

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

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## 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 clinical 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.<sup>1</sup> 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.<sup>2</sup> MSCs are self-renewal cells which are able to differentiate into different endodermal, mesodermal and ectodermal cell lineages in particular culture systems.<sup>3-6</sup> MSCs are capable to be divided up to 50 times in about 10 weeks *in vitro*.<sup>7</sup> 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.<sup>8-10</sup> 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.<sup>11-17</sup> 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 or CD11b, CD19 or CD79 $\alpha$  and HLA-II markers as measured by flow cytometry. 3) Differentiation capability in to adipogenic, osteogenic and chondrogenic lineage cells *in vitro*.<sup>18,19</sup> Recent publication is considered exceptions for identifying adipose tissue-derived stromal cells (ASC) and adipose tissue's stromal vascular fraction (SVF) cells.<sup>20</sup> It has been revealed that ASC, similar to the other MSCs, have tri-lineage differentiation potency with a set of markers phenotype (CD73<sup>+</sup>, CD90<sup>+</sup>, CD105<sup>+</sup>, CD36<sup>+</sup>, CD44<sup>+</sup>, CD106<sup>+</sup>, CD45<sup>-</sup>, and CD31<sup>-</sup>) to distinguish them from bone marrow MSCs. In order to identify the SVFs, these cells are characterizes by the (CD34<sup>+</sup>, CD45<sup>-</sup>, CD31<sup>-</sup>, CD235a<sup>-</sup>) phenotype and fibroblastoid colony-forming unit assay.<sup>20</sup> In addition to the bone marrow,<sup>21,22</sup> MSCs have been found in other sources, including liver,<sup>23</sup> lung,<sup>24,25</sup> brain,<sup>26</sup> adipose tissue,<sup>22,27-29</sup> peripheral blood,<sup>30</sup> cornea,<sup>31</sup> synovium,<sup>32</sup> thymus,<sup>33</sup> dental pulp,<sup>34,35</sup> periosteum,<sup>36</sup> tendon,<sup>37</sup> spleen,<sup>33</sup> fallopian tube,<sup>38</sup> placenta,<sup>39,40</sup> amniotic fluid,<sup>41</sup> Wharton's jelly,<sup>42</sup> umbilical cord<sup>43,44</sup> and umbilical cord blood.<sup>22,45</sup>

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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)- $\beta$ , prostaglandin E2 (PGE-2), hepatocyte growth factor (HGF), nitric oxide (NO)<sup>46,47</sup> and heme oxygenase-1 (HO-1).<sup>48-54</sup> 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,<sup>55,56</sup> co-stimulatory molecules B7-1 (CD80), B7-2 (CD86), CD40 or CD40L and FasL.<sup>15,57</sup> 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.<sup>58</sup> Recent findings revealed that depending on which TLR is stimulated, there will be a possibility of two distinct phenotypes of MSCs.<sup>59</sup> 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.<sup>60</sup> 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 phytohaemagglutinin (PHA)] stimulated T-cells with irradiated MSC in mixed lymphocyte reaction (MLR). They found that MSCs can suppress the activation and proliferation of CD4<sup>+</sup> and CD8<sup>+</sup> T-cells.<sup>61</sup> Following studies revealed that proliferation of CD3<sup>+</sup>/CD4<sup>+</sup> T- cells and production of IFN- $\gamma$  and IL-2, in the presence of MSCs were suppressed.<sup>62</sup> The suppression of these cytokines could inhibit the differentiation of naive CD8<sup>+</sup> T-cells into cytotoxic effector cells.<sup>63</sup> 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- $\alpha$  and IFN- $\gamma$ , 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.<sup>64</sup> 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.<sup>65</sup> 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<sup>+</sup>, Foxp3<sup>+</sup>, CD25<sup>+</sup>) by up-regulating HLA-G5 molecules when they co-cultured with activated CD4<sup>+</sup> T-cells.<sup>66</sup> The MSCs not only are able to inhibit natural killer cells (NK-cells) activity and IFN- $\gamma$  production, by secreting soluble mediators

including IDO, PGE2 and HLA-G<sup>67,68</sup> but also they can hinder the cell cycle of dendritic cells (DC-cells) and subsequently inhibit maturation and function of these cells.<sup>69</sup>

