Review Article

Mesenchymal Stem Cells

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Stem cells are special type of cells, which can be found almost in each type of tissue and through entire life span in multicellular organism. Their main functions are to provide tissue development, homeostasis and reparation of tissue damage. Stem cells are defined as cells that have the capacity to self renewal, multipotency/pluriopotency, clonality, and are divided into embryonic stem cells and adult stem cells.

Mesenchymal Stem Cells (MSCs)

Mesenchymal Stem Cells (MSCs) are a group of adult stem cells naturally found in the body. Adult stem cells are undifferentiated cells found in numerous tissues throughout the body that divide to replenish dying cells and regenerate damaged tissues. To date, other than bone marrow stem cells, MSCs have been identified in a variety of tissues[1-3], such as adipose tissue, peripheral blood, spleen, brain, synovial fluid, dermis, muscle, dental pulp, umbilical cord, placenta, skin, liver, pancreas and intestines that differentiate along several mesenchymal lineages. On the other hand, there are significant differences in the proliferation and differentiation abilities, and in harvesting procedures among these MSCs.

In 2007, The International Society for Cellular Therapy (ISCT) have agreed that a MSC should adhere to plastic in standard culture conditions; express ($\geq$95%+) CD105, CD73, CD90 and not express ($\leq$2%+) CD45, CD34, CD14 or CD11b, CD79a or CD19, HLA-DR and should give at least three differentiated lineages: osteoblastic, adipogenic, chondroblastic (needs to be demonstrated by staining of in vitro differentiated cell cultures)[4]. However, isolation of stem cells remains the major obstacle because of the lack of universally accepted markers. There are still controversies in obtaining reproducible results by the published methods, especially when the differentiation protocols are concerned[5-9]. Meanwhile, different isolation methods cause striking impacts on the differentiation potential of adult stem cells[10-11]. There is a limited number studies comparing the differentiation capacity of stem cells obtained from various sources

using the same differentiation protocols[12-17]. Since there is no consistency between the established protocols of different labs, it is also quite challenging to interpret the previously reported data.

MSCs have generated considerable biomedical interest since their multi-lineage potential was first identified in 1999[18]. MSCs can differentiate to several cell types and produce important growth factors and cytokines[19-20]. MSCs have ability to modify the response of immune cells thereby associating with immune-related disorders, especially autoimmune diseases[21-22]. Despite the wide distribution of MSCs in the body, the bone marrow remains the principal source for the most MSC-based pre-clinical and clinical studies where MSCs have mainly been characterized after isolation[19]. Actually, MSCs are a rare population in bone marrow aspirates. The frequency of MSCs is approximately $1/10^6$ nucleated cells in adult bone marrow and $1/10^4$ nucleated cells in umbilical cord [23]. The number of MSCs has been noted to decrease with age[24]. Later on, more primitive MSCs were discovered. Those immunomagnetically separated cells were named mesodermal progemitor cells (MPCs)[25] or multipotent adult progenitor cells (MAPCs)[26].

Expansion of Mesenchymal Stem Cells

Expansion of MSCs is a necessity for clinical use. MSCs are rare in the human body but can be expanded *in vitro* to hundreds of millions of cells, isolated from the other cells by adherence to plastic and consecutive passaging. MSCs proliferate to spindle-shaped cells in confluent cultures. Although homogeneous by light microscopy, even single cell– derived colonies form a molecularly heterogeneous population of cells that vary to some extent in their differentiative capacity. Even if MSCs rapidly expand 1 billion-fold, individual cells in a culture exhibit a highly variable expansion potential. Furthermore, the cell yield after expansion varies with the age and condition of the donor and with the harvesting techniques. Naturally, differences in isolation techniques, culture conditions, media additives, and sub-culturing techniques greatly affect cell yield and possibly also the phenotype of the expanded cell product. The gene expression/ proteomics of MSCs that have been culture expanded depend on the culture conditions, passage, species, and other factors or may or may not reflect *in vivo* events. Moderate subcultivation will not change the karyotype or telomerase activity of MSCs, but if the cells are cultured many passages, signs of senescence and apoptosis appear[27].

