Mesenchymal Stem Cells in Cardiac Repair: Effects on Myocytes, Vasculature, and Fibroblasts

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ABSTRACT

Purpose: Cardiac pathologies remain a dominant cause of morbidity and mortality within the community. The drive to develop therapies capable of repairing damaged heart tissue to achieve clinically significant restoration of function has motivated the pursuit of novel approaches such as cell therapy. To this end, evidence of therapeutic benefits achieved by using mesenchymal stem cells (MSCs) has captured considerable interest despite a relative lack of information regarding the mechanisms involved. This narrative review synthesizes and interprets the current literature describing mechanisms by which MSCs can elicit cardiac repair, thereby directing attention to avenues of further inquiry.

Methods: OVID versions of MEDLINE and EMBASE were searched for studies describing the role of MSCs in mammalian cardiac repair. Additional studies were sourced from the reference lists of relevant articles and other personal files.

Findings: MSCs elicit cardiac repair in a range of *in vitro* systems and animal models of diseases such as myocardial infarction and heart failure. Important mechanisms include the preservation of myocardial contractility, the promotion of angiogenesis, and the modulation of fibrosis. Exposing *in vitro* MSCs to a microenvironment reflective of that encountered in the injured heart seems to potentiate these therapeutic mechanisms.

Implications: Promising results in animal studies warrant continuation of clinical MSC cardiac therapy studies. Paracrine functions of MSCs seem to be the dominant mechanism of cardiac repair over direct cellular effects. Although integral, the MSC secretome remains poorly defined. In addition, most of the mechanistic data within the literature have been derived from animal MSC research, necessitating more human MSC-based work. (*Clin Ther.* 2020;42:1880–1891) © 2020 Elsevier Inc.

Key words: cardiac repair, mesenchymal stem cells, regenerative medicine, repair mechanisms.

INTRODUCTION

Despite a downtrend in the prevalence of cardiovascular disease since the 1960s, its population burden remains substantial and is threatening to worsen as the global obesity epidemic continues to evolve.[1] Central to the issue of treating established cardiac pathology is the mammalian heart's inherently poor capacity to regenerate after injury. The physician's toolkit is currently bereft of therapies capable of inducing clinically significant cardiac repair. For this reason, the field of regenerative cardiovascular medicine, which features both cellular and exogenous factor-based therapies, remains of great interest to clinicians and scientists alike. Although a plethora of stem and progenitor-like cells have been studied in a range of cardiac indications,[2–6] conflicting data and controversy have impeded progress.[7,8]

Adult mesenchymal stem cells (MSCs) have emerged as promising candidates for cardiac cell therapy. First formally described by Friedenstein in 1970,[9] MSCs are multipotent stromal cells with unlimited self-renewal capacity that can be classified according to origin and differentiation potential. Stroma of bone marrow, adipose tissue, muscle, and dental pulp are

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all rich MSC sources.[10] Furthermore, MSC-like cells have been directly isolated from adult and embryonic hearts of both mice and humans.[11,12] MSCs exhibit source-specific surface marker expression, which characterizes their differentiation potential and immunomodulatory capacity.[13] MSCs are capable of differentiating both *in vitro* and *in vivo* into mesenchymal lineages, including fibroblasts, vascular endothelial cells, osteoblasts, chondrocytes, and adipocytes.[14] Although originally believed to have cardiomyocyte differentiation capacity, this view has now been questioned (discussed later).[15] MSCs also exhibit broadly described immunomodulatory effects while not innately being immunosuppressive.[16–19] This topic falls beyond the scope of the present review but has shown benefit in the heart after injury.[20] Furthermore, autologous nongenetically modified MSCs offer the advantage of mitigating graft rejection given their lack of immunogenic foreign antigens.[21] MSCs are easily cultured *in vitro*,[22] giving rise to relative ease of manufacture scalability.[23] For these reasons, MSCs have been thoroughly investigated as a source of therapy for cardiac repair.

The goal of the present review was to synthesize and interpret current data regarding the mechanisms underpinning MSC-mediated cardiac repair in a number of disease states. This involves critical analysis of unresolved controversies within the field and their relevant implications, as well as the identification of knowledge gaps that necessitate further research.

MATERIALS AND METHODS

In this narrative review, OVID versions of MEDLINE (1946 through to April 2020, week 1) and EMBASE (1947 through to 2020, week 14) were searched for relevant full-text articles and abstracts using the terms "Heart," "cardiac," "Mesenchymal Stem Cells," "mesenchymal stem cell," "msc_," "mesenchymal stromal cell," and "medicinal signalling cell." In addition, the reference lists of relevant articles and other personal files were scanned for further studies. Studies that were not based on the interaction between MSCs and the mammalian heart or its constituent cells in normal or pathological states were excluded.

RESULTS AND DISCUSSION

Current State-of-the-Art in Cell Therapy for Myocardial Repair

The concept of therapeutically boosting the heart's intrinsically limited regenerative capacity to elicit clinically relevant myocardial repair was promoted by multiple reports[24,25] (including a retracted study by Kajstura et al[26]) describing adult human cardiomyocyte turnover and the presence of endogenous cardiac stem cells (CSCs). During the mid-2000s, a number of different adult stem/progenitor-like cells, including MSCs,[3] bone marrow–derived stem cells,[2] and cardiac resident cells (including Sca-1^{+}[5,27] and c-Kit^{+}[28] expressing cells and cardiosphere-derived cells[29]), were reported to confer benefit in rodent models of myocardial disease. These promising preclinical data resulted in escalation of cardiac cell therapy to early-phase clinical trials, many of which had inadequate randomization, lack of control groups, and other methodologic flaws.[30] Subsequent progression of various cardiac cell therapies to larger randomized controlled trials has consistently yielded marginal therapeutic benefit despite providing satisfactory safety profiles.[6,31,32]

The use of adult stem/progenitor cell therapy for myocardial repair is currently at a crossroads. Some proponents suggest that repeated cell dosing protocols in human studies may provide a way forward.[33] However, the stark inability to translate preclinical efficacy to clinical trials requires closer scrutiny before further resources and funding are dedicated to future trials. Pluripotent stem cells have recently emerged as a leading candidate for regenerative cardiac cell therapy owing to their capacity to divide indefinitely and differentiate into nearly any mature cell type, including cardiomyocytes. In contrast, reproducible and robust differentiation into cardiomyocytes has been a significant hurdle for adult stem/progenitor-like cells. Pluripotent stem cell–derived cardiomyocytes have provided functionally relevant remuscularization of infarcted myocardium in numerous large animal models.[34–38] However, given the history of adult stem/progenitor-like cell therapy trials, it will be essential to address remaining issues such as arrhythmogenicity in clinically relevant preclinical models to facilitate safe and effective clinical translation.

