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The role of multipotent marrow stromal cells (MSCs) in tissue regeneration

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Abbreviations: AKI, acute kidney injury; AM, adrenomedullin; CKD, chronic kidney disease; HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor-1; iPCs, induced pluripotent cells MSCs, multipotent marrow stromal cells; VEGF, vascular endothelial growth factor;

An extensive body of preclinical and clinical data has shown that administration of adult multipotent marrow stromal cells (MSCs) effectively ameliorates experimental and clinical conditions of many different organ systems. Differentiation into organ parenchymal cells, however, is very rare, and the main mechanism for organ protection and regeneration from different types of injury is the exertion of paracrine effects and stimulation of tissue repair. A large number of clinical trials have been conducted and are ongoing to investigate the safety and efficacy of MSCs in different organs after various types of organ injury. This article intends to give a brief overview about current applications of MSCs and mechanisms involved in organ protection and regeneration.

Introduction

The common final denominator of disease is tissue damage from any number of pathomechanisms, with ischemia being the most common, followed by distinct mechanisms such as drug toxicities, autoimmune mechanisms, mechanical trauma, degenerative changes, genetic mechanisms and many others. The hard endpoint of tissue damage is cell death, either as a consequence of apoptosis, frank necrosis or both. After cell death has occurred, the organism initiates a complex repair process that may be more or less effective, but which not infrequently results in incomplete repair or harmful tissue fibrosis. Common reactions to cell death are inflammation, scar formation with fibrosis and collagen deposition.

To optimize the repair process, therapeutic efforts should ideally target both the prevention of cell death and the replacement and repair of cells that are lost and injured in the process of tissue damage, respectively. And importantly, when designing and delivering such organ protective and repair stimulating therapy, it is critical that deleterious late outcomes, such as tissue fibrosis and scar formation, are avoided.

Stem cells are ideally suited as agents of tissue repair, given that they are both undifferentiated and have the ability to form many if not all differentiated cell types. In addition, stem cells also secrete bioactive proteins and molecules that guide and coordinate processes in tissue repair, orchestrating ordered rather than random reconstruction of an injured organ.

This commentary aims at giving a brief overview of the role of administered multipotent marrow stromal cells and their hitherto defined mechanisms that they employ in organ repair.

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Tissue Response to Injury

The physiological and coordinated response to injury is fairly similar in all tissues and involves several overlapping phases that can be divided into inflammation, new tissue formation and remodeling.[1]

Inflammation. Damaged cells express and secrete a variety of factors that elicit and coordinate responses aimed at removal of damaged cells and regeneration of the original tissue architecture. These include, amongst many others, inflammatory cytokines such as TNFα, interleukins, chemokines and growth factors.

This initial response then triggers and is followed by recruitment of inflammatory cells, mainly neutrophils and macrophages but also lymphocytes and other immune cells that carry out the removal of damaged tissue and that limit the extent of further damage. Macrophages are thought to be important for coordination of the later stages of tissue repair.

New tissue formation. Surviving and sub-lethally damaged parenchymal cells start to proliferate and migrate to sites of injury. This process is highly variable in different tissues, with some organs being highly capable of regeneration, while others do have a limited capacity to regenerate parenchymal cells and mainly develop detrimental responses, such as scar tissue formation or fibrosis.

Angiogenesis is also an important part of this repair phase, since tissues require blood supply for adequate delivery of oxygen and growth factors as well as for removal of waste products.

Fibroblasts also migrate towards damaged sites and produce extracellular matrix to support tissue architecture.

Remodeling. Once the acute injury phase is over and initial repair processes are winding down, organs start to remodel their structure, and recruited cells that are no longer needed at the site either undergo apoptosis or migrate out of the site of injury.

Proteins and factors involved in this phase include extracellular matrix proteins and their receptors, proteases, such as matrix metalloproteinases, and their inhibitors as well as enzymes regulating metabolism.

