Chapter 10
Heterogeneity of Human Mesenchymal Stromal/Stem Cells

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Abstract Increasing evidence has shown that mesenchymal stem cells (MSCs) isolated from body tissues are heterogeneous while being examined in vitro and in vivo. Besides some common characteristics, MSCs derived from different tissues exhibit unique biological properties. In addition, the therapeutic effects of MSCs may vary widely due to their heterogeneity and the technical differences in large-scale ex vivo expansion. In this chapter, the heterogeneity of MSCs will be discussed in three levels: the individual donors, the tissue sources, and the cell surface markers.

Keywords Mesenchymal stem cells · Heterogeneity · Surface markers · Biological property · Cell therapy · Subpopulation · Regenerative property · Immunomodulatory ability · Individual donor · Tissue source

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Introduction

In 1970, Friedenstein et al. found a group of osteoprogenitor cells in bone marrow that were capable of developing fibroblast colonies in vitro and ectopic bone formation in vivo [1]. Further investigation demonstrated that these adult bone marrow stem cells, named as mesenchymal stem cells (MSCs), can replicate as undifferentiated cells and have the potential to differentiate to lineages of mesenchymal tissues, including the bone, cartilage, fat, tendon, muscle, and marrow stroma [2]. Later findings suggest that the ability of MSCs to alter the tissue microenvironment via secretion of soluble factors may contribute more significantly than their capacity for transdifferentiation in tissue repair [3]. Moreover, MSCs mediate immune modulation by interacting with innate and adaptive immunity [4]. The promising features of MSCs, including their regenerative properties and immunomodulatory ability, have generated great interest among researchers whose work has offered intriguing perspectives on cell-based therapies for various diseases. By July 2018, 677 MSC-based clinical trials are registered on clinical.org, either completed or ongoing.

In 2006, heterogeneous procedures for isolating and cultivating MSCs among laboratories have prompted the International Society for Cellular Therapy (ISCT) to issue criteria for identifying unique populations of these cells [5]. However, the isolation of MSCs according to ISCT criteria has produced heterogeneous, non-clonal cultures of stromal cells containing multipotent stem cells, committed progenitors, and differentiated cells. The intrinsic differences and large-scale preclinical amplification have led to distinct biological properties of the MSC population, which may partly explain the differences in the outcomes of the clinical trials with MSCs. More precise molecular and cellular markers to define subsets of MSCs and to standardize the protocols for expansion of MSCs are urgently needed.

The present chapter will discuss the heterogeneity of MSCs with reference to four major aspects: heterogeneity among various individual donors, different tissue origins, differential cell surface markers, and different microenvironment and culture conditions. The schematic diagram of MSCs heterogeneity is demonstrated in Fig. 10.1.

Heterogeneity Among Individual Donors

Plenty of studies have shown that there is heterogeneity in MSCs among different individuals. For instance, Phinney et al. analyzed the heterogeneity of MSCs isolated from posterior iliac crest marrow aspirates of 17 healthy donors and found that MSCs populations showed dramatic differences in growth rates, levels of alkaline phosphatase enzyme activity, and levels of bone-specific gene induction [6]. Significant strain differences were also noted in the properties of mouse MSCs [7]. In addition, Peltzer et al. compared adult bone marrow MSCs with perinatal tissues-derived MSCs (cord blood, umbilical cord, amnion, and chorion) on their in vitro

