heart failure. Although it was originally assumed that MSCs differentiated into heart cells such as cardiomyocytes, recent evidence suggests that the differentiation capacity of MSCs is minimal and that injected MSCs restore cardiac function via the secretion of paracrine factors. MSCs secrete paracrine factors in not only naked forms but also membrane vesicles including exosomes containing bioactive substances such as proteins, messenger RNAs, and microRNAs. Although the details remain unclear, these bioactive molecules are selectively sorted in exosomes that are then released from donor cells in a regulated manner. Furthermore, exosomes are specifically internalized by recipient cells via ligand-receptor interactions. Thus, exosomes are promising natural vehicles that stably and specifically transport bioactive molecules to recipient cells. Indeed, stem cell-derived exosomes have been successfully used to treat cardiovascular disease (CVD), such as AMI, stroke, and pulmonary hypertension, in animal models, and their efficacy has been demonstrated. Therefore, exosome administration may be a promising strategy for the treatment of CVD. Furthermore, modifications of exosomal contents may enhance their therapeutic effects. Future clinical studies are required to confirm the efficacy of exosome treatment for CVD.

Vrijsen, K. R., et al. (2016). "Exosomes from Cardiomyocyte Progenitor Cells and Mesenchymal Stem Cells Stimulate Angiogenesis Via EMMPRIN." Adv Healthc Mater 5(19): 2555-2565.

To date, cellular transplantation therapy has not yet fulfilled its high expectations for cardiac repair. A major limiting factor is lack of long-term engraftment of the transplanted cells. Interestingly, transplanted cells can positively affect their environment via secreted paracrine factors, among which are extracellular vesicles, including exosomes: small bi-lipid-layered vesicles containing proteins, mRNAs, and miRNAs. An exosome-based

therapy will therefore relay a plethora of effects, without some of the limiting factors of cell therapy. Since cardiomyocyte progenitor cells (CMPC) and mesenchymal stem cells (MSC) induce vessel formation and are frequently investigated for cardiac-related therapies, the pro-angiogenic properties of CMPC and MSC-derived exosome-like vesicles are investigated. Both cell types secrete exosome-like vesicles, which are efficiently taken up by endothelial cells. Endothelial cell migration and vessel formation are stimulated by these exosomes in in vitro models, mediated via ERK/Akt-signaling. Additionally, these exosomes stimulated blood vessel formation into matrigel plugs. Analysis of pro-angiogenic factors revealed high levels of extracellular matrix metalloproteinase inducer (EMMPRIN). Knockdown of EMMPRIN on CMPCs leads to a diminished pro-angiogenic effect, both in vitro and in vivo. Therefore, CMPC and MSC exosomes have powerful pro-angiogenic effects, and this effect is largely mediated via the presence of EMMPRIN on exosomes.

Zhang, H., et al. (2016). "Inhibition of Myocardial Ischemia/Reperfusion Injury by Exosomes Secreted from Mesenchymal Stem Cells." Stem Cells Int 2016: 4328362.

Exosomes secreted by mesenchymal stem cells have shown great therapeutic potential in regenerative medicine. In this study, we performed meta-analysis to assess the clinical effectiveness of using exosomes in ischemia/reperfusion injury based on the reports published between January 2000 and September 2015 and indexed in the PUBMED and Web of Science databases. The effect of exosomes on heart function was evaluated according to the following parameters: the area at risk as a percentage of the left ventricle, infarct size as a percentage of the area at risk, infarct size as a percentage of the left ventricle, left ventricular ejection fraction, left ventricular fraction shortening, end-diastolic volume, and end-systolic volume. Our analysis indicated that the currently available evidence confirmed the therapeutic potential of mesenchymal stem cell-secreted exosomes in the improvement of heart function. However, further mechanistic studies, therapeutic safety, and clinical trials are required for optimization and validation of this approach to cardiac regeneration after ischemia/reperfusion injury.

Zhang, Z., et al. (2016). "Pretreatment of Cardiac Stem Cells With Exosomes Derived From Mesenchymal Stem Cells Enhances Myocardial Repair." J Am Heart Assoc 5(1).

BACKGROUND: Exosomes derived from mesenchymal stem cells (MSCs) were proved to boost cell proliferation and angiogenic potency. We explored whether cardiac stem cells (CSCs) preconditioned with MSC exosomes could survive and function better in a myocardial infarction model. METHODS AND RESULTS: DiI-labeled exosomes were internalized with CSCs. They stimulated proliferation, migration, and angiotube formation of CSCs in a dose-dependent manner. In a rat myocardial infarction model, MSC exosome-preconditioned CSCs had significantly better survival, enhanced capillary density, reduced cardiac fibrosis,

and restored long-term cardiac function. MicroRNA profiling analysis revealed that a set of microRNAs were significantly changed in CSCs after MSC exosome treatment. CONCLUSIONS: Pretreatment of CSCs with MSC exosomes provided a promising strategy to improve survival and angiogenic potency of CSCs.

Barile, L., et al. (2017). "Beneficial effects of exosomes secreted by cardiac-derived progenitor cells and other cell types in myocardial ischemia." Stem Cell Investig 4: 93.

When injected into acutely infarcted rodent or pig hearts, naturally secreted nanovesicles known as exosomes from cardiac-derived progenitor cells (CPCs) reduce scar size and improve cardiac function. In this regard, exosomes fully mimic the benefits of injecting their parent cells. This recognition paves the way to the development of exosome-based, cell-free treatments for heart disease that could possibly supplant cell-based therapies. Mechanisms of benefit of these vesicles are incompletely understood but cytoprotection, stimulation of angiogenesis, induction of antifibrotic cardiac fibroblasts, and modulation of M1/M2 polarization of macrophages infiltrating the infarcted region can all play important roles. Accordingly, the beneficial molecules carried by CPC-secreted exosomes have been identified only in part but cytoprotective and proangiogenic microRNAs (miRNA) and proteins have been described. Besides CPC-secreted exosomes, vesicles released from other cell types including mesenchymal stem cells (MSCs), embryonic stem cells (ESCs), and induced pluripotent stem cells (iSPCs) have also been associated with cardioprotection. This review aims to discuss recent advances in our understanding of the role of secreted vesicles in cardiac repair, with a focus on CPC-derived exosomes.

Chen, Y., et al. (2017). "MicroRNA-133 overexpression promotes the therapeutic efficacy of mesenchymal stem cells on acute myocardial infarction." Stem Cell Res Ther 8(1): 268.

BACKGROUND: Our study aim was to evaluate the therapeutic efficacy and mechanisms of miR-133-overexpressing mesenchymal stem cells (MSCs) on acute myocardial infarction. METHODS: Rat MSCs were isolated and purified by whole bone marrow adherent culturing. After transfection with the agomir or antagomir of miR-133, MSCs were collected for assay of cell vitality, apoptosis, and cell cycle progression. At the same time, exosomes were isolated from the supernatant to analyze the paracrine miR-133. For in-vivo studies, constitutive activation of miR-133 in MSCs was achieved by lentivirus-mediated miR-133 overexpression. A rat myocardial infarction model was created by ligating the left anterior descending coronary artery, while control MSCs (vector-MSCs) or miR-133-overexpressed MSCs (miR-133-MSCs) were injected into the zone around the myocardial infarction. Subsequently, myocardial function was evaluated by echocardiography on days 7 and 28 post infarction. Finally the infarcted hearts were collected on days 7 and 28 for myocardial infarct size measurement and detection of snail 1 expression. RESULTS:

Hypoxia-induced apoptosis of MSCs obviously reduced, along with enhanced expression of total poly ADP-ribose polymerase protein, after miR-133 agomir transfection, while the apoptosis rate increased in MSCs transfected with miR-133 antagomir. However, no change in cell viability and cell-cycle distribution was observed in control, miR-133-overexpressed, and miR-133-interfered MSCs. Importantly, rats transplanted with miR-133-MSCs displayed more improved cardiac function after acute myocardial infarction, compared with those that received vector-MSC injection. Further studies indicated that cardiac expression of snail 1 was significantly repressed by adjacent miR-133-overexpressing MSCs, and both the inflammatory level and the infarct size decreased in miR-133-MSC-injected rat hearts. CONCLUSIONS: miR-133-MSCs obviously improved cardiac function in a rat model of myocardial infarction. Transplantation of miR-133-overexpressing MSCs provides an effective strategy for cardiac repair and modulation of cardiac-related diseases.

Cui, X., et al. (2017). "Exosomes From Adipose-derived Mesenchymal Stem Cells Protect the Myocardium Against Ischemia/Reperfusion Injury Through Wnt/beta-Catenin Signaling Pathway." J Cardiovasc Pharmacol 70(4): 225-231.

Mesenchymal stem cells (MSCs) and their secreted exosomes exert a cardioprotective role in jeopardized myocardium. However, the specific effects and underlying mechanisms of exosomes derived from adipose-derived MSCs (ADMSCs) on myocardial ischemia/reperfusion (I/R) injury remain largely unclear. In this study, ADMSC-derived exosomes (ADMSCs-ex) were administrated into the rats subjected to I/R injury and H9c2 cells exposed to hypoxia/reoxygenation (H/R). Consequently, administration of ADMSCs-ex significantly reduced I/R-induced myocardial infarction, accompanied with a decrease in serum levels of creatine kinase-myocardial band, lactate dehydrogenase, and cardiac troponin I (cTnI). Simultaneously, ADMSCs-ex dramatically antagonized I/R-induced myocardial apoptosis, along with the upregulation of Bcl-2 and downregulation of Bax, and inhibition of Caspase 3 activity in rat myocardium. Similarly, ADMSCs-ex significantly reduced cell apoptosis and the expression of Bax, but markedly increased cell viability and the expression of Bcl-2 and Cyclin D1 under H/R. Furthermore, ADMSCs-ex observably induced the activation of Wnt/beta-catenin signaling by attenuating I/R- and H/R-induced inhibition of Wnt3a, p-GSK-3beta (Ser9), and beta-catenin expression. Importantly, treatment with Wnt/beta-catenin inhibitor XAV939 partly neutralized ADMSC-ex-induced antiapoptotic and prosurvival effects in H9c2 cells. In conclusion, we confirmed that ADMSCs-ex protect ischemic myocardium from I/R injury through the activation of Wnt/beta-catenin signaling pathway.

Fish, K. M. and J. S. Hulot (2017). "In Vitro Adherence Defines Therapeutic Cardiac Mesenchymal Cell Subpopulation." J Am Coll Cardiol 69(14): 1839-1841.

Huang, Q. and B. Cai (2017). "Exosomes as New Intercellular Mediators

in Development and Therapeutics of Cardiomyocyte Hypertrophy." Adv Exp Med Biol 998: 91-100.