### 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.<sup>70</sup> This similarity is due to the chemokine receptors are expressed on MSCs, are the same as the ones, acting in homing of leukocytes.<sup>71</sup> For instance MSCs express CCR8, CCR2, CXCR1, CXCR2, CXCR3, CCR1, CCR3 and CCR4 which are likely up-regulated under inflammatory conditions.<sup>72,73</sup> Numerous studies have proved MSC homing into tumors. Nakamura *et al.* demonstrated the migration of MSC to tumor by administration of MSCs into the rat model with gliomas.<sup>74</sup> Since tumor cells release various chemokines, cytokines and different inflammatory mediators, they have a potency to recruit respondent cells such as MSCs.<sup>75</sup> It is found that tumor cells and adjacent inflamed tissue, secrete different types of mediators, including IFN- $\gamma$ , TNF- $\alpha$ , IL-1, IL-1 $\beta$ , IL-10, monocyte chemoattractant protein-1 (MCP-1) and TGF- $\beta$ <sup>76,77</sup> and MSCs express receptors for these mediators.<sup>73,78</sup> 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-1 $\alpha$  (SDF-1 $\alpha$ ), 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.<sup>79-83</sup> 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,<sup>84</sup> malignant melanoma,<sup>85</sup> glioma<sup>74</sup> and colon carcinoma.<sup>86</sup> The results indicate that by secreting soluble mediators, MSCs reduce the progression of tumor growth.<sup>74,85</sup> Another study in this field has demonstrated that MSCs have a potency to induce down-regulation of NF $\kappa$ B in breast cancer and hepato-cellular carcinoma and subsequently reduce their proliferation.<sup>87</sup>

### 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.<sup>88</sup> 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.<sup>74,89-91</sup> 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.<sup>92</sup> 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.<sup>93</sup> Secretion of IFN- $\beta$  (which induces apoptosis) by genetically engineered MSC suppresses prostate cancers, melanomas, pancreatic tumors and breast cancers in animal models.<sup>94-97</sup> It has been also demonstrated that IL-12-expressing MSCs have similar effect on renal cell carcinoma.<sup>98</sup> 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.<sup>99</sup> 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.<sup>99</sup> A comparable study has used MSCs expressing cytosine deaminase enzyme to convert 5-fluorocytosine to 5-fluorouracil in colon carcinoma<sup>91</sup> and melanoma models.<sup>100</sup> 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.<sup>101</sup> In attempts to produce a good cellular vehicles, MSCs have been modified genetically to secrete nano-sized exosomes<sup>102</sup> to deliver different types of therapeutic agents such as siRNAs<sup>103</sup> and MHC class I/peptide complexes.<sup>104</sup> 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.<sup>105</sup> As other pioneering plan, engineered MSCs which constitutively express TRAIL have been used in different models, such as pancreatic cancer,<sup>106</sup> lung metastasis<sup>91</sup> and glioma model.<sup>107</sup> 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,<sup>108</sup> lung and breast metastases<sup>109,110</sup> 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.<sup>111,112</sup> 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.<sup>113</sup> Another model also has been suggested that the effect of administrated MSCs was synergized with rapamycin in prolonging allograft survival.<sup>114</sup> 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,<sup>115,116</sup> production of mediators that initiate or support tissue repair<sup>117,118</sup> and tissue replacement through multipotent differentiation potency.<sup>3,119</sup> 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<sup>120,121</sup> and decreased infarct size<sup>122</sup> 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).<sup>123</sup> 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.<sup>124</sup>

### 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 tumorigenic potential.<sup>125</sup> 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.

### References

- Friedenstein AJ, Chailakhyan RK, Latsinik NV, Panasyuk AF, Keiliss-Borok IV. Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues. Cloning in vitro and retransplantation in vivo. *Transplantation* 1974;17(4):331-40.
- Friedenstein AJ, Gorskaja JF, Kulagina NN. Fibroblast precursors in normal and irradiated mouse hematopoietic organs. *Exp Hematol* 1976;4(5):267-74.
- Mafi R, Hindocha S, Mafi P, Griffin M, Khan WS. Sources of adult mesenchymal stem cells applicable for musculoskeletal applications - a systematic review of the literature. *Open Orthop J* 2011;5 Suppl 2:242-8.
- Paunescu V, Deak E, Herman D, Siska IR, Tanasie G, Bunu C, et al. In vitro differentiation of human mesenchymal stem cells to epithelial lineage. *J Cell Mol Med* 2007;11(3):502-8.
- Oswald J, Boxberger S, Jørgensen B, Feldmann S, Ehninger G, Bornhäuser M, et al. Mesenchymal stem cells can be differentiated into endothelial cells in vitro. *Stem Cells* 2004;22(3):377-84.
- Tran TC, Kimura K, Nagano M, Yamashita T, Ohneda K, Sugimori H, et al. Identification of human placenta-derived mesenchymal stem cells involved in re-endothelialization. *J Cell Physiol* 2011;226(1):224-35.