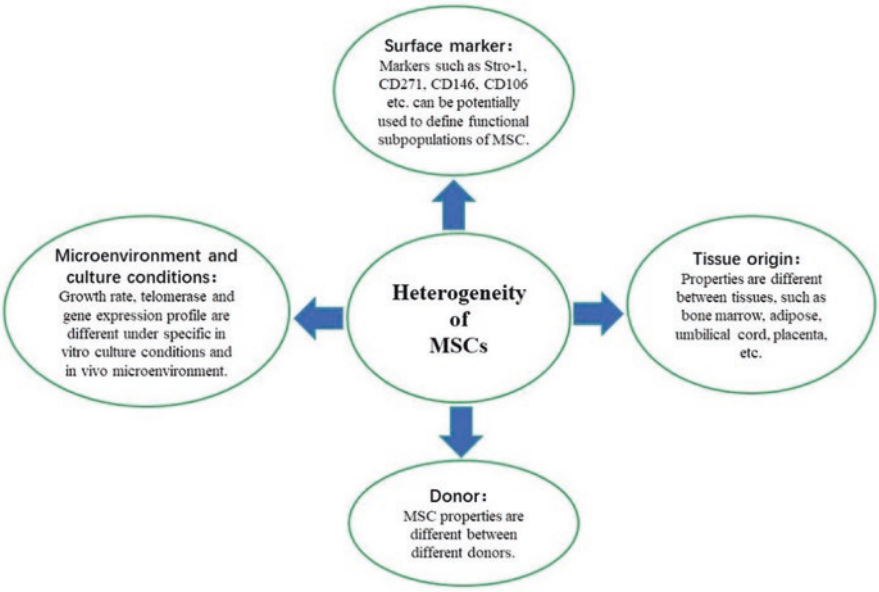

Fig. 10.1 Profile of MSCs heterogeneity

immunomodulatory activities under different priming conditions such as interferon gamma or tumor necrosis factor alpha, and the results showed contrasted effects of cytokine priming embedded in an important between-donor variability [8]. Our unpublished results also demonstrated the heterogeneity in the ability of differentiation and cytokine secretion of MSCs derived from the same kind of tissues but different individuals. Moreover, the age of donor [9] and the method of amplification in vitro [10] also affect the heterogeneity of MSC populations. Kang et al. suggest that sensitivity to hypoxic conditions is different between human umbilical cord blood MSCs originating from different donors and this difference affects the contribution to angiogenesis. The bioinformatics analysis of different donors under hypoxic culture conditions identified intrinsic variability in gene expression patterns and suggests alternative potential genetic factors, ANGPTL4, ADM, SLC2A3, and CDON, as guaranteed general indicators for further stem cell therapy [11].

Heterogeneity Among Different Tissue Origins

MSCs derived from different tissues demonstrated heterogeneity of MSCs properties. In 2006, we established a protocol to isolate abundant MSCs from human umbilical cords (UC-MSCs) with a 100% success rate. The biological characteristics of UC-MSCs were further determined and compared with normal adult bone marrow-derived MSCs (BM-MSCs). We found that UC-MSCs shared most of the

characteristics of BM-MSCs, including fibroblastic-like morphology, immunophenotype, cell cycle status, adipogenic and osteogenic differentiation potentials, and hematopoiesis-supportive function. However, in comparison with BM-MSCs, the UC-MSCs had a higher proliferation capacity and lower levels of expression of CD106 and HLA-ABC. Furthermore, UC-MSCs had a higher percentage of neuron-specific enolase-positive cells than BM-MSCs after neuronal induction [12]. Baksh D compared the proliferation and multilineage differentiation potential of MSCs derived from umbilical cord and bone marrow, which were referred to as human umbilical cord perivascular cells (HUCPVCs) and bone marrow MSCs (BMSCs), respectively. HUCPVCs showed a higher proliferative potential than BMSCs and were capable of osteogenic, chondrogenic, and adipogenic differentiation. Interestingly, osteogenic differentiation of HUCPVCs proceeded more rapidly than BMSCs. Additionally, HUCPVCs expressed higher levels of CD146, a putative MSC marker, relative to BMSCs [13]. Furthermore, the heterogeneity of human MSCs from bone marrow (BM), adipose tissue (AT), and Wharton's jelly (WJ) was evaluated in terms of proliferation, in vitro differentiation (osteogenic, adipogenic, and chondrogenic potential), expression of cell surface markers, and protein secretion using Luminex and ELISA assays. Cell proliferation was higher for WJ-MSCs, followed by AT-MSCs. WJ-MSCs secreted higher concentrations of chemokines, pro-inflammatory proteins, and growth factors. AT-MSCs showed a better pro-angiogenic profile and secreted higher amounts of extracellular matrix components and metalloproteinases [14].