Myocardial hypertrophy is a common cardiac condition in response to hemodynamic and neurohormonal alterations. Pathological hypertrophic growth in hearts caused the decline of cardiac functions, and finally developed into congestive heart failure. The exosomes are small membrane vesicles which are secreted by various cells and play important roles in cellular communication, migration, proliferation and differentiation. Recent studies uncovered that the exosomes from cardiac fibroblasts and other tissues participates in the development of myocardial hypertrophy. Nevertheless, cardiac progenitor cells and mesenchymal stem cells-derived exosomes confer protective action on myocardial hypertrophy. Thus, the exosomes serve as new intercellular mediators between cardiomyocytes and other cells, and show broad application potential in the diagnostic and therapy of cardiomyocyte hypertrophy.

Liu, L., et al. (2017). "Exosomes Derived from Mesenchymal Stem Cells Rescue Myocardial Ischaemia/Reperfusion Injury by Inducing Cardiomyocyte Autophagy Via AMPK and Akt Pathways." Cell Physiol Biochem 43(1): 52-68.

BACKGROUND/AIMS: Reperfusion after an ischaemic insult might cause infarct extension. Mesenchymal stem cell (MSC)-derived exosomes could attenuate myocardial remodelling in animal models of myocardial ischaemia reperfusion injury (MIRI), and the present study aimed to explore the related mechanisms. METHODS: In vitro, rat H9C2 cardiomyocytes (H9C2s) were exposed to H2O2. Cell viability was detected by the CCK-8 assay, apoptosis was detected by Annexin V-PE/7-AAD staining, ROS production was detected by fluorescence microscopy and flow cytometry, and apoptosis-related proteins and signalling pathway-related proteins were detected by western blot analysis. Autophagic flux was measured using the tandem fluorescent mRFG-GFP-LC3 assay. MSC-derived exosomes were extracted using the total exosome isolation reagent. Apoptosis, myocardial infarction size, heart function and myocardial LC3B expression were examined in an in vivo I/R model by the TUNEL assay, TTC/Evan blue staining, echocardiography and immunohistochemicalstaining, respectively. RESULTS: In vitro, H2O2 dose-dependently increased ROS production and cell apoptosis in H9C2s and blocked autophagic flux after 3 h of exposure; autophagy gradually decreased thereafter, and the lowest level was detected at 12 h after exposure. MSC-derived exosomes reduced H2O2-induced ROS production and cell apoptosis and enhanced autophagy at 12 h after exposure. In H9C2 cells exposed to H2O2 for 12 h, treatment with exosomes enhanced autophagy via the AMPK/mTOR and Akt/mTOR pathways. Likewise, in vivo exosome injections in rats that underwent I/R injury significantly reduced apoptosis and the myocardial infarct size and upregulated myocardial LC3B expression as well as improved heart function. CONCLUSIONS: Our results indicate that MSC-derived exosomes could reduce MIRI by inducing cardiomyocyte autophagy via AMPK/mTOR and Akt/mTOR pathways.

Ma, J., et al. (2017). "Exosomes Derived from Akt-Modified Human Umbilical Cord Mesenchymal Stem Cells Improve Cardiac Regeneration and Promote Angiogenesis via Activating Platelet-Derived Growth Factor D." Stem Cells Transl Med 6(1): 51-59.

We have previously demonstrated the cardioprotective effects of exosomes derived from mesenchymal stem cells (MSCs). It is well known that the activation of Akt is involved in stem cell-induced cardioprotection. In the present study, we investigated whether exosomes released from Akt-overexpressing MSCs showed a beneficial effect on cardioprotection and angiogenesis. MSCs were collected from human umbilical cord (hucMSCs), and Akt was transfected into hucMSCs (Akt-hucMSCs) by using an adenovirus transfection system. Exosomes were isolated from control hucMSCs (Exo) and Akt-hucMSCs (Akt-Exo). An acute myocardial infarction model was created by ligation of the left anterior decedent coronary artery (LAD) in rats. Various source exosomes (400 microg of protein) were infused via the tail vein immediately after LAD ligation. The cardiac function was evaluated by using echocardiography after different treatments for 1 and 5 weeks, respectively. Endothelial cell proliferation, migration, and tube-like structure formation, as well as chick allantoic membrane assay, were used to evaluate the angiogenetic effects of Akt-Exo. The results indicated that cardiac function was significantly improved in the animals treated with Akt-Exo. In addition, Akt-Exo significantly accelerated endothelial cell proliferation and migration, tube-like structure formation in vitro, and blood vessel formation in vivo. The expression of platelet-derived growth factor D (PDGF-D) was significantly upregulated in Akt-Exo. However, the angiogenesis was abrogated in endothelial cells treated with the exosomes obtained from MSCs transfected with PDGF-D-siRNA. Our studies suggest that exosomes obtained from Akt-modified hucMSCs are more effective in myocardial infarction therapy through promoting angiogenesis. PDGF-D plays an important role in Akt-Exo-mediated angiogenesis. Stem Cells Translational Medicine 2017;6:51-59.

Mayourian, J., et al. (2017). "Experimental and Computational Insight Into Human Mesenchymal Stem Cell Paracrine Signaling and Heterocellular Coupling Effects on Cardiac Contractility and Arrhythmogenicity." Circ Res 121(4): 411-423.

RATIONALE: Myocardial delivery of human mesenchymal stem cells (hMSCs) is an emerging therapy for treating the failing heart. However, the relative effects of hMSC-mediated heterocellular coupling (HC) and paracrine signaling (PS) on human cardiac contractility and arrhythmogenicity remain unresolved. OBJECTIVE: The objective is to better understand hMSC PS and HC effects on human cardiac contractility and arrhythmogenicity by integrating experimental and computational approaches. METHODS AND RESULTS: Extending our previous hMSC-cardiomyocyte HC computational model, we incorporated experimentally calibrated hMSC PS effects on cardiomyocyte L-type calcium channel/sarcoendoplasmic reticulum calcium-ATPase activity and

cardiac tissue fibrosis. Excitation-contraction simulations of hMSC PS-only and combined HC+PS effects on human cardiomyocytes were representative of human engineered cardiac tissue (hECT) contractile function measurements under matched experimental treatments. Model simulations and hECTs both demonstrated that hMSC-mediated effects were most pronounced under PS-only conditions, where developed force increased approximately 4-fold compared with non-hMSC-supplemented controls during physiological 1-Hz pacing. Simulations predicted contractility of isolated healthy and ischemic adult human cardiomyocytes would be minimally sensitive to hMSC HC, driven primarily by PS. Dominance of hMSC PS was also revealed in simulations of fibrotic cardiac tissue, where hMSC PS protected from potential proarrhythmic effects of HC at various levels of engraftment. Finally, to study the nature of the hMSC paracrine effects on contractility, proteomic analysis of hECT/hMSC conditioned media predicted activation of PI3K/Akt signaling, a recognized target of both soluble and exosomal fractions of the hMSC secretome. Treating hECTs with exosome-enriched, but not exosome-depleted, fractions of the hMSC secretome recapitulated the effects observed with hMSC conditioned media on hECT-developed force and expression of calcium-handling genes (eg, SERCA2a, L-type calcium channel). CONCLUSIONS: Collectively, this integrated experimental and computational study helps unravel relative hMSC PS and HC effects on human cardiac contractility and arrhythmogenicity, and provides novel insight into the role of exosomes in hMSC paracrine-mediated effects on contractility.

Monteiro, V. V. S., et al. (2017). "Dual Behavior of Exosomes in Septic Cardiomyopathy." Adv Exp Med Biol 998: 101-112.

Sepsis is one of the main causes of ICU hospitalization worldwide, with a high mortality rate, and is associated with a large number of comorbidities. One of the main comorbidities associated with sepsis is septic cardiomyopathy. This process occurs mainly due to mechanisms of damage in the cardiovascular system that will lead to changes in cardiovascular physiology, such as decreased Ca(2+) response, mitochondrial dysfunction and decreased beta-adrenergic receptor response. Within this process the exosomes play an important role in the pathophysiology of this disease, in which the exosomal content is related to mechanisms that will trigger its development. After platelet activation through ROS exposition, exosomes containing high concentrations of NADPH are released in heart blood vessels, those exosomes will be internalized in endothelial cells leading to cell death and cardiac dysfunction. On the opposite, exosomes derived from mesenchymal stem cells contain miR-223, that have anti-inflammatory properties, are released in less quantities in septic patients causing an imbalance that leads to cardiac dysfunction.

Poe, A. J. and A. A. Knowlton (2017). "Exosomes as agents of change in the cardiovascular system." J Mol Cell Cardiol 111: 40-50.

Exosomes have an evolving role in paracrine and autocrine signaling, which is enhanced because these lipid vesicles are quite

stable and can deliver miRNA, DNA, protein and other molecules to cells throughout the body. Most cell types release exosomes, and exosomes are found in all biological fluids, making them accessible biomarkers. Significantly, exosomes can carry a biologically potent cargo, which can alter the phenotype of recipient cells. In the cardiovascular system exosomes have been primarily studied for their role in mediating the beneficial effects of mesenchymal stem cells after myocardial injury. Exosomes released by cardiac cells in disease states, such as myocardial ischemia, can potentially have important pathophysiologic effects on other cardiac cells as well as on distant organs.

Rosca, A. M., et al. (2017). "Emerging Role of Stem Cells - Derived Exosomes as Valuable Tools for Cardiovascular Therapy." Curr Stem Cell Res Ther 12(2): 134-138.

In modern society, myocardial infarction is a major cause of mortality, morbidity and deterioration of quality of life. Although various therapeutic approaches are available, none of them lead to the regeneration of infarcted tissue. The use of mesenchymal stem cells in cell therapy for myocardial infarction showed a beneficial effect consisting in reduced infarcted area and improved cardiac function, which can be explained by paracrine mechanism. It has been shown that stem cells are able to release a very complex range of factors including growth factors, cytokines and chemokines, along with an abundant mixture of membrane vesicles. These secreted elements contribute to the beneficial effect of stem cells therapy observed both in vitro and in vivo. Recent studies have shown that exosomes, which are small membrane vesicles originating in the endocytic pathway of the cells and carry a complex cargo consisting in mRNA, microRNA and various other anti-apoptotic and pro-angiogenic factors, are the main mediators of stem cells paracrine effect. In this review, we discuss the capacity of mesenchymal stem cells to protect the ischemic myocardium, the role of exosomes as protective factors secreted by stem cells and the possibility to use these vesicles in developing a novel approach in cardiovascular therapy, involving a non-cellular use of mesenchymal stem cells paracrine activity.

Shafei, A. E., et al. (2017). "Mesenchymal stem cell therapy: A promising cell-based therapy for treatment of myocardial infarction." J Gene Med 19(12).

For decades, mesenchymal stem (MSCs) cells have been used for cardiovascular diseases as regenerative therapy. This review is an attempt to summarize the types of MSCs involved in myocardial infarction (MI) therapy, as well as its possible mechanisms effects, especially the paracrine one in MI focusing on the studies (human and animal) conducted within the last 10 years. Recently, reports showed that MSC therapy could have infarct-limiting effects after MI in both experimental and clinical trials. In this context, various types of MSCs can help cardiac regeneration by either revitalizing the cardiac stem cells or revascularizing the arteries and veins of the heart.

