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REVIEW ARTICLE

Heterogeneity of mesenchymal stem cells: characterization and application in cell therapy

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Abstract

Mesenchymal stem cells (MSCs) have shown great potentials in regenerative medicine for their low immunogenicity, multilineage differentiation potential, and extensive sources. However, the heterogeneity of MSCs limits their clinical application and industrial prospects. In this review, we introduced the heterogeneity of MSCs in terms of their applications, sources, functions, and surface markers; discussed the major factors leading to the heterogeneity in MSCs; summarized the main approaches to study the MSC heterogeneity, and addressed the clinical challenges resulting from heterogeneity. Finally, we proposed the strategies that might be used to purify the MSCs and to eliminate the heterogeneity of MSCs for their standardized production and reliable clinical application.

Keywords: *heterogeneity*; *mesenchymal stem cell*; *regenerative medicine*; *cell therapy*

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Mesenchymal stem cells (MSCs) are one kind of trophoblast cells that exists in most adult connective tissues. MSCs contribute to the maintenance and regeneration of diverse tissue by differentiating into bone, fat, cartilage, fibers and so on. These cells can secrete cytokines to support the survival and growth of various cells (1).

Mesenchymal stem cells are isolated from various tissues, such as amniotic membrane, dental pulp, fat, umbilical cord blood, umbilical cord, thymus, peripheral blood, and bone marrow (Table 1). The MSCs are defined by the International Society of Cellular Therapy as a heterogeneous population of spindle-shaped cells having minimum characteristics: (1) adherent growth; (2) expression of CD73, CD1053, and CD90 (≥95%, flow cytometry detection) and negative expression of CD14, HLA-DR, CD34, CD11b or CD45, CD19 or CD79α (≤2%, flow cytometry detection); and (3) differentiation into chondrocytes, osteoblasts, and adipocytes (2).

Numerous studies proved that MSCs could maintain stem cell properties even after multiple passages and have anti-bacterial, anti-fibrosis, and anti-transplant rejection properties (3–5). MSCs continuously secrete anti-apoptotic and anti-inflammatory cytokines to construct a favorable microenvironment that promotes tissue repair and regeneration *in vivo* (6). These cells are easily harvested, separated, cultured, and amplified *in vitro*. Benefiting from these advantages, MSCs are hopefully applied for tissue engineering and cell reprogramming. MSCs have been the most widely used adult stem cells in clinic. So far, there have been over 1000 registered clinical trials regarding MSCs around the world (www.clinicaltrials.gov), including pulmonary fibrosis (7), bone tissue engineering (8, 9), cartilage tissue engineering (10–12), myocardial infarction (MI) (13, 14), systemic lupus erythematosus (15), graft-versus-host disease (GVHD) (16), spinal cord injury (17, 18), hematopoiesis (19–21), diabetic foot ulcer (22), and multiple sclerosis (23). It is noteworthy that for their

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immune-modulatory properties, MSCs have been used for clinical therapy of coronavirus disease 2019 (COVID-19), a contagious disease induced by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (24–30). Preliminary clinical data indicated that the MSCs alleviated the clinical symptoms of COVID-19 patients by reducing cytokine storms, increasing oxygen saturation, and regenerating lung tissue (25, 31–36).

For the great potential of clinical application, the market of MSCs is now booming. According to the data released by Research and Markets, the global market of MSCs is approximately $172.9 million in 2020 and is expected to grow at a compound annual growth rate of 4.1% in the next 7 years. The market is expected to reach a revised size of $229 million by 2027. In February 2018, the company of Mesoblast Limited announced that the Phase 3 trial of the allogeneic MSCs for treating children with steroid-refractory acute graft-versus-host disease (aGVHD) achieved success. Although people are optimistic about the commercial prospects of MSCs, so far, most applications of MSCs have been in clinical trials (37). In comparison with the great advantages of MSCs for cell therapy, the clinical application and industrial development of MSCs are still far from satisfactory. One of the main impeding factors is heterogeneity. Usually, cell-based therapeutic products require stable biological characteristics to obtain long-lasting therapeutic effects among patients undergoing treatment. So, the safety and curative effect of MSCs have always been the most critical criterion in clinical applications. Due to MSC heterogeneity, it is difficult to establish a criterion for patient selection and provide repeatable treatment plans for patients. In manufacture, the heterogeneity impedes the large-scale standardized production of MSCs with high and uniform quality.