We identified human MSCs from adult bone marrow (ABM), fetal pancreas (FPan), and umbilical cord (UC), and their abilities to support megakaryocyte (MK) differentiation from $CD34^+$ hematopoietic progenitor cells (HPCs) were comparatively studied. FPan-MSCs and UC-MSCs showed the ability to promote megakaryocytopoiesis, while ABM-MSCs expanded more MK progenitor cells from $CD34^+$ HPCs [15]. Hsiao et al. investigated the paracrine factor expression patterns in MSCs isolated from adipose tissue (ASCs), bone marrow (BMSCs), and dermal tissues [dermal sheath cells (DSCs) and dermal papilla cells (DPCs)]. Specifically, mRNA expression analysis identified insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor-D (VEGF-D), and interleukin-8 (IL-8) were expressed at higher levels in ASCs compared with other MSCs populations, whereas VEGF-A, angiogenin, basic fibroblast growth factor (bFGF), and nerve growth factor (NGF) were expressed at comparable levels among the MSCs populations examined. Analysis of conditioned media (CM) protein confirmed the comparable level of angiogenin and VEGF-A secretion in all MSCs populations and showed that DSCs and DPCs produced significantly higher concentrations of leptin. Functional assays examining in vitro angiogenic paracrine activity showed that incubation of endothelial cells in ASCs resulted in increased tubulogenic efficiency compared with that observed in DPCs. Using neutralizing antibodies, they concluded that VEGF-A and VEGF-D were two of the major growth factors secreted by ASCs that supported endothelial tubulogenesis. Therefore, ASCs may

be preferred over other MSCs populations for augmenting therapeutic approaches dependent upon angiogenesis [16].

Though no significant differences in growth rate, colony-forming efficiency, and immunophenotype were observed between MSCs derived from the bone marrow, adipose tissue, the placenta, and umbilical cord blood, MSCs derived from bone marrow and adipose tissue shared not only in vitro tri-lineage differentiation potential but also gene expression profiles. While there was considerable inter-donor variation in DLX5 expression between MSCs derived from different tissues, its expression appears to be associated with the osteogenic potential of MSCs [17]. Similarly, Stubbendorff et al. compared the phenotype, proliferation rate, migration, immunogenicity, and immunomodulatory capabilities of human MSCs derived from umbilical cord lining (CL-MSCs), umbilical cord blood (CB-MSCs), placenta (P-MSCs), and Wharton's jelly (WJ-MSCs). Differences were noted in differentiation, proliferation, and migration, with CL-MSCs showing the highest proliferation and migration rates resulting in prolonged survival in immunodeficient mice. Moreover, CL-MSCs showed a prolongation in survival in xenogeneic BALB/c mice, which was attributed to their ability to dampen TH1 and TH2 responses. Weaker human cellular immune responses were detected against CL-MSCs and P-MSCs, which were correlated with their lower HLA I expression. Furthermore, HLA II was upregulated less substantially by CL-MSCs and CB-MSCs after IFN-γ stimulation. Despite their lower IDO, HLA-G, and TGF-β1 expression, only CL-MSCs were able to reduce the release of IFN-γ by lymphocytes in a mixed lymphocyte reaction. They concluded that CL-MSCs showed the best characteristics for cell-based strategies, as they are hypo-immunogenic and show high proliferation and migration rates [18]. Zhu et al. investigated the differences in human placental MSCs (P-MSCs) of fetal and maternal origins in the aspects of clinical importance. Although all P-MSCs express typical MSCs phenotype, fetal but not maternal P-MSCs express high levels of CD200 and HGF. Compared with HGF- and CD200-negative P-MSCs, HGF- and CD200-positive cells demonstrated significantly higher potentials in promoting angiogenesis in vitro and increasing immunosuppressive function in vivo [19]. In 2017, we reported that placental chorionic villi (CV)-derived MSCs exhibited superior activities of immunomodulation and pro-angiogenesis compared to MSCs derived from the bone marrow (BM), adipose tissue, and umbilical cord (UC). Furthermore, we identified a subpopulation of CD106 (VCAM-1)$^+$ MSCs, which are present richly in placental CV, moderately in BM, and lowly in adipose tissue and UC. The CD106$^+$ MSCs possess significantly increased immunomodulatory and pro-angiogenic activities compared to CD106$^-$MSCs. Analysis of gene expression and cytokine secretion revealed that CD106$^+$MSCs highly expressed several immunomodulatory and pro-angiogenic cytokines. Our data offer new insights on the identification and selection of suitable source or population of MSCs for clinical applications [20].