Reasons for the discrepancy in efficacy between preclinical studies and subsequent human trials of cardiac cell therapy are likely multifactorial. The excitement generated by data from rodent models saw rapid escalation to clinical trials, resulting in a paucity of information from preclinical studies using clinically relevant large animal models. This is significant because considerable differences in heart rate, coronary architecture, myocardial mechanical properties, and capillary density between the rodent and human heart limit the potential to extrapolate data from rodent studies. Using large animal models also offers the advantage of being able to use the same imaging modalities as used for humans, which produces similar measures and outcome parameters. In addition, species-specific differences in stem or progenitor cell gene expression may also limit the relevance of animal models.[39,40] Another barrier to efficacy in human studies is poor engraftment and retention of cells[41,42] due to ineffective delivery route, the harsh microenvironment elicited by ischemia-reperfusion injury,[43] and mechanical factors such as blood flow and movement of the beating heart.[44] A strong correlation between cell engraftment and long-term benefit has been observed for multiple cell types,[44–46] which highlights the need to improve retention. Repeated cell dosing is a potential strategy but would increase risk associated with invasive intramyocardial or intracoronary delivery approaches. An alternative approach currently being developed is encapsulation of cells within biomaterials such as hydrogels[47,48] and patches.[49,50]

Future Potential of Cardiac MSC Therapy

The disappointing performance of a number of cardiac stem and progenitor cell types in clinical trials has justifiably raised concerns regarding the escalation of MSCs to human studies. However, MSCs offer several properties that may enable them to overcome the lack of translation that has plagued other cell types. In fact, recent meta-reviews of early clinical trials suggest MSCs provide benefit in both acute[51] and chronic[52] myocardial disease states. MSCs are unique in that they can be harvested from readily accessible sites such as peripheral blood,[53] adipose,[54] and bone marrow.[55] This makes MSCs a prime candidate for autologous transplant, which is significant given that allogeneic cardiac cell transfer is associated with immunogenicity.[56,57] Furthermore, unlike other stem and progenitor cell types, MSCs have a broadly described immunomodulatory phenotype that may contribute to establishing a more pro-regenerative myocardial microenvironment after injury. For instance, human-induced pluripotent stem cell–derived MSCs were shown to activate regulatory T cells and suppress the number of proinflammatory cells in a porcine model of heart failure.[20] In this context, escalation of MSC therapy for myocardial repair to larger scale randomized controlled trials may be warranted. However, a clear lesson from trials on other cell types is that the potential overarching mechanism of reparative effects must be kept in mind.

Can MSCs Improve Contractile Function by Direct Cardiomyocyte Differentiation?

The potential for human MSCs to differentiate into functional adult mammalian cardiomyocytes capable of restoring myocardial contractility remains controversial (Figure 1). Myogenic cells were first differentiated from rat bone marrow–derived MSCs exposed to 5-azacytidine.[58] A subsequent study described a low rate of rat bone marrow–derived MSC differentiation into cardiomyocyte-like cells expressing cardiac markers such as cardiac troponin T, α-cardiac actin, connexin-43, and Mef-2c.[59] Over time, MSC differentiation strategies evolved to involve a range of agents, including 5-azacytidine,[58] bone morphogenetic protein,[60,61] and angiotensin II.[62] Co-culture protocols in which MSCs were directly exposed to primary cardiomyocytes[63,64] and ventricular myocardium[65] were also used. Although providing a more physiologically relevant means for differentiation, co-culture methods make identification of initial input MSCs difficult without the use of indelible genetic tags and confocal microscopy.[66] Encouraged by documented *in vitro* plasticity, rodent studies were performed, appearing to effect successful cardiac repair after systemic or local delivery of MSCs.[67,68] However, initial attempts to elicit cardiomyogenic differentiation of human MSCs were unsuccessful.[69] This failure was attributed to multiple technical factors, including 5-azacytidine cytotoxicity, the use of primitive isolation methods such as plastic adherence, and a tendency for human MSCs to lose multipotency during *ex vivo* expansion.[70] Improved understanding of the microenvironment conducive to cardiomyogenic

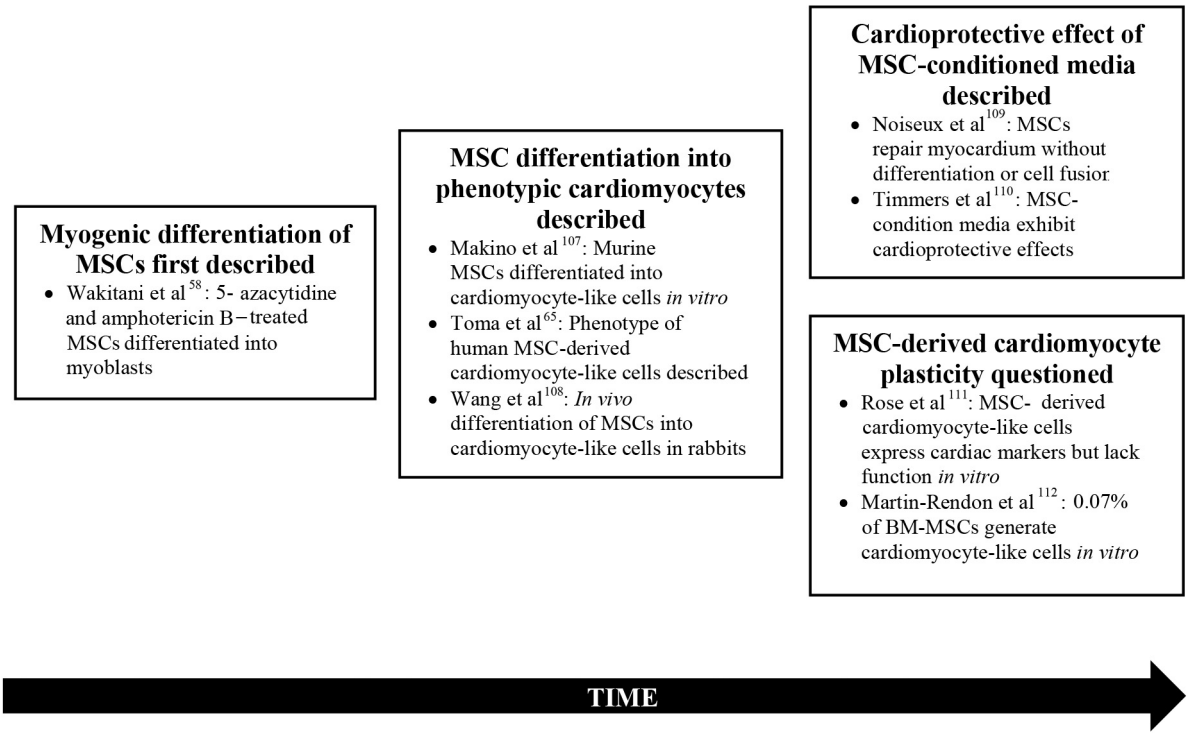

Figure 1. Mesenchymal stem cell (MSC) cardioprotective effects were initially attributed to differentiation into cardiomyocyte-like cells but have more recently been attributed to the MSC secretome. After the initial description of MSC myogenic differentiation (left panel), emphasis was placed on the capacity of MSCs to differentiate into cardiomyocyte-like cells (middle panel) and the relevant implications for management of cardiac pathologies. However, the therapeutic significance of MSC-derived cardiomyocyte-like cells has since been heavily disputed (right panel). The past decade has seen the research focus instead become directed toward the cardioprotective mechanism–elicited elements of the MSC secretome (right panel). BM-MSC = bone marrow–derived mesenchymal stem cell.