In this review, we discuss the major factors leading to the heterogeneity in MSCs, summarize the main approaches to study the MSC heterogeneity, and address the clinical challenges resulting from heterogeneity. Finally, we propose the strategies that may be used to purify MSCs and to eliminate the heterogeneity of MSCs for their standardized production and reliable clinical application.

Heterogeneity of MSCs

Mesenchymal stem cell heterogeneity means the differences in cell morphology and function among the heterogeneous subpopulation. This heterogeneity is inherent among donors (sex and age, etc.), source tissues (umbilical cord, gums, placenta, bone marrow, dental pulp, and adipose tissue, etc.), isolation and culture methods, passage times, and treatment modality (cryopreserved, resuscitation, etc.). These factors contribute to the primary cultured MSCs' high heterogeneity of proliferation potential, secretion of factors, and immune regulation capacity (Fig. 1) (38, 39).

It has been proved that there are differences in the immunoregulatory function of MSCs from different tissues and individuals (40). For example, MSCs derived from high-risk myelodysplastic syndrome were associated with higher immunosuppressive and apoptosis rates compared with MSCs derived from low-risk myelodysplastic syndrome (41). The individual heterogeneity of MSCs indicates their different therapeutic effects on different diseases. Therefore, many efforts were made to uncover the heterogeneity of MSCs.

Heterogeneity among individual donors

Numerous studies have revealed that MSCs are heterogeneous among different individuals. MSCs derived from the same tissue from different individuals also have heterogeneity. Xie et al. analyzed the features of hUC-MSCs derived from multiple donors, including cell activity, surface marker, multi-differentiation potential, immunoregulatory capability, and so on. Next, they treated mouse liver fibrosis using the hUC-MSCs with distinct immunomodulatory effects. The results showed that hUC-MSCs from multiple donors displayed a substantial heterogeneity in multi-differentiation potential and immunoregulatory capability; however, they have similar surface marker expression patterns, survival rate, growth curve, and tumorigenicity. *In vivo* results showed that three hUC-MSCs alleviated liver fibrosis; however, three hUC-MSCs have different therapeutic effects. The repair effects were dependent on the immune regulation ability of MSCs (40).

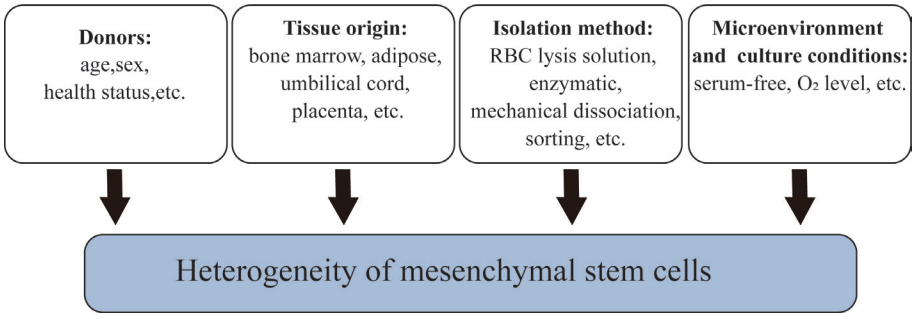

Fig. 1. Heterogeneity of mesenchymal stem cells.

