Mesenchymal Stem Cells

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Abstract

Mesenchymal stem cells (MSCs) are a heterogeneous subset of stromal stem cells which are multipotent in nature. MSCs that are isolated from human bone marrow are capable of self-renewal and differentiation into multiple mesodermal lineage cells including osteocytes, chondrocytes, adipocytes and cardiomyocytes. Intense research effort has been made in understanding about the isolation and mechanisms underneath differentiation of MSCs. Until now, promising results have been achieved from numerous pre-clinical and clinical studies where the therapeutic potentials of MSCs are noticeable. Beside multipotency, the immunomodulatory effects of human bone marrow-derived MSCs to both the innate and adaptive immune systems are also observed, which could facilitate the induction of immunological tolerance during the process of bone marrow and organ transplantation. This is due to the fact that *in vivo* administration of MSCs can successfully reduce graft rejection through the inhibition of inflammatory actions by the cytokines released from T-lymphocytes as well as the enhancement of the MSC microenvironment in the grafted tissues. Cytokines are believed to be responsible for most migration (homing) actions of MSCs into the site of tissue implantation and inflammation where the numerous cytokine receptors expressed in MSCs are attracted by the signals generated through the cytokine gradients. Although MSCs hold a great promise in tissue engineering and regenerative medicine, several potential limitations are still needed to be overcome, which ensure the safety, efficacy and feasibility of MSC-based therapies in the future.

What Are Mesenchymal Stem Cells?

Scientists have long been fascinated by the ability of certain organisms such as starfish and earthworms to regenerate their body tissues or organs under certain conditions. Although more complex organisms like humans can also continuously regenerate blood cells and skin cells once they are inadequate, their regenerating abilities are limited and often inconsistent. In human adults, cells that can reproduce themselves

(self-renewal) as well as give rise to a variety of other cell types in tissues (differentiation) are collectively known as adult stem cells. Adult stem cells can be either pluripotent or multipotent. Pluripotent stem cells can give rise to any type of cell in adult body. While multipotent stem cells are lineage restricted, that is, they have limited ability to differentiate, often confined to their tissue origin. A particular type of multipotent stem cells isolated from human bone marrow, called mesenchymal stem cells (MSCs), has aroused scientist's interest because of its proliferative potential and capacity to differentiate into multiple cell lineages of connective tissues, including adipocytes, chondrocytes, osteoblasts, myocytes and neuron-like cells.[1] The multipotent MSCs are isolated primarily within the stromal compartment of bone marrow, as well as the adipose tissue, umbilical cord blood, fetal tissues such as the placenta, amniotic fluid and amniotic membrane.[2,3] The unique properties of human MSCs are considered to have therapeutic potentials in regenerative medicine and tissue engineering and this has been reflected by an enormous number of promising results from pre-clinical and clinical studies, even though there are still a lot of questions remain to be solved.

Characterization and Isolation of Human MSCs

Non-hematopoietic human mesenchymal stem cells (MSCs) in bone marrow were first investigated by Friedenstein *et al*.[4] MSCs can be defined as a heterogeneous population of cells that are capable of long-term proliferation and self-renewal into discrete colonies of plastic-adherent, spindle-shaped, fibroblast-like clongenic cells (colony forming unit-fibroblast, CFU-F) *in vitro*, and at the same time remain multipotent differentiation capacity to become certain lineages of mesenchymal tissues including bone, cartilage, tendon and fat.[5-7] In order to define human MSCs clearly, general consensus on minimal criteria for MSCs must be reached by scientists. In accordance with the standards proposed by the International Society for Cellular Therapy (ISCT) in 2006, human MSCs must be able to adhere to plastic in standard culture conditions, as well as expressing specific surface markers and be able to differentiate into mesenchymal lineages.[7]

In fact, plastic adherence is one of the most distinguishable properties of human MSCs. Human MSCs resemble a population of spindle-shaped cells with fibroblastic morphology when cultured *in vitro*, usually with basal medium such as Dulbecco's modified Eagle's medium with 10% qualified sterile fetal bovine serum.[5] The cell population tends to adhere to the plastic surface of the culture flask and undergoes expansion until confluency is reached, where undifferentiated MSCs exhibit contact inhibition with flattened monolayer morphology under microscope. However, it is known that only a very small fraction of MSCs are present in the bone marrow, approximately 0.001-0.01% of the total population of nucleated cells in bone marrow

and with the ability to form CFU-F.[5] Zuk *et al*. have revealed that human adipose tissue is one of the sources of multipotent MSCs where it is rich in processed lipoaspirate (PLA) cells, a population of cells behaves like MSCs (with trilineage differentiation capacity and expression of multiple surface antigens consistent with MSCs).[8] Those adipose tissue-derived MSCs can therefore be an ideal source of uncultured MSCs for *in vitro* study.

But for the purpose of MSCs purification the plastic-adherent property is not the only criteria to be concerned with. Typically enriched MSCs from bone marrow via standard culture techniques may contain a mixture of other bone marrow-derived progenitor cells such as hematopoietic and fibroblastoid cell types, which are also plastic-adherent cells. Several methods have been reported for the separation of pure MSCs from populations of fibroblastoids and hematopoietic cells in plastic adherent cells elaborated from murine bone marrow cultures but none of them are perfectly efficient and consistent.[9,10] Simmons and Torok-Storb successfully demonstrated that bone marrow stromal cells expressing surface markers such as CD105 (endoglin), CD73 (ecto-5'-nucleotidase) and CD90 (Thy-1) are recognized by murine IgM monoclonal antibody STRO-1, which are all cell surface markers present in non-hematopoietic human MSCs.[11] Phinney has also documented the use of monoclonal antibody 'cocktails' to negatively select cell populations without cell surface phenotypes of typical MSCs from murine bone marrow.[12] Those selected immunodepleted MSCs after immunodepletion treatment has proven to retain their proliferation and differentiation capacity. The same principle has now been adopted by many scientists for long-term *in vitro* expansion of primitive human MSCs. However, in general only a small amount of human cells can be claimed as human MSCs through isolation because of the minimal criteria set up by ISCT: at least 95% expression of surface markers and at the same time no more than 2% expression of CD11b, CD14, CD34, CD45, CD79α and human leukocyte antigen HLA-DR, which are all surface antigens for hematopoietic stem cells.[7] Even human MSCs can be successfully isolated, the life-span of the harvested MSCs is always limited with an inevitable age-related decline. Together with the invasive techniques generally required for human MSC isolation (especially MSCs derived from bone marrow), a continuous supply of human MSCs for pre-clinical studies is difficult to achieve and it further impedes the functional practicality of human MSCs.[13]