Furthermore, MSCs could produce paracrine growth factors that increase the survival of nearby cardiomyocytes, as well as increase angiogenesis through recruitment of stem cell from bone marrow or inducing vessel growth from existing capillaries. Recent research suggests that the paracrine effects of MSCs could be mediated by extracellular vesicles including exosomes. Exosomal microRNAs (miRNAs) released by MSCs are promising therapeutic hotspot target for MI. This could be attributed to the role of miRNA in cardiac biology, including cardiac regeneration, stem cell differentiation, apoptosis, neovascularization, cardiac contractility and cardiac remodeling. Furthermore, gene-modified MSCs could be a recent promising therapy for MI to enhance the paracrine effects of MSCs, including better homing and effective cell targeted tissue regeneration. Although MSC therapy has achieved considerable attention and progress, there are critical challenges that remains to be overcome to achieve the most effective successful cell-based therapy in MI.

Shao, L., et al. (2017). "MiRNA-Sequence Indicates That Mesenchymal Stem Cells and Exosomes Have Similar Mechanism to Enhance Cardiac Repair." Biomed Res Int 2017: 4150705.

Mesenchymal stem cells (MSCs) repair infarcted heart through paracrine mechanism. We sought to compare the effectiveness of MSCs and MSC-derived exosomes (MSC-Exo) in repairing infarcted hearts and to identify how MSC-Exo mediated cardiac repair is regulated. In a rat myocardial infarction model, we found that MSC-Exo inhibited cardiac fibrosis, inflammation, and improved cardiac function. The beneficial effects of MSC-Exo were significantly superior compared to that of MSCs. To explore the potential mechanisms underlying MSC-Exo's effects, we performed several in vitro experiments and miRNA-sequence analysis. MSC-Exo stimulated cardiomyocyte H9C2 cell proliferation, inhibited apoptosis induced by H2O2, and inhibited TGF-beta induced transformation of fibroblast cell into myofibroblast. Importantly, novel miRNA sequencing results indicated that MSC-Exo and MSCs have similar miRNA expression profile, which could be one of the reasons that MSC-Exo can replace MSCs for cardiac repair. In addition, the expression of several miRNAs from MSC-Exo was significantly different from that of MSCs, which may explain why MSC-Exo has better therapeutic effect than MSCs. In conclusion, this study demonstrates that MSC-Exo could be used alone to promote cardiac repair and are superior to MSCs in repairing injured myocardium.

Suzuki, E., et al. (2017). "Therapeutic Effects of Mesenchymal Stem Cell-Derived Exosomes in Cardiovascular Disease." Adv Exp Med Biol 998: 179-185.

Mesenchymal stem cells (MSCs) are multipotent stem cells that reside in various organs. They have the capacity to differentiate into various cell types, including cardiomyocytes, vascular endothelial cells, and vascular smooth muscle cells. Among the various MSCs, bone marrow-derived MSCs (BMMSCs) have been widely used for treating acute myocardial infarction (AMI) and ischemic heart failure (IHF) in

preclinical and clinical studies. Although the beneficial effects of BMMSCs in treating AMI and IHF were originally attributed to their capacity to differentiate into cardiac cell types, recent evidence suggests that the differentiation capacity of BMMSCs appears to be minimal and that BMMSCs exert cardioprotective effects by secreting paracrine factors. In this context, MSC-derived exosomes have recently gained much attention. In this chapter, we introduce preclinical studies in which MSC-derived exosomes are used for treating cardiovascular diseases (CVDs) such as AMI, stroke, pulmonary hypertension, and septic cardiomyopathy. Future clinical studies are required to confirm the efficacy of exosome administration in treating CVDs.

Vanhaverbeke, M., et al. (2017). "Functional Role of Cardiovascular Exosomes in Myocardial Injury and Atherosclerosis." Adv Exp Med Biol 998: 45-58.

Extracellular vesicles are now widely recognized as key players in the prevention, repair or progression of cardiovascular disease. Here we first focus on the functional roles of extracellular vesicles in the cross-talk between cardiomyocytes and endothelial cells, important for maintaining normal development and function of the heart. Second, we discuss the role of extracellular vesicles secreted by embryonic and non-embryonic stem cells in repairing cardiomyocyte function and in restoring angiogenic potential after myocardial ischemia-reperfusion injury. Third, we focus on the role of extracellular vesicles in Endothelial to Mesenchymal Transition (EndMT), leading to conversion of endothelial cells to fibroblasts, secretion of extracellular proteins collagen and fibronectin, and fibrosis. Finally, we discuss the role of extracellular vesicles secreted under stress by endothelial cells, macrophages and vascular smooth muscle cells in atherosclerosis. On aggregate, the reviewed preclinical studies present evidence that extracellular vesicles secreted by cardiomyocytes, fibroblasts, endothelial cells, immune-system-related cells, vascular smooth muscle cells and stem cells play an important role in the pathogenesis of cardiovascular disease. However, further studies are needed to gain better insight into the mechanisms used to select specific content to transfer to specific target cells, and to induce or repress signaling pathways in their target cells.

Wang, K., et al. (2017). "Enhanced Cardioprotection by Human Endometrium Mesenchymal Stem Cells Driven by Exosomal MicroRNA-21." Stem Cells Transl Med 6(1): 209-222.

Our group recently reported positive therapeutic benefit of human endometrium-derived mesenchymal stem cells (EnMSCs) delivered to infarcted rat myocardium, an effect that correlated with enhanced secretion of protective cytokines and growth factors compared with parallel cultures of human bone marrow MSCs (BMMSCs). To define more precisely the molecular mechanisms of EnMSC therapy, in the present study, we assessed in parallel the paracrine and therapeutic

properties of MSCs derived from endometrium, bone marrow, and adipose tissues in a rat model of myocardial infarction (MI). EnMSCs, BMMSCs, and adipose-derived MSCs (AdMSCs) were characterized by fluorescence-activated cell sorting (FACS). Paracrine and cytoprotective actions were assessed in vitro by coculture with neonatal cardiomyocytes and human umbilical vein endothelial cells. A rat MI model was used to compare cell therapy by intramyocardial injection of BMMSCs, AdMSCs, and EnMSCs. We found that EnMSCs conferred superior cardioprotection relative to BMMSCs or AdMSCs and supported enhanced microvessel density. Inhibitor studies indicated that the enhanced paracrine actions of EnMSCs were mediated by secreted exosomes. Analyses of exosomal microRNAs (miRs) by miR array and quantitative polymerase chain reaction revealed that miR-21 expression was selectively enhanced in exosomes derived from EnMSCs. Selective antagonism of miR-21 by anti-miR treatment abolished the antiapoptotic and angiogenic effects of EnMSCs with parallel effects on phosphatase and tensin homolog (PTEN), a miR-21 target and downstream Akt. The results of the present study confirm the superior cardioprotection by EnMSCs relative to BMMSCs or AdMSCs and implicates miR-21 as a potential mediator of EnMSC therapy by enhancing cell survival through the PTEN/Akt pathway. The endometrium might be a preferential source of MSCs for cardiovascular cell therapy. Stem Cells Translational Medicine 2017;6:209-222.

Yu, H., et al. (2017). "Stem cell therapy for ischemic heart diseases." Br Med Bull 121(1): 135-154.

Introduction: Ischemic heart diseases, especially the myocardial infarction, is a major hazard problem to human health. Despite substantial advances in control of risk factors and therapies with drugs and interventions including bypass surgery and stent placement, the ischemic heart diseases usually result in heart failure (HF), which could aggravate social burden and increase the mortality rate. The current therapeutic methods to treat HF stay at delaying the disease progression without repair and regeneration of the damaged myocardium. While heart transplantation is the only effective therapy for end-stage patients, limited supply of donor heart makes it impossible to meet the substantial demand from patients with HF. Stem cell-based transplantation is one of the most promising treatment for the damaged myocardial tissue. Sources of data: Key recent published literatures and ClinicalTrials.gov. Areas of agreement: Stem cell-based therapy is a promising strategy for the damaged myocardial tissue. Different kinds of stem cells have their advantages for treatment of Ischemic heart diseases. Areas of controversy: The efficacy and potency of cell therapies vary significantly from trial to trial; some clinical trials did not show benefit. Diverged effects of cell therapy could be affected by cell types, sources, delivery methods, dose and their mechanisms by which delivered cells exert their effects. Growing points: Understanding the origin of the regenerated cardiomyocytes, exploring the therapeutic effects of stem cell-derived exosomes and using the cell reprogram technology to

improve the efficacy of cell therapy for cardiovascular diseases. Areas timely for developing research: Recently, stem cell-derived exosomes emerge as a critical player in paracrine mechanism of stem cell-based therapy. It is promising to exploit exosomes-based cell-free therapy for ischemic heart diseases in the future.

Bagno, L., et al. (2018). "Mesenchymal Stem Cell-Based Therapy for Cardiovascular Disease: Progress and Challenges." Mol Ther 26(7): 1610-1623.

Administration of mesenchymal stem cells (MSCs) to diseased hearts improves cardiac function and reduces scar size. These effects occur via the stimulation of endogenous repair mechanisms, including regulation of immune responses, tissue perfusion, inhibition of fibrosis, and proliferation of resident cardiac cells, although rare events of transdifferentiation into cardiomyocytes and vascular components are also described in animal models. While these improvements demonstrate the potential of stem cell therapy, the goal of full cardiac recovery has yet to be realized in either preclinical or clinical studies. To reach this goal, novel cell-based therapeutic approaches are needed. Ongoing studies include cell combinations, incorporation of MSCs into biomaterials, or pre-conditioning or genetic manipulation of MSCs to boost their release of paracrine factors, such as exosomes, growth factors, microRNAs, etc. All of these approaches can augment therapeutic efficacy. Further study of the optimal route of administration, the correct dose, the best cell population(s), and timing for treatment are parameters that still need to be addressed in order to achieve the goal of complete cardiac regeneration. Despite significant progress, many challenges remain.

Barile, L., et al. (2018). "Cardioprotection by cardiac progenitor cell-secreted exosomes: role of pregnancy-associated plasma protein-A." Cardiovasc Res 114(7): 992-1005.