It was reported that there are significant differences in the growth rate, alkaline phosphatase activity, and bone-specific gene level of bone marrow MSCs (BM-MSCs) from 17 healthy donors (42). MSCs in the bone marrow of inbred strains of mice exhibit markedly disparate expression levels of alkaline phosphatase that is an early marker of osteoblast differentiation (43). The age of the donors also affects the heterogeneity of MSCs (44). Zhou et al. found that in the older subjects (>55 years), the proliferation and osteoblast differentiation of MSCs were significantly decreased. The senescence-associated β-galactosidase (SA-β-gal) activity and the expression of p53 pathway genes in older subjects were higher than in young subjects. The proportion of SA-β-gal$^+$ MSCs derived from older subjects were four times higher than that of younger subjects. The proliferation rate of MSCs derived from the older subjects is 1.7 times less than that of the younger subjects (44). The human umbilical cord MSCs (hUC-MSCs) from different people have different sensitivity to hypoxic conditions. The result of bioinformatics analysis demonstrated the intrinsic variability and suggested that the alternative potential genetic factors, such as ADM, ANGPTL4, CDON, and SLC2A3, may be considered as potential indicators for cell therapy (45). Genders can affect the heterogeneity of MSCs. Xie et al. revealed that the osteogenic potential of male infant hUC-MSCs was approximately 10 times higher than that of female infant hUC-MSCs *in vitro*. On the contrary, there was no apparent heterogeneity in cartilage and adipogenesis differentiation capacity (40).

Heterogeneity of MSCs under different culturing environments

A critical factor determining the heterogeneity of MSCs may be the microenvironment of MSCs (46). MSC functions are extremely diverse and depend on the special microenvironment in which MSCs are embedded (47). A hypoxic environment would induce hypoxia inducible factor-1a (HIF-1a) expression of MSCs. In a 1% hypoxic environment, MSCs tend to be elliptical and up-regulate the expression of stemness-associated markers, such as octamer-binding transcription factor 4 (OCT4), NANOG, and SRY-related high-mobility-group-box protein 2 (SOX2) (48). Studies had shown that the growth rate of MSCs and stemness were significantly enhanced when adipose-derived MSCs (AD-MSCs) were cultured under 1% hypoxic conditions (49). However, hypoxic culture could inhibit the osteogenic differentiation and adipogenic differentiation of MSCs. When the oxygen content was increased from 1 to 3%, the osteogenic differentiation ability of MSCs was restored (50). The serum may affect the heterogeneity of MSCs. There were differences in the cell proliferation, telomerase, and gene expression profile when hUC-MSCs were cultured in serum or serum-free medium (SFM). In the SFM, the hUC-MSCs had a slower proliferation rate, increased cell apoptosis, and higher pluripotency. Both SFM- and serum-containing medium-expanded hUC-MSCs finally obtained copy number variant in the long-term *in vitro* culture (51). In addition, adding a small amount of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interferon-γ (IFN-γ) to the culture medium enhanced the immunosuppressive function of AD-MSCs (52, 53). Otherwise, studies reported that the mechanical forces from extracellular matrix (ECM) materials and three-dimensional (3D) culture environment would affect cell polarity and cell–cell interactions, and further affect biochemical signaling, gene expression, and cell phenotype, finally contributing to the heterogeneity of MSCs (54, 55).

Heterogeneity among different tissue origins

Mesenchymal stem cells derived from various tissues will exhibit different cytological characteristics. So far, many sources of MSCs have been applied for cellular therapy (Table 1).

Araújo et al. interrogated the biological characteristics of different MSCs, such as AM-MSCs, PD-MSCs, CM-MSCs, and UC-MSCs, and analyzed their differentiation ability, immunophenotype, cell complexity, cell size,