Evidence also supports that none of those above mentioned markers are expressed exclusively on MSCs and the expression levels of them are often varied, probably due to different tissue sources and culture conditions.[14] The lack of specific surface antigen expression profiles for MSCs identification implies that positive selection and isolation of MSCs would be difficult and it is not surprised to see more novel surface

markers will be identified in the future, leading to modifications of current criteria. In fact, among all candidate markers, cells expressing antigen CD271 (CD271+) was reported as the most versatile marker for bone marrow-derived MSCs based on their clonogenic capacity analyzed by the MSC CFU-F assay.[15] When compared with MSCs obtained by plastic adherence, the expansion rate of CD271+ is 200-fold higher (while no CFU-F is detected in the CD271-cell fraction). This finding is accompanied with an array of well-established and novel MSC markers that are also co-expressed alongside CD271+ cell populations. Still, no one single and unique antigen expression profile can be used for isolation of highly purified MSCs.

Studies of Differentiation of MSCs

A large number of studies have been performed after the reveal of multipotency and differentiation ability of bone marrow-derived MSCs investigated by multiple laboratories. It has been hypothesized that MSCs derived from tissues are often tissue-specific and only cell lineages with characteristics of those tissues of residence can be formed. Of the studies undertaken, this hypothesis has been proven wrong as evidence suggested that MSCs pre-committed to a given mesenchymal cell lineage can transdifferentiate (cross lineage barriers) into other cell types under certain conditions.[16] The study of trans-differentiation potential of bone-marrow MSCs has largely focused on mesoderm-derived cell types such as osteoblasts, adipocytes, chondroblasts and cardiomyocytes where inductive signals and biomimetic materials are found to play key roles in driving the transdifferentiation between lineages.[17]

The Importance of Culturing Environment

Although it has long been thought that the multipotency of MSCs would gradually become more restricted as they under different lineage differentiation pathways, certain degrees of 'plasticity' do exist from the observations of transdifferentiation between different terminally differentiated connective tissue cells derived from MSCs both *in vivo* and *in vitro*. Several induction conditions of human MSCs along adipogenic, chondrogenic and osteogenic pathways have been described[18] where the importance of using inductive (growth) factors and mimicking of the native extracellular matrix (ECM) has been mentioned repeatedly by numerous reports. An early report mentioned by Hurle *et al*. indicates that the induction conditions for *in vitro* differentiation of mesenchymal precursor cells along the osteogenic and chondrogenic pathways often requires incubation with three-dimensional tissue constructs where a 'threshold' of cell density must be reached in order to promote the formation of specific ECM in the local monolayer microenvironment.[19]

In fact, the need of three-dimensional culture format in human MSC differentiation is based on the theory of micromass technique where it is postulated that the presence of proteinaceous extracellular matrix in culture

would have both direct and indirect effects on the cultured cells and thus the subsequent modulation of their behaviors (i.e. differentiation potentials). This can be seen on the chondrocyte differentiation pathway where in a serum-free medium supplemented with transforming growth factor-β1, differentiation was found most prominent when collagen type II hydrogel is used as the three-dimensional natural scaffold, even though other biomaterials are also reported as possible biocompatible scaffolds/polymer gels for chondrogenic induction.[20] Evidently, chondrogenic differentiation is also accompanied by a rapid loss of fibroblastic phenotype and production of various cartilage-specific extracellular matrix components such as glycosaminoglycan and sulfated proteoglycan. These components in the cartilaginous microenvironment are believed to be able to recruit precursor cells to condensation, thus leading to tissue (chondrocytes) differentiation. The importance of collagen type II extracellular matrix for MSC chondrogenesis *in vitro* was further supported by the up-regulation of a series of chondrocyte-specific mRNA transcripts detected by immunohistochemical, histological, *in situ* hybridization and quantitative real-time PCR methods.[21] This implies that by understanding the cellular responses to ECM in culture, a proper design of scaffolds or hydrogels can be made which ensures the chondrogenic differentiation of MSC can be applied to cartilage tissue engineering one day.

The Flexibility of Differentiation Pathways

Similarly, investigations on osteogenic pathway have revealed the use of ascorbic acid, dexamethasone and β-glycerophosphate added in fetal bovine serum can result in loss of fibroblastic phenotype in culture and at the same time an increase in alkaline phosphatase level and deposition of calcium-rich mineralized extracellular matrix, which are characteristics of oestogenic differentiation.[5] Fully differentiated osteoblasts, however, according to the experiments conducted by Song and Tuan, have demonstrated the ability to transdifferentiate into chondrocytes and adipocytes when appropriately cultured.[16] This switching of cell lineage from the pre-committed cell type involves reprogramming of genome and phenotype in response to inductive extracellular signals. However, the altered phenotype of MSCs after transdifferentiation may in fact the outcome of spontaneous cell fusion where the MSCs could be contaminated with other precursor cells and the resulting hybrid cells may still possess the characteristics (e.g. phenotype, surface makers) that would be interpreted as the result of transdifferentiation.[22,23] This suggests that clear examinations (e.g. genetic analysis) are necessary when considering the real cell plasticity of any putative products claiming to be generated from MSC transdifferentiation in the future.[24]

Interestingly, the reversal of terminated differentiated human MSCs to their primitive progenitor cells with multipotency *in vitro* is also possible. In an article mentioned by