Heterogeneity of Cell Surface Markers

Stro-1

Stro-1 is the best-known MSCs marker. However, Stro-1 is not expressed on MSCs populations derived from all kinds of tissues. So far, Stro-1 is reported to be expressed on MSCs derived from dental tissues [21], synovial membranes [22], and choriodecidua [23] but barely or at low level expressed on MSCs derived from adipose tissue [24], human umbilical cord blood [25], human umbilical cord [26], etc. Hongxiu Ning et al. suggested that Stro-1 is intrinsically an endothelial antigen and its expression on MSCs is probably an induced event [27]. Immunoselection with monoclonal antibodies against Stro-1 and CD106 prior to expansion resulted in a 1000-fold enrichment of mesenchymal precursors compared to standard isolation techniques. Moreover, intramyocardial injection of human Stro-1-selected precursors in an athymic rat model of acute myocardial infarction resulted in induction of vascular network formation and arteriogenesis coupled with global functional cardiac recovery [28]. Stro-1^+ cells may rather be used for gene delivery in tissues due to their stronger homing capabilities, while Stro-1^- cells may rather be used to support hematopoietic engraftment [29]. Compared to plastic adherence-isolated MSC (PA-MSCs), Stro-1-MSCs displayed greater clonogenicity, proliferative capacity, multilineage differentiation potential, and mRNA expression of MSC-related transcripts. In vitro assays demonstrated that conditioned medium from Stro-1-MSC had greater paracrine activity than PA-MSCs, with respect to cardiac cell proliferation and migration and endothelial cell migration and tube formation [30].

Thus, Stro-1 may get involved in MSCs colony forming, homing, and angiogenesis.

CD271

CD271, also called the low-affinity nerve growth factor receptor (LNGFR), is one of the two receptor types for the neurotrophins, a family of protein growth factors that stimulate neuronal cells to survive and differentiate. In vivo studies showed that CD271$^+$ MSCs promoted significantly greater lymphoid engraftment than did plastic adherence MSCs when co-transplanted with CD133$^+$ hematopoietic stem cells at a ratio of 8:1 in immunodeficient NOD/SCID-IL2Rgamma(null) mice. Therefore, CD271 antigen provides a versatile marker for prospective isolation and expansion of a subset of MSCs with immunosuppressive and lymphohematopoietic engraftment-promoting properties [31]. Hermida-Gómez et al. revealed that synovial membranes from human osteoarthritic patients contain more cells expressing CD271 antigen than those from healthy joints, and the cell subset CD271$^+$ MSCs provide higher-quality chondral repair than the CD271$^-$ subset [32]. CD271 is highly expressed on MSCs derived from the bone marrow [33], adipose tissue [34],

and periodontal ligament [35], lowly expressed on placental MSC [36, 37], and not expressed on MSCs derived from the synovial membrane [38], umbilical cord [39], and umbilical cord blood [40].