differentiation of MSCs has prompted protocol refinement, with one group recently reporting aggregate-based differentiation of first-trimester human umbilical cord perivascular cells into clusters of synchronously contracting cardiomyocyte-like cells.[71] However, despite this finding, the current sentiment remains that human MSCs are not able to differentiate into bona fide adult cardiomyocytes and that their role in mediating cardiac repair is largely though paracrine actions on newly injured tissue.[15]

What MSC Paracrine Effects Could Improve Cardiomyocyte Function?

Given a relative lack of direct cardiomyocyte differentiation potential, protection of injured cardiomyocytes has been deemed a key mechanism by which MSCs can promote improved myocardial contractile function. Co-culture with bone marrow–derived MSCs enhances survival among H9C2 cardiomyocytes exposed to ischemia-reperfusion injury.[72] This antiapoptotic effect is at least partially attributable to the formation of tunneling nanotubes and subsequent mitochondrial transfer between MSCs and cardiomyocytes.[72] This has been recognized as a common mechanism of tissue repair elicited by MSCs across a number of organ systems. Evidence supporting a paracrine antiapoptotic role for MSCs includes the visualization of 50- to 100-nm exosomes secreted by human embryonic stem cell–derived MSCs in culture

medium.[73] Lai et al[73] elegantly described a direct cardioprotective role for MSC-derived exosomes by fractionating them away from the rest of the MSC secretome, delivering them to an *ex vivo* murine myocardial ischemia-reperfusion model, and reporting significantly decreased myocardial infarction. MSCs also can regulate apoptosis via oxygen-dependent ubiquitylation of the alpha regulatory subunit of hypoxia-inducible factor-1α(HIF-1α).[74] HIF-1α is inhibited in the low-oxygen bone marrow microenvironment, and hypoxia-preconditioned murine MSCs display high HIF-1α activity. This is associated with upregulation of antiapoptotic proteins such as phosphorylated Akt, an important regulator of phosphatidylinositol 3-kinase–mediated cell survival.[75] Murine MSCs overexpressing Akt exhibit enhanced antiapoptotic capacity through paracrine secretion of secreted frizzled related protein-2, which upregulates β-catenin in hypoxic cardiomyocytes and thereby mimics canonical Wnt signalling.[76]

Consistent with these proposed antiapoptotic signaling mechanisms, intramyocardial delivery of MSCs overexpressing Akt into ischemic rat hearts produced a cell number–dependent improvement in infarct volume as well as systolic and diastolic function.[67] Although the MSC secretome seems to serve a number of important antiapoptotic functions in the injured heart, comprehensively profiling it has remained elusive due to technical limitations such as dependence on antibody availability in ELISAs, as well as the relative insensitivity of gel-based assays impairing detection of MSC factors secreted in low quantities. Newly developed direct immunofluorescence assays using quantum dots offer higher sensitivity and may therefore allow for a more complete assessment of *in vivo* MSC paracrine secretions. Once possible, screening the MSC secretome to identify the components that confer the greatest therapeutic benefit in different cardiac pathologies would provide a logical direction for further investigation.

Do MSC Interactions With Endogenous Cardiac Progenitors Modulate Cardiac Function?

MSCs also interact with a population of c-kit$^+$ cells in the mammalian heart that were previously believed to represent endogenous adult CSCs. The concept of an adult CSC population capable of differentiating into cardiomyocytes (and therefore potentially regenerating injured myocardium) arose following reports that adult hematopoietic[2,77] and epithelial[78] stem cells could self-renew and differentiate into mature cardiac cell types, including cardiomyocytes. Investigators used well-established adult hematopoietic stem cell surface markers such as Sca-1 and c-kit to identify endogenous CSCs in the mammalian heart and used cell culture and lineage mapping techniques to observe these cells fusing with and differentiating into cardiomyocytes.[79,80] However, multiple independent laboratories have recently used Cre recombinase-dependent lineage mapping to show that, in the adult heart, differentiation of c-kit$^+$ cells into cardiomyocytes occurs extremely rarely[81–84] and, in fact, pales into insignificance compared with the physiological rate of cardiomyocyte turnover.[85] The majority of new adult cardiomyocytes are believed to differentiate from pre-existing cardiomyocytes, and the predominant fate of Sca-1$^+$ and c-kit$^+$ cells in the heart seems to be endothelial,[82] which is not surprising given these markers were originally recognized in hematopoietic stem cells. Therefore, although it was previously hypothesized that MSCs could facilitate myocardial regeneration by expanding an endogenous CSC population and strengthening its commitment to the cardiomyocyte lineage, this no longer seems likely.

The interaction between MSCs and c-kit$^+$ cells in the heart involves both direct contact and paracrine actions. Hatzistergos et al[86] observed the formation of N-cadherin mechanical connections and connexin-43 gap junctions between MSCs and c-kit$^+$ cells in the infarcted swine heart. Interestingly, this interaction was not only associated with a 20-fold expansion of cardiac c-kit$^+$ cells but also a 6-fold increase in the number of cells expressing GATA-4, which is a transcription factor expressed by adult cardiomyocytes.[86] Cardiac c-kit$^+$ cells can also become activated in response to components of the MSC secretome such as insulin-like growth factor and fibroblast growth factor,[87] and undergo migration according to hepatocyte growth factor (HGF) gradients established by MSCs.[88] However, a murine myocardial infarction study with a randomized controlled design found that MSCs do not result in expansion of the cardiomyocyte pool.[89] It thus follows that despite MSCs being capable of inducing c-kit$^+$ cell proliferation in the heart, any associated therapeutic effect is unlikely to be

attributed to the production of a functionally significant number of new cardiomyocytes. Given that the overwhelming majority of cardiac c-kit$^+$ cells differentiate into endothelial cells,[82] it is indeed plausible that MSC-mediated mobilization of the cardiac c-kit$^+$ population yields benefit through angiogenesis.