Tsonis, a dedifferentiation process was observed in the studies of urodele amphibians for their regenerative capabilities.[25] The amputated limbs in amphibians can cause the stump tissues (e.g. muscle and cartilage) to lose their tissue characteristics and become undifferentiated again. The undifferentiated cells are in the form of a blastema (a cell mass capable of regeneration and growth into an organ or appendage) and lately redifferentiate to reconstitute a replica of the lost limbs. The dedifferentiation of stump tissues to become blastema at some points is similar to the transdifferentiation of human MSCs to become myocytes, osteoblasts, chondrocytes and adipocytes in culture. Again, like transdifferentiation, the induction of dedifferentiation is signal-dependent. Chen *et al*. used the murine C2C12 myogenic cell line to generate lineage-committed cells myotubes, which are multinucleated, differentiated muscle cells in development.[26] By screening the cell line with a huge number of small molecules, 2-(4-morpho-linoanilino)-6-cyclohexylaminopurine (also known as reversine) was found to stop the proliferation of myotubes and trigger dedifferentiation to become mesenchymal progenitor cells. Those uncommitted progenitor cells are able to redifferentiate into either adipocytes or osteoblasts when supplied with specific inducing substances. The reversal of lineage by reversine is in fact similar to the blastema cells generated during the process of limb regeneration in urodele amphibians through dedifferentiation and transdifferentiation. The use of those molecules *in vitro* can provide insight into the molecular mechanisms of cell dedifferentiation and transdifferentiation in developing mammalian tissues and thus the possibility of regulation of MSC differentiation. The understanding of the mechanisms mediating the differentiation of MSC is fundamentally essential for MSCs to be used as a therapeutic tool in the future.

Possible Therapeutic Applications of MSCs

The therapeutic potentials of human MSCs for a wide range of human diseases have drawn a lot of attention to physicians because of the unique features of MSCs. It is well known that human MSCs are capable of differentiating into cells of the mesodermal lineages (whether cells can transdifferentiate into other lineages remains controversial), but they also show immunomodulatory effects on immune cells. The immuno-modulatory function of MSCs on T lymphocytes is of particular interest since self-reactive T lymphocytes in the adaptive immunity are associated with medical conditions including inflammatory auto-immune diseases, graft-versus-host diseases (GVHD) and allograft rejection. The induction of immunological tolerance by MSCs therefore holds a great promise for tackling serious complications arise in bone marrow and organ transplantation, even though it still requires a lot more scientific evidence to prove the usefulness of MSCs for therapeutic use.

Role of MSCs in Bone Marrow Transplantation

Bone marrow transplantation is one of the best known treatments to treat leukemia by using hematopoietic stem cells (HSCs). HSCs are commonly derived from sources such as bone marrow, peripheral blood, umbilical cord blood and even blood vessels. The infusion of either autologous or allogenic HSCs is generally accepted as a safe procedure and is capable of reconstituting the hematopoietic micro-environment in bone marrow of patients after chemotherapy (or with radiation together). However, the infused cell dose of HSCs is often inadequate (with relatively low cellularity) from those sources and the transplantation of HSCs to patients with acute leukemia would thus slow down the replenishment process of various blood cells (e.g. blood platelets and neutrophils). The slow recovery process of clonogenic growth may in part be due to residual graft resistance and altered microenvironment that supports hematopoiesis.[27,28] Because of this MacMillan *et al*. have suggested that at the time of HSC transplantation a concomitant systemic infusion of cultured-expanded haploidentical MSCs should be accompanied with since it is believed that the injection of haploidentical MSCs along with alloegneic HSC transplantation after high–dose chemotherapy would lead to enhanced engraftment.[29]

The usefulness of MSCs in bone marrow transplantation was evidenced by the formation of 'HSC niche' in the bone marrow of murine model with non-obese diabetic-severe combined immunodeficiency (NOD-SCID) after intramedullary trans-plantation of human MSCs.[30] The engrafted MSCs were integrated into the hematopoietic microenvironment of murine bone marrow where they showed the ability to differentiate into functional components (e.g. myo-fibroblasts, pericytes, osteocytes, bone marrow stromal cells and endothelial cells, etc.) that constituted the hematopoietic microenvironment, as well as interactions with other primitive hematopoietic cells. Based on the histological identification of the increasing number of the primitive human hematopoietic cells in murine bone marrow, the ability of human MSCs in enhancing hematopoietic cell engraftment (and the subsequent bone marrow recovery) was demonstrated. This finding has led to the idea of 'molecular crosstalk' between HSCs and the surrounding cellular constituents in the HSC niche.[31]

It is thought that a state of homeostasis is maintained in the HSC niche through the interactions between niche stromal pro-genitor cells and the mobilized HSCs, so that a balance between self-renewal and differentiation of HSCs could be established accordingly. In fact, Wilson and Trumpp had mentioned a complex mechanism to explain the importance of HSC niche.[31] They postulated that the stromal cells in different niche microenvironments provided a sheltering environment for HSCs from being stimulated to undergo differentiation (or apoptosis) where HSCs could remain

'quiescent'. While under certain conditions specialized stromal cells would induce signals for HSCs to differentiate and proliferate, letting mature hematopoietic cells to release into circulation. And no matter how complex the mechanism is, human MSCs have already been put into clinical trials. Le Blanc *et al*. reported using haploidentical human MSCs alongside with allogenic HSC transplantation in patients with graft failure (a condition where the transplanted bone marrow is rejected by the body without normal production of blood cells).[32] Although patients in this treatment were found to have different grades of GVHD, and one was dead because of *Aspergillus* infection, a fast engraftment of neutrophil and platelets without severe side effects was noticed in all patients. Together with a similar result obtained from the experiment conducted by Ball *et al*. targeting children with hematologic malignancies, it is highly likely that the use of autologous MSCs could enhance engraftment of allogenic HSCs and could probably reduce the risk of graft failure.[33]

Role of MSCs in Graft-Versus-Host Disease (GVHD)