Aims: Cell therapy trials using cardiac-resident progenitor cells (CPCs) and bone marrow-derived mesenchymal stem/progenitor cells (BMCs) in patients after myocardial infarction have provided encouraging results. Exosomes, nanosized extracellular vesicles of endosomal origin, figure prominently in the bioactivities of these cells. However, a head-to-head comparison of exosomes from the two cell types has not been performed yet. Methods and results: CPCs and BMCs were derived from cardiac atrial appendage specimens and sternal bone marrow, respectively, from patients (n = 20; age, 69.9 +/- 10.9) undergoing heart surgery for aortic valve disease and/or coronary artery disease. Vesicles were purified from cell conditioned media by centrifugation/filtration and ultracentrifugation. Vesicle preparations were predominantly composed of exosomes based on particle size and marker expression (CD9, CD63, CD81, Alix, and TSG-101). CPC-secreted exosomes prevented staurosporine-induced cardiomyocyte apoptosis more effectively than BMC-secreted exosomes. In vivo, CPC-secreted exosomes reduced scar size and improved ventricular function after permanent coronary occlusion in rats more efficiently than BMC-

secreted exosomes. Both types of exosomes stimulated blood vessel formation. CPC-secreted exosomes, but not BMC-derived exosomes, enhanced ventricular function after ischaemia/reperfusion. Proteomics profiling identified pregnancy-associated plasma protein-A (PAPP-A) as one of the most highly enriched proteins in CPC vs. BMC exosomes. The active form of PAPP-A was detected on CPC exosome surfaces. These vesicles released insulin-like growth factor-1 (IGF-1) via proteolytic cleavage of IGF-binding protein-4 (IGFBP-4), resulting in IGF-1 receptor activation, intracellular Akt and ERK1/2 phosphorylation, decreased caspase activation, and reduced cardiomyocyte apoptosis. PAPP-A knockdown prevented CPC exosome-mediated cardioprotection both in vitro and in vivo. Conclusion: These results suggest that CPC-secreted exosomes may be more cardioprotective than BMC-secreted exosomes, and that PAPP-A-mediated IGF-1 release may explain the benefit. They illustrate a general mechanism whereby exosomes may function via an active protease on their surface, which releases a ligand in proximity to the transmembrane receptor bound by the ligand.

Chang, Y. M., et al. (2018). "Alpinate Oxyphyllae extracts enhance the longevity and homing of mesenchymal stem cells and augment their protection against senescence in H9c2 cells." J Cell Physiol.

Adipose-derived mesenchymal stem cells (ADMSCs) are easily accessible and are attractive mesenchymal stem cells for use in regenerative medicine; however their application is frequently restricted due to various challenges present in the environment they are administered. Therefore ADMSCs are preferably preconditioned with various stimulating factors to overcome the barriers developed in any pathological conditions. Here we used ADMSCs from rat adipose based on the abundance of positive markers and preconditioned the cells with extracts from Alpinate Oxyphyllae Fructus (AOF), a traditional Chinese herb used for antiaging, associated various health benefits. The preconditioned stem cells were tested for their potential to drive H9c2 from doxorubicin (Dox)-induced aging. The AOF-treated stem cells enriched stemness in ADMSCs with respect to their stem cells' positive marker, and enhanced their longevity mechanism and elevated the stem cell homing-associated C-X-C chemokine receptor type 7 (CXCR7). The AOF preconditioned stem cells, when cocultured with H9c2 cells, showed effective protection to Dox-induced senescence and stem cell homing to damaged H9c2 cells. The presence of AOF provided greater protective effects in the Dox environment. In addition, AOF-pretreated ADMSCs showed enhanced migration than those treated with AOF in Dox environment. Therefore, our results show that administration of AOF preconditioned stem cells is potentially an effective strategy in the management of aging-associated cardiac disorders.

Fang, L., et al. (2018). "[Effects of Exosomes Derived from miR-486 Gene Modified Umbilical Cord Mesenchymal Stem Cells on Biological Characteristics of Rat Cardiomyocytes]." Zhongguo Shi Yan Xue Ye Xue Za Zhi 26(5): 1531-1537.

OBJECTIVE: To investigate the effects of exosomes derived from

miR-486 gene-modified umbilical cord mesenchymal stem cells (UC-MSCs) on biological characteristics of rat cardiomyocytes. METHODS: The human umbilical cord mesenchymal stem cells (UC-MSCs) were isolated and cultured, then the immunophenotypes and ability of osteogenic and adipogenic differentiation of UC-MSC were identified. The structure of exosomes was observed by electron microscopy; the effect of exosomes on cell migration was detected by transwell cell migration test; the miR-486 high expression of UC-MSC was mediated by using recombinant adenovirus vector, moreover the UC-MSC with high expression of miR-486 were identified by qPT-PCR. The exosomes were isolated from cell culture supernatant by ultracentrifugation and the miR-486 expression level of UC MSC exosomes was detected by qRT-PCR. The effect of exosomes on the proliferation of cardiomyocytes was evaluated by Dye670 marking. The H2O2-induced cardiomyocyte apoptosis model was established, and the effect of exosomes on apoptosis of cardiomyocytes was detected by flow cytometry with Annexin V/PI double staining. RESULTS: The exosomes derived from UC-MSCs had the diameter between 40-100 nm and double membrane stracture. The recombinant adenovirus could effectively mediate the expression of miR-486 in UC-MSC, and the expression level of miR-486 in exosomes of miR-486-modified UC-MSC significantly increased. The exosomes with miR-486 high expression possessed the pro-proliferation and pro-migration effects on cardiomyocytes, moreover the preventive effect on apoptosis of cardiomyocytes. CONCLUSION: The exosomes derived from UC-MSC and accompamied by high expression of miR-486 can promote the proliferation and migration of cardio myocytes, yet can prevent the apoptosis of cardiomyocytes.

He, J. G., et al. (2018). "GATA-4-expressing mouse bone marrow mesenchymal stem cells improve cardiac function after myocardial infarction via secreted exosomes." Sci Rep 8(1): 9047.

This study aimed to investigate whether exosomes secreted by mouse GATA-4-expressing bone marrow mesenchymal stem cells (BMSCs) could induce BMSC differentiation into myocyte precursors, decrease cardiomyocyte apoptosis, and improve cardiac function following myocardial infarction (MI). BMSCs were transduced with a lentivirus carrying a doxycycline (DOX)-inducible GATA-4 or control lentivirus, and secreted exosomes from these BMSCs were collected and co-cultured with BMSCs or cardiomyocytes under hypoxic and serum free conditions. Furthermore, exosomes were injected into mice 48 h after MI. Cardiac function was evaluated by echocardiography at 48, 72, and 96 h after exosome treatment. Quantitative PCR showed that co-culture of BMSCs with GATA-4-BMSC exosomes increased cardiomyocyte-related marker expression. Co-culture of GATA-4-BMSC exosomes with cardiomyocytes in anoxic conditions decreased apoptosis as detected by flow cytometry. Injection of GATA-4-BMSC exosomes in mice 48 h after MI increased cardiac function over the next 96 h; increased cardiac blood vessel density and number of c-kit-positive cells and decreased apoptotic cardiomyocyte cells were also observed. Differential expression of candidate differentiation- and apoptosis-related miRNAs and proteins

that may mediate these effects was also identified. Exosomes isolated from GATA-4-expressing BMSCs induce differentiation of BMSCs into cardiomyocyte-like cells, decrease anoxia-induced cardiomyocyte apoptosis, and improve myocardial function after infarction.

Henning, R. J. (2018). "Current status of stem cells in cardiac repair." Future Cardiol 14(2): 181-192.

One out of every two men and one out of every three women greater than the age of 40 will experience an acute myocardial infarction (AMI) at some time during their lifetime. As more patients survive their AMIs, the incidence of congestive heart failure (CHF) is increasing. 6 million people in the USA have ischemic cardiomyopathies and CHF. The search for new and innovative treatments for patients with AMI and CHF has led to investigations and use of human embryonic stem cells, cardiac stem/progenitor cells, bone marrow-derived mononuclear cells and mesenchymal stem cells for treatment of these heart conditions. This paper reviews current investigations with human embryonic, cardiac, bone marrow and mesenchymal stem cells, and also stem cell paracrine factors and exosomes.

Hu, M., et al. (2018). "The harsh microenvironment in infarcted heart accelerates transplanted bone marrow mesenchymal stem cells injury: the role of injured cardiomyocytes-derived exosomes." Cell Death Dis 9(3): 357.

Stem cell therapy can be used to repair and regenerate damaged hearts tissue; nevertheless, the low survival rate of transplanted cells limits their therapeutic efficacy. Recently, it has been proposed that exosomes regulate multiple cellular processes by mediating cell survival and communication among cells. The following study investigates whether injured cardiomyocytes-derived exosomes (cardiac exosomes) affect the survival of transplanted bone marrow mesenchymal stem cells (BMSCs) in infarcted heart. To mimic the harsh microenvironment in infarcted heart that the cardiomyocytes or transplanted BMSCs encounter in vivo, cardiomyocytes conditioned medium and cardiac exosomes collected from H2O2-treated cardiomyocytes culture medium were cultured with BMSCs under oxidative stress in vitro. Cardiomyocytes conditioned medium and cardiac exosomes significantly accelerated the injury of BMSCs induced by H2O2; increased cleaved caspase-3/caspase-3 and apoptotic percentage, and decreased the ratio of Bcl-2/Bax and cell viability in those cells. Next, we explored the role of cardiac exosomes in the survival of transplanted BMSCs in vivo by constructing a Rab27a knockout (KO) mice model by a transcription activator-like effector nuclease (TALEN) genome-editing technique; Rab27a is a family of GTPases, which has critical role in secretion of exosomes. Male mouse GFP-modified BMSCs were implanted into the viable myocardium bordering the infarction in Rab27a KO and wild-type female mice. The obtained results showed that the transplanted BMSCs survival in infarcted heart was increased in Rab27a KO mice by the higher level of Y-chromosome Sry DNA, GFP mRNA, and the GFP fluorescence signal intensity. To sum up, these findings

revealed that the injured cardiomyocytes-derived exosomes accelerate transplanted BMSCs injury in infarcted heart, thus highlighting a new mechanism underlying the survival of transplanted cells after myocardial infarction.

Ju, C., et al. (2018). "Transplantation of Cardiac Mesenchymal Stem Cell-Derived Exosomes Promotes Repair in Ischemic Myocardium." J Cardiovasc Transl Res 11(5): 420-428.

Our previous study demonstrated the beneficial effects of exosomes secreted by cardiac mesenchymal stem cells (C-MSC-Exo) in protecting acute ischemic myocardium from reperfusion injury. Here, we investigated the effect of exosomes from C-MSC on angiogenesis in ischemic myocardium. We intramyocardially injected C-MSC-Exo or PBS into the infarct border zone after induction of acute mouse myocardial infarction (MI). We observed that hearts treated with C-MSC-Exo exhibit improved cardiac function compared to control hearts treated with PBS at one month after MI. Capillary density and Ki67-postive cells were significantly higher following treatment with C-MSC-Exo as compared with PBS. Moreover, C-MSC-Exo treatment increased cardiomyocyte proliferation in infarcted hearts. In conclusion, intramyocardial delivery of C-MSC-Exo after myocardial infarction enhances cardiac angiogenesis, promotes cardiomyocyte proliferation, and preserves heart function. C-MSC-Exo constitute a novel form of cell-free therapy for cardiac repair.

Lazar, E., et al. (2018). "Stem cell-derived exosomes - an emerging tool for myocardial regeneration." World J Stem Cells 10(8): 106-115.