Does MSC-Induced Angiogenesis Contribute to Improved Cardiac Function?

MSCs express a variety of angiogenic paracrine factors that can facilitate revascularization of injured myocardium by inducing endothelial cell survival, proliferation, and migration. Human MSCs conditioned in normal atmospheric oxygen levels (21% oxygen) do not increase expression of angiogenic factors such as vascular endothelial growth factor (VEGF).[90] However, given that the tissues from which MSCs are generally extracted are relatively hypoxic, preconditioning MSCs in low-oxygen environments (2%–7% oxygen) *in vitro* enables more accurate emulation of their natural microenvironment. Real-time polymerase chain reaction analysis showed that hypoxic pretreatment (2%–7% oxygen) of rat MSCs upregulated HIF-1α expression[91] and increased transcription of HIF-1α–responsive angiogenic factors fibroblast growth factor and VEGF by 2- to 8-fold and 10- to 20-fold, respectively. Furthermore, murine MSCs preconditioned with inflammatory mediator transforming growth factor-α (TGF-α) were shown to increase VEGF expression by 30% through a mitogen-activated protein kinase–mediated pathway.[92] Therefore, the low-oxygen and proinflammatory microenvironment of injured myocardium is likely to potentiate MSC secretion of angiogenic factors. Intramyocardial delivery of MSCs into a canine model of chronic myocardial ischemia significantly increased vascular density and was associated with a 5% absolute increase in left ventricular ejection fraction (LVEF) after 30 days compared with control-treated animals, who experienced a 9% decrease in LVEF.[93]

The CD146$^+$ CD34$^-$ subpopulation of MSCs has potential to differentiate into vascular smooth muscle cells (vSMCs) and pericytes, enabling them to support endothelial cells during angiogenesis.[94] Contact-dependent TGF-β activation is a common driver of MSC differentiation into both vSMCs and pericytes. Differentiation into vSMCs is believed to involve a TGF-β/ALK signaling mechanism that is deficient in disease states such as hereditary hemorrhagic telangiectasia.[95] Interestingly, human cardiac MSC-like cells injected directly into the infarcted rat heart directly induced host vasculogenesis, and this action was associated with improved left ventricular function.[96] Furthermore, bovine MSCs exposed to platelet-derived growth factor (PDGF) gradients produced by endothelial cells in culture differentiate into spindle-shaped cells resembling vSMCs and undergo migration toward the endothelial cells.[97] Collectively, these data indicate that MSCs may respond to endothelial TGF-β in a concentration-dependent nature or that MSC differentiation is directed by TGF-β acting in conjunction with other factors (eg, PDGF).

Some MSCs also have the capacity to differentiate into endothelial-like cells during neovascularization of injured myocardium. Endothelial-like cells derived from human bone marrow–derived MSCs cultured in the presence of VEGF express the endothelial markers KDR and VEGF receptor-1 and form capillary-like structures, supporting their angiogenic role in infarcted tissue.[98] A separate *in vitro* study in which MSCs were cultured in endothelial growth medium and exposed to shear forces found that differentiated cells lacked expression of the endothelial markers platelet endothelial cell adhesion molecule 1 (PECAM-1) or kinase insert domain receptor (KDR),[99] which was possibly attributable to lower growth medium VEGF content. However, the endothelial-like cells still exhibited typical endothelial morphology and gene expression profile, and formed a capillary network in three-dimensional culture in *in vivo* conditions,[99] indicating that they were still able to participate in neovascularization. A possible explanation for this phenomenon may be that MSCs produce functional hybrid endothelial-like cells through ROCK-actin/myosin pathway-dependent fusion or entosis with resident cells.[100]

Is Modulation of Fibrosis Responsible for MSC-Derived Cardiac Improvement?

MSCs exhibit extracellular matrix (ECM)-protective effects during the early reparative response to tissue injury, preventing extensive degradation of intact ECM. Human MSCs exposed to proinflammatory cytokines IL-1β and soluble TNF-α *in vitro* abrogate

matrix metalloproteinase (MMP) activity by expressing tissue inhibitor of metalloproteinase (TIMP).[101] MSC secretion of TIMP reduced MMP-mediated degradation of the ECM components such as fibronectin, type IV collagen, and laminin-5, preventing an increase in human endothelial cell tube permeability. Although a topic of uncertainty, inflammatory mediator-induced MSC upregulation of TIMP may occur through nuclear factor κB signaling, given that the nuclear factor κB inhibitor IkB kinase-β was shown to impair human MSC antifibrotic signaling in response to binding IL-1βα.[102]

During the fibrotic phase of myocardial repair, MSCs modulate the deposition of new ECM through the secretion of antifibrotic paracrine factors. *In vitro* data suggest that rat MSC-conditioned medium reduces cardiac fibroblast viability by 30% and impairs differentiation of cardiac fibroblasts into myofibroblasts, resulting in significantly reduced deposition of type I and type III collagen fibers.[103] Rats administered intramyocardial bone marrow–derived MSCs 4 weeks after receiving isoproterenol-induced global heart failure upregulated antifibrotic factor HGF, strongly correlating with reduced type I and III collagen expression,[104] and indicating the possible involvement of an HGF-mediated mechanism. Furthermore, TGF-β–stimulated rat MSCs significantly reduced murine vocal fold fibroblast type I and III collagen production and increased MMP-1 expression relative to a non–TGF-β–treated MSC group,[105] indicating that inflammatory mediators such as TGF-β may potentiate the MSC antifibrotic response. Moreover, given that type I collagen accumulation reduces MSC expression of VEGF,[106] MSC antifibrotic mechanisms may indirectly facilitate angiogenesis by removing suppression of VEGF. The functional significance of the antifibrotic action of MSCs in the heart was highlighted by increased LVEF after 2 weeks and a 15% reduction in ventricular fibrosis sustained after 2 months in a murine postinfarct heart failure model administered with intramyocardial MSCs.[103]