Another problem that is commonly associated with allogenic HSC transplantation is the occurrence of graft-versus-host disease (GVHD). GVHD is a condition when the mature post-thymic T cells in the donor's bone marrow recognize the transplanted stem cells as foreign and attack them, leading to possible graft failure. Since GVHD is a life-threatening complication, a lot of effort has been put on testing the therapeutic potential of MSCs in preventing GVHD for long-term HSC engraftment. A promising improvement in patients with treatment-resistant acute GVHD was noticed when *ex vivo* expanded haploidentical MSCs were injected following continuous immunosuppressive treatment.[34] Despite the fact that only 30 out of 55 patients had shown full recovery from acute GVHD (while some showed re-emergence of acute GVHD later after treatment), the *in vivo* immunosuppressive ability of the infused MSCs on effector T-lymphocytes to alloantigens has evidently demonstrated. The immunosuppressive effect of MSCs is thought to inhibit the proliferation of T-lymphocytes probably through a network of cytokine secretion, which in turn reduces the host alloreactivity to donor alloantigens on the surface of HSCs, resulting in less severe GVHD. The beneficial effects of MSCs however still remain uncertain as another report investigating the correlation between co-transplantation of MSCs with MHC-identical allogeneic HSCs and recurrence rate has discovered that for patients with leukemia the chance of having GVHD relapse is higher than control group without co-transplantation.[35]

According to Bleakley and Riddell, unless the T-lymphocytes in donor HSC grafts are removed, there is a chance that the immune system of the recipient recognizes the minor histocompatibility antigens on the donated HSCs even the donor and recipient are MHC-identical.[36] As leukemia cells also

possess minor histocompatibility antigens, the HSC transplantation of allogeneic T-lymphocytes would cause immunological rejection of leukemia cells in the recipients, a phenomenon known as 'graft-versus-leukemia' (GVL) effect. The GVL effect can be seen in patients with transfusion of donor lymphocytes to treat relapsed leukemia after receiving a transplant.[37] The major mediators contributed to GVL are probably T-lymphocytes (both CD4+ and CD8+ cells) where cytokines secreted by those cells interact with malignant blood cell clones.[38] As a result, the GVL effect can be regarded as the beneficial side of GVHD from allogenic HSC transplantation and thus it would be interesting to see if GVL effect from the allogenic HST transplantation can be used as an alternative method of immunotherapy, since no high dosage of immunosuppressive drugs are required for leukemia patients after HSC transplant. All these results indicate the mechanisms underlying immunomodulatory effect of MSCs on the improvement of GVHD are likely to be complex and a better understanding of the relationship between the co-transplanted MSCs and GVL effect in immune system modulation of patients with GVHD is necessary before more clinical trials are put into practice.

Role of MSCs in Autoimmune Diseases

The promising results in co-transplantation of MSCs with alloegneic HSCs in leukemia patients imply the possibility of MSCs to immunomodulate the immune cells responsible for a variety of autoimmune diseases including rheumatoid arthritis, systemic lupus erythematosus, encephalo-myelitis and Crohn's disease. Although the immunosuppressive activities of MSCs to the cells of the innate and adaptive immune systems have been well documented in many studies, the underlying mechanisms of how MSCs can become immunoregulatory following the persistent antigenic stimulation in autoimmune diseases still remain unclear.[14,39]

Taking rheumatoid arthritis (RA) as an example, this autoimmune disease is characterized by joint inflammation that is normally triggered by auto-reactive T-lymphocyte proliferation in conjunction with B-lymphocytes and macrophages. The pathogenesis of RA is the outcome of infiltration of tumor necrosis factor α (TNF-α) secreted by macrophages in the synovial membrane. TNF-α induces a cascade of interleukins which subsequently causes the symptoms of RA. González *et al*. have stated that the use of human adipose-derived MSCs could effectively reduce the severity of T-lymphocyte-mediated auto-reactive response in murine model with RA by inducing anti-inflammatory cytokine IL-10 in joints and lymph nodes.[40] This result is consistent with another experiment where increased levels of serum pro-inflammatory cytokines as well as inflammatory cell infiltration dropped significantly in mice injected with MSCs.[41] The production of anti-inflammatory IL-10 (and other anti-proliferative mediators) means that MSCs not only can reduce the clinical signs of RA

by secreting anti-inflammatory mediators, but also inhibiting the perpetuation of RA by shifting the cytokine profile of T-lymphocytes from type 1 to type 2. The type 2 cytokines favours the humoral immune response which counteracts the actions triggered by type 1 cytokines, including the pro-inflammatory TNF-α responsible for the immunopathogenesis of RA.

Apart from rheumatoid arthritis, the association between autoimmune diseases and MSC-mediated immunoregulation was also observed in autoimmune experimental models induced with encephalomyelitis (i.e. experimental autoimmune encephalomyelitis, EAE). EAE is often used to study human CNS demyelinating diseases such as multiple sclerosis (MS) where systemic injection with MSCs was found to decrease the formation of inflammatory lesions in the brain and spinal cords of murine models, therefore leading to a lesser degree of CNS demyelination. Moreover, the infiltration of T-lymphocytes and macrophage into CNS parenchyma was also reduced in treated mice.[42] With less secretion of inflammatory cytokines from these immune cells, the subsequent *in vivo* production of specific antibodies against myelin components is inhibited, resulting in less severe form of demyelinating diseases. The less number of relapses and degrees of axonal loss in MSC-treated mice compared with the control has further proven the immunomodulatory properties and therapeutic value of MSCs in the treatment of encephalomyelitis.[43]

Similarly, the MSC-based therapy could probably be applied in the future for patients with systemic lupus erythematosus (SLE) since experimental murine models of SLE following allogenic infusion of bone marrow-derived MSCs were shown to have reduced level of serum auto-antibody as well as the reconstruction of osteoblastic niche which plays role in the pathogenesis of SLE.[44] The efficacy of allogeneic MSC transplantation was demonstrated in patients with treatment-refractory SLE. Previous studies have indicated that the inhibitory signals from regulatory T-lymphocytes (CD4+ Foxp3+ regulatory T lymphocytes, Treg) are necessary for maintaining the immune tolerance in humans, thus the restoration of Treg cells by MSC transplantation can absolutely enhance the protection from autoimmune diseases such as SLE.[45] The absence of adverse effects over the clinical trial period in SLE patients has further supported the safety of using allogeneic MSC transplantation. However, such promising results are still not adequate for this MSC-based therapy to become a clinical protocol as some studies reveal no beneficial therapeutic effects (and even worsen the disease) after systemic administration of MSCs in murine models of SLE.[46] This highlights the importance of understanding the immunoregulatory mechanisms of MSCs when bringing appropriate applications of MSC therapy to tackle autoimmune diseases.

