APB *Advanced Pharmaceutical Bulletin,* **2014**, 4(1), 5-13
doi: http://dx.doi.org/10.5681/apb.2014.002
http://apb.tbzmed.ac.ir/

Immunomodulatory Nature and Site Specific Affinity of Mesenchymal Stem Cells: a Hope in Cell Therapy

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ARTICLEINFO

Article Type:
Review

Article History:
Received: 15 August 2013
Revised: 5 September 2013
Accepted: 9 September 2013
ePublished: 23 December 2013

Keywords:
Mesenchymal stem cells
Immunomodulation
Cell migration
Cell therapy

ABSTRACT

Immunosuppressive ability of mesenchymal stem cells (MSCs), their differentiation properties to various specialized tissue types, ease of *in vitro* and *in vivo* expansion and specific migration capacity, make them to be tested in different clinical trials for the treatment of various diseases. The immunomodulatory effects of MSCs are less identified which probably has high clinically significance. The clinical trials based on primary research will cause better understanding the ability of MSCs in immunomodulatory applications and site specific migration in the optimization of therapy. So, this review focus on MSCs functional role in modulating immune responses, their ability in homing to tumor, their potency as delivery vehicle and their medical importance.

Introduction

Mesenchymal stem cells (MSCs) which also recognized as multipotent stromal or mesenchymal cells were discovered by Friedenstein and his colleagues in 1970.[1] They used an innovative method for isolation of MSCs from bone marrow based on their intrinsic adhesion features. The bone marrow derived fibroblastoid adherent cells were clonogenic without phagocytic activity.[2] MSCs are self-renewal cells which are able to differentiate into different endodermal, mesodermal and ectodermal cell lineages in particular culture systems.[3-6] MSCs are capable to be divided up to 50 times in about 10 weeks *in vitro*.[7] The possibility of MSCs isolation from different sources is giving promising confidence to establish mesenchymal stem cell banks in future.

It is believed that MSCs are residues of embryonic stem cells which remain in adult human body and express embryonic stem cell markers, including SSEA-1, Nanog, Oct-4, Rex-1 and GATA-4.[8-10] Despite of numerous attempts, researchers have found that there is no individual specific marker for MSCs identification. MSCs do not express hematopoietic markers such as CD34, CD45, CD11 or CD14 or co-stimulatory molecules, CD40, CD80, and CD86 while express CD166, CD29, CD106, and ICAM-1 in various status.[11-17] The International Society for Cellular Therapy (ISCT) has offered several criteria to identify MSCs which are listed as: 1) Plastic adherence while maintaining these cells in standard conditions. 2) Expression of CD73, CD90 and CD105 markers in at least 95% of cell population and lack expression of CD34, CD45, CD14 orCD11b, CD19 or CD79α and HLA-II markers as measured by flow cytometry. 3) Differentiation capability in to adipogenic, osteogenic and chondrogenic lineage cells in vitro.[18,19] Recent publication is considered exceptions for identifying adipose tissue-derived stromal cells (ASC) and adipose tissue's stromal vascular fraction (SVF) cells.[20] It has been revealed that ASC, similar to the other MSCs, have tri-lineage differentiation potency with a set of markers phenotype ($CD73^+$, $CD90^+$, $CD105^+$, $CD36^+$, $CD44^+$, $CD106^-$, $CD45^-$, and $CD31^-$) to distinguish them from bone marrow MSCs. In order to identify the SVFs, these cells are characterizes by the (CD34+, CD45-, CD31-, CD235a-) phenotype and fibroblastoid colony-forming unit assay.[20] In addition to the bone marrow,[21,22] MSCs have been found in other sources, including liver,[23] lung,[24,25] brain,[26] adipose tissue,[22,27-29] peripheral blood,[30] cornea,[31] synovium,[32] thymus,[33] dental pulp,[34,35] periosteum,[36] tendon,[37] spleen,[33] fallopian tube,[38] placenta,[39,40] amniotic fluid,[41] Wharton's jelly,[42] umbilical cord[43,44] and umbilical cord blood.[22,45]