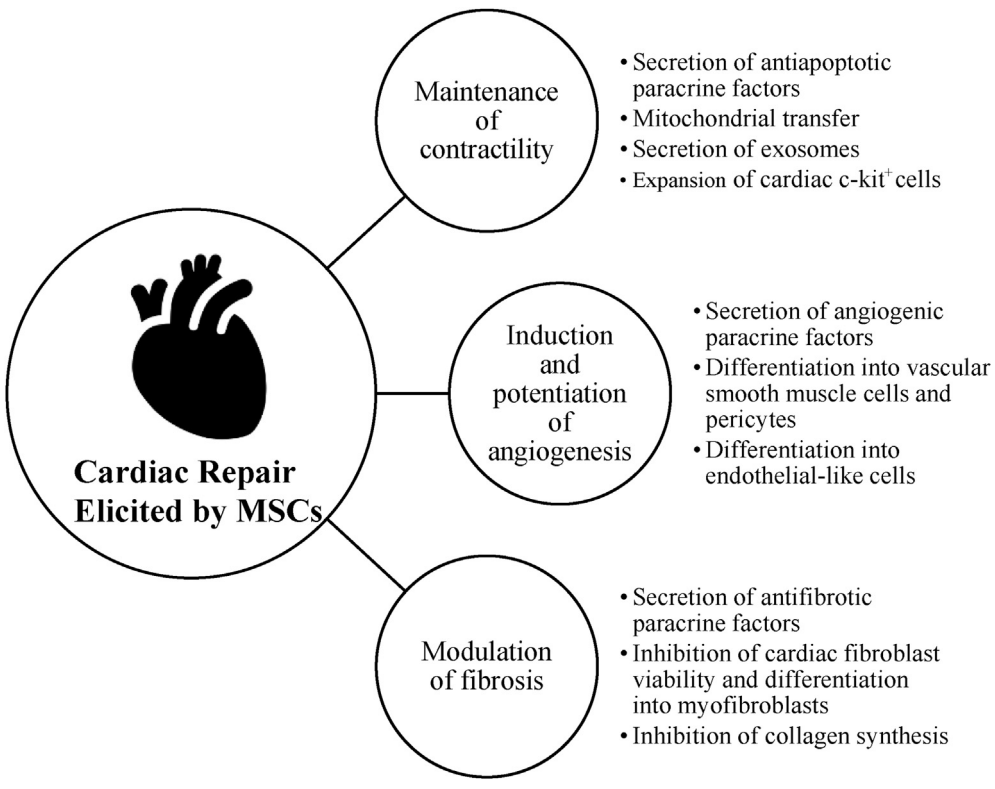

Figure 2. Key mechanisms of cardiac repair elicited by mesenchymal stem cells (MSCs).

CONCLUSIONS

Despite new insights into mechanisms involved, the use of MSCs remains a promising potential therapeutic approach to elicit cardiac repair in pathologic states. MSCs facilitate cardiac repair through 3 broad effects: (1) maintenance of myocardial contractility; (2) induction and potentiation of angiogenesis; and (3) modulation of fibrosis (Figure 2). Mechanisms such as MSC differentiation into mature adult cardiomyocytes as well as MSC-directed expansion and differentiation of endogenous CSCs into cardiomyocytes were historically favored but now seem less plausible; rather, much of the MSC cardioprotective role seems to arise from paracrine secretion and direct contact with local cells within the heart. Furthermore, the efficacy of MSCs as mediators of cardiac repair can be enhanced by emulating the microenvironment of the injured heart by preconditioning MSCs in the presence of low-oxygen conditions and inflammatory mediators such as TGF-β. This should be taken into consideration during the future development of MSC-based therapeutic protocols. Remaining challenges include comprehensively evaluating the entire MSC secretome in the injured heart microenvironment and developing a detailed understanding of relevant downstream intracellular signaling pathways. In contrast to some other cardiac stem/progenitor cell therapies, MSCs have shown more consistent promise at the level of early-phase clinical trials. Cardiac MSC therapy therefore merits escalation to larger scale randomized controlled trials.

DISCLOSURES

The authors have indicated that they have no conflicts of interest regarding the content of this article.

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REFERENCES

1. Mensah GA, Wei GS, Sorlie PD, et al. Decline in cardiovascular mortality: possible causes and implications. *Circ Res*. 2017;120:366–380.
2. Orlic D, Kajstura J, Chimenti S, et al. Bone marrow cells regenerate infarcted myocardium. *Nature*. 2001;410:701–705.
3. Gnecchi M, He H, Noiseux N, et al. Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. *FASEB J*. 2006;20:661–669.
4. Bolli R, Tang XL, Sanganalmath SK, et al. Intracoronary delivery of autologous cardiac stem cells improves cardiac function in a porcine model of chronic ischemic cardiomyopathy. *Circulation*. 2013;128:122–131.
5. Matsuura K, Honda A, Nagai T, et al. Transplantation of cardiac progenitor cells ameliorates cardiac dysfunction after myocardial infarction in mice. *J Clin Invest*. 2009;119:2204–2217.
6. Makkar RR, Smith RR, Cheng K, et al. Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial. *Lancet*. 2012;379:895–904.
7. Tang JN, Cores J, Huang K, et al. Concise review: is cardiac cell therapy dead? Embarrassing trial outcomes and new directions for the future. *Stem Cell Transl Med*. 2018;7:354–359.
8. A futile cycle in cell therapy. *Nat Biotechnol*. 2017;35:291.
9. Friedenstein AJ, Chailakhjan RK, Lalykina KS. The development of fibroblast colonies in monolayer cultures of Guinea-pig bone marrow and spleen cells. *Cell Tissue Kinet*. 1970;3:393–403.
10. Hass R, Kasper C, Bohm S, Jacobs R. Different populations and sources of human mesenchymal stem cells (MSC): a comparison of adult and neonatal tissue-derived MSC. *Cell Commun Signal*. 2011;9:12.
11. Chong JJ, Chandrakanthan V, Xaymardan M, et al. Adult cardiac-resident MSC-like stem cells with a proepicardial origin. *Cell Stem Cell*. 2011;9:527–540.
12. Le TYL, Pickett HA, dos Remedios CG, Barbaro PM, Kizana E, Chong JJH. Platelet-derived growth factor receptor-alpha expressing cardiac progenitor cells can be derived from previously cryopreserved human heart samples. *Stem Cell Develop*. 2018;27:184–198.
13. Maleki M, Ghanbarvand F, Reza Behvarz M, Ejtemaei M, Ghadirkhomi E. Comparison of mesenchymal stem cell markers in multiple human adult stem cells. *Int J Stem Cell*. 2014;7:118–126.
14. Augello A, De Bari C. The regulation of differentiation in mesenchymal stem cells. *Hum Gene Ther*. 2010;21:1226–1238.