Role of MSCs in Cardiology

Myocardial infarction (heart attack) is one of

the leading causes of deaths in the world. The blockage of blood flow to the coronary arteries often results in permanent damage of the cardiac muscles, leading to possible heart failure. Whether injection of human MSCs can replenish the massive loss of cardiac muscles cells (cardiomyocytes) has been intensively studied in recent years. Bone marrow-derived human MSCs is well known for its ability to differentiate into multilineage cells of the mesodermal origin, including cells exhibiting features of cardiomyocytes. Irrespective of some arguments by mentioning that the use of human MSCs as the treatment of myocardial infarction is ineffective or with no therapeutic value,[47,48] both intravenous and intramyocardial injection of bone marrow-derived human MSCs in murine models with infracted myocardium can promote angio-genesis and differentiation of myocytes, thus ameliorating the heart function after myocardial infarction.[49]

The experiment performed by Rogers *et al*. found that the stress-induced neonatal mouse ventricular cardiomyocytes (nMCM) are protected by the bone marrow-derived human MSCs (hMSCs) in transwell cultures at the transcriptional level, where the hMSCs release soluble factors blocking the cardiac receptor-mediated activation of the NF-κB signaling cascade responsible for causing cardiac damage through secretion of cytokines (e.g. TNF-α and IL-8).[50] At the same time hMSCs stimulate the repair by releasing soluble factors (by an unknown mechanism) to restore the intracellular Ca2+ signals that are impeded in damaged myocytes. All findings suggest that hMSCs can directly protect the stressed cardio-myocytes and this provides a new insight of whether the hMSCs-based therapy can be used as a therapeutic tool in cardiac tissue repair following cardiac infarction.

Role of MSCs in Oncology

The relationship between MSCs and cancer has been well described by many literatures. The therapeutic value of MSCs has been subjected to a large number of discussions and whether it is safe to administrate MSCs to patients is largely based on the *in vitro* and *in vivo* clinical trials using animal models and patients with specific illness (e.g. myocardial damage and GVHD). Although so far no concrete evidence to prove any harmful effects of MSCs in MSC-based therapies, an increasing number of studies indicate the oncogenic potential of MSCs for favouring malignant transformation. Different mechanisms have been proposed to account for the interactions between MSCs and tumors and it relies on the fact that MSCs exhibit tumor-directed migration and incorporation independent of tumor type, immunocompetence of patients and delivery routes of MSCs.[51]

How MSCs migrate to and proliferate in the tumor microenvironment remains unclear, while it is postulated that the various kinds of soluble molecules like different cytokines and growth factors produced by tumor cells can act as chemoattractants to MSCs. A non-specific ‘tropism’ of MSCs is believed

to exist in tumor microenvironment as MSCs have shown to express a number of chemokine receptors that are responsive to chemoattractant stimuli.[52] In fact, numerous cytokine/receptor pairs (SDF-1/CXCR4, SCF-c-Kit, HGF/c-Met, VEGH/VEGFR, etc.) and adhesion molecules have been reported to be associated with the migration of MSCs non-specifically[53] to different locations of the body where their directional migration is probably following a concentration gradient of chemoattractants generated from the tumor microenvironment during the development of cancer.[54]

According to Snoussi *et al.*, the homing and trafficking properties of MSCs are believed to be involved in the metastasis of cancer cells from primary to secondary sites as well.[55] From the migration assay using murine mammary cancer cell lines, the bone marrow-derived MSCs were proven to be responsible for the metastasis of the cancer cells from mammary tissues to the bones, a chemokine-dependent migration. The chemokines secreted by the MSCs (e.g. CXCL1 and CXCL5) mediate the migration where tumor cells with the corresponding chemokine receptors will be targeted for metastasis. The chemokine receptor CXCR2 expressed by the several breast cancer cell lines are of particular interest since it has been implicated to the aggressiveness of cancer.[55] However, whether a chemokine gradient can be established by the small population of MSCs in the bone marrow microenvironment for recruiting CXCR2+ circulating tumor cells remains controversial.[56] On the other hand, the immunosuppressive effect of exogenous MSCs is also considered as another major factor contributing malignant transformation of cells. In animal tumor models MSCs are capable of interacting with both T and B lymphocytes of the innate and adaptive immune system where the division and proliferation of activated T lymphocytes are arrested by MSCs in a cytokine-dependent fashion.[53] Interferon-γ (INF-γ) and other several pro-inflammatory cytokines mediate the secretion of inducible nitric oxide synthase (iNOS) as well as other cytokines (including CXCL-9 and CXCL-10) which drive the migration of T lymphocytes into close proximity with MSCs. It is thought that the nitric oxide generated by MSCs can suppress the immunological functions of T lymphocytes which in turn facilitates the escape of tumor cells from immunological surveillance by both the innate and adaptive immune systems.[57]

Indeed, it is only one of the possible mechanisms explaining the relationship between MSCs and tumor metastasis. The above-mentioned cytokines are not the only factors affecting the behaviors of immune cells responsible for malignant transformation. The interactions between MSCs and immune cells are in fact more complicated than expected. It is observed that the expression of cell surface receptors, adhesion molecules, ligands as well as the inhibitions of several immunological processes by the cells of the immune system may be all related to the immunomodulatory

actions of MSCs and are linked to the onset of tumor metastasis. In fact, it is also hypothesized that the oncogenic potential of MSCs may arise from their multipotency. Recently bone-marrow derived MSCs are being proved to be a possible source of carcinoma-associated fibroblasts (CAF) where they differentiate to resemble the CAF-like myofibroblastic phenotype when cultured in tumor-conditioned media (TCM) for a long period of time with similar functional properties of CAF.[58] For instance, sustained expression of stromal-cell derived factor-1 (SDF-1), morphological change of MSCs in response to transforming growth factor beta (TGF-β), expression of myofibroblast markers and *in vitro* and *in vivo* tumor growth-enhancing ability all appear in the bone marrow-derived MSCs exhibiting CAF-like myofibroblastic phenotype.[59,60] Since CAF constitutes the majority of stromal cells within the breast carcinoma by providing structural support, enhancing angiogenesis and constructing microvascular structures during tumori-genesis, it is therefore reasonable to deduce that the transition of MSCs into CAF-like phenotype within the tumor micro-environment is the causative agent for tumor growth and neoplastic progression of various types of epithelial tumors.[60-62]

