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EDITORIAL

Next-Generation Stem Cell Therapy: Genetically Modified Mesenchymal Stem Cells for Cardiac Repair

Editorial to: "Mesenchymal Stem Cells with eNOS Over-Expression Enhance Cardiac Repair in Rats with Myocardial Infarction" by Leilei Chen et al.

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Although therapeutic advances have progressively reduced annual deaths from heart disease, cardiovascular disorders remain the leading cause of mortality and morbidity worldwide [1]. A main new therapeutic target is actual regeneration, fully repairing infarcted regions and restoring functioning myocardium following injury. In this regard, over the last ~15 years, the use of cell-based therapy has emerged as a leading approach to promoting myocardial regeneration and reducing myocardial infarct scar size.

A variety of stem cell populations including mesenchymal, cardiac and bone marrow-derived mononuclear cells have been identified and evaluated for their regenerative potential for the treatment of heart disease. One of the most favorable candidates for cellular therapy is the mesenchymal stem cell (MSC). There is substantial data from in vitro [2], preclinical [3–5] and clinical [6–9] studies supporting a multifactorial mechanism of action for the cardioreparative effects of MSCs. Main mechanisms include: reducing fibrosis and inflammation; stimulating angiogenesis; restoring contractile function and stimulating proliferation and activity of endogenous cardiomyocytes and cardiac stem cells. Although MSC therapy is promising, numerous challenges remain, including the source of donor cells, methods of cell delivery, and survival of transplanted cells in vivo. As such, enhancing the therapeutic effects of MSCs either by preconditioning with growth factors, hypoxia and/or drugs or through genetic modification is under active investigation. MSCs pre-conditioned by exposure to pro-angiogenic or anti-apoptotic growth factors [10, 11] such as hypoxia inducible factor-1 (HIF-1), vascular endothelial growth factor (VEGF), insulin-like growth factor [12], heme-oxygenase-1 or protein kinase B (Akt) show enhanced left ventricular (LV) function in animal models of MI. Similarly, stem cells transfected with angiopoietin-1, CXC chemokine receptor 4, PIM-1 kinase or stromal-derived factor-1 showed enhanced engraftment and myocardial function, thereby preventing cardiac remodeling, in animal models of MI [10, 11, 13].

In this issue of Cardiovascular Drugs and Therapy, Chen et al. examined the therapeutic potential of MSCs overexpressing endothelial nitric oxide (NO) synthase (eNOS/NOS3) for the treatment of ischemic cardiac injury in rats [14]. NO produced by eNOS plays a broad range of regulatory roles in the cardiovascular system [15–17] including promotion of vasodilation, modulation of myocardial contractile responses, nitroso-redox imbalance, angiogenesis and inflammation. eNOS-derived NO plays a cardioprotective role following MI as shown by eNOS knockout (KO) mice [18] which exhibited left ventricular (LV) dysfunction and enhanced interstitial fibrosis whereas cardiomyocyte-specific eNOS overexpressing mice [19] showed enhanced LV function and decreased myocyte hypertrophy following MI. Furthermore, local transfer of eNOS into ischemic rat hearts increased NO bioavailability, stimulated neovascularization, attenuated cardiac remodeling and suppressed oxidative stress associated apoptosis [20, 21]. Thus, eNOS may be an ideal candidate to enhance the cellular and therapeutic effects of MSCs.

Chen et al. [14] showed that adenoviral delivery of the human eNOS gene into mouse bone marrow-derived MSCs (BM-MSCs) ameliorated the ischemic injury in rats. The combination of eNOS gene delivery and MSCs reduced infarct

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size, improved hemodynamic parameters and increased capillary density [14]. These findings are a first step in using genetically modified MSCs that overexpress eNOS for the treatment of ischemic injury.

What are the mechanisms by which overexpressing eNOS can lead to improved therapeutic potential of MSCs in cardiac protection and tissue regeneration? Secretion of pro-angiogenic and pro-arteriogenic factors by MSCs is thought to be essential for the regenerative process following myocardial injury and NO plays an important regulatory role in angiogenesis and vasculogenesis. eNOS-derived NO, coupled with VEGF signaling, promotes endothelial cell-mediated angiogenesis [22, 23]. Both cGMP signaling and S-nitrosylation mediate the pro-angiogenic effects of NO. Furthermore, environments with nitroso-redox imbalance, such as found during ischemia and heart failure, induce regenerative processes. Hypoxia triggers angiogenesis via upregulation of HIF1α, which stimulates transcription of VEGF and heme-oxygenase (HO-1), a mechanism that is enhanced by eNOS-mediated protein S-nitrosylation [24, 25]. Male mice lacking S-nitrosoglutathione reductase ($GSNOR^{-/-}$ mice), a denitrosylase that regulates S-nitrosylation, exhibit constitutively S-nitrosylated HIF-1α with increased binding to the VEGF gene [26]. These mice also manifest cardio-protection after MI that is associated with increased myocardial capillary density [26, 27]. Paradoxically, bone marrow-derived MSCs from $GSNOR^{-/-}$ mice exhibit reduced endothelial differentiation capacity ex vivo [28], illustrating the complicated and cell lineage-specific relationship between cardiac remodeling and nitroso-redox state. In addition, $GSNOR^{-/-}$ mice exhibit improved post-myocardial infarction regenerative activity, characterized by enhanced turnover of cardiomyocytes and cardiac stem cells (CSCs) [27]. Endogenous CSCs and other resident cardiac progenitor cells are thought to proliferate after MI to support cardiac repair. Furthermore, both MSCs and eNOS possess anti-inflammatory capacities, thus the combined treatment may work to modulate inflammation, thereby preventing extensive cell death and promoting the capacity for the cells to proliferate and renew.

Cell pre-conditioning and genetic modification are promising options in augmenting MSC- and other stem cell-based therapy. Chen et al. [14] showed that the combination of eNOS and MSCs may represent a promising cocktail with the potential to improve cardiac repair following ischemic injury. Direct, in vivo comparisons are needed to ascertain the most effective therapeutic approach for different types of heart disease. For example, the best cell pre-conditioning/genetic modification cocktail protocol for treatment of acute myocardial infarction may not be optimal therapeutic treatment for chronic myocardial infarction or heart failure with preserved ejection fraction. The next step will be to address efficiency and safety concerns by performing appropriate large animal studies, before this combined treatment can be taken into the clinical setting.

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