Applications of Mesenchymal Stem Cells: An Updated Review

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Mesenchymal stem cells (MSCs) can be readily isolated from a number of adult and fetal tissues, and have the capacity of expansion in vitro on a clinical scale. Bone marrow MSCs are able to differentiate into multiple cell lineages that resemble osteoblasts, chondrocytes, myoblasts, adipocytes, endothelial cells, neuron-like cells, cardiomyocytes and hepatocytes. Preclinical findings from animal experiments are promising and have shown that human multipotent MSCs may have considerable therapeutic potential in a wide variety of human diseases. Research into the role that MSCs play in the induction of tolerance in bone marrow and organ transplantation holds great for future therapeutic strategies. Clinical trials are underway to assess the safety, feasibility and efficacy of MSC transplantation in a variety of human diseases. Clinicians need to know the recent progress and rationale for performing these clinical studies. As such, this review focuses on the background of MSCs and medical research in this area, bridging bench and bedside applications. Conflicting preclinical results and published data from our laboratory are discussed. *(Chang Gung Med J 2008;31:228-36)*

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Mesenchymal stem cells (MSCs) are defined as adherent cells which possess a proliferative potential and an ability to differentiate *in vitro* into chondrogenic, osteogenic, adipogenic and myogenic lineages. Recently, under proper conditions, MSCs have been demonstrated capable of differentiating into hepatocyte-like[1] and neuron-like[2] cells. Apart from bone marrow, MSCs can be isolated from adipose tissue,[3,4] umbilical cord blood[5] and various fetal tissues such as the placenta,[6] amniotic fluid and amniotic membrane.[7] Many studies have shown that Wharton's jelly in the human umbilical cord is also a rich source of primitive MSCs.[8-10] Regardless of their sources, undifferentiated MSCs are adherent cells with a fibroblast-like morphology and are capable of self-replication through many passages. Therefore, they can potentially be expanded to sufficient numbers for tissue and organ regeneration.

Isolation and culture of MSCs

The protocol for MSC isolation and expansion has not yet been standardized. Many laboratories used their own protocols for studying MSCs and thus the observations in one laboratory may not be seen in others. Recent studies have shown various cell isolation protocols have a major impact on the functional

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activity of bone marrow-derived progenitor cells and can affect the results of clinical trials.[11] In our laboratory, MSCs were isolated from bone marrow aspirates by negative immuno-depletion of CD3, CD14, CD19, CD38, CD66b, and glycophorin-A positive cells, followed by Ficoll-Paque density gradient centrifugation, and were then plated in plastic culture flasks.[1,12] MSCs were allowed to adhere overnight and non-adherent cells were washed out with medium changes. The colony-forming units of MSCs were grown in medium consisting of Iscove's modified Dulbecco's medium and 10% fetal bovine serum supplemented with 10 ng/ml epidermal growth factor (EGF), 10 ng/ml fibroblast growth factor-2 (FGF2), 100 U penicillin, 1000 U streptomycin, and 2 mM L-glutamine.[1,12] In our experience, this protocol works consistently for MSC isolation and long-tem culture expansion, even when using bone marrow, of people up to 80 years old.

Characterization of MSCs

Although MSCs have been studied for decades, a true MSC marker has not yet been identified. The cells are characterized by the expression of numerous surface antigens. Unfortunately, none of them appears to be exclusively expressed on MSCs which makes the definition of MSCs difficult. To better define human MSCs, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy has reached a consensus on minimal criteria.[13] First, MSCs must be plastic-adherent when maintained in standard culture conditions. Second, MSCs must express markers CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79alpha or CD19 and HLA-DR surface molecules. Third, MSCs must at least be able to differentiate into lineages of osteoblasts, adipocytes and chondroblasts in vitro. Many studies continue to search for novel markers to isolate highly purified MSCs. Recently, CD271 was reported as the most specific marker for bone marrow -derived MSCs.[14]

Overview of clinical applications

In the past few years, both *in vivo* and *in vitro* reports have shown a greater plasticity in MSCs than previously thought. The source of MSCs carries fewer ethical concerns than that of embryonic stem cells and therefore, attention has been drawn to MSCs because of their potential use in cell therapy and regenerative medicine. In this review, we will present their progress in different clinical entities.