CD146

CD146, also known as the melanoma cell adhesion molecule (MCAM) or cell surface glycoprotein MUC18, is a cell adhesion molecule which gets involved in the process of angiogenesis. CD146 is extensively expressed by MSCs derived from a variety of sources, such as the bone marrow [41], adipose tissue [42], umbilical cord [43], synovium [38], umbilical cord blood [40], placenta [37], dermis [44], etc. Human endometrial stromal $CD146^{+}PDGF\text{-}R\beta^{+}$ cells were enriched for colony-forming cells compared with $CD146^{-}PDGF\text{-}R\beta^{-}$ cells and also underwent differentiation into adipogenic, osteogenic, myogenic, and chondrogenic lineages [45]. Sorrentino et al. found that the $CD146^{+}$ MSCs represent a subset of stromal cells supporting hematopoiesis and secrete a complex combination of growth factors controlling hematopoietic stem cells (HSCs) function while providing a >2-log increase in the long-term culture (LTC) colony output in 8-week LTC over conventional assays. Thus $CD146^{+}$ MSCs may represent a tool to explore the MSC-HSC cross talk in an in vitro surrogate model for HSC "niches" and for regenerative therapy studies [46]. Moreover, CD146 expressing, subendothelial cells in human bone marrow stroma are capable of transferring, upon transplantation, the hematopoietic microenvironment to heterotopic sites [47].

CD106

CD106, also known as vascular cell adhesion protein 1 or vascular cell adhesion molecule 1 (VCAM-1), is a protein that functions as a cell adhesion molecule. CD106 is critical for MSC-mediated immunosuppression [48] and for the binding of hematopoietic progenitor cells [49]. Martens et al. reported that immunoselection with monoclonal antibodies against Stro-1 and CD106 prior to expansion resulted in a 1000-fold enrichment of mesenchymal precursors compared to standard isolation techniques [28]. Moreover, the combination of three cell surface markers (LNGFR, THY-1, and CD106) allows for the selection of highly enriched clonogenic cells (one out of three isolated cells) [50]. Fukiage et al. showed that the CD106-positive fraction contained less osteogenic and more adipogenic cells than the CD106-negative fraction, indicating the usefulness of CD106 as a differentiation-predicting marker of bone marrow stromal cells [51]. Our research team compared the phenotype and biological properties among different MSCs isolated from human placental chorionic villi (CV), umbilical cord (UC), adult bone marrow (BM), and adipose (AD) tissue. We found that CD106 (VCAM-1) was expressed highest on

the CV-MSCs, moderately on BM-MSCs, lightly on UC-MSCs, and absent on AD-MSCs. CV-MSCs also showed unique immune-associated gene expression and immunomodulation. We thus separated CD106$^+$ cells and CD106$^-$ cells from CV-MSCs and compared their biological activities. Both two subpopulations were capable of osteogenic and adipogenic differentiation, while CD106$^+$ CV-MSCs were more effective to modulate T-helper subsets but possessed decreased colony formation capacity. In addition, CD106$^+$ CV-MSCs expressed more cytokines than CD106$^-$ CV-MSCs. These data demonstrate that CD106 identifies a subpopulation of CV-MSCs with unique immunoregulatory activity and reveals a previously unrecognized mechanism underlying immunomodulation of MSCs [52]. Furthermore, we found that angiogenic genes, including HGF, ANG, IL8, IL6, VEGF-A, TGFβ, MMP2, and bFGF, were upregulated in CD106$^+$ CV-MSCs. Consistently, angiogenic cytokines especially HGF, IL8, angiogenin, angiopoitin-2, μPAR, CXCL1, IL-1β, IL-1α, CSF2, CSF3, MCP-3, CTACK, and OPG were found to be significantly increased in CD106$^+$ CV-MSCs. CD106$^+$ CV-MSCs showed remarkable vasculo-angiogenic abilities by angiogenesis analysis with Matrigel in vitro and in vivo, and the conditioned medium of CD106$^+$ CV-MSCs exerted markedly pro-proliferative and pro-migratory effects on endothelial cells compared to CD106$^-$ CV-MSCs. Finally, transplantation of CD106$^+$ CV-MSCs into the ischemic hind limb of BALB/c nude mice resulted in a significantly functional improvement in comparison with CD106$^-$ CV-MSCs transplantation. CD106$^+$ CV-MSCs possessed a favorable angiogenic paracrine activity and displayed therapeutic efficacy on hindlimb ischemia. Our results suggested that CD106$^+$ CV-MSCs may represent an important subpopulation of MSC for efficient therapeutic angiogenesis [53].