But despite the experimental evidence demonstrating the tumorigenic potential of MSCs, the lack of relevant information from clinical trial often imposes limitations when considering whether MSCs could be one of the possible causes of cancer.[53] The current trends in the study of MSCs mainly focus on the safety and efficacy of MSCs within the context of non-cancerous (benign) conditions such as myocardial infraction, graft-versus-host disease and bone marrow transplantation. The general public is most concerned about the reliability of MSC treatment for those non-cancerous conditions but not the potential carcinogenic threat to patients' health. The absence of long-term surveillance of patients for carcinogenesis and any other adverse outcomes simply provide inadequate evidence to rule out the safety concerns of MSC-based therapies in humans. The reliance on animal models for the investigation of carcinogenic mechanisms of MSCs further affects the accuracy of evaluation for oncogenic risks due to the basic structural differences between humans and animals in the case of malignant transformation. Because of such limit in clinical trials, many of the current claims of usefulness of MSC-based therapies are still in doubt.

Instead of the negative effects of bone marrow-derived MSCs in cancer metastasis, the homing and trafficking properties of MSCs allows them to be used as possible anti-tumor drug delivery vehicles to control cancer progression.[63] Cancer-specific anti-cancer genes have been engineered into MSCs where anti-cancer effect has been observed in murine models induced with different kinds of cancers including leukemia, hepatoma, glioma etc.[51,64] Since the actions of engineered MSCs are tumor-specific, the effectiveness of cancer treatment by the

engineered MSCs can therefore be guaranteed. Surprisingly, the migration of MSCs is also promoted through irradiation. Local irradiation of NOD/SCID mice following human MSCs infusion showed specific homing of human MSCs to different tissues and organs with good engraftment.[65] The synergistic effect of human MSCs in response to local irradiation distant from the exposed sites suggests that it might be possible to use human MSCs to repair irradiation-damaged tissues in cancer patients after radiotherapy or people subjected to irradiation by accident.

Future Directions of MSCs

Up to date, multipotent MSCs have been intensively studied in many aspects: from tissue engineering, bone marrow trans-plantation, therapy for autoimmune diseases to the treatment of cancers, and so on. Early clinical applications of MSCs mainly focus in the field of hemato-oncology, like allogenic transplantation of HSCs to leukemia patients. More therapeutic potentials of MSCs are revealed later, while there are still many questions left to be answered before MSCs can be put into practical use. For example, safety issue is always a concern when MSCs are administrated to humans in clinical trials. In murine models with subcutaneous xenograft tumor and xenogeneic MSCs, MSCs are able to promote tumor growth by stimulating angiogenesis around the tumor micro-environment.[66] The metastasis of tumor cells because of such pro-tumorigenic effect of MSCs would definitely impede the *in vitro* applications in humans. The possible malignant transformation of MSCs observed in murine models poses another fear to the cell-based clinical therapies of MSCs. However, so far there is no concrete evidence that bone marrow-derived MSCs can cause tumor formation in humans[53,67] and MSCs are generally accepted to be safe when cultured *in vitro* with no increased risk of malignant transformation.[68] It is obvious that more clinical trials and laboratory-based experiments are required in order to clarify the therapeutic efficacy of bone marrow-derived MSCs transplantation and the underlying mechanisms for engraftment, homing ability as well as the *in vitro* differentiation of MSCs. This should be followed by appropriate toxicology testing and long-term follow-up in patients with any MSC-based treatments in order to demonstrate the long-term safety of MSC-based therapies. The widespread use of MSC-based therapy also depends on whether MSCs can be cultured in a large-scale by validated methods; well-designed engineered devices for storage and distribution of MSCs can further enhance the popularity of clinical applications of MSCs in the future. The therapeutic potentials of MSCs are undeniable and more promising results can be expected in the near future with the advances in molecular technology and methodology.

References

1. Kim JY, Jeon HB, Yang YS, *et al*.

Application of human umbilical cord blood-derived mesenchymal stem cells in disease models. World J Stem Cells 2010; 2(2): 34-8.

2. Frank P, Barry J, Mary M. Mesenchymal stem cells: clinical applications and biological characterization. Int J Biochem & Cell Biol 2004; 36: 568-84.
3. Ghannam S, Bouffi C, Djouad F, *et al*. Immunosuppression by mesenchymal stem cells: mechanisms and clinical applications. Stem Cell Res & Therapy 2010; 1(1): 2.
4. Friedenstein AJ, Petrakova KV, Kurolesova AI, Frolova GP. Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. Transplantation 1968; 6: 230-47.
5. Pittenger MF, Mackay AM, Beck SC, *et al*. Multilineage potential of adult human mesenchymal stem cells. Science 1999; 284(5411): 143-7.
6. Horwitz EM, Le Blanc K, Dominici M, *et al*. Classification of the nomenclature for MSCs: The international society for cellular therapy position statement. Cytotherapy 2005; 7(5): 393-5.
7. Dominici M, Le Blanc K, Mueller I, *et al*. Minimal criteria for defining multipotent mesenchymal stromal cells. The international society for cellular therapy position statement. Cytotherapy 2006; 8(4): 315-7.
8. Zuk PA, Zhu M, Ashjian P, *et al*. Human adipose tissue is a source of multipotent stem cells. Am Soc for Cell Biol 2002; 13(2): 4279-95.
9. Prowse KR, Greider CW. Developmental and tissue-specific regulation of mouse telomerase and telomere length. Pro Natl Acad Sci of USA 1995; 92(11): 4818-22.
10. Baddoo M, Hill K, Wilkinson R, *et al*. Characterization of mesenchymal stem cells isolated from murine bone marrow by negative selection. J Cellular Biochem 2003; 89(6): 1235-49.
11. Simmons PJ, Torok-Storb B. Identification of stromal cell precursors in human bone marrow by a novel monoclonal antibody, STRO-1. Am Soc Hematol 1991; 78(1): 55-62.
12. Phinney DG. Isolation of mesenchymal stem cells from murine blood marrow by immunodepletion. Methods in Molecular Biol 2008; 449: 171-86.
13. Shah K. Mesenchymal stem cells engineered for cancer therapy. Adv Drug Delivery Rev 2011; 64(8): 739-48.
14. Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. Nature Rev Immunol 2008; 8: 726-36.
15. Böhring HJ, Battula VL, Treml S, *et al*. Novel markers for the perspective isolation of human MSC. Annals New York Acad Sci 2007; 1106: 262-71.
16. Song L, Tuan RS. Transdifferentiation potential of human mesenchymal stem cells derived from bone marrow. J FASEB 2004; 18:980-2.