MSCs in hepatology

At present, liver transplantation is hampered by the limited availability of suitable donor organs. Hence, novel cell sources are required for clinical therapy. We were the first to demonstrate that MSCs isolated from human bone marrow and umbilical cord blood can be induced into hepatic differentiation.[1] These cells have a cuboidal morphology, which is characteristic of hepatocytes, and functions characteristic of liver cells, including albumin production, glycogen storage, urea secretion, uptake of low-density lipoprotein, and phenobarbital-inducible cytochrome P450 activity. Adipose-derived MSCs, like bone marrow, were later shown to have a hepatogenic differentiation potential.[15] In vivo, human mesenchymal stem cells xenografted directly to allylalcohol-treated rat liver[16] as well as immunodeficient Pfp/Rag2 mice,[17] can be differentiated into human hepatocytes without cell fusion. Therefore, human MSCs from different sources are able to differentiate into functional hepatocyte-like cells and may serve as an alternative for hepatocyte transplantation, cell-based therapy for liver injury and preclinical drug testing. In the rat model of CCl_4 induced liver fibrosis, MSCs showed a potential therapeutic effect against the fibrotic process through their effect in inhibiting collagen deposition in addition to their capacity to differentiate into hepatocytes.[18,19] This animal study which suggested bone marrow stem cell transplantation could lead to regression of liver fibrosis has evoked great interest in the treatment of decompensated liver cirrhosis. A phase I study of bone marrow MSC transplantation in 4 patients with cirrhosis was completed and the procedure was safe, and feasible, with somewhat promising results (Mohamadnejad M, et al. unpublished). Therefore, a multicenter, randomized placebo controlled trial recruiting more patients with decompensated cirrhosis (Child-Pugh class B and C) is underway at the University of Tehran, Iran. In the treatment arm autologous bone marrow from the patients was aspirated, cultured and infused through the peripheral veins. Another phase I/II clinical trial is currently enrolling patients with end-stage liver disease for salvage treatment. In this study MSCs

will first be differentiated *in vitro* into progenitors of hepatocytes and then autografted into the portal vein under ultrasound guidance to determine the effects of injected cells in the reestablishment of liver function.

MSCs in tolerance against allograft rejection and graft-versus-host disease (GVHD)

MSCs have been shown to have profound immunomodulatory effects both in vitro and in vivo. The mechanisms that govern these functions remain elusive. Some studies have indicated that soluble factors such as prostaglandin E2 and transforming growth factor beta (TGFβ) play an important role, while others support a role for cell-cell contact.[20] Bone marrow-derived MSCs from healthy donors and patients with auto-immune disease have anti-proliferation of autologous- and allogeneic-stimulated T-lymphocytes in mixed-lymphocyte reactions.[21,22] Therefore, MSCs seem to have implications for treatment of allograft rejection, graft-versus-host disease (GVHD) and autoimmune inflammatory diseases in which immunomodulation is required.[23] The role of MSCs in these issues remains to be clarified.

Research into the role that MSCs can play in the induction of tolerance of bone marrow and organ transplantation should have significant implications for therapeutic strategies in the future. Unfortunately, current data are conflicting. For example, two studies showed infusion of allogeneic MSCs facilitated the induction of islet allograft tolerance in streptozotocin-diabetic rats[24] but failed to induce tolerance against rejection of allogeneic skin grafts in C57BL/6 (B6) mice. A group in Germany showed MSC injection did not prolong cardiac allograft survival in rat heart transplant models but tended to accelerate allograft rejection.[25] In contrast another report showed MSCs suppressed allogeneic T-cell responses both in vitro and in vivo and prolonged the survival of transplanted hearts.[26] In a pilot study, co-transplantation of MSCs enhanced engraftment of allogeneic hematopoietic stem cells in humans.[27]