Mesenchymal Stem Cells from Bone Marrow

MSCs were fist identified in the stromal compartment of bone marrow by Friedenstein and colleagues in 1960s[28-31]. MSC are conventionally extracted from bone marrow sources as a cellular therapy

for inflammatory associated conditions. Specifically, the most advanced clinical trials in the area of regenerative medicine have been performed by the company Osiris, whose main product is a "universal donor" MSC, termed "Prochymal". This cellular product has entered Phase III trials in graft versus host disease, and is currently being tested for heart failure [32]. Other bone marrow derived MSC-like products are in clinical trials, for example, Mesoblast is in Phase III assessing its Mesenchymal Precursor Cell for efficacy in post hematopoietic transplant graft failure, as well as in Phase II for heart failure [33]. Therapeutic advantages of MSC include their ability to migrate to injured tissue, in part via detections of hypoxia through the CXCR4-SDF-1 axis[34-35] differentiation activity into multiple tissues[36-37] release of trophic factors[38] inhibition of apoptosis[39-41] stimulation of angiogenesis [42], inhibition of inflammation[43], and stimulation of Treg activity[44]. Despite the advantages of the current approaches, bone marrow contains relatively small numbers of MSC, thus, as previously mentioned, therapeutics with bone marrow for systemic applications requires ex vivo expansion.

Mesenchymal Stem Cells from Adipose Tissue

Adipose tissue contains approximately 100-1000 fold higher MSC concentration, or approximately 50-100,000 MSC per ml[45]. Given the relative ease of extracting 500 ml of lipoaspirate, it is conceptually feasible to generate a 25-50 million cell dose of MSC, which is close to the systemic doses of MSC that are typically used in clinical trials of allogeneic expanded cells (eg. 50-100 million cells in various clinical trials)[46]. Conceptually, given that the MSC present in the stromal vascular fraction (SVF) are autologous, one could envision higher therapeutic potential due to the lack of allo-immune clearance as compared to allogeneic MSC, although this needs to be assessed experimentally.

Adipose MSC contain several similarities and differences as compared to bone marrow derived MSC, although this area is still considered to be controversial. Specifically, in animal cardiac infarct models it has been demonstrated that expanded adipose MSC are superior to bone marrow MSC in terms of stimulating angiogenesis, decreasing cardiac pathology, and stimulating VEGF and FGF secretion[47]. Using an in vivo lentiviral-labeled system, it was demonstrated that adipose derived MSC (ASC) have a superior ability to BM derived MSC (BDSC) to integrate into cardiac muscle after injury, as well as to restore function[48]. In addition to specific propensities for differentiation, adipose tissue-derived MSC appear to be superior to bone marrow in terms of proliferative potential without loss of telomere length. Vidal et al. demonstrated that adipose MSC could multiply for almost twice as many cell passages without undergoing senescence as compared to bone marrow MSC[49].

A much simpler procedure, for which adipose tissue is uniquely suited, is the administration of autologous, non-expanded cellular fraction. The rationale behind this derives from observations that: a) adipose tissue contains substantially higher numbers of MSC compared to bone marrow[50] b) MSC from adipose tissue do not appear to decrease in number as a result of age[51-52]. It has also been reported that the expression level of 5 chemokine receptors (CCR1, CXCR4, CCR7, CXCR6, and CXCR3) is higher in ASC than BDSC, which indicated ASC might show a better migration and homing capacity following transplantation[53]. These distinct characteristics will determine the strategy for cell-based therapy.