Cardiovascular diseases (CVDs) continue to represent the number one cause of death and disability in industrialized countries. The most severe form of CVD is acute myocardial infarction (AMI), a devastating disease associated with high mortality and disability. In a substantial proportion of patients who survive AMI, loss of functional cardiomyocytes as a result of ischaemic injury leads to ventricular failure, resulting in significant alteration to quality of life and increased mortality. Therefore, many attempts have been made in recent years to identify new tools for the regeneration of functional cardiomyocytes. Regenerative therapy currently represents the ultimate goal for restoring the function of damaged myocardium by stimulating the regeneration of the infarcted tissue or by providing cells that can generate new myocardial tissue to replace the damaged tissue. Stem cells (SCs) have been proposed as a viable therapy option in these cases. However, despite the great enthusiasm at the beginning of the SC era, justified by promising initial results, this therapy has failed to demonstrate a significant benefit in large clinical trials. One interesting finding of SC studies is that exosomes released by mesenchymal SCs (MSCs) are able to enhance the viability of cardiomyocytes after ischaemia/reperfusion injury, suggesting that the beneficial effects of MSCs in the recovery of functional myocardium could be related to their capacity to secrete exosomes. Ten years ago, it was discovered that exosomes have the unique property of

transferring miRNA between cells, acting as miRNA nanocarriers. Therefore, exosome-based therapy has recently been proposed as an emerging tool for cardiac regeneration as an alternative to SC therapy in the post-infarction period. This review aims to discuss the emerging role of exosomes in developing innovative therapies for cardiac regeneration as well as their potential role as candidate biomarkers or for developing new diagnostic tools.

Luther, K. M., et al. (2018). "Exosomal miR-21a-5p mediates cardioprotection by mesenchymal stem cells." J Mol Cell Cardiol 119: 125-137.

Though experimental, stem cell transplantation has the potential to improve the condition of the heart after myocardial infarction. It does so by reducing infarct size and inducing repair of heart muscle and its blood supply. Mesenchymal stem cells (MSC) have been found to be effective in pre-clinical animal models and clinical trials, but the mechanisms by which they induce cardioprotection and repair are still not fully understood. Small extracellular vesicles known as exosomes are now recognized to be key mediators of beneficial MSC paracrine effects, and the concept that they transfer miRNA to change gene expression in recipient cells is of current therapeutic interest. We present complete deep miRNA sequencing of MSC exosome cargo, and found that of several cardioprotective miRNAs, miR-21a-5p was the most abundant. Because miR-21a-5p is a well-known cardioprotective miRNA, we investigated the hypothesis that MSC exosomes can cardioprotect the heart by increasing the level of miR-21a-5p in recipient cardiac cells, thereby downregulating expression of the pro-apoptotic gene products PDCD4, PTEN, Peli1 and FasL in the myocardium. Using miR-21 mimic transfection and treatment with wild type and miR-21a knockout MSC exosomes, we confirmed that exosomal miR-21a-5p is transferred into myocardium and is a major cardioprotective paracrine factor produced by MSCs acting via synergistic activity on multiple pathways. The data supports that residual cardioprotective effect may be due to other ncRNA or protein cargo. In silico analyses support that MSC exosomes may also contribute to angiogenesis, cell proliferation and other aspects of cardiac repair.

Ma, T., et al. (2018). "MicroRNA-132, Delivered by Mesenchymal Stem Cell-Derived Exosomes, Promote Angiogenesis in Myocardial Infarction." Stem Cells Int 2018: 3290372.

Background: To cure ischemic diseases, angiogenesis needs to be improved by various strategies in ischemic area. Considering that microRNA-132 (miR-132) regulates endothelial cell behavior during angiogenesis and the safe and efficacious delivery of microRNAs in vivo is rarely achieved, an ideal vehicle for miR-132 delivery could bring the promise for ischemic diseases. As a natural carrier of biological molecules, exosomes are more and more developed as an ideal vehicle for miRNA transfer. Meanwhile, mesenchymal stem cells could release large amounts of exosomes. Thus, this study aimed to

investigate whether MSC-derived exosomes can be used for miR-132 delivery in the treatment of myocardial ischemia. Methods: MSC-derived exosomes were electroporated with miR-132 mimics and inhibitors. After electroporation, miR-132 exosomes were labelled with DiI and added to HUVECs. Internalization of DiI-labelled exosomes was examined by fluorescent microscopy. Expression levels of miR-132 in exosomes and HUVECs were quantified by real-time PCR. The mRNA levels of miR-132 target gene RASA1 in HUVECs were quantified by real-time PCR. Luciferase reporter assay was performed to examine the targeting relationship between miR-132 and RASA1. The effects of miR-132 exosomes on the angiogenic ability of endothelial cells were evaluated by tube formation assay. Matrigel plug assay and myocardial infarction model were used to determine whether miR-132 exosomes can promote angiogenesis in vivo. Results: miR-132 mimics were effectively electroporated and highly detected in MSC-derived exosomes. The expression level of miR-132 was high in HUVECs preincubated with miR-132 mimic-electroporated exosomes and low in HUVECs preincubated with miR-132 inhibitor-electroporated exosomes. The expression level of RASA1, miR-132 target gene, was reversely correlated with miR-132 expression in HUVECs pretreated with exosomes. Luciferase reporter assay further confirmed that RASA1 was a direct target of miR-132. Exosomes loaded with miR-132, as a vehicle for miRNA transfer, significantly increased tube formation of endothelial cells. Moreover, subcutaneous injection of HUVECs pretreated with miR-132 exosomes in nude mice significantly increased their angiogenesis capacity in vivo. In addition, transplantation of miR-132 exosomes in the ischemic hearts of mice markedly enhanced the neovascularization in the peri-infarct zone and preserved heart functions. Conclusions: The findings suggest that the export of miR-132 via MSC-derived exosomes represents a novel strategy to enhance angiogenesis in ischemic diseases.

Mayourian, J., et al. (2018). "Exosomal microRNA-21-5p Mediates Mesenchymal Stem Cell Paracrine Effects on Human Cardiac Tissue Contractility." Circ Res 122(7): 933-944.

RATIONALE: The promising clinical benefits of delivering human mesenchymal stem cells (hMSCs) for treating heart disease warrant a better understanding of underlying mechanisms of action. hMSC exosomes increase myocardial contractility; however, the exosomal cargo responsible for these effects remains unresolved. OBJECTIVE: This study aims to identify lead cardioactive hMSC exosomal microRNAs to provide a mechanistic basis for optimizing future stem cell-based cardiotherapies. METHODS AND RESULTS: Integrating systems biology and human engineered cardiac tissue (hECT) technologies, partial least squares regression analysis of exosomal microRNA profiling data predicted microRNA-21-5p (miR-21-5p) levels positively correlate with contractile force and calcium handling gene expression responses in hECTs treated with conditioned media from multiple cell types. Furthermore, miR-21-5p levels were significantly elevated in hECTs treated with the exosome-enriched fraction of the hMSC secretome (hMSC-exo) versus untreated controls. This motivated experimentally

testing the human-specific role of miR-21-5p in hMSC-exo-mediated increases of cardiac tissue contractility. Treating hECTs with miR-21-5p alone was sufficient to recapitulate effects observed with hMSC-exo on hECT developed force and expression of associated calcium handling genes (eg, SERCA2a and L-type calcium channel). Conversely, knockdown of miR-21-5p in hMSCs significantly diminished exosomal procontractile and associated calcium handling gene expression effects on hECTs. Western blots supported miR-21-5p effects on calcium handling gene expression at the protein level, corresponding to significantly increased calcium transient amplitude and decreased decay time constant in comparison to miR-scramble control. Mechanistically, cotreating with miR-21-5p and LY294002, a PI3K inhibitor, suppressed these effects. Finally, mathematical simulations predicted the translational capacity for miR-21-5p treatment to restore calcium handling in mature ischemic adult human cardiomyocytes. CONCLUSIONS: miR-21-5p plays a key role in hMSC-exo-mediated effects on cardiac contractility and calcium handling, likely via PI3K signaling. These findings may open new avenues of research to harness the role of miR-21-5p in optimizing future stem cell-based cardiotherapies.

Ruan, X. F., et al. (2018). "Suxiao Jiuxin pill promotes exosome secretion from mouse cardiac mesenchymal stem cells in vitro." Acta Pharmacol Sin 39(4): 569-578.

Cardiac mesenchymal stem cells (C-MSCs) are endogenous cardiac stromal cells that play a role in heart repair after injury. C-MSC-derived exosomes (Exo) have shown protective effects against apoptosis induced by acute myocardial ischemia/reperfusion. Suxiao Jiuxin pill (SJP) is a traditional Chinese medicine (TCM) formula used in China for the treatment of acute myocardial ischemia, which contains tetramethylpyrazine (TMP) and borneol (BOR) as major components. In this study, we investigated whether SJP treatment affected exosome release from C-MSCs in vitro. C-MSCs prepared from mice were treated with SJP (62.5 mug/mL), TMP (25 mug/mL) or BOR (15 mug/mL). Using an acetylcholinesterase activity assay, we found that both SJP and TMP treatment significantly increased exosome secretion compared to the control ethanol treatment. The neutral sphingomyelinase 2 (nSMase2) pathway was important in exosome formation and packaging. But neither the level of nSMase2 mRNA nor the level of protein changed following SJP, TMP or BOR treatment, suggesting that SJP stimulated exosome release via an nSMase2-independent pathway. The Rab27a and Rab27b GTPases controlled different steps of the exosome secretion pathway. We showed that SJP treatment significantly increased the protein levels of Rab27a, SYTL4 (Rab27a effector) and Rab27b compared with the control treatment. SJP treatment also significantly upregulated the mRNA level of Rab27b, rather than Rab27a. Moreover, SJP-induced increase of C-MSC-exosome release was inhibited by Rab27b knockdown, suggesting that SJP promotes exosome secretion from C-MSCs via a GTPase-dependent pathway. This study reveals a novel mechanism for SJP in modulating cardiac homeostasis.

Ruan, X. F., et al. (2018). "Exosomes from Suxiao Jiuxin pill-treated cardiac mesenchymal stem cells decrease H3K27 demethylase UTX expression in mouse cardiomyocytes in vitro." Acta Pharmacol Sin 39(4): 579-586.