The immunosuppressive properties of MSCs make them particularly attractive in GVHD. However, controversial results were also seen in pre-clinical models. MSCs were shown effective at preventing but not treating GVHD in sublethally irradiatiated mice with non-obese diabetic/severe combined immunodeficiency (NOD/SCID) which had been transplanted with human peripheral blood mononuclear cells.[28] However they failed to prevent GVHD in two other murine models.[29,30] In a small clinical trial, MSCs seemed promising in treating steroid-refractory grade III-IV acute GVHD.[31] A phase II clinical trial using cotransplantation of human leukocyte antigen (HLA)-identical sibling culture-expanded MSCs with HLA-identical sibling hematopoietic stem cells in patients with hematologic malignancy was performed at multiple centers. Patients were given intravenously culture-expanded MSCs (1.0-5.0 x 10^6/kg) 4 hours before infusion of either bone marrow or peripheral blood stem cells on day 0. There were no infusion-related adverse events. However, grade II to IV acute GVHD was still observed in 13 (28%) of 46 patients and chronic GVHD was observed in 22 (61%) of 36 patients who survived at least 90 days.[32] More phase II trials with MSCs in the treatment of GVHD are currently underway.[33] A multi-center Phase I/II trial was started in January 2007 in Spain to study a single dose of allogenic MSCs (1-2 x 10^6/kg) in patients with GVHD refractory to first-line or subsequent treatment. In this trial, MSC suspension will be obtained from the bone marrow of a family member and expanded *in vitro* in a specific culture medium with autologous donor serum and with no animal-derived products.

MSCs in cardiology

MSCs produce a variety of cardio-protective signaling molecules, and under *in vitro* conditions, MSCs differentiate into cells exhibiting features of cardiomyocytes. MSCs have been injected directly into infarcts, or administered intravenously after which they migrated to the site of heart injury. Animal studies support the concept that therapeutically delivered MSCs can safely improve heart function after an acute myocardial infarction. Intravenous delivery of MSCs improved myocardial perfusion in a pig model of myocardial infarction;[34] however, the underlying mechanisms are poorly understood. In fact, the beneficial effects of MSC therapy may involve multiple mechanisms. In rat model of myocardial ischemia with reperfusion, implanted MSCs improved cardiac structure and function through the combined effects of myogenesis and angiogenesis.[35] Transplantation of VEGF gene-transfected MSCs brought better improvement in myocardial perfusion and in restoration of heart

function after myocardial infarction than cellular or gene therapy alone.[36] MSC transplantation has an anti-inflammatory role by decreasing gene expression of the inflammation cytokines tumor necrosis factor (TNF)-alpha, IL-1β and IL-6.[37] It also inhibited deposition of type I and III collagen, as well as gene and protein expression of matrix metalloproteinase-1 and tissue inhibitor of metalloproteinase-1,[38] consequently interrupting the progress of adverse left ventricle remodeling in heart failure following acute myocardial infarction. MSCs overexpressing Akt dramatically repaired infarcted myocardium and improve cardiac function despite infrequent cellular fusion or differentiation.[39] These new observations further confirm that paracrine mechanisms mediated by MSC are responsible for enhancing the survival of existing myocytes and that Akt can alter the secretion of various cytokines and growth factors. Based on these preclinical data showing that MSCs from the bone marrow can be stimulated to differentiate into endothelial cells that participate in the development of new blood vessels and cardiomyocytes in ischemic tissue, a phase I/II safety and efficacy study is ongoing in Denmark to evaluate the clinical effect of autologous MSC cell therapy in patients with severe chronic myocardial ischemia. In this study, patients with reversible ischemia on a single photon emission computerized tomography (SPECT) image will be treated with direct intramyocardial injections of autologous isolated and expanded MSCs. A prospective double blind trial of intraoperative transmyocardial bone marrow derived mesenchymal cell transplantation versus placebo in patients with a low left ventricular ejection fraction who are scheduled for coronary bypass surgery is also underway at Helsinki University, Finland. These two trials will investigate the role of bone marrow MSC transplantation in heart failure and coronary artery disease treatments.