- Giordano A, Galderisi U, Marino IR. From the laboratory bench to the patient's bedside: an update on clinical trials with mesenchymal stem cells. *J Cell Physiol* 2007;211(1):27-35.
- Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* 2002;418(6893):41-9.
- Riekstina U, Cakstina I, Parfejevs V, Hoogduijn M, Jankovskis G, Muiznieks I, et al. Embryonic stem cell marker expression pattern in human mesenchymal stem cells derived from bone marrow, adipose tissue, heart and dermis. *Stem cell Rev* 2009;5(4):378-86.
- Movassagh Pour AA, Salehnia M, Pourfatollah AA, Soleimani M. CFU-GM like colonies derived from embryonic stem cells cultured on the bone marrow stromal cells. *Iran Biomed J* 2004;8(1):1-5.
- Covas DT, Panepucci RA, Fontes AM, Silva WA, Jr., Orellana MD, Freitas MC, et al. Multipotent mesenchymal stromal cells obtained from diverse human tissues share functional properties and gene-expression profile with CD146+ perivascular cells and fibroblasts. *Exp Hematol* 2008;36(5):642-54.
- Da Silva Meirelles L, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. *J Cell Sci* 2006;119(Pt 11):2204-13.
- Haynesworth SE, Baber MA, Caplan AI. Cell surface antigens on human marrow-derived mesenchymal cells are detected by monoclonal antibodies. *Bone* 1992;13(1):69-80.
- Galmiche MC, Koteliensky VE, Briere J, Herve P, Charbord P. Stromal cells from human long-term marrow cultures are mesenchymal cells that differentiate following a vascular smooth muscle differentiation pathway. *Blood* 1993;82(1):66-76.
- Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999;284(5411):143-7.
- Sordi V, Malosio ML, Marchesi F, Mercalli A, Melzi R, Giordano T, et al. Bone marrow mesenchymal stem cells express a restricted set of functionally active chemokine receptors capable of promoting migration to pancreatic islets. *Blood* 2005;106(2):419-27.
- Le Blanc K, Tammik C, Rosendahl K, Zetterberg E, Ringden O. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. *Exp Hematol* 2003;31(10):890-6.
- Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal

- criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006;8(4):315-7.
19. Horwitz EM, Le Blanc K, Dominici M, Mueller I, Slaper-Cortenbach I, Marini FC, et al. Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. *Cytotherapy* 2005;7(5):393-5.
  20. Bourin P, Bunnell BA, Casteilla L, Dominici M, Katz AJ, March KL, et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). *Cytotherapy* 2013;15(6):641-8.
  21. Li M, Ikehara S. Bone-marrow-derived mesenchymal stem cells for organ repair. *Stem Cells Int* 2013;2013:132642.
  22. Kern S, Eichler H, Stoeve J, Kluter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells* 2006;24(5):1294-301.
  23. Najimi M, Khuu DN, Lysy PA, Jazouli N, Abarca J, Sempoux C, et al. Adult-derived human liver mesenchymal-like cells as a potential progenitor reservoir of hepatocytes? *Cell Transplant* 2007;16(7):717-28.
  24. Sabatini F, Petecchia L, Taviani M, Jodon De Villeroche V, Rossi GA, Brouty-Boye D. Human bronchial fibroblasts exhibit a mesenchymal stem cell phenotype and multilineage differentiating potentialities. *Lab Invest* 2005;85(8):962-71.
  25. Lama VN, Smith L, Badri L, Flint A, Andrei AC, Murray S, et al. Evidence for tissue-resident mesenchymal stem cells in human adult lung from studies of transplanted allografts. *J Clin Invest* 2007;117(4):989-96.
  26. Kang SG, Shinojima N, Hossain A, Gumin J, Yong RL, Colman H, et al. Isolation and perivascular localization of mesenchymal stem cells from mouse brain. *Neurosurgery* 2010;67(3):711-20.
  27. Pawitan JA. Prospect of adipose tissue derived mesenchymal stem cells in regenerative medicine. *Cell Tissue Transplant Ther* 2009;2:7-9.
  28. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, et al. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell* 2002;13(12):4279-95.
  29. Zannettino AC, Paton S, Arthur A, Khor F, Itescu S, Gimble JM, et al. Multipotential human adipose-derived stromal stem cells exhibit a perivascular phenotype in vitro and in vivo. *J Cell Physiol* 2008;214(2):413-21.
  30. Chong PP, Selvaratnam L, Abbas AA, Kamarul T. Human peripheral blood derived mesenchymal stem cells demonstrate similar characteristics and chondrogenic differentiation potential to bone marrow derived mesenchymal stem cells. *J Orthop Res* 2012;30(4):634-42.