Detrimental effects of tissue repair. Tissue repair is aimed at reducing damage and restoring original parenchymal structure. However, the human body has no ability to completely restore damaged tissue to its former functional state, and regenerative processes are more or less effective, depending on the tissue of origin, with highly evolved and effective repair in the liver on one side and extensive and detrimental scar production on the other side in tissues such as brain and heart.

The heart has no or only an extremely limited capacity for cardiomyocyte proliferation and tissue restoration after a myocardial infarction, resulting in the formation of scar tissue, which can then lead to congestive heart failure and arrhythmias.

The liver, on the other hand, can regenerate up to 70% of itself without evidence of significant scar formation, although this depends on the type of injury as well as on its acuity, with chronic injury from toxins leading to extensive fibrosis, while acute toxic liver injury can result in complete regeneration.[2]

Role of Stem Cells

Single-agent therapies, e.g., administration of growth factors or pharmacological agents, have been shown to only possess a limited impact on tissue regeneration and usually affect only one or a limited number of the various pathways that characterize pathogenesis and organ repair. Stem cells have the capability to secrete a large number of cytokines and factors and are able to directly interact with the microenvironment once they are located in the area of tissue damage. Historically, with the discovery of the possibilities of stem cells as therapeutic agents, it has been proposed that differentiation into organ parenchymal cells is the main mechanism of action. Once it was recognized that the number of differentiated stem cells that contribute to organ parenchyma is actually quite low, alternative mechanisms, such as paracrine actions, came to be appreciated as mediators of tissue protection and regeneration.

The ability of stem cells to differentiate into a wide variety of terminally differentiated structures and specialized cells, as well as the secretion of proteins and their interactions with tissues and cells at the molecular level, made them attractive targets for the development of effective therapies that are targeted at tissue regeneration and repair.

Multipotent Marrow Stromal Cells

Multipotent marrow stromal cells are easily derived from the bone marrow but also from adipose tissue and other tissues. These cells are able to self-renew; they readily expand in culture and can differentiate into mesenchymal cell types, such as cartilage, bone and fat.[3] During the past decades,MSCs have generated a great deal of interest in many clinical settings, including regenerative medicine, immune modulation and tissue engineering.

Bone marrow-derived MSCs are not contributing to hematopoietic lineages and were initially described and cultured in vitro by Friedenstein.[4] Because their culture expansion in vitro is rapid and relatively easy and due to their ability to differentiate into several tissue lineages, a large body of data has been created regarding their use in regenerative medicine.[5] They are isolated from a bone marrow aspirate by virtue of their selective plastic adherence in tissue culture flasks (compared to the non-adherent hematopoietic progenitor cells) and are characterized by their differentiation potential into adipocytes, chondrocytes and osteocytes as well as their surface marker expression profile. A consensus article defined them as expressing the markers CD73, CD90 and CD105, while lacking the surface marker expression of CD45, CD34, CD14 or CDllb, Cd79a or CD19 and HLA class II.[6]

Use of MSCs as Therapeutic Vehicles

Because of their unique properties in vitro and in vivo, MSCs have been explored as therapeutic tools for a large variety of indications. Their first use in humans dates back to the 1990s, when they were used as adjunct therapy to hasten and improve engraftment and recovery in patients receiving an autologous bone marrow transplant after chemotherapy.[7] Since then a substantial number of other conditions are being considered or have been targeted for MSC therapy.

Inflammatory diseases. MSCs have been shown to modify (in vitro or in vivo) to terminate inflammatory responses through modulation of cytokine production, suppression of T-cell proliferation, modulation of B-cell function, suppression of NK proliferation and cytotoxicity as well as inhibition of dendritic cell maturation.[8]

Based on these broad immune modulating and anti-inflammatory activities, MSCs have been used in a number of inflammatory conditions, including acute and chronic graft versus host disease, Crohn disease, Sjögren syndrome, ulcerative colitis, multiple sclerosis, transplant rejection, systemic sclerosis and systemic lupus erythematodes.

