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Genetically Modified Mesenchymal Stem Cells and Their Clinical Potential in Acute Cardiovascular Disease

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Abstract

Adult mesenchymal stem cells (MSCs) are non-hematopoietic cells with multi-lineage potential to differentiate into various tissues of mesodermal origin. They can be isolated from bone marrow and other tissues and have the capacity to extensively proliferate in vitro. Moreover, MSCs have also been shown to produce anti-inflammatory molecules which can modulate humoral and cellular immune responses. Considering their regenerative potential and immunoregulatory effect, MSC therapy is a promising tool in the treatment of degenerative, inflammatory and autoimmune diseases. However, the current understanding from results of clinical trials is that MSC-therapy is safe but its therapeutic efficiency needs to be improved. In this article we will focus on options for genetic manipulation of MSCs and on current progress in adapting genetically-modified MSCs for clinical use in acute cardiovascular disease.
Properties of mesenchymal stem cells

Mesenchymal stem cells (MSCs) are non-hematopoietic cells with multi-lineage potential (Barry and Murphy 2004). They have been shown to differentiate into various tissues of mesodermal origin, such as adipocytes, osteoblasts, chondrocytes, tenocytes, and skeletal myocytes. They can be isolated from bone marrow (BM) and various other sources such as umbilical cord blood or adipose tissues and have the capacity to extensively proliferate in vitro. Their capacity to differentiate into various lineages and their in vitro proliferative potential makes them attractive targets for regenerative medicine applications. Interestingly, it has been demonstrated in vitro that MSCs also possess immunomodulatory properties. MSCs are shown to inhibit T cell proliferation and to influence the maturation and expression profile of professional antigen presenting cells such as dendritic cells (DCs). Moreover, MSCs modulate B cell functions and are able to inhibit proinflammatory cytokine producing CD4 Th17 cells in a CC chemokine Ligand 2-dependent (also known as MCP-1) manner (Rafei et al. 2009). At least some of these effects seem to be operational in vivo as MSC infusion has been shown to significantly prolong the survival of MHC mismatched skin grafts in baboons (Bartholomew et al. 2002) and lower the incidence of graft-versus-host disease (GvHD) after allogeneic hematopoietic stem cell transplantation in humans (Lazarus et al. 2005). More recent data suggest that MSCs attenuate septic complications by reprogramming host macrophages to increase production of Interleukin (IL)-10 (Nemeth et al. 2009) and decrease neuronal death in global ischemia by modulation of inflammatory immune responses (Ohtaki et al. 2008).

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Isolation and characterisation of MSCs
As discussed earlier MSCs can be isolated from various tissues including bone marrow. Typically, bone marrow cell suspensions are layered onto a density gradient and the nucleated cell fraction is collected, washed and resuspended in MSC culture medium. After 24 h of cultivation non-adherent cells are removed, fresh medium added and individual colonies of fibroblast like cells are allowed to expand and approach confluence prior to passage. One important criterion during the isolation and expansion of MSCs is to characterize the expanded cells for multi-potency. This is routinely performed by testing the isolated cells for their capacity to differentiate into chondrocytes, adipocytes or osteocytes. More recently protocols for characterisation of expression cell surface molecules using flow cytometry have been developed. MSC preparations are analysed for the expression of a characteristic profile of cell surface markers (CD29⁺, CD44⁺, CD90⁺, CD105⁺, CD14⁻, CD31⁻, CD34⁻, CD45⁻) by flow cytometry. However, up to now no single marker has been identified to definitively distinguish human MSCs from all other cell types.