Table 1. The source of MSCs in the body

Source	
1. Bone marrow	(BM-MSC)
2. Placenta	(PD-MSC)
3. Peripheral blood	(PB-MSC)
4. Wharton's Jelly	(WJ-MSC)
5. Adipose tissue	(AD-MSC)
6. Fetal pancreas	(FPan-MSC)
7. Chorionic plate	(CP-MSC)
8. Breast milk	(Br-MSC)
9. Chorionic villi	(CP-MSC)
10. Placental villi	(PV-MSC)
11. Dermal sheath cell	(DSC)
12. Placental Decidua basalis	(PDB-MSC)
13. Amniotic membrane	(AM-MSC)
14. Ligamentum flavum	(LF-MSC)
15. Amniotic fluid	(AF-MSC)
16. Dental pulp	(DP-MSC)
17. Umbilical cord	(UC-MSC)
18. Menstrual blood	(Men-MSC)
19. Umbilical cord blood	(UCB-MSC)
20. Arthroscopic flushing fluid	(AFF-MSC)
21. Umbilical cord lining	(UCL-MSC)
22. Dermal papilla	(DPC)
23. Umbilical cord matrix	(UCM-MSC)

polarity index, and growth kinetics (56). Although the surface marker profile and differentiation ability of all sources of MSCs were consistent with the characteristics of human MSCs, they had far differences in cell size, morphology, polarity, growth capacity, proliferation, and lifespan. The other research study reported that there were significant differences in phenotype, proliferation, migration, and immunogen among Wharton's jelly MSCs (WJ-MSCs), UCB-MSCs, PD-MSCs, and human umbilical cord lining MSCs (UCL-MSCs). UCL-MSCs showed the highest proliferation and migration rate but a lower cellular immune response. UCB-MSCs and WJ-MSCs significantly enhanced the release of IFN-γ (57). Kern et al. revealed that BM-MSCs had the shortest proliferation capacity, followed by AD-MSCs. However, UCB-MSCs showed the proliferation capacity in all passages analyzed (58).

The expression patterns of paracrine factors are also different among the different sources of MSCs. One of the research studies reported that although the secretion levels of angiogenin and vascular endothelial growth factor-A (VEGF-A) in MSC populations isolated from AD-MSCs, BM-MSCs, dermal sheath cells (DSCs), and dermal papillary cells (DPCs) were almost similar, the expression levels of insulin-like growth factor-1 (IGF-1), VEGF-D, and IL-8 in AD-MSCs were higher than other MSCs, suggesting their different therapeutic potential in clinic (59).

Otherwise, studies reported that MSCs derived from placental villi (PV-MSCs) had better immunomodulatory and pro-angiogenic activities than BM-MSCs, AD-MSCs, and UC-MSCs (60); UC-MSCs had a higher proportion of neuron-specific enolase-positive cells than BM-MSCs when they were induced to neurons (61). Human umbilical cord perivascular cells (hUCPVCs) had higher pluripotency and the expression of CD146 than BM-MSCs and could be more easily differentiated into osteogenic, cartilage, and adipogenesis (62); UC-MSCs promoted megakaryocyte production, while BM-MSCs could expand more megakaryocyte progenitor cells from $CD34^+$ hematopoietic precursor cells (63).

Heterogeneity of MSCs caused by different isolation methods

Different techniques have been used for MSC isolation. These techniques can affect the quantity and quality of the MSCs, and contribute to MSC heterogeneity (64). There are two main methods to obtain MSCs, including the enzymatic digestion and explant method. Hua et al. found that the proliferation rate of MSCs in the explant cure treated was higher than the MSCs in the enzymatic group. They found that the MSCs in the 10-mm size pieces group (explant cure methods) exhibited the highest number of cells, shortest primary culture time, and highest proliferation rates (65).

In another study, Horn et al. compared the isolation of BM-MSCs based on red blood cell (RBC) lysis with ammonium chloride, Ficoll density fractionation, and colony formation from untreated whole bone marrow. The colonies were larger through the RBC lysis method than through Ficoll density gradient separation, which might arise from the platelets maintained after the RBC lysis isolation. RBC lysis can be standardized more efficiently and faster than the Ficoll procedure for the clinical application of MSCs (66). The MSCs isolated with human platelet lysate (HPF) showed a higher osteogenic capability and proliferation ability than those treated with the fetal calf serum (67).