17. Reddi AH. Morphogenesis and tissue engineering of bone and cartilage: inductive signals, stem cells, and biomimetic biomaterials. Tissue Engineering 2000; 6(4): 351-9.
18. Nombela-Arrieta C, Ritz J, Silberstein LE. The elusive nature and function of mesenchymal stem cells. Nature Rev Molecular Cell Biol 2011; 12: 126-31.
19. Hurle JM, Gana Y, Marcias D. Experimental analysis of the *in vivo* chondrogenic potential of the interdigital mesenchyme of the chick leg bud subjected to local ectodermal removal. Dev Biol 1989; 132: 368-74.
20. Bosnakovski D, Mizuno M, Kim G, *et al*. Chondrogenic differentiation of bovine marrow mesenchymal stem cells (MSCs) in different hydrogels: Influence of collagen type II extracellular matrix on MSC chondrogenesis. Biotech & Bio-engineering 2005; 93(6): 1152-63.
21. Bosnakovski D, Mizuno M, Kim G, *et al*. Chondrogenic differentiation of bovine bone marrow mesenchymal stem cells in pellet cultural system. Int Soc Exp Hematol 2004; 32(5): 502-9.
22. Terada N, Hamazaki T, Oka M, *et al*. Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. Nature 2002; 416: 542-5.
23. Wurmser AW, Gage FH. Cell fusion causes confusion. Nature 2002; 416: 485-6.
24. Ying QL, Nichols J, Evans EP, Smith AG. Changing potency by spontaneous fusion. Nature 2002; 416: 545-8.
25. Tsonis PA. Stem cells from differentiated cells. Molecular Interventions 2004; 4(2): 81-3.
26. Chen S, Zhang Q, Wu X, *et al*. Dedifferentiation of lineage-committed cells by a small molecule. J Am Chem Soc 2004; 126(2): 410-1.
27. Rice A, Reiffers J, Bernard P, *et al*. Incomplete stroma formation after allogenic marrow or autologous blood stem cell transplantation. Nouv Rev Fr Hematol 1992; 34(2): 167-74.
28. Novitzky N, Mohamed R. Alternations in both the hematopoietic microenvironment and the progenitor cell population follow the recovery from myeloablative therapy and bone marrow transplantation. Experimental Hematol 1995; 23(14): 1661-6.
29. MacMillan ML, Blazar BR, DeFor TE, Wagner JE. Transplantation of ex-vivo culture-expanded parental haploidentical mesenchymal stem cells to promoter engraftment in pediatric recipients of unrelated donor umbilical cord blood: results of a phase I-II clinical trial. Bone Marrow Transplantation 2008; 43: 447-54.
30. Ando K, Muguruma Y, Yahata T. Humanizing bone marrow in immune-deficient mice. Curr Topics Microbiol & Immunol 2008; 324: 77-86.
31. Wilson A, Trumpp A. Bone-marrow haematopoietic-stem-cell niches. Nature Reviews. Immunology 2006; 6(2): 93-106.
32. Le Blanc K, Samuelsson H, Gustafsson B, *et al*. Transplantation of mesen-

chymal stem cells to enhance engraftment of hematopoietic stem cells. Leukemia 2007; 21(8): 1733-8.

33. Ball LM, Bernardo ME, Roelofs H, *et al*. Cotransplantation of ex vivo-expanded mesenchymal stem cells accelerates lymphocyte recovery and may reduce the risk of graft failure in haploidentical hematopoietic stem-cell transplantation. Am Soc Hematol 2007; 110(7): 2764-7.
34. Le Blanc K, Frassoni F, Locatelli F, *et al*. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host-disease: a phase II study. Lancet 2004; 371(9624): 1579-86.
35. Ning H, Yang F, Jiang M, *et al*. The correlation between cotransplantation of mesenchymal stem cells and higher recurrence rate in hematologic malignancy patients: outcome of a pilot clinical study. Leukemia 2008; 22(3): 593-9.
36. Bleakley M, Riddle SR. Molecules and mechanisms of the graft-versus-leukemia effect. Nature Rev Cancer 2004; 4(5): 371-80.
37. Kolb HJ. Graft-versus-leukemia effects of transplantation and donor lymphocytes. J Am Soc Hematol 2008; 112(12): 4371-83.
38. Mavroudis D, Barrett J. The graft-versus-leukemia effect. Curr Opinion Hematol 1996; 3(6): 423-9.
39. Dazzi F, Krampera M. Mesenchymal stem cells and autoimmune diseases. Best Practice & Res Clin Hematol 2011; 24(2011): 49-57.
40. González MA, González-Rey E, Rico L, *et al*. Treatment of experimental arthritis by inducing immune tolerance with human adipose-derived mesenchymal stem cells. Arthritis & Rheumatism 2009; 60(4): 1006-19.
41. Mao F, Xu WR, Qian H, *et al*. Immunosuppressive effects of mesenchymal stem cells in collagen-induced mouse arthritis. Inflamm Res 2010; 59(3): 219-25.
42. Ben-Ami E, Berrih-Aknin S, Miller A. Mesenchymal stem cells as immunomodulatory therapeutic strategy for autoimmune diseases. Autoimmunity Rev 2011; 10: 410-5.
43. Gerdoni E, Gallo B, Casazza S, *et al*. Mesenchymal stem cells effectively modulate pathogenic immune response in experimental autoimmune encephalomyelitis. Ann Neurol 2007; 61(3): 219-27.
44. Sun L, Akiyama K, Zhang H, *et al*. Mesenchymal stem cell transplantation reverses mulitorgan dysfunction in systemic lupus erythematosus mice and humans. Stem Cells 2009; 27(6): 1421-32.
45. Suri-Payer E, Fritzsching B. Regulatory T cells in experimental autoimmune disease. Springer Seminars in Immunopathol 2006; 28(1): 3-16.
46. Youd M, Blickarz C, Woodworth L, *et al*. Allogeneic mesenchymal stem cells do not protect NZBxNZW F1 mice from developing lupus disease. Clin & Experimental Immunol 2010; 161(1):