  31. Choong PF, Mok PL, Cheong SK, Then KY. Mesenchymal stromal cell-like characteristics of corneal keratocytes. *Cytotherapy* 2007;9(3):252-8.
  32. Jones E, Churchman SM, English A, Buch MH, Horner EA, Burgoyne CH, et al. Mesenchymal stem cells in rheumatoid synovium: enumeration and functional assessment in relation to synovial inflammation level. *Ann Rheum Dis* 2010;69(2):450-7.
  33. Krampera M, Sartoris S, Liotta F, Pasini A, Angeli R, Cosmi L, et al. Immune regulation by mesenchymal stem cells derived from adult spleen and thymus. *Stem Cells Dev* 2007;16(5):797-810.
  34. Gronthos S. The therapeutic potential of dental pulp cells: more than pulp fiction? *Cytotherapy* 2011;13(10):1162-3.
  35. Gronthos S, Mankani M, Brahimi J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proc Natl Acad Sci U S A* 2000;97(25):13625-30.
  36. Nakahara H, Bruder SP, Haynesworth SE, Holecck JJ, Baber MA, Goldberg VM, et al. Bone and cartilage formation in diffusion chambers by subcultured cells derived from the periosteum. *Bone* 1990;11(3):181-8.
  37. Bi Y, Ehrlichou D, Kilts TM, Inkson CA, Embree MC, Sonoyama W, et al. Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. *Nat Med* 2007;13(10):1219-27.
  38. Jazedje T, Perin PM, Czeresnia CE, Maluf M, Halpern S, Secco M, et al. Human fallopian tube: a new source of multipotent adult mesenchymal stem cells discarded in surgical procedures. *J Transl Med* 2009;7:46.
  39. Sabapathy V, Ravi S, Srivastava V, Srivastava A, Kumar S. Long-term cultured human term placenta-derived mesenchymal stem cells of maternal origin displays plasticity. *Stem Cells Int* 2012;2012:174328.
  40. Igura K, Zhang X, Takahashi K, Mitsuru A, Yamaguchi S, Takashi TA. Isolation and characterization of mesenchymal progenitor cells from chorionic villi of human placenta. *Cytotherapy* 2004;6(6):543-53.
  41. You Q, Cai L, Zheng J, Tong X, Zhang D, Zhang Y. Isolation of human mesenchymal stem cells from third-trimester amniotic fluid. *Int J Gynaecol Obstet* 2008;103(2):149-52.
  42. Wang HS, Hung SC, Peng ST, Huang CC, Wei HM, Guo YJ, et al. Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord. *Stem Cells* 2004;22(7):1330-7.
  43. Capelli C, Gotti E, Morigi M, Rota C, Weng L, Dazzi F, et al. Minimally manipulated whole human umbilical cord is a rich source of clinical-grade

- human mesenchymal stromal cells expanded in human platelet lysate. *Cytotherapy* 2011;13(7):786-801.
44. Romanov YA, Svintsitskaya VA, Smirnov VN. Searching for alternative sources of postnatal human mesenchymal stem cells: candidate MSC-like cells from umbilical cord. *Stem Cells* 2003;21(1):105-10.
  45. Kim HS, Shin TH, Yang SR, Seo MS, Kim DJ, Kang SK, et al. Implication of NOD1 and NOD2 for the differentiation of multipotent mesenchymal stem cells derived from human umbilical cord blood. *PLoS One* 2010;5(10):e15369.
  46. Azadmehr A, Afshari A, Baradaran B, Hajiaghae R, Rezazadeh S, Monsef-Esfahani H. Suppression of nitric oxide production in activated murine peritoneal macrophages in vitro and ex vivo by *Scrophularia striata* ethanolic extract. *J Ethnopharmacol* 2009;124(1):166-9.
  47. Ren G, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, et al. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. *Cell Stem Cell* 2008;2(2):141-50.
  48. Krampera M, Glennie S, Dyson J, Scott D, Laylor R, Simpson E, et al. Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. *Blood* 2003;101(9):3722-9.
  49. Bartholomew A, Sturgeon C, Siatskas M, Ferrer K, McIntosh K, Patil S, et al. Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. *Exp Hematol* 2002;30(1):42-8.
  50. Zhang W, Ge W, Li C, You S, Liao L, Han Q, et al. Effects of mesenchymal stem cells on differentiation, maturation, and function of human monocyte-derived dendritic cells. *Stem Cells Dev* 2004;13(3):263-71.
  51. Yanez R, Lamana ML, Garcia-Castro J, Colmenero I, Ramirez M, Bueren JA. Adipose tissue-derived mesenchymal stem cells have in vivo immunosuppressive properties applicable for the control of the graft-versus-host disease. *Stem Cells* 2006;24(11):2582-91.