MSCs in radiotherapy

The ability of MSCs to help the regeneration of the abdominal wall after irradiation-induced small intestine injury was established by transplanting human MSCs into immune-tolerant NOD/SCID mice.[40] Although the use of MSC therapy to repair damaged gastrointestinal tracts in patients who undergo pelvic or abdominal radiotherapy is promising, the biologic responses of bone marrow MSCs to ionizing radiation have rarely been described in the literature. The clinical observation that MSCs obtained from bone marrow transplantation recipients were found to originate from the host suggested that MSCs in their niches could be resistant to irradiation. To delineate the response and intracellular mechanisms of MSCs to ionic radiation, we were the first to demonstrate that MSCs possess a favorable antioxidant reactive oxygen species-scavenging capacity with normal ataxia-telangiectasia mutated (ATM) protein phosphorylation, activation of cell-cycle checkpoints and double-strand break repair to facilitate their radioresistance. These findings provide a much better understanding of radiation-induced biologic responses in MSCs and may lead to the development of better strategies for MSC treatment in cancer therapy.

MSCs in oncology and cell therapy

The relationship of MSCs with cancer has seldom been addressed in the literature. Recently, MSCs, which form the microenvironment where leukemic cells grow, were found to express asparagine synthetase 20 times higher than levels in acute lymphoblastic leukemia (ALL) cells, and thus protected ALL cells from asparaginase cytotoxicity.[41] MSCs can behave as potent antigen-presenting cells to amplify immune responses against tumor-specific antigens, which could theoretically be exploited as a new therapeutic tool in cancer therapy. Clinical use of cultured human MSCs has been launched for cancer patients. Genetically-modified MSCs such as IL2-producing MSCs have been tested as an anticancer agent in preclinical studies. MSCs have tropism to gliomas *in vitro* and *in vivo* because gliomas secrete MSC-attracting factors such as interleukin-8, transforming growth factor-ss1 and neurotrophin-3.[42] MSCs can be transduced efficiently by adeno-associated virus, an ideal vector for human gene therapy primarily due to its lack of pathogenicity and low risk of insertional mutagenesis, and these transduced MSCs retain multipotential activity.[43] As these findings indicate, MSCs are promising as vehicles for gene transfer and anti-cancer therapy.

MSCs in neurology

Transplantation of bone marrow MSCs in rodent models has been reported to ameliorate functional deficits in several central nervous diseases and spinal

cord injury.[44-47] MSCs can be induced to form functional neuronal cells, which are transplanted to animal models of neurodegenerative disorders, including Parkinson's disease and ischemic brain injury, resulting in the successful integration of transplanted cells and improvement in function in the transplanted animals.[48] These observations have raised interest in the potential use of MSCs in cell therapy strategies for neurodegenerative diseases and traumatic injuries.[49] Twenty patients with complete spinal cord injury (SCI) received unmanipulated autologous bone marrow transplants 10 to 467 days post-injury.[50] Intra-arterial versus intravenous administration of all mononuclear cells were infused in groups of acute (10-30 days post-SCI, n = 7) and chronic patients (2-17 months postinjury, n = 13). Improvement in motor and/or sensory functions was observed within 3 months in 5 of 6 patients with intra-arterial application, in 5 of 7 acute patients, and in 1 of 13 chronic patients. Thus transplantation within 3-4 weeks following injury seems to play an important role in stem cell therapy. Although the observed beneficial effects cannot be confirmed to be due wholly to the cell therapy, the implantation of autologous bone marrow cells appears to be safe (11 patients followed up for more than 2 years). Stem cells also offer great promise as a therapy for Parkinson's disease, but data on which type of stem cells are best is inconclusive. Neural stem/precursor cells, which are obtained from the midbrain, can give rise to tyrosine-hydroxylase (TH)-positive neurons. However, the growth of the cells is slow and the differentiation rate of dopaminergic (DA) neurons is still too low for clinical application. Embryonic stem cells (ESCs) are also candidates for potential donor cells in transplantation. Monkey ESCs give rise to midbrain DA neurons,[51] and the transplanted ESC-derived neurospheres function as DA neurons, attenuating the neurological symptoms in a monkey Parkinson's disease model. These results suggest the possibility of using ESCs for Parkinsonism, but the problems of low survival rate in vivo and tumor formation remain to be solved.[52] In a 6-hydroxydopamine (6-OHDA) rat model of Parkinson's disease, transplanted MSCs were engrafted better in the 6-OHDA-induced lesioned hemisphere than in the unlesioned side. They migrated through the corpus callosum to populate the striatum, thalamic nuclei and substantia nigra area.[53] However, as Parkinson's disease involves degeneration of both dopaminergic and non-dopaminergic neurons, many problems remain to be solved before clinical application of MSCs. A phase I/IIA trial of bone marrow-derived autologous adult human MSCs will open to patients with multiple sclerosis at the University of Cambridge, UK. In this trial, a single dose of 2 x 10^6/kg MSCs will be infused intravenously.