Nestin

Nestin (acronym for neuroectodermal stem cell marker) is a type VI intermediate filament protein expressed in the early stages of development [54]. Increasing studies show a particular association between Nestin and MSCs. Nestin could characterize a subset of bone marrow perivascular MSCs which contributed to bone development and closely contacted with hematopoietic stem cells (HSCs) [55]. Nestin$^+$ MSCs contain all the bone-marrow colony-forming-unit fibroblastic activity and can be propagated as non-adherent "mesenspheres" that can self-renew and expand in serial transplantations. Nestin$^+$ MSCs are spatially associated with HSCs and adrenergic nerve fibers and highly express HSCs maintenance genes. In addition, in vivo Nestin$^+$ cell depletion rapidly reduces HSCs content in the bone marrow and purified HSCs home near Nestin$^+$ MSCs in the bone marrow of lethally irradiated mice [56]. However, the intracellular location of Nestin prevents its use for prospective live cell isolation. The combination of surface markers PDGFRα and CD51 could be used for identifying Nestin$^+$ cells. PDGFRα$^+$ CD51$^+$ cells in the human fetal bone marrow represent a small subset of CD146$^+$ cells expressing

Nestin and enriched for MSCs and HSCs niche activities. Importantly, cultured human PDGFRα$^+$ CD51$^+$ non-adherent mesenspheres that could significantly expand multipotent hematopoietic progenitors were able to engraft immunodeficient mice [57].

Except for the above described specific markers for MSCs, there are some other surface molecules that have been found to be useful for identification of specific subset of MSCs, such as CD349 [58], CD49f [59], GD2 [60], 3G5 [61], SSEA-4 [62], etc.

Heterogeneity of Human MSCs Under Specific Conditions

Except for the individual donors and the tissue sources, the culture condition and microenvironment also contribute to the heterogeneity of MSC characteristics. For instance, if adipose-derived MSCs (ADMSCs) were cultured under hypoxic (1% O^2) conditions, ADMSCs proliferation and the expression of stemness genes, i.e., Nanog and Sox2, were significantly favored [63]. The heterogeneity of human umbilical cord MSCs (hUC-MSCs) cultured in serum-free medium (SFM) and serum-containing medium (SCM) was investigated by us. SFM-expanded hUC-MSCs were different from SCM-expanded hUC-MSCs in growth rate, telomerase, and gene expression profile. hUC-MSCs propagated more slowly and senesce ultimately in SFM. However, SFM-expanded hUC-MSCs maintained multipotency and the profile of surface antigen which were used to define human MSCs. Both SFM- and SCM-expanded hUC-MSCs gained copy number variation (CNV) in long-term in vitro culture [64]. Moreover, we found that bone marrow microenvironment of acquired aplastic anemia (AA) affects the heterogeneity of human bone marrow-derived MSCs (BM-MSCs). BM-MSCs from AA patients exhibited down-regulation of the CD106 gene and low expression of CD106 in vitro. The expression of NF-κB was decreased in AA MSCs, and NF-κB regulated the CD106 gene which supported hematopoiesis [65].

Conclusion and Perspective

Though the minimal criteria to define MSCs were proposed by the Tissue Stem Cell Committee of International Society for Cellular Therapy in 2006, the isolation of MSCs produces heterogeneous, nonclonal cultures of stromal cells containing stem cells with different multipotential properties, committed progenitors, and differentiated cells. In addition to the common immunophenotypic markers of the isolated MSCs, there are some special surface molecules that may be used to define different functional MSC subgroups. Analysis of different subpopulations of MSC can enhance the understanding of MSCs' biological characteristics.

In the future, to establish stem cell banks based on the heterogeneity of MSC subpopulations is quite necessary. In addition, selective application of different MSCs subgroups with one or two unique advantage functions, such as osteogenesis, adipogenesis, chondrogenesis, immunomodulation, angiogenesis, and hematopoiesis support, for the treatment of differential diseases might be promising in the field of stem cell therapy. However, it remains elusive whether application of MSCs that show heterogeneity while being cultured in vitro will function differently in vivo. Therefore, the in vivo heterogeneity of MSCs warrants further investigation.

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