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Immunomodulatory mechanism of MSCs on immune cells

Regard to enormous conducted researches; it has been found that the potency of MSCs to modulate immune responses is resulting from both cell-cell interactions and paracrine effects. The paracrine effects are caused by the release of soluble immune modulators such as IL-6, IL-10, indoleamine 2,3 dioxygenase (IDO), transforming growth factor (TGF)-β, prostaglandin E2 (PGE-2), hepatocyte growth factor (HGF), nitric oxide (NO)[46,47] and heme oxygenase-1 (HO-1).[48-54] In parallel, MSCs induce an immune tolerant phenotype by cell-cell interaction, which is characterized by intermediate or low levels of MHC class I and lack of MHC class II antigens expression,[55,56] co-stimulatory molecules B7-1 (CD80), B7-2 (CD86), CD40 or CD40L and FasL.[15,57] In a well-known reports, it has been shown that MSCs express Toll-like receptors(TLR) 2, 3, 4, 7 and 9, which involve in immunomodulatory properties.[58] Recent findings reveled that depending on which TLR is stimulated, there will be a possibility of two distinct phenotypes of MSCs.[59] It is widely accepted that MSCs possess immunomodulatory effects on immune cells in vitro, and they can arrest the immune cells cycle in G0/G1 phases and hinder subsequent cell proliferation.[60] For the first time, Di Nicola et al. reported the suppression of cell-mediated immune interactions by co-culturing dendritic cells [(DC cells), irradiated allogeneic lymphocytes or phytohaemaglutinin (PHA)] stimulated T-cells with irradiated MSC in mixed lymphocyte reaction (MLR). They found that MSCs can suppress the activation and proliferation of $CD4^+$ and $CD8^+$ T-cells.[61] Following studies revealed that proliferation of $CD3^+/CD4^+$ T- cells and production of IFN-γ and IL-2, in the presence of MSCs were suppressed.[62] The suppression of these cytokines could inhibit the differentiation of naive $CD8^+$ T-cells into cytotoxic effector cells.[63] Ghannam et al have also shown that MSCs have a potency to induce T-reg cell activity in the presence of pro-inflammatory cytokines including TNF-α and IFN-γ, in Th17 cells. When Th17 cells were co-cultured with MSC, the secretion of stored PGE2 in MSCs were increased and raised the suppressive effect of MSCs.[64] MSCs also are able to modulate the immune response of B-cells. It has been reported that in a co-culture system of stimulated B-cells and MSCs, the B-cells proliferation and antibody secretion (IgA, IgG, and IgM) were inhibited. Chemotactic ability of B-cells also could be modulate by MSCs via CXCR5 and CXCR4 down-regulation.[65] The effect of MSCs on inducing regulatory T-cells has also been investigated, in which MSCs induce T-cells differentiation into T-reg phenotype (CD4+, Foxp3+, CD25+) by up-regulating HLA-G5 molecules when they co-cultured with activated CD4+ T-cells.[66] The MSCs not only are able to inhibit natural killer cells (NK-cells) activity and IFN-γ production, by secreting soluble mediators including IDO, PGE2 and HLA-G[67,68] but also they can hinder the cell cycle of dendritic cells (DC-cells)and subsequently inhibit maturation and function of these cells.[69]