Organ repair. MSCs contribute directly to tissue repair and have been shown to be incorporated into organ structures in the long term, although the numbers of directly engrafted MSCs have been low in most animal studies, and it has been estimated in a human study that the contribution of MSCs to the cells of the bone was not more than 2%.[9] Direct incorporation is thought to be important in the following diseases or conditions: osteoarthritis, osteogenesis imperfecta, for foot and ankle fusion, liver cirrhosis, myocardial infarction and burn injuries.

The extent of their direct contribution to the replacement of cells in injured tissues is, however, controversial, and it is likely that mechanisms other than direct differentiation and engraftment contribute significantly to actual regeneration. Some studies

have shown that the contribution of MSCs to tissue is less than 2% of total cells in human organs.[9]

MSCs for gene therapy and protein delivery. MSCs are readily transduced with gene expression vectors and are therefore an attractive vehicle for systemic gene therapy and delivery of proteins to site-specific locations. It has been shown in several model systems that transduced MSCs successfully deliver the intended therapeutic protein to target tissues, where beneficial effects can be readily observed. An example would be erythropoietin-transduced MSCs, which have been shown to correct anemia in a mouse model of chronic kidney disease (CKD).[10]

To date, human studies have not yet been conducted with this technology.

Tumor therapy with MSCs. Transplantation experiments have provided evidence that MSCs are homing to tumor tissue.

One study showed that up to 40% of the myofibroblasts in a tumor were bone marrow-derived in a mouse model, and 20% of the cells in lung cancer after sex-mismatched bone marrow transplantation were bone marrow-derived in a patient.[11]

The most likely cause of preferential migration to tumor tissue is the release of chemotactic factors, such as SDF-1 (CXCL12) from the tumors that attract circulating MSCs.

Heart. It has been shown in a number of animal models that myocardial injection of MSCs improves cardiac function after myocardial infarction and in cardiomyopathy. Although MSCs have been shown to differentiate in cardiomyocytes in vitro and in vivo, their contribution to the actual myocardium after injury was not enough to explain the observed benefits. MSCs secrete a number of angiogenic, antiapoptotic and mitogenic factors, amongst them vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), adrenomedullin (AM) and insulin-like growth factor-1 (IGF-1).[12] Because direct differentiation into target cells and their engraftment was insufficient to explain obtained benefits, it was hypothesized and shown in various model systems that cardiac remodeling, contractility and metabolism after injury are targeted in paracrine fashion. Therapeutic responses in animal models included stimulated angiogenesis with increased capillary density as well as enhanced myogenesis and inhibition of fibrosis.[13] The currently accepted view is that MSCs located in ischemic or damaged myocardium secrete VEGF and thereby increase vascular density and blood flow; furthermore, they decrease apoptosis and may differentiate directly into endothelial cells.[14]

Brain. MSCs have been shown to improve function by inhibiting T-cell proliferation in a model of experimental autoimmune encephalomyelitis.[15]

MSCs have been administered into the ischemic brain in animal models and have been shown to induce neurogenesis, angiogenesis and to effect neuroprotection, all of which contributed to an improved outcome as compared to controls.[16]

First trials with autologous MSC therapy in stroke patients have shown promising results.[17]

Kidney. The demonstration of hematopoietic stem cell (HSC) differentiation into cells outside their common lineage boundaries, including hepatocytes, cardiomyocytes as well as renal cells, initiated investigation of stem cell therapy in all fields of organ regeneration, including the kidney.[18] In humans, it has been shown, by analyzing the results from sex-mismatched transplantations, that bone marrow-derived cells do contribute to renal tubular cells, but overall accounted for only about 1% of tubular cells.[19]

Because hematopoietic stem cells are relatively difficult to obtain and cannot be cultured and expanded to sufficient quantities in vitro, the focus of renal regenerative therapy has shifted towards MSCs as a promising cell type for renal therapies. At present, MSCs have become a central tool in the development of novel therapies in nephrology.