MSCs in orthopedics

Hyaline articular cartilage has very limited repair and regeneration capacities. Cartilage tissue engineering has been attempted by combining cells, scaffold and environmental factors, including growth factors, signaling molecules, and mechanic stimuli. Various cell types have been used in cell-based approaches for cartilage lesion repair, including autologous chondrocytes, perichondrial or periosteal cells, and mesenchymal progenitor cells from bone marrow and other sources. To date, only autologous chondrocytes are used in clinical practice. Recently, MSCs have provided an attractive alternative to chondrocytes because unlike mature chondrocytes, which must be surgically harvested from a very limited supply of non-weight-bearing articular cartilage, MSCs can be easily obtained and expanded and will maintain their multilineage potential with passage.

MSCs are multipotent cells that are able to differentiate into chondrogenic and osteogenic precursors *in vitro* and *in vivo*. Chondrogenic differentiation was achieved using micromass culture in which insulin-like growth factor (IGF-I) and TGF-β1 played critical roles.[54] Phenotypic maintenance of articular chondrocytes in vitro requires bone morphogenetic protein (BMP) activity.[55] MSC-based tissue engineering is a promising technology for the development of a transplantable cartilage replacement to improve joint function. The major step for MSCs in articular cartilage repair is how to promote their differentiation toward chondrogenesis and maintenance of an articular cartilage phenotype without ossification or fibrinogenesis. Although repair of full-thickness articular cartilage defects using autologous bone marrow MSCs has been reported in case reports with some levels of success,[56,57] it is far from clinical practice. Many experimental approaches are underway to enhance the clinical outcome of this type of procedure. For example, studies are presently researching how to promote chondrogenic differenti-

ation and maintenance of the chondrocyte phenotype by adding growth factors such as the TGF-β superfamily, including TGF-β 1, 2, 3, and several BMPs, IGF-1, fibroblast growth factors (FGFs) and epidermal growth factor (EGF), or by gene transfer approaches *in vivo* including IGF-1, BMP-2, BMP-7, FGF-2, and SOX9. In addition, issues such as the use of BMP inhibitors (noggin, chordin) to prevent osteogenesis and protection cells transplanted for cartilage repair are being investigated to enhance the utility of MSCs in orthopedic medicine. MSC-based tissue engineering has also been applied in animal and human studies in osteochondral defects,[58,59] large bone defects,[60] and ligament repair[61] with promising results.

Conclusion

To date, multipotent mesenchymal stem cells are considered the cell type of choice for tissue engineering because of their multilineage differentiation capabilities and because of the ease with which they can be isolated and expanded from a small aspirate of bone marrow or unwanted Wharton's jelly in the umbilical cord. Preclinical findings from animal experiments are promising and have shown that human MSCs have considerable therapeutic potential in a wide variety of human diseases. However, some *in vivo* data are conflicting and more clinical trials are required to clarify the precise therapeutic effects of MSCs.

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間質幹細胞臨床應用的新進展

李冠德

成人與胎兒的許多器官組織都可分離出間質幹細胞 (mesenchymal stem cells)，在體外並能大量培養至臨床治療所需的數量。骨髓中的間質幹細胞在適當條件下可以分化成不同功能細胞，包括骨頭、軟骨、脂肪、血管上皮、神經、心肌與肝臟細胞。許多動物實驗顯示間質幹細胞很可能可應用於許多不同人類疾病的治療。目前已有一些人體試驗開始研究間質幹細胞治療的安全性及可行性。這些進步也使得一般的臨床醫師，很想瞭解幹細胞最新研究進展與這些人體試驗的背景和科學依據究竟爲何。針對這個需要，本文對間質幹細胞在實驗室的研究成果如何轉譯成臨床應用做一整理介紹，內容亦包含我們實驗室已發表之有關間質幹細胞的成果。(長庚醫誌 2008;31:228-36)

關鍵詞：間質幹細胞，骨髓

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