MSCs homing to tumor and inflammatory sites

Although many studies have been conducted on the homing of MSC, the exact mechanism of migration and homing of MSC to the tumor and the site of injury is still not well known. It has been suggested that the method MSCs migrate to the site of injury is same as the leukocytes recruitment to the site of inflammation.[70] This similarity is due to the chemokine receptors are expressed on MSCs, are the same as the ones, acting in homing of leukocytes.[71] For instance MSCs express CCR8, CCR2, CXCR1, CXCR2, CXCR3, CCR1, CCR3 and CCR4 which are likely up-regulated under inflammatory conditions.[72,73] Numerous studies have proved MSC homing into tumors. Nakamuraet al. demonstrated the migration of MSC to tumor by administration of MSCs into the rat model with gliomas.[74] Since tumor cells release various chemokines, cytokines and different inflammatory mediators, they have a potency to recruit respondent cells such as MSCs.[75] It is found that tumor cells and adjacent inflamed tissue, secrete different types of mediators, including IFN-γ, TNF-α, IL-1, IL-1β, IL-10, monocyte chemoattractant protein-1(MCP-1) and TGF-β[76,77] and MSCs express receptors for these mediators.[73,78] Expressing these receptors plays key role in the mediator specific homing of MSCs to the tumor. In addition to previously mentioned mediators, It has been identified that other important factors are involved in migration and homing of MSCs such as vascular endothelial growth factor (VEGF), stromal cell-derived factor-1α (SDF-1α), urokinase plasminogen activator (uPA), transmembrane protein 18 (TMEM18) and epidermal growth factor (EGF). All aforementioned factors act in MSCs homing into tumor and exerting their anti-tumor properties.[79-83] Scientists have utilized the migration potency of MSCs to inhibit tumor cells growth. This has been tried in different kinds of malignancies, such as Kaposi's sarcoma,[84] malignant melanoma,[85] glioma[74] and colon carcinoma.[86] The results indicate that by secreting soluble mediators, MSCs reduce the progression of tumor growth.[74,85] Another study in this field has demonstrated that MSCs have a potency to induce down-regulation of NFκB in breast cancer and hepato-cellular carcinoma and subsequently reduce their proliferation.[87]

MSCs as delivery vehicles

The self-renewing capability of MSCs, uncomplicated isolation procedure, migratory capacity toward inflammatory sites and tumors which make them an appropriate option as cell therapy vehicles for the delivery of mediators into tumors and damaged tissues.[88] Up to now, many animal model studies have

been directed to show the potential of genetically engineered MSCs in delivering therapeutic agents into the tumor sites, which consequently, prevent tumor growth. The anti-tumor activity of genetically manipulated MSCs has been proved in various kinds of subcutaneous, lung and brain tumors.[74,89-91] By different approaches, several studies have used engineered MSC to produce and deliver a variety of chemokines and cytokines. It has been demonstrated that the intravenous injection of IL-2-expressing MSCs, enhances immune surveillance against tumors and reduces metastasis of subcutaneous tumor model.[92] Similarly, delivery of CX3CL1 (a chemokine which activates both T-cells and NK-cells) by manipulated MSCs, causes a considerable reduction in lung tumors established by intravenous administered of melanoma cells.[93] Secretion of IFN-β (which induces apoptosis) by genetically engineered MSC suppresses prostate cancers, melanomas, pancreatic tumors and breast cancers in animal models.[94-97] It has been also demonstrated that IL-12-expressing MSCs have similar effect on renal cell carcinoma.[98] Pro-drugs converting MSCs have also been engineered by expressing particular enzymes which converts a pro-drug into a cytotoxic factor at the site of tumors. It has been elegantly revealed in a glioma model.[99] In the similar approach, MSCs have been engineered to express thymidine kinase of herpes simplex virus, which converts the ganciclovir at the tumor site. Although the toxicity of final product to the carrier MSCs is limited the efficiency of this technique.[99] A comparable study has used MSCs expressing cytosine deaminase enzyme to convert 5-fluorocytosine to 5-fluorouracil in colon carcinoma[91] and melanoma models.[100] In efforts to find effective plan by the same strategy, MSCs engineered to express rabbit carboxylesterase enzyme which convert the pro-drug CPT-11 into the active drug SN-38, which acts as topoisomerase-I inhibitor.[101] In attempts to produce a good cellular vehicles, MSCs have been modified genetically to secrete nano-sized exosomes[102] to deliver different types of therapeutic agents such as siRNAs[103] and MHC class I/peptide complexes.[104] Delivery of erythropoietin (EPO) by genetically-engineered MSCs was another challenge which has been done in a murine model of chronic kidney failure (CKF) and could reduce progression of red blood cells aplasia.[105] As other pioneering plan, engineered MSCs which constitutively express TRAIL have been used in different models, such as pancreatic cancer,[106] lung metastasis[91] and glioma model.[107] MSCs-expressing TRAIL home into the tumors and induce selective apoptosis of tumor cells with no obvious cytotoxicity to the adjacent tissue. Finally, a new possible method of virus delivery is considering MSCs as carrier vectors for virus delivery. The advantage of this delivery method is the reduction immune response of the recipient to the virus. This mechanism has been applied in several tumor models, such as ovarian cancer,[108] lung and breast metastases[109,110] with different level of success.