  52. Cui L, Yin S, Liu W, Li N, Zhang W, Cao Y. Expanded adipose-derived stem cells suppress mixed lymphocyte reaction by secretion of prostaglandin E2. *Tissue Eng* 2007;13(6):1185-95.
  53. Chabannes D, Hill M, Merieau E, Rossignol J, Brion R, Soulillou JP, et al. A role for heme oxygenase-1 in the immunosuppressive effect of adult rat and human mesenchymal stem cells. *Blood* 2007;110(10):3691-4.
  54. Oh I, Ozaki K, Sato K, Meguro A, Tataru R, Hatanaka K, et al. Interferon-gamma and NF-kappaB mediate nitric oxide production by mesenchymal stromal cells. *Biochem Biophys Res Commun* 2007;355(4):956-62.
  55. Morandi F, Raffaghello L, Bianchi G, Meloni F, Salis A, Millo E, et al. Immunogenicity of human mesenchymal stem cells in HLA-class I-restricted T-cell responses against viral or tumor-associated antigens. *Stem Cells* 2008;26(5):1275-87.
  56. Le Blanc K, Tammik L, Sundberg B, Haynesworth SE, Ringden O. Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex. *Scand J Immunol* 2003;57(1):11-20.
  57. Deans RJ, Moseley AB. Mesenchymal stem cells: biology and potential clinical uses. *Exp Hematol* 2000;28(8):875-84.
  58. Delarosa O, Lombardo E. Modulation of adult mesenchymal stem cells activity by toll-like receptors: implications on therapeutic potential. *Mediators Inflamm* 2010;2010:865601.
  59. Waterman RS, Tomchuck SL, Henkle SL, Betancourt AM. A new mesenchymal stem cell (MSC) paradigm: polarization into a pro-inflammatory MSC1 or an immunosuppressive MSC2 phenotype. *PLoS One* 2010;5(4):e10088.
  60. Glennie S, Soeiro I, Dyson PJ, Lam EW, Dazzi F. Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. *Blood* 2005;105(7):2821-7.
  61. Di Nicola M, Carlo-Stella C, Magni M, Milanese M, Longoni PD, Matteucci P, et al. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood* 2002;99(10):3838-43.
  62. Parekkadan B, Milwid JM. Mesenchymal stem cells as therapeutics. *Annu Rev Biomed Eng* 2010;12:87-117.
  63. Sad S, Marcotte R, Mosmann TR. Cytokine-induced differentiation of precursor mouse CD8+ T cells into cytotoxic CD8+ T cells secreting Th1 or Th2 cytokines. *Immunity* 1995;2(3):271-9.
  64. Ghannam S, Pene J, Torcy-Moquet G, Jorgensen C, Yssel H. Mesenchymal stem cells inhibit human Th17 cell differentiation and function and induce a T regulatory cell phenotype. *J Immunol* 2010;185(1):302-12.
  65. Corcione A, Benvenuto F, Ferretti E, Giunti D, Cappiello V, Cazzanti F, et al. Human mesenchymal stem cells modulate B-cell functions. *Blood* 2006;107(1):367-72.
  66. Selmani Z, Naji A, Zidi I, Favier B, Gaiffe E, Obert L, et al. Human leukocyte antigen-G5 secretion by human mesenchymal stem cells is required to suppress T lymphocyte and natural killer function and to induce CD4+CD25highFOXP3+ regulatory T cells. *Stem Cells* 2008;26(1):212-22.
  67. Spaggiari GM, Capobianco A, Abdelrazik H, Becchetti F, Mingari MC, Moretta L. Mesenchymal stem cells inhibit natural killer-cell proliferation, cytotoxicity, and cytokine production: role of

- indoleamine 2,3-dioxygenase and prostaglandin E2. *Blood* 2008;111(3):1327-33.
68. Gonen-Gross T, Goldman-Wohl D, Huppertz B, Lankry D, Greenfield C, Natanson-Yaron S, et al. Inhibitory NK receptor recognition of HLA-G: regulation by contact residues and by cell specific expression at the fetal-maternal interface. *PLoS One* 2010;5(1):e8941.
  69. Jiang XX, Zhang Y, Liu B, Zhang SX, Wu Y, Yu XD, et al. Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. *Blood* 2005;105(10):4120-6.
  70. Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 1994;76(2):301-14.
  71. Ruster B, Göttig S, Ludwig RJ, Bistrrian R, Müller S, Seifried E, et al. Mesenchymal stem cells (MSCs) display coordinated rolling and adhesion behavior on endothelial cells. *Blood* 2006;108(12):3938-44.