Thus it appears that the MSC component of adipose tissue possesses numerous preclinical and clinical therapeutic properties and may be an important component of the SVF cell population that is responsible for therapeutic effects observed after administration. Patients received the indicated amount of cells by intravenous injection ($2x10^6$ cells per ml diluted in Saline solution), intra-articular injection ($2.5x10^6$ cells per ml in each injured joint, diluted in Saline solution and the patient's own serum). Multiple injections of cells were given to increase the therapeutic efficacy. Followups were performed for all patients at 1, 3, 6 and 12 months. SVF cells were isolated and prepared under the guidelines of Good Tissue Practices 21 CFR 1271 as relates to sample screening and processing in the sterile flow hood, inside of a class 10000 clean room[54]. Thirteen patients with rheumatoid arthritis were treated with 38-148 million SVF cells intravenously and intra-articularly. Although no hematopoietic or biological abnormalities were noted, one of the patients reported facial flushing, fever and myalgia after a third of four injections. These symptoms all resolved spontaneously.

Mesenchymal Stem Cells from Dental Pulp (DPSC)

Dental pulp (DP) is well defined compartment of soft tissue, which keeps primitive structure similar to gelatinous tissue of umbilical cord. Dental pulp represents well delimited and from other tissues separated compartment, which retains unique histological structure and stem cell niche. Since there are two sources for dental pulp development (dental mesenchyme of neural crest origin and vascular mesenchyme) there are two different lines of DPSCs inside the DP. DPSCs can be isolated from two DP compartments. Jakub Suchánek and co-workers[55] named these compartments according to their localization within the DP – subodontoblastic compartment (inner surface of tooth and outer part of DP;SOc) and perivascular compartment (the inner part of DP;PVc). DPSCs isolated from PVc were spindle-shaped with long processes. Conversely, DPSCs from SOc were more rounded.

In a year 2000, Gronthos and co-workers isolated stem cells from the human dental pulp (DPSCs)[56]. The pulp tissue was extracted from impacted third molars. In the year 2003, Miura *et al*[57] have isolated stem cells from human exfoliated deciduous teeth (SHED). DPSCs can be cultivated for long time, over 60 population doublings in cultivation media designed for bone marrow MPCs[55]. After reaching Hayflick's limit, they still have normal karyotype. Initial doubling time of the cultures was from 12 to 50 hours for first 40 population doublings, after reaching 50 population doublings, doubling time had increased to 60–90 hours. Regression analysis of uncumulated population doublings proved tight dependence of population doublings on passage number and slow decrease of proliferation potential. In comparison with bone marrow MPCs, DPSCs share similar biological characteristics and stem cell properties. The results of our experiments proved that the DPSCs and MPCs are highly proliferative, clonogenic cells that can be expanded beyond Hayflick's limit and remain cytogenetically stable. Moreover two different populations of DPSCs can be isolated. These DPSCs lines differed one from another in morphology. Because of their high proliferative and differentiation potential, DPSCs can become more attractive, easily accessible source of adult stem cells for therapeutic purposes

Cultivated DPSCs and SHED were higly proliferative and cytogenetically stable stem cells. Morphological differences of cells isolated from both defined compartments were not related to changes in proliferation potential. Over the entire cultivation period, Jakub Suchánek and co-workers[55] did not observe any changes in cell viability and cells remained undifferentiated. Not only for mentioned reasons, dental pulp represents an alternative and easily accessible source for obtaining tissue-specific stem cells which are histocompatible with tissues of the individual patient. In comparison with bone marrow MPCs, DPSCs share similar biological characteristics and stem cell properties. DNA analysis proved that DPSCs have more cells in S-G2 phase than bone marrow MPCs[55]. Higher proliferation activity of DPSCs was confirmed by DT trend analysis. In addition,any signs of spontaneous differentiation was not observed during DPSCs long term cultivation.

Stem cells from human exfoliated deciduous teeth show higher proliferation rates and increased population doubling time than stem cells from human permanent teeth pulp[9,57]. Apart from deciduous teeth, the umbilical cord is another postnatal organ discarded after birth and the collection of cells does not require an invasive procedure with ethical concerns. Stromal cells, as the dominant cells of this fetus-derived tissue, possess multipotent properties between embryonic stem cells and adult stem cells. They bear a relatively higher proliferation rate and self-renewal capacity[58]. The suitable cells should be chosen

for specific tissue engineering trials The most reliable cell source for dental tissue engineering is that of autologous pulp stem/progenitor cells isolated from deciduous teeth, which have been exfoliated naturally.