Suxiao Jiuxin pill (SJP) is a traditional Chinese medicine for the treatment of acute coronary syndrome in China, which contains two principal components, tetramethylpyrazine (TMP) and borneol (BOR). Thus far, however, the molecular mechanisms underlying the beneficial effects of SJP on the cardiac microenvironment are unknown. Cardiac mesenchymal stem cells (C-MSCs) communicate with cardiomyocytes (CMs) through the release of microvesicles (exosomes) to restore cardiac homeostasis and elicit repair, in part through epigenetic regulatory mechanisms. In this study, we examined whether SJP treatment altered C-MSC-derived exosomes (SJP-Exos) to cause epigenetic chromatic remodeling in recipient CMs. C-MSC isolated from mouse hearts were pretreated with SJP (SJP-Exos), TMP (TMP-Exos) or BOR (BOR-Exos). Then, HL-1 cells, a mouse cardiomyocyte line, were treated with exosomes from control C-MSCs (Ctrl-Exos), SJP-Exos, TMP-Exos or BOR-Exos. Treatment with SJP-Exos significantly increased the protein levels of histone 3 lysine 27 trimethylation (H3K27me3), a key epigenetic chromatin marker for cardiac transcriptional suppression, in the HL-1 cells. To further explore the mechanisms of SJP-Exo-mediated H3K27me3 upregulation, we assessed the mRNA expression levels of key histone methylases (EZH1, EZH2 and EED) and demethylases (JMJD3 and UTX) in the exosome-treated HL-1 cells. Treatment with SJP-Exo selectively suppressed UTX expression in the recipient HL-1 cells. Furthermore, PCNA, an endogenous marker of cell replication, was significantly higher in SJP-Exo-treated HL-1 cells than in Ctrl-Exo-treated HL-1 cells. These results show that SJP-Exos increase cardiomyocyte proliferation and demonstrate that SJP can modulate C-MSC-derived exosomes to cause epigenetic chromatin remodeling in recipient cardiomyocytes; consequently, SJP-Exos might be used to promote cardiomyocyte proliferation.

Shafei, A. E., et al. (2018). "Mechanistic effects of mesenchymal and hematopoietic stem cells: New therapeutic targets in myocardial infarction." J Cell Biochem 119(7): 5274-5286.

Myocardial infarction (MI) results in dysfunction and irreversible loss of cardiomyocytes and is of the most serious health threats today. Mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs) have been explored as promising cell therapy in MI and regenerative therapy. Recently, reports investigated the potential therapeutic effects of MSCs or HSCs transplantation after MI in numerous experimental and clinical studies; however, their results are controversy and needs more explorations. The current review is an attempt to clarify the therapeutic potentials of MSCs and HSCs in MI therapy, as well as their possible effects; especially the paracrine one and the exosome-derived stem cell among animal models as well as clinical trials conducted within the last 10 years. In this context,

various sources of MSCs and HSCs have been addressed in helping cardiac regeneration by either revitalizing the cardiac stem cells niche or revascularizing the arteries and veins of the heart. In addition, both MSCs and HSCs could produce paracrine mediators and growth factors which led to cardiomyocytes protection, angiogenesis, immunemodulation, antioxidants, anti-apoptotic, anti-inflammatory, antifibrotic, as well as increasing cardiac contractility. Recently, microRNAs (miRNAs), post-transcriptional regulators of gene expression, and long non-coding RNA (lncRNA), a miRNA sponge, are recent stem cell-derived mediators can be promising targets of MSCs and HSCs through their paracrine effects. Although MSCs and HSCs have achieved considerable achievements, however, some challenges still remain that need to be overcome in order to establish it as a successful technique. The present review clarified the mechanistic potentials of MSCs and HSCs especially paracrine effects involved in MI including human and animal studies and the challenges challenges regarding type, differentiation, route, and number of injections.

Shi, B., et al. (2018). "Bone marrow mesenchymal stem cell-derived exosomal miR-21 protects C-kit+ cardiac stem cells from oxidative injury through the PTEN/PI3K/Akt axis." PLoS One 13(2): e0191616.

Stem cell (SC) therapy for ischemic cardiomyopathy is hampered by poor survival of the implanted cells. Recently, SC-derived exosomes have been shown to facilitate cell proliferation and survival by transporting various proteins and non-coding RNAs (such as microRNAs and lncRNAs). In this study, miR-21 was highly enriched in exosomes derived from bone marrow mesenchymal stem cells (MSCs). Interestingly, exosomes collected from hydrogen peroxide (H2O2)-treated MSCs (H-Exo) contained higher levels of miR-21 than exosomes released from MSCs under normal conditions (N-Exo). The pre-treatment of C-kit+ cardiac stem cells (CSCs) with H-Exos resulted in significantly increased levels of miR-21 and phosphor-Akt (pAkt) and decreased levels of PTEN, which is a known target of miR-21. AnnexinV-FITC/PI analysis further demonstrated that the degree of oxidative stress-induced apoptosis was markedly lower in H-Exo-treated C-kit+ CSCs than that in N-Exo-treated cells. These protective effects could be blocked by both a miR-21 inhibitor and the PI3K/Akt inhibitor LY294002. Therefore, exosomal miR-21 derived from H2O2-treated MSCs could be transported to C-kit+ cardiac stem cells to functionally inhibit PTEN expression, thereby activating PI3K/AKT signaling and leading to protection against oxidative stress-triggered cell death. Thus, exosomes derived from MSCs could be used as a new therapeutic vehicle to facilitate C-kit+ CSC therapies in the ischemic myocardium.

Spinosa, M., et al. (2018). "Human mesenchymal stromal cell-derived extracellular vesicles attenuate aortic aneurysm formation and macrophage activation via microRNA-147." Faseb j: fj201701138RR.

The formation of an abdominal aortic aneurysm (AAA) is characterized by inflammation, macrophage infiltration, and vascular remodeling. In this study, we tested the hypothesis that mesenchymal

stromal cell (MSC)-derived extracellular vesicles (EVs) immunomodulate aortic inflammation, to mitigate AAA formation via modulation of microRNA-147. An elastase-treatment model of AAA was used in male C57BL/6 wild-type (WT) mice. Administration of EVs in elastase-treated WT mice caused a significant attenuation of aortic diameter and mitigated proinflammatory cytokines, inflammatory cell infiltration, an increase in smooth muscle cell alpha-actin expression, and a decrease in elastic fiber disruption, compared with untreated mice. A 10-fold up-regulation of microRNA (miR)-147, a key mediator of macrophage inflammatory responses, was observed in murine aortic tissue in elastase-treated mice compared with controls on d 14. EVs derived from MSCs transfected with miR-147 mimic, but not with miR-147 inhibitor, attenuated aortic diameter, inflammation, and leukocyte infiltration in elastase-treated mice. In vitro studies of human aortic tissue explants and murine-derived CD11b(+) macrophages induced proinflammatory cytokines after elastase treatment, and the expression was attenuated by cocultures with EVs transfected with miR-147 mimic, but not with miR-147 inhibitor. Thus, our findings define a critical role of MSC-derived EVs in attenuation of aortic inflammation and macrophage activation via miR-147 during AAA formation.-Spinosa, M., Lu, G., Su, G., Bontha, S. V., Gehrau, R., Salmon, M. D., Smith, J. R., Weiss, M. L., Mas, V. R., Upchurch, G. R., Sharma, A. K. Human mesenchymal stromal cell-derived extracellular vesicles attenuate aortic aneurysm formation and macrophage activation via microRNA-147.

Sun, X., et al. (2018). "Intravenous mesenchymal stem cell-derived exosomes ameliorate myocardial inflammation in the dilated cardiomyopathy." Biochem Biophys Res Commun 503(4): 2611-2618.

Mesenchymal stem cells (MSCs) have been shown to be efficacy to attenuating cardiovascular inflammation; however, there are many limitations to stem cell treatment. Present study was to prove MSC-derived exosomes (MSC-Exos) could alleviating inflammatory cardiomyopathy by improving the inflammatory microenvironment of myocardium, especially by regulating the activity of macrophages. Mice were intraperitoneal injected of doxorubicin (DOX) to establish a dilated cardiomyopathy (DCM) model, and then received intravenous injection of either MSC-Exos or PBS as control. Mice receiving MSC-Exos showed improved cardiac function via echocardiography and attenuated cardiac dilation via HE staining, as well as reduced cardiomyocytes apoptosis. Expression levels of inflammatory factors were reduced. And there was a significant decrease of the inflammatory cells infiltration in the MSC-Exos treatment group comparing to the PBS group. Meanwhile, MSC-Exos could remarkably attenuate the pro-inflammatory macrophages amount in both blood and heart, which was proved that MSC-Exos relied on the JAK2-STAT6 pathway mediating macrophages activation. MSC-Exos improved the inflammatory microenvironment of dilated cardiomyopathy by regulating the polarization of the macrophage, which may hold promise for dilated cardiomyopathy clinical therapy.

Terashvili, M. and Z. J. Bosnjak (2018). "Stem Cell Therapies in Cardiovascular Disease." J Cardiothorac Vasc Anesth.

Despite considerable advances in medicine, cardiovascular disease is still rising, with ischemic heart disease being the leading cause of death and disability worldwide. Thus extensive efforts are continuing to establish effective therapeutic modalities that would improve both quality of life and survival in this patient population. Novel therapies are being investigated not only to protect the myocardium against ischemia-reperfusion injury but also to regenerate the heart. Stem cell therapy, such as potential use of human mesenchymal stem cells and induced pluripotent stem cells and their exosomes, will make it possible not only to address molecular mechanisms of cardiac conditioning, but also to develop new therapies for ischemic heart disease. Despite the studies and progress made over the last 15 years on the use of stem cell therapy for cardiovascular disease, the efforts are still in their infancy. Even though the expectations have been high, the findings indicate that most of the clinical trials generally have been small and the results inconclusive. Because of many negative findings, there is certain pessimism that cardiac cell therapy is likely to yield any meaningful results over the next decade or so. Similar to other new technologies, early failures are not unusual and they may be followed by impressive success. Nevertheless, there has been considerable attention to safety by the clinical investigators because the adverse events of stem cell therapy have been impressively rare. In summary, although regenerative biology might not help the cardiovascular patient in the near term, it is destined to do so over the next several decades.

Wang, X., et al. (2018). "Engineered Exosomes With Ischemic Myocardium-Targeting Peptide for Targeted Therapy in Myocardial Infarction." J Am Heart Assoc 7(15): e008737.

Background Exosomes are membranous vesicles generated by almost all cells. Recent studies demonstrated that mesenchymal stem cell-derived exosomes possessed many effects, including antiapoptosis, anti-inflammatory effects, stimulation of angiogenesis, anticardiac remodeling, and recovery of cardiac function on cardiovascular diseases. However, targeting of exosomes to recipient cells precisely in vivo still remains a problem. Ligand fragments or homing peptides discovered by phage display and in vivo biopanning methods fused to the enriched molecules on the external part of exosomes have been exploited to improve the ability of exosomes to target specific tissues or organs carrying cognate receptors. Herein, we briefly elucidated how to improve targeting ability of exosomes to ischemic myocardium. Methods and Results We used technology of molecular cloning and lentivirus packaging to engineer exosomal enriched membrane protein (Lamp2b) fused with ischemic myocardium-targeting peptide CSTSMLKAC (IMTP). In vitro results showed that IMTP-exosomes could be internalized by hypoxia-injured H9C2 cells more efficiently than blank-exosomes. Compared with blank-exosomes, IMTP-exosomes were observed to be increasingly accumulated in ischemic heart area

(P<0.05). Meanwhile, attenuated inflammation and apoptosis, reduced fibrosis, enhanced vasculogenesis, and cardiac function were detected by mesenchymal stem cell-derived IMTP-exosome treatment in ischemic heart area. Conclusions Our research concludes that exosomes engineered by IMTP can specially target ischemic myocardium, and mesenchymal stem cell-derived IMTP-exosomes exert enhanced therapeutic effects on acute myocardial infarction.