MSCs and immunosuppressant drugs

Some conducted studies are intended to complement or even replace the use of immunosuppressants with MSCs in the future. Since MSCs and some drugs have the common targets there is a possibility that immunosuppressants and MSCs have synergic or inhibitory effects on each other. Several studies have revealed that the mTOR inhibitors, rapamycin, and calcineurin inhibitor, tacrolimus, cause reduction of immunomodulatory activity of MSCs.[111,112] In contrast, mycophenolic acid (MPA) (potent inhibitor of the cell cycle) and MSCs have synergetic immunosuppressive effects. These results are confirmed in animal model studies and have been showed that MPA in combination with MSCs have a higher effect on the survival of transplanted heart than each MPA and MSC alone.[113] Another model also has been suggested that the effect of administrated MSCs was synergized with rapamycin in prolonging allograft survival.[114] In summary, more studies are required to find the most appropriate immunosuppressant medicine to be combined with MSCs in various situations.

Potential clinical applications of MSCs

Principal functions of MSCs is related to their various therapeutic properties; anti-inflammatory and immunomodulatory effects,[115,116] production of mediators that initiate or support tissue repair[117,118] and tissue replacement through multipotent differentiation potency.[3,119] Recently these properties have been subjugated in the treatment of a variety of disorders in preclinical and clinical studies. The anti-inflammatory effects of MSCs have been studied in inflammatory disorders, including chronic pulmonary disease and inflammatory bowel disease, and in other diseases, such as cardiac disease. Several studies demonstrated an improved cardiac function[120,121] and decreased infarct size[122] by administrating MSCs after chronic ischemic heart failure and myocardial infarction. In other human trial study, MSC-induced suppression of T-cell mediated immunity has revealed that single intra-arterial MSC injection significantly improves the survival rate of the graft versus host disease (GVHD).[123] Many phase I and II clinical trials relating to MSCs for treatment of various diseases are available in the http://clinicaltrials.gov database, which probably is the largest clinical trial database. The most important therapeutic areas include ischemic cardiac disease, graft-versus-host disease, chronic obstructive pulmonary disease and Crohn's disease. There have been about 339 clinical trials in http://clinicaltrials.gov injecting MSC for cell therapy with no reported incidence of MSCs malignant transformation. But by the time of writing this review (July 2013) there weren't no reported trials about the use of MSCs as delivery mediators for anti-tumor therapy.[124]

Concluding remarks and future perspectives for using MSCs in therapy

Although the potential anti-proliferative and immunomodulatory roles of MSCs are currently being studied by different groups, and in spite of increasing hopes to consider MSCs application as a new treatments candidate for various human diseases, a better understanding of their immunosuppressive ability is now required. Despite the immunosuppressive characteristics and differentiation potential, which certificate their clinical application, several obstacles with the use of autologous or allogeneic MSCs have been raised in the clinical settings. Whereas autologous MSCs will engraft with a high efficacy, theoretically they could induce tumors. This was further supported by the fact that during in vitro expansion, MSCs can undergo spontaneous transformation that exhibits a tumorogenic potential.[125] In inflammatory conditions, MSCs might express MHC class I and class II surface antigens, and therefore act as APCs for T cells, resulting in MSCs rejection. It is important to accomplish a better understanding of these mechanisms by further studies especially in animal models to clarify many unanswered questions about the overall effect of MSCs administration on systemic and local immunity. Thus, a precise definition and characterization of MSCs phenotype is required to make possible well-designed preclinical studies that should be performed to determine the in vivo biological properties of MSCs and further explore their clinical applications.

Conflict of Interest

The authors report no conflicts of interest.

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