The Potential Clinical Use of Mesenchymal Stem Cells

A significant improvement in understanding MSC biology in recent years has paved the way to their potential clinical use. A new era has begun in the treatment of diseases with the discovery that stem cells from diverse organs and tissues. Increasing evidence suggests that one mechanism of action by which cells provide tissue protection and repair may involve paracrine factors, including cytokines and growth factors, released from transplanted stem cells into the surrounding tissue[59]. There is increasing evidence that stem cells themselves, especifically MSCs, secrete a variety of pro-inflammatory and anti-inflammatory cytokines. MSCs represent an advantageous cell type for allogeneic transplantation as well because MSCs are immune-privileged with low major histocompatibility complex I (MHC I) and no MHC II expression, therefore possessing a reduced risk of allogeneic transplant rejection[19].

Different tissue-originated MSCs may have variance in their differentiation capacity even if cultured in the exact same microenvironment. While investigators report studies of MSCs using different methods to isolate the cells and using different approaches to characterize the cells, the considerable therapeutic potential of human MSCs has generated markedly increasing interest in a wide variety of biomedical disciplines. Thus it is increasingly difficult to compare study outcomes, which hinders progress in the field. Obviously, it is critical to have an acknowledged standard to evaluate the characteristics of MSCs.

Cardiovascular therapeutic potential

The cardiovascular therapeutic potential of bone marrow mesenchymal stromal/stem cells (MSC) is largely mediated by paracrine effects. Traditional preparation of MSC has involved plastic adherence-isolation. In contrast, prospective immunoselection aims to improve cell isolation by enriching for mesenchymal precursor cells (MPC) at higher purity. This study compared the biological characteristics and cardiovascular trophic activity of plastic adherence-isolated MSC (PA-MSC) and MPC prepared from the same human donors by immunoselection for stromal precursor antigen-1 (STRO-1). Compared to PA-MSC, STRO-1-MPC displayed greater (1) clonogenicity, proliferative capacity, multilineage differentiation potential, and mRNA expression of mesenchymal stem cell-related transcripts. In vitro assays demonstrated that conditioned medium from STRO-1-MPC had greater paracrine activity than PA-MSC, with respect to cardiac cell proliferation and migration and endothelial cell migration and tube formation.

Enrichment for STRO-1 is also accompanied by increased expression of cardiovascular-relevant cytokines and enhanced trophic activity[60]. Over the last decade, cellular therapy has emerged as a potential adjunct in the management of ischemic heart disease and congestive heart failure[61]. Preclinical and clinical studies have shown that bone marrow (BM)-derived MSC are capable of mediating cardiovascular reparative effects[62-64], predominantly through indirect, paracrine mechanisms that target endogenous cardiomyocytes and vascular cells[65]. The field of MSC research remains hindered by a lack of uniformity in the methods used for cell isolation, culture, and characterization. Until now, the majority of in vitro and in vivo cardiovascular studies have utilized BM MSC prepared by plastic adherence-isolation[18,66]. However, this non-selective technique is limited both by the low frequency of clonogenic colony forming units-fibroblastic (CFU-F) in adult human BM and the contamination of immature mesenchymal precursor cells (MPC) with more mature stromal and non-mesenchymal cell types[67]. Prospective immunoselection has been advocated as an alternative strategy for isolating pure populations of immature MPC, based on their expression of cell surface antigens to which specific monoclonal antibodies (mAb) may be directed. One such example is the murine IgM mAb that identifies stromal precursor antigen-1 (STRO-1). The STRO-1 antigen is expressed on the surface of approximately 10–20% of adult human BM that includes all CFU-F, Glycophorin-A^+ nucleated red cells, and a small subset of $CD19^+$ B-cells, but is not expressed on hematopoietic stem and progenitor cells (HSC)[68] STRO-1 is widely regarded as a marker of early mesenchymal/ stromal precursor cells, because it has been strongly linked to mesenchymal cell clonogenicity, plasticity, and other progenitor cell characteristics[69-74]. This study also presents new findings to show that the presence of STRO-1^+ precursors is an important indicator of the cardiovascular paracrine properties of mesenchymal cells. Many of the limitations of MSC therapy for cardiovascular disease arise from the inadequate engraftment and transdifferentiation of transplanted cells in recipient myocardium[75]. Crucially, by comparison to plastic adherence-isolation,the expanded progeny of STRO-1-MPC displays biological characteristics indicative of a higher retention of immature precursor cells supporting the notion that improving the precision and quality of STRO-1-MPC isolation is an important consideration in optimizing mesenchymal cell biology and repair.