  72. Ringe J, Strassburg S, Neumann K, Endres M, Notter M, Burmester GR, et al. Towards in situ tissue repair: human mesenchymal stem cells express chemokine receptors CXCR1, CXCR2 and CCR2, and migrate upon stimulation with CXCL8 but not CCL2. *J Cell Biochem* 2007;101(1):135-46.
  73. Ponte AL, Marais E, Gallay N, Langonne A, Delorme B, Herault O, et al. The in vitro migration capacity of human bone marrow mesenchymal stem cells: comparison of chemokine and growth factor chemotactic activities. *Stem Cells* 2007;25(7):1737-45.
  74. Nakamura K, Ito Y, Kawano Y, Kurozumi K, Kobune M, Tsuda H, et al. Anti-tumor effect of genetically engineered mesenchymal stem cells in a rat glioma model. *Gene Ther* 2004;11(14):1155-64.
  75. Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *N Engl J Med* 1986;315(26):1650-9.
  76. Yamanaka R, Tanaka R, Saitoh T, Okoshi S. Cytokine gene expression on glioma cell lines and specimens. *J Neurooncol* 1994;21(3):243-7.
  77. Kielian T, Van Rooijen N, Hickey WF. MCP-1 expression in CNS-1 astrocytoma cells: implications for macrophage infiltration into tumors in vivo. *J Neurooncol* 2002;56(1):1-12.
  78. Chamberlain G, Wright K, Rot A, Ashton B, Middleton J. Murine mesenchymal stem cells exhibit a restricted repertoire of functional chemokine receptors: comparison with human. *PLoS One* 2008;3(8):e2934.
  79. Van Der Meulen AA, Biber K, Lukovac S, Balasubramanian V, Den Dunnen WF, Boddeke HW, et al. The role of CXC chemokine ligand (CXCL)12-CXC chemokine receptor (CXCR)4 signalling in the migration of neural stem cells towards a brain tumour. *Neuropathol Appl Neurobiol* 2009;35(6):579-91.
  80. Schmidt NO, Przylecki W, Yang W, Ziu M, Teng Y, Kim SU, et al. Brain tumor tropism of transplanted human neural stem cells is induced by vascular endothelial growth factor. *Neoplasia* 2005;7(6):623-9.
  81. Gutova M, Najbauer J, Frank Rt, Kendall SE, Gevorgyan A, Metz MZ, et al. Urokinase plasminogen activator and urokinase plasminogen activator receptor mediate human stem cell tropism to malignant solid tumors. *Stem Cells* 2008;26(6):1406-13.
  82. Sato H, Kuwashima N, Sakaida T, Hatano M, Dusak JE, Fellows-Mayle WK, et al. Epidermal growth factor receptor-transfected bone marrow stromal cells exhibit enhanced migratory response and therapeutic potential against murine brain tumors. *Cancer Gene Ther* 2005;12(9):757-68.
  83. Jurvansuu J, Zhao Y, Leung DS, Boulaire J, Yu YH, Ahmed S, et al. Transmembrane protein 18 enhances the tropism of neural stem cells for glioma cells. *Cancer Res* 2008;68(12):4614-22.
  84. Khakoo AY, Pati S, Anderson SA, Reid W, Elshal MF, Rovira, Ii, et al. Human mesenchymal stem cells exert potent antitumorigenic effects in a model of Kaposi's sarcoma. *J Exp Med* 2006;203(5):1235-47.
  85. Maestroni GJ, Hertens E, Galli P. Factor(s) from nonmacrophage bone marrow stromal cells inhibit Lewis lung carcinoma and B16 melanoma growth in mice. *Cell Mol Life Sci* 1999;55(4):663-7.
  86. Ohlsson LB, Varas L, Kjellman C, Edvardsen K, Lindvall M. Mesenchymal progenitor cell-mediated inhibition of tumor growth in vivo and in vitro in gelatin matrix. *Exp Mol Pathol* 2003;75(3):248-55.
  87. Qiao L, Zhao TJ, Wang FZ, Shan CL, Ye LH, Zhang XD. NF- $\kappa$ B downregulation downregulation may be involved the depression of tumor cell proliferation mediated by human mesenchymal stem cells. *Acta Pharmacol Sin* 2008;29:333-40.
  88. Dennis JE, Cohen N, Goldberg VM, Caplan AI. Targeted delivery of progenitor cells for cartilage repair. *J Orthop Res* 2004;22(4):735-41.
  89. Nakamizo A, Marini F, Amano T, Khan A, Studeny M, Gumin J, et al. Human bone marrow-derived mesenchymal stem cells in the treatment of gliomas. *Cancer Res* 2005;65(8):3307-18.