176-86.

47. Lunde K, Solheim S, Aakhus S, *et al*. Intracoronary Injection of mononuclear bone marrow cells in acute myocardial infarction. New Eng J Med 2006; 355: 1199-209.
48. Janssens S, Dubois C, Bogaert J, *et al*. Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomized controlled trials. Lancet 2006; 367(9505): 113-21.
49. Tang J, Xie Q, Pan G, *et al*. Mesenchymal stem cells participate in angiogenesis and improve heart function in rat model of myocardial ischemia with reperfusion. Euro J Cardio-Thoracic Surg 2006; 30(2): 242-361.
50. Rogers TB, Pati S, Gaa S, *et al*. Mesenchymal stem cells stimulate protective genetic reprogramming of injured cardiac ventricular myocytes. J Molecular & Cellular Cardiology 2011; 50(2): 346-56.
51. Dai LJ, Moniri MR, Zeng ZR, *et al*. Potential implications of mesenchymal stem cells in cancer therapy. Cancer Letters 2011; 305(1): 8-20.
52. Löttichau V, Notohamiprodjo M, Wechselberger A, *et al*. Human adult CD34-progenitor cells functionally express the chemokine receptors CCR1, CCR4, CCR7, CXCR5, and CCR10 but not CXCR4. Stem Cells & Dev 2005; 14(3): 329-36.
53. Momin EN, Vela G, Zaidi HA, Quinones-Hinojosa A. The Oncogenic potential of mesenchymal stem cells in the treatment of cancers: directions for future research. Cur Immunol Rev 2010; 6(2): 137-48.
54. Ramon D, Baugher PJ, Thu YM, Richmond A. Role of chemokines in tumor growth. Cancer Letters 2007; 256(2): 137-65.
55. Snoussi K, Mahfoundh W, Boiapioma N, *et al*. Combined effect of IL-8 and CXCR2 gene polymorphisms on breast cancer susceptibility and aggressiveness. BMC Cancer 2010; 10: 283-94.
56. Shiozawa Y, Haven AM, Pienta KJ, Taichman RS. The bone marrow niche: habitat to hematopoietic and mesen-chymal stem cells, and unwitting host to molecular diseases. Leukemia 2008; 22(5): 941-50.
57. Ren G, Zhang L, Zhao X, *et al*. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. Cell Stem Cell 2008; 2(2): 141-50.
58. Mishra PJ, Mishra PJ, Glod JW, Banerjee D. Mesenchymal stem cells: flip side of the coin. Cancer Res 2009; 69(4): 1255-8.
59. Wang D, Park JS, Chu JS, *et al*. Proteomic profiling of bone marrow mesenchymal stem cells upon transforming growth factor beta1 stimulation. J Biol Chem 2004; 279(42): 43725-34.
60. Mishra PJ, Mishra PJ, Humeniuk R, *et al*. Carcinoma-associated fibroblast-

like differentiation of human mesenchymal stem cells. Cancer Res 2008; 68(11): 4331-9.

61. Orimo A, Gupta PB, Sgroi DC, *et al*. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell 2005; 121(3): 335-48.
62. Spaeth EL, Dembinski JL, Sasser AK, *et al*. Mesenchymal stem cell transition to tumor-associated fibroblasts contributes to fibrovascular network expansion and tumor progression. PLoS ONE 2009; 4(4): e4992. doi:10.1371/journal.pone.0004992
63. Kidd S, Caldwell L, Dietrich M, *et al*. Mesenchymal stromal cells alone or expressing interferon-beta suppress pancreatic tumors *in vivo*, an effect countered by anti-inflammatory treatment. Cytotherapy 2011; 12(5): 615-25.
64. Madeira C, Ribeiro SC, Pinheiro ISM, *et al*. Gene delivery to human bone marrow mesenchymal stem cells by microporation. J Biotech 2011; 151: 130-6.
65. Francois S, Bensidhoum M, Mouiseddine M, *et al*. Local irradiation not only induces homing of human mesenchymal stem cells at exposed sites but promotes their widespread engraftment to multiple organs: a study of their quantitative distribution after irradiation damage. Stem Cells 2006; 24(4): 1020-9.
66. Zhu W, Xu W, Jiang R, *et al*. Mesenchymal stem cells derived from bone marrow favor tumor cell growth *in vivo*. Experimental Molecular & Pathol 2006; 80(3): 264-74.
67. Lepperdinger G, Brunauer R, Jamnig A, *et al*. Controversial issue: is it safe to employ mesenchymal stem cells in cell-based therapies? Experimental Gerontology 2008; 43(11): 1018-23.
68. Bernardo ME, Zaffaroni N, Novara F, *et al*. Human bone marrow-derived mesenchymal stem cells do not undergo transformation after long-term *in vitro* culture and do not exhibit telomere maintenance mechanisms. Cancer Res 2007; 67(19): 9142-9.