Genetic modification of MSCs to improve their therapeutic potential
The genetic modification of MSCs is an interesting option to improve their therapeutic potential (Kumar et al. 2008). Most pre-clinical and clinical applications of gene therapy have utilised virus-based transfer of genetic material. Viral vectors
are generally characterized by high infectivity and broad tropism, although transduction efficiency may vary, depending on the target cell. The most frequently used carriers for the transfer of genetic information in human gene therapy trials have been adenoviruses (Ad) and retroviruses (RV) including lentiviruses (Edelstein et al. 2007). We have recently shown that MSCs of different species can be efficiently transduced with both adenoviral and retroviral vectors (McMahon et al. 2006). It is believed that genetic modification with RV-vectors leads to long-term expression of the therapeutic gene due to the integration of the viral vector into the cellular DNA. However, insertional mutagenesis due to integration events after RV-mediated gene transfer into hematopoietic stem cells have been recently reported and this concern may render retroviral vectors unsafe for human clinical applications (Nienhuis et al. 2006). In contrast, Ad-vectors do not integrate into the cellular DNA but are known to induce both humoral and cellular immune responses at least after direct injection in animals or humans. Moreover, transgene expression in proliferating stem cells will only be transient due to the non-integrative properties of Ad-vectors. Nonetheless, short-term therapeutic gene expression may be sufficient or even desirable for treatment of diseases such as myocardial infarction and in which transient paracrine effects of MSCs enhance tissue healing and repair responses. Furthermore, therapy with Ad-transduced MSCs is likely to be less immunogenic than direct administration of Ad-vectors to affected tissues as a result of anti-inflammatory properties of MSCs. Recently, Adeno-associated viruses (AAV) have been also developed as gene therapeutic vehicle based on its low immunogenic profile upon in vivo administration. Although it has been reported that AAV can efficiently transduce human MSCs very high titres seem to be required for in vitro transduction of MSCs (Stender et al. 2007).
Beside viral gene therapy vectors many attempts have been undertaken to develop efficient techniques for non-viral genetic modification of cells or tissues. In contrast to viral gene delivery, synthetic vectors are superior in terms of toxicity and their potential to induce immune responses. They are also industrially reproducible allowing easier pharmaceutical development, quality control and scalable production – features that cannot be underestimated when “good manufacturing practices” (GMP) are to be translated to large patient populations. Currently, however, ex vivo utilised non-viral gene delivery systems in MSCs such as lipoplexes or polymers, electroporation (McMahon et al. 2006) and nucleofection must be substantially improved to match viral gene transfer efficiencies while maintaining low levels of cytotoxicity. Interestingly, lipoplexes modified with recombinant peptides such as TAT peptides (reviewed by Torchilin 2008) or nuclear localization sequence (NLS) peptides (Hoare et al.) have been used successfully to enhance gene expression by addressing some of the barriers to non-viral gene delivery - cellular uptake and nuclear localization, respectively. While these modifications to non-viral gene delivery systems hold considerable potential to displace viral vectors in clinical gene therapy protocols, they will also require rigorous in vivo testing for immunogenicity, duration of expression and other toxicities.

Pre-clinical investigation of gene-modified MSCs in animal models of acute cardiovascular disease.

With the realization in the past decade that MSCs have significant disease-modifying effects and are relatively permissive to a range of gene transfer methodologies, there has been a strong impetus toward pre-clinical testing of MSCs transduced with a range of potentially therapeutic gene products (Wagner et al. 2009). Among the
applications that have received most attention are the targeting of therapeutic products to cancers, the repair of neural injury and degeneration, the promotion of bone and cartilage regeneration and the amelioration of acute cardiovascular events. In this section of the review we will highlight a number of recent representative studies in the area of cardiovascular disease. Emphasis is placed on experimental approaches that would appear to hold most promise for clinical translation.

In the realm of cardiovascular disease, acute myocardial infarction (AMI) in the rat has been by far the most commonly utilized model for testing in vivo efficacy of genetically-modified MSCs. The majority of such studies have employed intra-myocardial injection of transduced or control MSCs with variability in the choice of therapeutic gene, the gene transfer vector used and the timing of delivery. Among the most significant of these reports are those which have derived from specific mechanistic approaches to improving MSC migration, retention, survival or production of factors known to promote angiogenesis and tissue repair. One of the earliest and most striking examples was provided by Mangi et al. who predicted that over-expression of the anti-apoptotic signaling protein Akt in MSCs would improve myocardial repair following AMI through pro-survival effects (Mangi et al. 2003). As shown in this and subsequent studies, Akt over-expression by retroviral transduction of MSCs was associated with significantly reduced infarct size and myocardial remodeling and improved left ventricular function. Although regeneration via MSC differentiation into cardiomyocytes was initially considered, further studies demonstrated that the predominant beneficial mechanism was through enhanced production of anti-inflammatory, pro-repair factors (Noiseux et al. 2006). Lasting benefits (up to 3 months) of MSCs Ad-transduced with Akt and the pro-angiogenic
protein angiopoietin 1 were recently reported in a similar rat AMI model (Shujia et al. 2008).