Wang, X. L., et al. (2018). "Exosomes derived from human umbilical cord mesenchymal stem cells improve myocardial repair via upregulation of Smad7." Int J Mol Med 41(5): 3063-3072.

It has been previously reported that exosomes derived from human umbilical cord mesenchymal stem cells (hucMSC)exosomes exhibit cardioprotective effects on the rat acute myocardial infarction (AMI) models and cardiomyocyte hypoxia injury models in vitro, however the exact mechanisms involved require further investigation. The present study aimed to investigate the repair effects of hucMSCexosomes on myocardial injury via the regulation of mothers against decapentaplegic homolog 7 (Smad7) expression. Compared with sham or normoxia groups (in vivo and in vitro, respectively), western blotting demonstrated that Smad7 expression was significantly decreased in the borderline area of infraction myocardium and in H9C2(21) cells following hypoxiainduced injury. Additionally, microRNA (miR)125b5p expression was markedly increased using reverse transcriptionquantitative polymerase chain reaction, but was reversed by hucMSCexosomes. Trypan blue staining and lactate dehydrogenase release detection demonstrated that cell injury was significantly increased in the AMI + PBS and hypoxia group compared with in the sham and normoxia groups and was inhibited by hucMSCexosomes. A dual luciferase reporter gene assay confirmed that Smad7 is a target gene of miR125b5p. In addition, miR125b5p mimics promoted H9C2(21) cell injury following 48 h exposure to hypoxia. Downregulation of Smad7 expression under hypoxia was increased by miR125b5p mimics compared with the mimic negative control, and hucMSCexosomes partially alleviated this phenomenon. In conclusion, hucMSCexosomes may promote Smad7 expression by inhibiting miR125b5p to increase myocardial repair. The present study may provide a potential therapeutic approach to improve myocardial repair following AMI.

Wang, Y., et al. (2018). "Rapid Delivery of Hsa-miR-590-3p Using Targeted Exosomes to Treat Acute Myocardial Infarction Through Regulation of the Cell Cycle." J Biomed Nanotechnol 14(5): 968-977.

Acute myocardial infarction leads to heart failure due to inadequate regeneration of cardiomyocytes. Therefore, promotion of cardiomyocyte proliferation is the key for the restoration of cardiac function. Induction of the cell cycle and the downregulation of genes that inhibit cardiomyocyte proliferation could induce cardiomyocyte to re-enter into the proliferative state. Hsa-miR-590-3p has good application prospects in myocardial proliferation since it could downregulate the expression of genes inhibiting cell proliferation

such as Hopx. However, delivering sufficient hsa-miR-590-3p to the infarct area with non-invasive and non-viral methods efficiently and rapidly is challenging. Based on the high expression of cTnI in the microenvironment of infarct area, we used gene transfection to express a cTnI-targeted short peptide on the surface of mesenchymal stem cells to obtain cTnI-targeted exosomes. These exosomes could localize to infarct area along a cTnI concentration gradient. Exosomes carrying hsa-miR-590-3p were endocytosis by cardiomyocytes and thus promoted cardiomyocyte proliferation in the peri-infarct area and eventually restored cardiac function. Our results show that targeted exosome is a minimally invasive, non-viral, efficient, and rapid delivery system for the treatment of acute myocardial infarction.

Wang, Y., et al. (2018). "Exosomes Derived from miR-214-Enriched Bone Marrow-Derived Mesenchymal Stem Cells Regulate Oxidative Damage in Cardiac Stem Cells by Targeting CaMKII." Oxid Med Cell Longev 2018: 4971261.

Cardiac stem cells (CSCs) have emerged as one of the most promising stem cells for cardiac protection. Recently, exosomes from bone marrow-derived mesenchymal stem cells (BMSCs) have been found to facilitate cell proliferation and survival by transporting various bioactive molecules, including microRNAs (miRs). In this study, we found that BMSC-derived exosomes (BMSC-exos) significantly decreased apoptosis rates and reactive oxygen species (ROS) production in CSCs after oxidative stress injury. Moreover, a stronger effect was induced by exosomes collected from BMSCs cultured under hypoxic conditions (Hypoxic-exos) than those collected from BMSCs cultured under normal conditions (Nor-exos). We also observed greater miR-214 enrichment in Hypoxic-exos than in Nor-exos. In addition, a miR-214 inhibitor or mimics added to modulate miR-214 levels in BMSC-exos revealed that exosomes from miR-214-depleted BMSCs partially reversed the effects of hypoxia-induced exosomes on oxidative damage in CSCs. These data further confirmed that miR-214 is the main effector molecule in BMSC-exos that protects CSCs from oxidative damage. miR-214 mimic and inhibitor transfection assays verified that CaMKII is a target gene of miR-214 in CSCs, with exosome-pretreated CSCs exhibiting increased miR-214 levels but decreased CaMKII levels. Therefore, the miR-214/CaMKII axis regulates oxidative stress-related injury in CSCs, such as apoptosis, calcium homeostasis disequilibrium, and excessive ROS accumulation. Collectively, these findings suggest that BMSCs release miR-214-containing exosomes to suppress oxidative stress injury in CSCs through CaMKII silencing.

Ward, M. R., et al. (2018). "Concise Review: Rational Use of Mesenchymal Stem Cells in the Treatment of Ischemic Heart Disease." Stem Cells Transl Med 7(7): 543-550.

The capacity of stem and progenitor cells to stimulate cardiac regeneration has been studied for almost 20 years, with very promising preclinical data and mixed clinical results. Several cell types have been studied, identified by their cell surface markers,

differentiation capacity and their secreted growth factors. Bone marrow derived mesenchymal stem cells (MSCs) have been found to have potent regenerative capacity, through multiple mechanisms, including mesoderm lineage differentiation, immunomodulation, and paracrine stimulation. MSCs also secrete exosomes and microvesicles, which themselves contain potent angiogenic cytokines or mRNA molecules with effects on their local milieu. This concise review summarizes the mechanisms of MSC-based cardiac regeneration and highlighting results from molecular and preclinical studies. We also discuss clinical trial results to date, and ongoing studies. Furthermore, we discuss novel approaches for the enhancement of MSC based cardiac regeneration, such as genetic modification. Stem Cells Translational Medicine 2018;7:543-550.

Xiao, C., et al. (2018). "Transplanted Mesenchymal Stem Cells Reduce Autophagic Flux in Infarcted Hearts via the Exosomal Transfer of miR-125b." Circ Res 123(5): 564-578.

RATIONALE: Autophagy can preserve cell viability under conditions of mild ischemic stress by degrading damaged organelles for ATP production, but under conditions of severe ischemia, it can promote cell death and worsen cardiac performance. Mesenchymal stem cells (MSCs) are cardioprotective when tested in animal models of myocardial infarction, but whether these benefits occur through the regulation of autophagy is unknown. OBJECTIVE: To determine whether transplanted MSCs reduce the rate of autophagic degradation (autophagic flux) in infarcted hearts and if so, to characterize the mechanisms involved. METHODS AND RESULTS: Treatment with transplanted MSCs improved cardiac function and infarct size while reducing apoptosis and measures of autophagic flux (bafilomycin A1-induced LC3-II [microtubule-associated protein 1 light chain 3] accumulation and autophagosome/autolysosome prevalence) in infarcted mouse hearts. In hypoxia and serum deprivation-cultured neonatal mouse cardiomyocytes, autophagic flux and cell death, as well as p53-Bnip3 (B-cell lymphoma 2-interacting protein 3) signaling, declined when the cells were cultured with MSCs or MSC-secreted exosomes (MSC-exo), but the changes associated with MSC-exo were largely abolished by pretreatment with the exosomal inhibitor GW4869. Furthermore, a mimic of the exosomal oligonucleotide miR-125b reduced, whereas an anti-miR-125b oligonucleotide increased, autophagic flux and cell death, via modulating p53-Bnip3 signaling in hypoxia and serum deprivation-cultured neonatal mouse cardiomyocytes. In the in vivo mouse myocardial infarction model, MSC-exo, but not the exosomes obtained from MSCs pretreated with the anti-miR-125b oligonucleotide (MSC-exo(anti-miR-125b)), recapitulated the same results as the in vitro experiments. Moreover, measurements of infarct size and cardiac function were significantly better in groups that were treated with MSC-exo than the MSC-exo(anti-miR-125b) group. CONCLUSIONS: The beneficial effects offered by MSC transplantation after myocardial infarction are at least partially because of improved autophagic flux through excreted exosome containing mainly miR-125b-5p.

Yoshida, S., et al. (2018). "Maturation of Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes by Soluble Factors from Human Mesenchymal Stem Cells." Mol Ther 26(11): 2681-2695.

In this study, we proposed that the functionality or phenotype of differentiated cardiomyocytes derived from human induced pluripotent stem cells (iPSC-CMs) might be modified by co-culture with mesenchymal stem cells (MSCs), resulting in an improved therapeutic potential for failing myocardial tissues. Structural, motility, electrophysiological, and metabolic analyses revealed that iPSC-CMs co-cultured with MSCs displayed aligned myofibrils with A-, H-, and I-bands that could contract and relax quickly, indicating the promotion of differentiation and the establishment of the iPSC-CM structural framework, and showed clear gap junctions and an electric pacing of >2 Hz, indicating enhanced cell-cell interactions. In addition, soluble factors excreted by MSCs, including several cytokines and exosomes, enhanced cardiomyocyte-specific marker production, produced more energy under normal and stressed conditions, and reduced reactive oxygen species production by iPSC-CMs under stressed condition. Notably, gene ontology and pathway analysis revealed that microRNAs and proteins in the exosomes impacted the functionality and maturation of iPSC-CMs. Furthermore, cell sheets consisting of a mixture of iPSC-CMs and MSCs showed longer survival and enhanced therapeutic effects compared with those consisting of iPSC-CMs alone. This may lead to a new type of iPSC-based cardiomyogenesis therapy for patients with heart failure.

Zhong, J., et al. (2018). "The current status and future of cardiac stem/progenitor cell therapy for congenital heart defects from diabetic pregnancy." Pediatr Res 83(1-2): 275-282.

Pregestational maternal diabetes induces congenital heart defects (CHDs). Cardiac dysfunction after palliative surgical procedures contributes to the high mortality of CHD patients. Autologous or allogeneic stem cell therapies are effective for improving cardiac function in animal models and clinical trials. c-kit(+) cardiac progenitor cells (CPCs), the most recognized CPCs, have the following basic properties of stem cells: self-renewal, multicellular clone formation, and differentiation into multiple cardiac lineages. However, there is ongoing debate regarding whether c-kit(+) CPCs can give rise to sufficient cardiomyocytes. A new hypothesis to address the beneficial effect of c-kit(+) CPCs is that these cells stimulate endogenous cardiac cells through a paracrine function in producing a robust secretome and exosomes. The values of other cardiac CPCs, including Sca1(+) CPCs and cardiosphere-derived cells, are beginning to be revealed. These cells may be better choices than c-kit(+) CPCs for generating cardiomyocytes. Adult mesenchymal stem cells are considered immune-incompetent and effective for improving cardiac function. Autologous CPC therapy may be limited by the observation that maternal diabetes adversely affects the biological function of embryonic stem cells and CPCs. Future studies

should focus on determining the mechanistic action of these cells, identifying new CPC markers, selecting highly effective CPCs, and engineering cell-free products.