15. Le TY, Thavapalachandran S, Kizana E, Chong JJ. New developments in cardiac regeneration. *Heart Lung Circ*. 2017;26:316–322.
16. Abdi R, Fiorina P, Adra CN, Atkinson M, Sayegh MH. Immunomodulation by mesenchymal stem cells: a potential therapeutic strategy for type 1 diabetes. *Diabetes*. 2008;57:1759–1767.
17. Gao F, Chiu SM, Motan DA, et al. Mesenchymal stem cells and immunomodulation: current status and future prospects. *Cell Death Dis*. 2016;7, e2062.
18. Ma OK, Chan KH. Immunomodulation by mesenchymal stem cells: interplay between mesenchymal stem cells and regulatory lymphocytes. *World J Stem Cell*. 2016;8:268–278.
19. Wang Y, Chen X, Cao W, Shi Y. Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. *Nat Immunol*. 2014;15:1009–1016.
20. Liao S, Zhang Y, Ting S, et al. Potent immunomodulation and angiogenic effects of mesenchymal stem cells versus cardiomyocytes derived from pluripotent stem cells for treatment of heart failure. *Stem Cell Res Ther*. 2019;10:78.
21. Hare JM, Fishman JE, Gerstenblith G, et al. Comparison of allogeneic vs autologous bone marrow-derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: the POSEIDON randomized trial. *JAMA*. 2012;308:2369–2379.
22. Steens J, Klein D. Current strategies to generate human mesenchymal stem cells in vitro. *Stem Cell Int*. 2018;2018:6726185.
23. de Sousa Pinto D, Bandeiras C, de Almeida Fuzeta M, et al. Scalable manufacturing of human mesenchymal stromal cells in the vertical-wheel bioreactor system: an experimental and economic approach. *Biotechnol J*. 2019;14, e1800716.
24. Bearzi C, Rota M, Hosoda T, et al. Human cardiac stem cells. *Proc Natl Acad Sci U S A*. 2007;104:14068–14073.
25. van Vliet P, Roccio M, Smits AM, et al. Progenitor cells isolated from the human heart: a potential cell source for regenerative therapy. *Neth Heart J*. 2008;16:163–169.
26. Kajstura J, Gurusamy N, Ogorek B, et al. Myocyte turnover in the aging human heart. *Circ Res*. 2010;107:1374–1386.
27. Takamiya M, Haider KH, Ashraf M. Identification and characterization of a novel multipotent sub-population of Sca-1(+) cardiac progenitor cells for myocardial regeneration. *PLoS One*. 2011;6, e25265.
28. Rota M, Padin-Iruegas ME, Misao Y, et al. Local activation or implantation of cardiac progenitor cells rescues scarred infarcted myocardium improving cardiac function. *Circ Res*. 2008;103:107–116.
29. Smith RR, Barile L, Cho HC, et al. Regenerative potential of cardiosphere-derived cells expanded from percutaneous endomyocardial biopsy specimens. *Circulation*. 2007;115:896–908.
30. Bolli R, Chugh AR, D'Amario D, et al. Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. *Lancet*. 2011;378:1847–1857.
31. Perin EC, Borow KM, Silva GV, et al. A Phase II dose-escalation study of allogeneic mesenchymal precursor cells in patients with ischemic or nonischemic heart failure. *Circ Res*. 2015;117:576–584.
32. Hirsch A, Nijveldt R, van der Vleuten PA, et al. Intracoronary infusion of mononuclear cells from bone marrow or peripheral blood compared with standard therapy in patients after acute myocardial infarction treated by primary percutaneous coronary intervention: results of the randomized controlled HEBE trial. *Eur Heart J*. 2011;32:1736–1747.
33. Bolli R. Repeated cell therapy: a paradigm shift whose time has come. *Circ Res*. 2017;120:1072–1074.
34. Chong JJ, Yang X, Don CW, et al. Human embryonic-stem-cell-derived cardiomyocytes regenerate non-human primate hearts. *Nature*. 2014;510:273–277.
35. Shiba Y, Gomibuchi T, Seto T, et al. Allogeneic transplantation of iPS cell-derived cardiomyocytes regenerates primate hearts. *Nature*. 2016;538:388–391.
36. Liu YW, Chen B, Yang X, et al. Human embryonic stem cell-derived cardiomyocytes restore function in infarcted hearts of non-human primates. *Nat Biotechnol*. 2018;36:597–605.
37. Romagnuolo R, Masoudpour H, Porta-Sanchez A, et al. Human embryonic stem cell-derived cardiomyocytes regenerate the infarcted pig heart but induce ventricular tachyarrhythmias. *Stem Cell Rep*. 2019;12:967–981.
38. Fernandes S, Chong JJH, Paige SL, et al. Comparison of human embryonic stem cell-derived cardiomyocytes, cardiovascular progenitors, and bone marrow mononuclear cells for cardiac repair. *Stem Cell Rep*. 2015;5:753–762.
39. Ginis I, Luo Y, Miura T, et al. Differences between human and mouse embryonic stem cells. *Dev Biol*. 2004;269:360–380.
40. Chong JJ, Murry CE. Cardiac regeneration using pluripotent stem cells—progression to large animal models. *Stem Cell Res*. 2014;13:654–665.
41. Zeng L, Hu Q, Wang X, et al. Bioenergetic and functional