Novel wound – healing promotion therapy

Chronic wounds are difficult to heal, and little improvement has been made in preventing the associated morbidity and disability over the past few decades[76]. The best available treatment for chronic wounds achieves only a 50% healing rate Therefore innovative treatments to enhance wound healing and

regeneration are needed. The major goal of wound-healing biology is to discover how skin can be induced to reconstruct damaged parts more perfectly[77].

SHED and hMSCs can enhance wound healing by promoting re-epithelialization and the relationship with the extracellular matrix, especially HA[78] . Treatments using MSCs would be effective, but the number, proliferation and differentiation potential of MSCs decline with increasing age[79]. On the other hand, SHED can be obtained without any invasion and could be a substitute for MSCs[57]. SHED significantly promotes wound healing compared with hFibro and control groups[78]. Deciduous teeth, which are considered to be medical waste, could provide novel therapeutic approaches for the treatment of wounds and novel stem-cell sources for wound healing[78].

Implications of the immunoregulatory functions of mesenchymal stem cells in the treatment of human liver diseases

Transplantation of mesenchymal stem cells(MSCs)has been recently studied in animal models,and in clinical trials of patients with fulminant hepatic failure,end-stage liver diseases and inherited metabolic disorders.Modulatory cytokines produced by MSCs can inhibit immunocyte proliferation and migration to the liver,thereby attenuating inflammatory injury and reducing hepatocyte apoptosis.In addition,MSCs play an important role in regressing liver fibrosis and in supporting the function,proliferation and differentiation of endogenous hepatocytes under appropriate conditions[80]. These findings indicate that MSC treatment is promising in the therapy of liver diseases, Although remarkable progress has been achieved in basic and clinical MSC studies,optimal therapeutic regimens for the clinical application of MSCs,such as optimal doses,transplantation routine and interval period for transplantation,need to be elucidated in detail.

Anti-inflammatory and anti-tumor effects

It has been demonstrated that MSC exhibit innate anti-tumor effects against PANC-1 cells and can serve as delivery vehicles for IFN-β for the treatment of pancreatic cancer. However, these beneficial effects may be lost in therapies combining MSC with anti-inflammatory agents[81]. It is now clear that trophic modulation of inflammation, cell death, fibrosis, and tissue repair are the main mechanisms of MSC therapy. Delivery of growth factors, cytokines, and other signaling molecules secreted by MSCs is often sufficient to obtain the therapeutic effects[82].

Other Diseases

It has been shown that the transplantation of MSCs could be an effective therapy for many diseases[83-103] , including blood disease, diabetes type 1 and 2, osteoarthritis, lung disease, spinal cord injury, liver injury, stroke, myocardial infarction, amyotrophic lateral sclerosis, parkinson's disease, neural disease, acute graft-versus-host-disease (GVHD), systemic lupus erythematosus (SLE), kidney disease and cancers. To date, hundreds of clinical trials using MSCs have been registered in the database (http://www.clinicaltrials. gov/) of the US national institutes of health. However, it is essential to find out the specific adult stem cell with great potential for tissue engineering and transplantation, which require good survival rates and stable hemodynamic behavior. In addition, the differences between gene and protein expressions in different adult stem cells have to be clarified first. The success of stem cell-based therapy will depend on cell availability, the potential to differentiate into specific cell lineage, inflammation response after transplantation, etc. Mesenchymal stem cell types from different sources could partly fulfill the criteria to be a suitable candidate for a specific lineage, which in turn is very important in regenerative cell therapies.

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