  90. Studeny M, Marini FC, Dembinski JL, Zompetta C, Cabreira-Hansen M, Bekele BN, et al. Mesenchymal stem cells: potential precursors for tumor stroma and targeted-delivery vehicles for anticancer agents. *J Natl Cancer Inst* 2004;96(21):1593-603.
  91. Kucerova L, Altanerova V, Matuskova M, Tyciakova S, Altaner C. Adipose tissue-derived human mesenchymal stem cells mediated prodrug cancer gene therapy. *Cancer Res* 2007;67(13):6304-13.
  92. Chen X, Lin X, Zhao J, Shi W, Zhang H, Wang Y, et al. A tumor-selective biotherapy with prolonged

- impact on established metastases based on cytokine gene-engineered MSCs. *Mol Ther* 2008;16(4):749-56.
93. Xin H, Kanehira M, Mizuguchi H, Hayakawa T, Kikuchi T, Nukiwa T, et al. Targeted delivery of CX3CL1 to multiple lung tumors by mesenchymal stem cells. *Stem Cells* 2007;25(7):1618-26.
  94. Kidd S, Caldwell L, Dietrich M, Samudio I, Spaeth EL, Watson K, et al. Mesenchymal stromal cells alone or expressing interferon-beta suppress pancreatic tumors in vivo, an effect countered by anti-inflammatory treatment. *Cytotherapy* 2010;12(5):615-25.
  95. Studeny M, Marini FC, Champlin RE, Zompetta C, Fidler IJ, Andreeff M. Bone marrow-derived mesenchymal stem cells as vehicles for interferon-beta delivery into tumors. *Cancer Res* 2002;62(13):3603-8.
  96. Ahn JO, Lee HW, Seo KW, Kang SK, Ra JC, Youn HY. Anti-Tumor Effect of Adipose Tissue Derived-Mesenchymal Stem Cells Expressing Interferon-beta and Treatment with Cisplatin in a Xenograft Mouse Model for Canine Melanoma. *PLoS One* 2013;8(9):e74897.
  97. Ren C, Kumar S, Chanda D, Kallman L, Chen J, Mountz JD, et al. Cancer gene therapy using mesenchymal stem cells expressing interferon-beta in a mouse prostate cancer lung metastasis model. *Gene Ther* 2008;15(21):1446-53.
  98. Gao P, Ding Q, Wu Z, Jiang H, Fang Z. Therapeutic potential of human mesenchymal stem cells producing IL-12 in a mouse xenograft model of renal cell carcinoma. *Cancer Lett* 2010;290(2):157-66.
  99. Uchibori R, Okada T, Ito T, Urabe M, Mizukami H, Kume A, et al. Retroviral vector-producing mesenchymal stem cells for targeted suicide cancer gene therapy. *J Gene Med* 2009;11(5):373-81.
  100. Kucerova L, Matuskova M, Pastorakova A, Tyciakova S, Jakubikova J, Bohovic R, et al. Cytosine deaminase expressing human mesenchymal stem cells mediated tumour regression in melanoma bearing mice. *J Gene Med* 2008;10(10):1071-82.
  101. Choi SA, Lee JY, Wang KC, Phi JH, Song SH, Song J, et al. Human adipose tissue-derived mesenchymal stem cells: characteristics and therapeutic potential as cellular vehicles for prodrug gene therapy against brainstem gliomas. *Eur J Cancer* 2012;48(1):129-37.
  102. Yeo RW, Lai RC, Zhang B, Tan SS, Yin Y, Teh BJ, et al. Mesenchymal stem cell: an efficient mass producer of exosomes for drug delivery. *Adv Drug Deliv Rev* 2013;65(3):336-41.
  103. Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhali S, Wood MJ. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nat Biotechnol* 2011;29(4):341-5.
  104. Andre F, Chaput N, Scharz NE, Flament C, Aubert N, Bernard J, et al. Exosomes as potent cell-free peptide-based vaccine. I. Dendritic cell-derived exosomes transfer functional MHC class I/peptide complexes to dendritic cells. *J Immunol* 2004;172(4):2126-36.
  105. Mok PL, Cheong SK, Leong CF, Chua KH, Ainoon O. Human mesenchymal stromal cells could deliver erythropoietin and migrate to the basal layer of hair shaft when subcutaneously implanted in a murine model. *Tissue Cell* 2012;44(4):249-56.
  106. Mohr A, Albarenque SM, Deedigan L, Yu R, Reidy M, Fulda S, et al. Targeting of XIAP combined with systemic mesenchymal stem cell-mediated delivery of sTRAIL ligand inhibits metastatic growth of pancreatic carcinoma cells. *Stem Cells* 2010;28(11):2109-20.