Over-expression of another pro-survival protein, Bcl2, was similarly shown to result in prolonged retention of MSCs at the site of AMI with resulting reduction in infarct size and improvement in left ventricular function compared to control-transfected cells (Li et al. 2007). Of additional interest, this study employed a non-viral gene transfection system and certain therapeutic effects of Bcl2 over-expression were shown to be mediated by increased MSC production of vascular endothelial growth factor (VEGF). More recently, Tang et al, using Ad-transduction, demonstrated additive benefits in AMI of dual over-expression of VEGF and the stem cell homing/retention factor SDF-1 by MSCs compared to MSCs transduced with either gene alone. In this study, delivery of genetically modified MSCs was delayed until 7 days post-infarction. Other mechanistic approaches to enhancing MSC effects in acutely ischemic tissue have also been explored. For example, MSC transduction with an Ad-vector encoding prostacyclin synthetase, resulted in improved restoration of blood flow in a mouse model of acute limb ischemia (Ishii et al. 2009) and over-expression in MSCs of tumor necrosis factor receptor (TNFR) via AAV was associated with reduced cardiac inflammation and apoptosis and improved LV function in rat AMI (Bao et al. 2008).

Several additional reports have been published within the past 3 years describing beneficial effects of genetically modified MSCs in the rat AMI model. These have utilized additional viral and non-viral vector systems and have employed a range of therapeutic genes. Taken together with the examples highlighted here, the available literature supports several conclusions regarding the progress that has been made in developing genetically modified MSCs as a bona fide therapeutic option in human
acute myocardial infarction: 1. There is convincing in vivo proof of principal that MSCs modified to over-express proteins that enhance their retention, survival and production of anti-inflammatory and pro-angiogenic factors are therapeutically superior to non-transduced MSCs. 2. Combined over-expression of two or more genes with complementary mechanisms of action (e.g. pro-survival and pro-angiogenic) is feasible and holds promise for further optimizing therapeutic efficacy. 3. Significant benefits have been observed for MSCs transduced using vector systems (adenovirus, non-viral) that are, in principal, applicable to human therapeutics. 4. There remains a dearth of pre-clinical studies carried out in large animal models that more closely reproduce the typical pathobiology of human atherosclerosis and AMI. 5. Animal studies have been carried out predominantly by intra-myocardial injection rather than by systemic or intra-coronary routes which may be more broadly applicable to human AMI and other acute vascular conditions.

Path toward clinical application of gene-modified MSCs

As we have described here several gene transfer systems have been successfully applied to in vitro transduction and in vivo pre-clinical testing of therapeutic MSCs. In addition, however, each vector system has potential limitations as regards efficacy and safety for human clinical applications. The recent development of third generation Ad-vectors is an interesting option as these constructs only contain minimal residual adenoviral DNA (inverted repeats and packaging signal) resulting in reduced immunogenicity upon injection. The development of site-specific integration vectors for long-term gene expression has also seen recent advances. Transposable genetic elements such as the ‘sleeping beauty transposon’ have been proposed for the non-viral genetic modification of stem cells (reviewed by (Ivics et al. 2009)) although the
risk of insertional mutagenesis will require careful analysis. Other approaches to ensure extended gene delivery to MSCs *ex vivo* or *in vivo* may include the incorporation of cells and vectors into biomaterial scaffolds (reviewed by Kulkarni et al.). The selection of optimal therapeutic genes for individual disease processes will continue to be a large challenge although progress may be accelerated by *in silico* analysis of the genomic and proteomic profiles of bulk and cloned MSC cultures. An additional future goal for some therapeutic applications will be the development of strategies to detect and directly transfect MSCs *in vivo* in their physiological niche. Ultimately, the broad clinical potential for genetically modified MSCs in cardiovascular and orthopedic disease is likely to be realized by progress in three specific areas: 1. The development of a safe, highly efficient non-viral gene delivery system with flexible duration of expression. 2. The successful combination of genetically modified MSCs with biomaterial scaffolds that recapitulate the niche occupied by these cells *in vivo*. 3. The optimization of serum-free culture conditions for large-scale MSC production that permit economically-feasible large scale production while preserving therapeutic efficacy.
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Gene-modified MSC expressing therapeutic gene

Strategy:
Overexpression siRNA

Treatment of Myocardial infarct or osteochondral defects