consequences of bone marrow-derived multipotent progenitor cell transplantation in hearts with postinfarction left ventricular remodeling. *Circulation*. 2007;115:1866–1875.
42. Hong KU, Li QH, Guo Y, et al. A highly sensitive and accurate method to quantify absolute numbers of c-kit+ cardiac stem cells following transplantation in mice. *Basic Res Cardiol*. 2013;108:346.
43. Sanada S, Komuro I, Kitakaze M. Pathophysiology of myocardial reperfusion injury: preconditioning, postconditioning, and translational aspects of protective measures. *Am J Physiol Heart Circ Physiol*. 2011;301:H1723–H1741.
44. Terrovitis J, Lautamaki R, Bonios M, et al. Noninvasive quantification and optimization of acute cell retention by in vivo positron emission tomography after intramyocardial cardiac-derived stem cell delivery. *J Am Coll Cardiol*. 2009;54:1619–1626.
45. Cheng K, Li TS, Malliaras K, Davis DR, Zhang Y, Marban E. Magnetic targeting enhances engraftment and functional benefit of iron-labeled cardiosphere-derived cells in myocardial infarction. *Circ Res*. 2010;106:1570–1581.
46. Quevedo HC, Hatzistergos KE, Oskouei BN, et al. Allogeneic mesenchymal stem cells restore cardiac function in chronic ischemic cardiomyopathy via trilineage differentiating capacity. *Proc Natl Acad Sci U S A*. 2009;106:14022–14027.
47. Hernandez MJ, Christman KL. Designing acellular injectable biomaterial therapeutics for treating myocardial infarction and peripheral artery disease. *JACC Basic Transl Sci*. 2017;2:212–226.
48. Tang J, Cui X, Caranasos TG, et al. Heart repair using nanogel-encapsulated human cardiac stem cells in mice and pigs with myocardial infarction. *ACS Nano*. 2017;11:9738–9749.
49. Gao L, Kupfer ME, Jung JP, et al. Myocardial tissue engineering with cells derived from human-induced pluripotent stem cells and a native-like, high-resolution, 3-dimensionally printed scaffold. *Circ Res*. 2017;120:1318–1325.
50. Tang J, Vandergriff A, Wang Z, et al. A regenerative cardiac patch formed by spray painting of biomaterials onto the heart. *Tissue Eng C Methods*. 2017;23:146–155.
51. Jeong H, Yim HW, Park HJ, et al. Mesenchymal stem cell therapy for ischemic heart disease: systematic review and meta-analysis. *Int J Stem Cell*. 2018;11:1–12.
52. Fan M, Huang Y, Chen Z, et al. Efficacy of mesenchymal stem cell therapy in systolic heart failure: a systematic review and meta-analysis. *Stem Cell Res Ther*. 2019;10:150.
53. Chong PP, Selvaratnam L, Abbas AA, Kamarul T. Human peripheral blood derived mesenchymal stem cells demonstrate similar characteristics and chondrogenic differentiation potential to bone marrow derived mesenchymal stem cells. *J Orthop Res*. 2012;30:634–642.
54. Zuk PA, Zhu M, Ashjian P, et al. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell*. 2002;13:4279–4295.
55. Caplan AI. Mesenchymal stem cells. *J Orthop Res*. 1991;9:641–650.
56. Berglund AK, Fortier LA, Antczak DF, Schnabel LV. Immunoprivileged no more: measuring the immunogenicity of allogeneic adult mesenchymal stem cells. *Stem Cell Res Ther*. 2017;8:288.
57. Heslop JA, Hammond TG, Santeramo I, et al. Concise review: workshop review: understanding and assessing the risks of stem cell-based therapies. *Stem Cell Transl Med*. 2015;4:389–400.
58. Wakitani S, Saito T, Caplan AI. Myogenic cells derived from rat bone marrow mesenchymal stem cells exposed to 5-azacytidine. *Muscle Nerve*. 1995;18:1417–1426.
59. Gao Q, Guo M, Jiang X, Hu X, Wang Y, Fan Y. A cocktail method for promoting cardiomyocyte differentiation from bone marrow-derived mesenchymal stem cells. *Stem Cell Int*. 2014;2014:162024.
60. Zandstra PW, Bauwens C, Yin T, et al. Scalable production of embryonic stem cell-derived cardiomyocytes. *Tissue Eng*. 2003;9:767–778.
61. Hou J, Lu AL, Liu BW, et al. Combination of BMP-2 and 5-AZA is advantageous in rat bone marrow-derived mesenchymal stem cells differentiation into cardiomyocytes. *Cell Biol Int*. 2013;37:1291–1299.
62. Xing Y, Lv A, Wang L, Yan X. The combination of angiotensin II and 5-azacytidine promotes cardiomyocyte differentiation of rat bone marrow mesenchymal stem cells. *Mol Cell Biochem*. 2012;360:279–287.
63. Ramkisoensing AA, Pijnappels DA, Swildens J, et al. Gap junctional coupling with cardiomyocytes is necessary but not sufficient for cardiomyogenic differentiation of cocultured human mesenchymal stem cells. *Stem Cells*. 2012;30:1236–1245.
64. Rangappa S, Entwistle JW, Wechsler AS, Kresh JY. Cardiomyocyte-mediated contact programs human mesenchymal stem cells to express cardiogenic phenotype. *J Thorac Cardiovasc Surg*. 2003;126:124–132.
65. Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation*. 2002;105:93–98.
66. Chong JJ, Forte E, Harvey RP. Developmental origins and lineage descendants of endogenous adult

cardiac progenitor cells. *Stem Cell Res*. 2014;13:592—614.
67. Mangi AA, Noiseux N, Kong D, et al. Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. *Nat Med*. 2003;9:1195—1201.
68. Zhang S, Ge J, Sun A, et al. Comparison of various kinds of bone marrow stem cells for the repair of infarcted myocardium: single clonally purified non-hematopoietic mesenchymal stem cells serve as a superior source. *J Cell Biochem*. 2006;99:1132—1147.
69. Siegel G, Krause P, Wohrle S, et al. Bone marrow-derived human mesenchymal stem cells express cardiomyogenic proteins but do not exhibit functional cardiomyogenic differentiation potential. *Stem Cell Dev*. 2012;21:2457—2470.
70. Ng CP, Sharif AR, Heath DE, et al. Enhanced ex vivo expansion of adult mesenchymal stem cells by fetal mesenchymal stem cell ECM. *Biomaterials*. 2014;35:4046—4057.
71. Szaraz P, Gratch YS, Iqbal F, Librach CL. In vitro differentiation of human mesenchymal stem cells into functional cardiomyocyte-like cells. *J Vis Exp*. 2017;126:55757.
72. Han H, Hu J, Yan Q, et al. Bone marrow-derived mesenchymal stem cells rescue injured H9c2 cells via transferring intact mitochondria through tunneling nanotubes in an in vitro simulated ischemia/reperfusion model. *Mol Med Rep*. 2016;13:1517—1524.
73. Lai RC, Arslan F, Lee MM, et al. Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. *Stem Cell Res*. 2010;4:214—222.
74. Ziello JE, Jovin IS, Huang Y. Hypoxia-inducible factor (HIF)-1 regulatory pathway and its potential for therapeutic intervention in malignancy and ischemia. *Yale J Biol Med*. 2007;80:51—60.
75. Chacko SM, Ahmed S, Selvendiran K, Kuppusamy ML, Khan M, Kuppusamy P. Hypoxic preconditioning induces the expression of prosurvival and proangiogenic markers in mesenchymal stem cells. *Am J Physiol Cell Physiol*. 2010;299:C1562—C1570.
76. Mirotsou M, Zhang Z, Deb A, et al. Secreted frizzled related protein 2 (Sfrp2) is the key Akt-mesenchymal stem cell-released paracrine factor mediating myocardial survival and repair. *Proc Natl Acad Sci U S A*. 2007;104:1643—1648.
77. Jackson KA, Majka SM, Wang H, et al. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J Clin Invest*. 2001;107:1395—1402.
78. Miki T, Lehmann T, Cai H, Stolz DB, Strom SC. Stem cell characteristics of amniotic epithelial cells. *Stem Cells*. 2005;23:1549—1559.
79. Oh H, Bradfute SB, Gallardo TD, et al. Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. *Proc Natl Acad Sci U S A*. 2003;100:12313—12318.
80. Uchida S, De Gaspari P, Kostin S, et al. Sca1-derived cells are a source of myocardial renewal in the murine adult heart. *Stem Cell Rep*. 2013;1:397—410.
81. Li Y, He L, Huang X, et al. Genetic lineage tracing of nonmyocyte population by dual recombinases. *Circulation*. 2018;138:793—805.
82. van Berlo JH, Kanisicak O, Maillet M, et al. c-kit+ cells minimally contribute cardiomyocytes to the heart. *Nature*. 2014;509:337—341.
83. Sultana N, Zhang L, Yan J, et al. Resident c-kit(+) cells in the heart are not cardiac stem cells. *Nat Commun*. 2015;6:8701.
84. Liu Q, Yang R, Huang X, et al. Genetic lineage tracing identifies in situ Kit-expressing cardiomyocytes. *Cell Res*. 2016;26:119—130.
85. Graham E, Bergmann O. Dating the heart: exploring cardiomyocyte renewal in humans. *Physiology (Bethesda)*. 2017;32:33—41.
86. Hatzistergos KE, Quevedo H, Oskouei BN, et al. Bone marrow mesenchymal stem cells stimulate cardiac stem cell proliferation and differentiation. *Circ Res*. 2010;107:913—922.
87. Baraniak PR, McDevitt TC. Stem cell paracrine actions and tissue regeneration. *Regen Med*. 2010;5:121—143.
88. Linke A, Muller P, Nurzynska D, et al. Stem cells in the dog heart are self-renewing, clonogenic, and multipotent and regenerate infarcted myocardium, improving cardiac function. *Proc Natl Acad Sci U S A*. 2005;102:8966—8971.
89. Loffredo FS, Steinhauser ML, Gannon J, Lee RT. Bone marrow-derived cell therapy stimulates endogenous cardiomyocyte progenitors and promotes cardiac repair. *Cell Stem Cell*. 2011;8:389—398.
90. Hu X, Zhang L, Jin J, et al. Heparanase released from mesenchymal stem cells activates integrin beta1/HIF-2alpha/Flk-1 signaling and promotes endothelial cell migration and angiogenesis. *Stem Cells*. 2015;33:1850—1862.
91. Liu J, Hao H, Xia L, et al. Hypoxia pretreatment of bone marrow mesenchymal stem cells facilitates angiogenesis by improving the function of endothelial cells in diabetic rats with lower ischemia. *PLoS One*. 2015;10, e0126715.
92. Herrmann JL, Wang Y, Abarbanell AM, Weil BR, Tan J, Meldrum DR. Preconditioning mesenchymal stem cells with transforming growth factor-alpha improves mesenchymal stem cell-mediated cardioprotection. *Shock*. 2010;33:24—30.
93. Silva GV, Litovsky S, Assad JA, et al. Mesenchymal stem cells