Zhu, J., et al. (2018). "Myocardial reparative functions of exosomes from mesenchymal stem cells are enhanced by hypoxia treatment of the cells via transferring microRNA-210 in an nSMase2-dependent way." Artif Cells Nanomed Biotechnol 46(8): 1659-1670.

Hypoxia treatment enhances paracrine effect of mesenchymal stem cells (MSCs). The aim of this study was to investigate whether exosomes from hypoxia-treated MSCs (Exo(H)) are superior to those from normoxia-treated MSCs (Exo(N)) for myocardial repair. Mouse bone marrow-derived MSCs were cultured under hypoxia or normoxia for 24 h, and exosomes from conditioned media were intramyocardially injected into infarcted heart of C57BL/6 mouse. Exo(H) resulted in significantly higher survival, smaller scar size and better cardiac functions recovery. Exo(H) conferred increased vascular density, lower cardiomyocytes (CMs) apoptosis, reduced fibrosis and increased recruitment of cardiac progenitor cells in the infarcted heart relative to Exo(N). MicroRNA analysis revealed significantly higher levels of microRNA-210 (miR-210) in Exo(H) compared with Exo(N). Transfection of a miR-210 mimic into endothelial cells (ECs) and CMs conferred similar biological effects as Exo(H). Hypoxia treatment of MSCs increased the expression of neutral sphingomyelinase 2 (nSMase2) which is crucial for exosome secretion. Blocking the activity of nSMase2 resulted in reduced miR-210 secretion and abrogated the beneficial effects of Exo(H). In conclusion, hypoxic culture augments miR-210 and nSMase2 activities in MSCs and their secreted exosomes, and this is responsible at least in part for the enhanced cardioprotective actions of exosomes derived from hypoxia-treated cells.

Gong, X. H., et al. (2019). "Exosomes derived from SDF1-overexpressing mesenchymal stem cells inhibit ischemic myocardial cell apoptosis and promote cardiac endothelial microvascular regeneration in mice with myocardial infarction." J Cell Physiol.

Exosomes extracted from mesenchymal stem cells (MSCs) was reported to reduce myocardial ischemia/reperfusion damage. Besides, stromal-derived factor 1 (SDF1a) functions as cardiac repair after myocardial infarction (MI). Therefore, the present study aims to identify whether exosomes (Exo) released from SDF1-overexpressing MSCs display a beneficial effect on ischemic myocardial infarction. Initially, a gain-of-function study was performed to investigate the function of SDF1 in ischemic myocardial cells and cardiac endothelial cells. Coculture experiments were performed to measure potential exosomic transfer of SDF1 from MSCs to ischemic myocardial cells and cardiac endothelial cells. During the coculture experiments, exosome secretion was disrupted by neutral sphingomyelinase inhibitor GW4869 and upregulated exosomal SDF1 using SDF1 plasmid. Effects of Exo-SDF1 on cardiac function in MI mice were investigated in vivo. MSCs

suppressed myocardial cell apoptosis and promoted microvascular regeneration of endothelial cells through secretion of exosomes. The addition of GW4869 led to increased apoptotic capacity of myocardial cells, decreased microvascular formation ability of endothelial cells, enhanced autophagy ability, and elevated Beclin-1 level as well as ratio of LC3II/LC3I. Overexpression of SDF1 and Exo-SDF1 inhibited apoptosis and autophagy of myocardial cells, but promoted tube formation of endothelial cells. The interference of PI3K signaling pathway promoted apoptosis and autophagy of myocardial cells, but inhibited tube formation of endothelial cells. SDF1 activated the PI3K signaling pathway. Exo-SDF1 protected cardiac function of MI mice and inhibited myocardial tissue damage. This study provided evidence that SDF1 overexpression in MSCs-derived exosomes inhibited autophagy of ischemic myocardial cells and promoted microvascular production of endothelial cells.

Li, J., et al. (2019). "Exosomes derived from mesenchymal stem cells attenuate the progression of atherosclerosis in ApoE(-/-) mice via miR-let7 mediated infiltration and polarization of M2 macrophage." Biochem Biophys Res Commun 510(4): 565-572.

Atherosclerosis is a chronic inflammatory disease of the vasculature. Exosomes derived from mesenchymal stem cells (MSCs) exert immunomodulatory and immunosuppressive effects; however, the MSCs-exosomes administration on atherosclerosis was unknown. Here, our ApoE(-/-) mice were fed a high-fat diet and received intravenous injections of exosomes from MSCs for 12 weeks. After tail-vein injection, MSCs-exosomes were capable of migrating to atherosclerotic plaque and selectively taking up residence near macrophages. MSCs-exosomes treatment decreased the atherosclerotic plaque area of ApoE(-/-) mice and greatly reduced the infiltration of macrophages in the plaque, associating induced macrophage polarization towards M2. In vitro, MSCs-exosomes treatment markedly inhibited LPS-induced M1 markers expression, while increased M2 markers expression in macrophages. Moreover, miR-let7 family was found to be highly enriched in MSCs-exosomes. Endogenous miR-let7 expression was found in the aortic root of ApoE(-/-) mice, and MSCs-exosomes treatment further up-regulated miR-let7 levels. In addition, inhibition of miR-let7 in U937cells significantly inhibited the migration and M2 polarization via IGF2BP1 and HMGA2 pathway respectively in vitro. Our study demonstrates that MSCs-exosomes ameliorated atherosclerosis in ApoE(-/-) and promoted M2 macrophage polarization in the plaque through miR-let7/HMGA2/NF-kappaB pathway. In addition, MSCs-exosomes suppressed macrophage infiltration via miR-let7/IGF2BP1/PTEN pathway in the plaque. This finding extends our knowledge on MSCs-exosomes affect inflammation in atherosclerosis plaque and provides a potential method to prevent the atherosclerosis. Exosomes from MSCs hold promise as therapeutic agents to reduce the residual risk of coronary artery diseases.

Zhang, Y., et al. (2019). "Exosomes from human umbilical cord

mesenchymal stem cells enhance fracture healing through HIF-1alpha-mediated promotion of angiogenesis in a rat model of stabilized fracture." Cell Prolif: e12570.

OBJECTIVES: Exosomes, as important players in intercellular communication due to their ability to transfer certain molecules to target cells, are believed to take similar effects in promoting bone regeneration with their derived stem cells. Studies have suggested that umbilical cord mesenchymal stem cells (uMSCs) could promote angiogenesis. This study investigated whether exosomes derived from uMSCs (uMSC-Exos) could enhance fracture healing as primary factors by promoting angiogenesis. MATERIALS AND METHODS: uMSCs were obtained to isolate uMSC-Exos by ultrafiltration, with exosomes from human embryonic kidney 293 cells (HEK293) and phosphate-buffered saline (PBS) being used as control groups. NanoSight, laser light scattering spectrometer, transmission electron microscopy and Western blotting were used to identify exosomes. Next, uMSC-Exos combined with hydrogel were transplanted into the fracture site in a rat model of femoral fracture. Bone healing processes were monitored and evaluated by radiographic methods on days 7, 14, 21 and 31 after surgery; angiogenesis of the fracture sites was assessed by radiographic and histological strategies on post-operative day 14. In vitro, the expression levels of osteogenesis- or angiogenesis-related genes after being cultured with uMSC-Exos were identified by qRT-PCR. The internalization ability of exosomes was determined using the PKH67 assay. Cell cycle analysis, EdU incorporation and immunofluorescence staining, scratch wound assay and tube formation analysis were also used to determine the altered abilities of human umbilical vein endothelial cells (HUVECs) administered with uMSC-Exos in proliferation, migration and angiogenesis. Finally, to further explore the underlying molecular mechanisms, specific RNA inhibitors or siRNAs were used, and the subsequent effects were observed. RESULTS: uMSC-Exos had a diameter of approximately 100 nm, were spherical, meanwhile expressing CD9, CD63 and CD81. Transplantation of uMSC-Exos markedly enhanced angiogenesis and bone healing processes in a rat model of femoral fracture. In vitro, other than enhancing osteogenic differentiation, uMSC-Exos increased the expression of vascular endothelial growth factor (VEGF) and hypoxia inducible factor-1alpha (HIF-1alpha). uMSC-Exos were taken up by HUVECs and enhanced their proliferation, migration and tube formation. Finally, by using specific RNA inhibitors or siRNAs, it has been confirmed that HIF-1alpha played an important role in the uMSC-Exos-induced VEGF expression, pro-angiogenesis and enhanced fracture repair, which may be one of the underlying mechanisms. CONCLUSIONS: These results revealed a novel role of exosomes in uMSC-mediated therapy and suggested that implanted uMSC-Exos may represent a crucial clinical strategy to accelerate fracture healing via the promotion of angiogenesis. HIF-1alpha played an important role in this process.

Zhao, J., et al. (2019). "Mesenchymal Stromal Cell-Derived Exosomes Attenuate Myocardial Ischemia-Reperfusion Injury through miR-182-

Regulated Macrophage Polarization." Cardiovasc Res.

Aims: Mesenchymal stem cells (MSCs) gradually become attractive candidates for cardiac inflammation modulation, yet understanding of the mechanism remains elusive. Strikingly, recent studies indicated that exosomes secreted by MSCs might be a novel mechanism for the beneficial effect of MSCs transplantation after myocardial infarction. We therefore explored the role of MSC-derived exosomes (MSC-Exo) in the immunomodulation of macrophages after myocardial ischemia/reperfusion (I/R) and its implications in cardiac injury repair. Methods and Results: Exosomes were isolated from the supernatant of MSCs using gradient centrifugation method. Administration of MSC-Exo to mice through intramyocardial injection after myocardial I/R reduced infarct size and alleviated inflammation level in heart and serum. Systemic depletion of macrophages with clodronate liposomes abolished the curative effects of MSC-Exo. MSC-Exo modified the polarization of M1 macrophages to M2 macrophages both in vivo and in vitro. miRNA-sequencing of MSC-Exo and bioinformatics analysis implicated miR-182 as a potent candidate mediator of macrophage polarization and TLR4 as a downstream target. Diminishing miR-182 in MSC-Exo partially attenuated its modulation of macrophage polarization. Likewise, knock down of TLR4 also conferred cardioprotective efficacy and reduced inflammation level in a mouse model of myocardial I/R. Conclusion: Our data indicates that MSC-Exo attenuates myocardial I/R injury in mice via shuttling miR-182 that modifies the polarization status of macrophages. This study sheds new light on the application of MSC-Exo as a potential therapeutic tool for myocardial I/R injury.