MSCs were demonstrated to contribute to tubular epithelial cell regeneration in mice in a glycerol model of acute kidney injury (AKI), and another group confirmed these findings in a cisplatinum model of AKI.[20,21] The actual contribution of MSCs to the number of regenerating cells was, however, very variable in these models, and subsequent studies did not find engrafted cell numbers high enough to explain the significant functional improvements seen with MSC therapy.[22-24] Several groups hypothesized that paracrine mechanisms are more likely to explain observed improvements and started identifying factors that might confer renoprotection and regeneration. A number of key factors have been identified so far. VEGF has been shown to be directly renoprotective and also has important effects on renal vasculature, which is a key component in the pathophysiological cascade of AKI.[25,26] IGF-1, a potent proliferative and survival factor that has been shown to be renoprotective when infused as a protein in rats, has been demonstrated to be highly expressed in MSCs, and knockdown of IGF-1 expression limited the protective action of MSCs.[27] More recently, cord blood-derived human MSCs have been shown to exhibit potent renoprotective actions.[28] MSCs in this model were shown to induce protection by increasing expression of the survival factor Akt, thereby reducing apoptosis and increasing tubular cell proliferation. Furthermore, MSCs, when co-cultured with damaged tubular cells, inhibited IL-1β and TNFα synthesis, thereby demonstrating their anti-inflammatory properties.

In general, multiple key factors that are delivered by MSCs in paracrine fashion, carry out the complex actions whereby the different pathophysiological pathways of kidney injury are beneficially affected, thereby achieving superior renoprotection and repair when compared to single factor or other pharmacological therapies.

Bone marrow-derived cells have also been shown to be beneficial in other models of kidney disease, such as Alport syndrome, where they contributed to basement membrane repair and podocyte regeneration.[29,30]

Data for the treatment of chronic kidney disease (CKD) are still limited. In a rat remnant kidney model, MSCs demonstrated modulation of inflammation in the initial phase of CKD as well as decreased fibrosis later in the course.[31]

Based on a large body of data showing the effectiveness and safety of MSCs in animal models of AKI as well as the demonstrated safety and efficacy of these cells in several completed clinical trials, a dose-escalating phase I clinical trial has been conducted to test the safety and feasibility of MSCs in patients at

high risk of postoperative AKI.[32] These data demonstrate that the postoperative infusion of MSCs at incremental doses is safe, as no adverse events or severe adverse events attributable to infusion of MSCs were observed.

Potential Complications of MSCs

Although the benefits of MSCs in regeneration and repair of tissues are clearly demonstrated in animals and humans and although MSCs are the stem cell type that is most advanced in clinical development and application and even long-term data are available, there is still concern about potential side effects; highest amongst them is the development of organ fibrosis and tumorigenesis.[33]

Administration of stem cells into the blood stream can cause complications, such as pulmonary emboli or infarctions.

MSCs injected into rat hearts in high numbers induced intramyocardial calcifications,[34] and high numbers of MSCs infused directly into the renal arteries of rodents with anti-Thy-1-induced glomerulonephritis led to their intra-glomerular maldifferentiation into adipocytes and subsequent sclerosis.[35]

MSCs after lung irradiation contributed to fibroblast and myofibroblast accummulation in areas of pulmonary damage.[36]

Of note, there have been, to date, no reports in humans showing detrimental effects of autologous or allogeneic MSC infusions despite the adverse effects described in specific animal models above. Even limited long-term data have not yet demonstrated late adverse effects of infused MSCs in patients.

Overall, it is apparent that the significant clinical safety of MSCs raises considerably less concern than the use of embryonic stem cells or genetically modified cells.

Future Directions

Within a few decades, MSCs have made the transition from bench to bedside, with currently more than 150 studies ongoing and completed as listed in www.clinicaltrials.gov. This was accomplished in just about 40 years after the crucial discovery by Friedenstein. So far, no serious human side effects have been reported in the autologous and allogeneic setting.

MSCs, together with their bone marrow relatives, HSCs, represent truly the most successful stem cell therapy in use currently, and this for a large and growing number of different diseases. While the high expectations of embryonic stem cells and the recently developed iPCs are still in early pre-clinical and clinical stages, MSC therapy has already proven effective in humans.

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