differentiate into an endothelial phenotype, enhance vascular density, and improve heart function in a canine chronic ischemia model. *Circulation*. 2005;111:150–156.
94. Esteves CL, Sheldrake TA, Mesquita SP, et al. Isolation and characterization of equine native MSC populations. *Stem Cell Res Ther*. 2017;8:80.
95. Carvalho RL, Jonker L, Goumans MJ, et al. Defective paracrine signalling by TGFbeta in yolk sac vasculature of endoglin mutant mice: a paradigm for hereditary haemorrhagic telangiectasia. *Development*. 2004;131:6237–6247.
96. Le TYL, Pickett HA, Yang A, et al. Enhanced cardiac repair by telomerase reverse transcriptase over-expression in human cardiac mesenchymal stromal cells. *Sci Rep*. 2019;9:10579.
97. Hirschi KK, Rohovsky SA, D'Amore PA. PDGF, TGF-beta, and heterotypic cell-cell interactions mediate endothelial cell-induced recruitment of 10T1/2 cells and their differentiation to a smooth muscle fate. *J Cell Biol*. 1998;141:805–814.
98. Oswald J, Boxberger S, Jorgensen B, et al. Mesenchymal stem cells can be differentiated into endothelial cells in vitro. *Stem Cells*. 2004;22:377–384.
99. Portalska KJ, Leferink A, Groen N, et al. Endothelial differentiation of mesenchymal stromal cells. *PLOS One*. 2012;7, e46842.
100. Sottile F, Aulicino F, Theka I, Cosma MP. Mesenchymal stem cells generate distinct functional hybrids in vitro via cell fusion or entosis. *Sci Rep*. 2016;6:36863.
101. Lozito TP, Tuan RS. Mesenchymal stem cells inhibit both endogenous and exogenous MMPs via secreted TIMPs. *J Cell Physiol*. 2011;226:385–396.
102. Carrero R, Cerrada I, Lledo E, et al. IL1beta induces mesenchymal stem cells migration and leucocyte chemotaxis through NF-kappaB. *Stem Cell Rev Rep*. 2012;8:905–916.
103. Mias C, Lairez O, Trouche E, et al. Mesenchymal stem cells promote matrix metalloproteinase secretion by cardiac fibroblasts and reduce cardiac ventricular fibrosis after myocardial infarction. *Stem Cells*. 2009;27:2734–2743.
104. Li L, Zhang Y, Li Y, et al. Mesenchymal stem cell transplantation attenuates cardiac fibrosis associated with isoproterenol-induced global heart failure. *Transpl Int*. 2008;21:1181–1189.
105. Hiwatashi N, Bing R, Kraja I, Branski RC. Mesenchymal stem cells have antifibrotic effects on transforming growth factor-beta1-stimulated vocal fold fibroblasts. *Laryngoscope*. 2017;127:E35–E41.
106. De Lisio M, Jensen T, Sukiennik RA, Huntsman HD, Boppart MD. Substrate and strain alter the muscle-derived mesenchymal stem cell secretome to promote myogenesis. *Stem Cell Res Ther*. 2014;5:74.

FURTHER READING

107. Makino S, Fukuda K, Miyoshi S, et al. Cardiomyocytes can be generated from marrow stromal cells. *J Clin Invest*. 1999;103:697–705.
108. Wang JA, Li CL, Fan YQ, He H, Sun Y. Allograftic bone marrow-derived mesenchymal stem cells transplanted into heart infarcted model of rabbit to renovate infarcted heart. *J Zhejiang Univ Sci*. 2004;5:1279–1285.
109. Noiseux N, Gnecchi M, Lopez-Ilasaca M, et al. Mesenchymal stem cells overexpressing Akt dramatically repair infarcted myocardium and improve cardiac function despite infrequent cellular fusion or differentiation. *Mol Ther*. 2006;14:840–850.
110. Timmers L, Lim SK, Arslan F, et al. Reduction of myocardial infarct size by human mesenchymal stem cell conditioned medium. *Stem Cell Res*. 2007;1:129–137.
111. Rose RA, Jiang H, Wang X, et al. Bone marrow-derived mesenchymal stromal cells express cardiac-specific markers, retain the stromal phenotype, and do not become functional cardiomyocytes in vitro. *Stem Cells*. 2008;26:2884–2892.
112. Martin-Rendon E, Sweeney D, Lu F, Girdlestone J, Navarrete C, Watt SM. 5-Azacytidine-treated human mesenchymal stem/progenitor cells derived from umbilical cord, cord blood and bone marrow do not generate cardiomyocytes in vitro at high frequencies. *Vox Sang*. 2008;95:137–148.

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