  107. Menon LG, Kelly K, Yang HW, Kim SK, Black PM, Carroll RS. Human bone marrow-derived mesenchymal stromal cells expressing S-TRAIL as a cellular delivery vehicle for human glioma therapy. *Stem Cells* 2009;27(9):2320-30.
  108. Komarova S, Kawakami Y, Stoff-Khalili MA, Curiel DT, Pereboeva L. Mesenchymal progenitor cells as cellular vehicles for delivery of oncolytic adenoviruses. *Mol Cancer Ther* 2006;5(3):755-66.
  109. Hakkarainen T, Sarkioja M, Lehenkari P, Miettinen S, Ylikomi T, Suuronen R, et al. Human mesenchymal stem cells lack tumor tropism but enhance the antitumor activity of oncolytic adenoviruses in orthotopic lung and breast tumors. *Hum Gene Ther* 2007;18(7):627-41.
  110. Stoff-Khalili MA, Rivera AA, Mathis JM, Banerjee NS, Moon AS, Hess A, et al. Mesenchymal stem cells as a vehicle for targeted delivery of CRAds to lung metastases of breast carcinoma. *Breast Cancer Res Treat* 2007;105(2):157-67.
  111. Hoogduijn MJ, Crop MJ, Korevaar SS, Peeters AM, Eijken M, Maat LP, et al. Susceptibility of human mesenchymal stem cells to tacrolimus, mycophenolic acid, and rapamycin. *Transplantation* 2008;86(9):1283-91.
  112. Buron F, Perrin H, Malcus C, Hequet O, Thauinat O, Kholopp-Sarda MN, et al. Human mesenchymal stem cells and immunosuppressive drug interactions in allogeneic responses: an in vitro study using human cells. *Transplant Proc* 2009;41(8):3347-52.
  113. Caimi PF, Reese J, Lee Z, Lazarus HM. Emerging therapeutic approaches for multipotent mesenchymal stromal cells. *Curr Opin Hematol* 2010;17(6):505-13.
  114. Garcia-Gomez I, Elvira G, Zapata AG, Lamana ML, Ramirez M, Castro JG, et al. Mesenchymal stem cells: biological properties and clinical applications. *Expert Opin Biol Ther* 2010;10(10):1453-68.



115. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood* 2005;105(4):1815-22.
116. Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. *J Cell Biochem* 2006;98(5):1076-84.
117. Waszak P, Alphonse R, Vadivel A, Ionescu L, Eaton F, Thebaud B. Preconditioning enhances the paracrine effect of mesenchymal stem cells in preventing oxygen-induced neonatal lung injury in rats. *Stem Cells Dev* 2012;21(15):2789-97.
118. Du Z, Wei C, Cheng K, Han B, Yan J, Zhang M, et al. Mesenchymal stem cell-conditioned medium reduces liver injury and enhances regeneration in reduced-size rat liver transplantation. *J Surg Res* 2013;183(2):907-15.
119. Quevedo HC, Hatzistergos KE, Oskouei BN, Feigenbaum GS, Rodriguez JE, Valdes D, et al. Allogeneic mesenchymal stem cells restore cardiac function in chronic ischemic cardiomyopathy via trilineage differentiating capacity. *Proc Natl Acad Sci U S A* 2009;106(33):14022-7.
120. Assmus B, Honold J, Schächinger V, Britten MB, Fischer-Rasokat U, Lehmann R, et al. Transcoronary transplantation of progenitor cells after myocardial infarction. *N Engl J Med* 2006;355(12):1222-32.
121. Schächinger V, Erbs S, Elsässer A, Haberbosch W, Hambrecht R, Hölschermann H, et al. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. *N Engl J Med* 2006;355(12):1210-21.
122. Hare JM, Chaparro SV. Cardiac regeneration and stem cell therapy. *Curr Opin Organ Transplant* 2008;13(5):536-42.
123. Arima N, Nakamura F, Fukunaga A, Hirata H, Machida H, Kouno S, et al. Single intra-arterial injection of mesenchymal stromal cells for treatment of steroid-refractory acute graft-versus-host disease: a pilot study. *Cytotherapy* 2010;12(2):265-8.
124. ClinicalTrials.gov. Clinical trials related to search term Mesenchymal stem cells. Rockville Pike, Bethesda, MD: U.S. National Library of Medicine; 2013; Available from: <http://clinicaltrials.gov/ct2/results?term=mesechymal+stem+cells>.
125. Rubio D, Garcia S, Paz MF, De La Cueva T, Lopez-Fernandez LA, Lloyd AC, et al. Molecular characterization of spontaneous mesenchymal stem cell transformation. *PLoS One* 2008;3(1):e1398.