Markers involved in MSCs heterogeneity

Mesenchymal stem cells derived from bone marrow, fat, umbilical cord, and umbilical blood have significant differences in the expression of cell surface markers, such as CD271, Stro-1 (68), CD146, CD106 (69), nestin (70), GD2, and SSEA-4 (71, 72). Currently, these MSC surface markers may offer a breakthrough to reveal the heterogeneity of MSCs.

Stro-1 is one of well-known MSCs markers; however, it is not universally expressed in all reported types of MSCs (73). Immature MSCs have high proliferative rates and multi-differentiation potential and are associated with the high expression of Stro-1. The researcher showed that Stro-1^+ cells tend to have higher homing capabilities and gene delivery functions, while Stro-1^- cells support hematopoietic engraftment to a greater extent (74).

CD271 is highly expressed in BM- or AD-MSCs, not expressed in UC-MSCs and lowly expressed in placenta-derived MSCs (PD-MSCs). Studies have shown that $CD271^+$ hematopoietic stem cells (HSCs) and $CD133^+$ HSCs are co-transplanted into NOD/SCID-IL2Rγ (null) mice at a ratio of 8:1, $CD271^+$ HSCs have significantly promoting effects on lymphocyte engraftment compared with $CD133^+$ HSCs (75).

$CD146^+$ MSCs represented an MSC subpopulation that support hematopoiesis and secrete growth factors controlling the function of HSCs. $CD146^+PDGF\text{-}R\beta^+$ MSCs possessed a stronger self-renewal ability and can differentiate into adipocytes and osteoblasts compared with $CD146^-PDGF\text{-}R\beta^-$ cells (76).

CD106 is an adhesion protein, which is essential for the immunosuppression mediated by BM-MSCs and the binding of HPCs. Combining THY-1, LNGFR and CD106 can efficiently select BM-MSCs (77). Compared with the $CD106^-$ cells, the $CD106^+$ cells contained fewer osteoblasts and more adipocytes, indicating that CD106 can be used as a predictive indicator for the differentiation of BM-MSCs (78).

SSEA-4, an embryonic stem cell marker, is a marker of BM-MSCs and is used to isolate genuine MSCs from

bone marrow (79). On the contrary, the AT-MSCs, UC-MSCs, and UB-MSCs do not express SSEA-4 (80, 81). SSEA-4$^-$ BMSCs failed to grow, while SSEA-4$^+$ BMSCs expand extensively. Moreover, the expression of SSEA-4 in BMSCs gradually increased over time (79).

The pros and cons of heterogeneity in the clinical application of MSCs

So far, MSC-based therapy has been widely used in clinic. However, how the heterogeneity affects the clinical application of MSCs is still controversial (82). In 2009, the use of an industrial MSC product failed to meet its primary clinical end point of achieving a significant increase of complete response of SR-aGvHD (NCT00366145) (83). The failure may result from the limitation of MSCs diversity. All MSCs used in the phase-3 trial were derived from a single donor, and the MSCs were expanded to passages 3 and 4 to yield enough MSCs to treat all 240 participants. On the contrary, in the other research, Kuçi et al. treated 26 SR-aGvHD patients with pooled MSCs generated from multiple healthy donors. Finally, they got the result that a 77% overall response in GvHD patients was induced at the primary end point, which was much better than the result treated with MSCs derived from single donors (84). Although the pooled MSCs seem to circumvent the donor-to-donor heterogeneity, it is not as what the authors stated that this method can minimize the heterogeneity. In fact, this method certainly magnified the heterogeneity rather than solving the problem. The pooled method may be just expediency for therapy before clearly understanding the heterogeneity and purifying the MSCs.

For a long time, scientists have been dying to know whether different sources of MSCs are specifically beneficial to different indications. Thus, the study on the heterogeneity of MSCs derived from different tissue sources is quite significant to answer these questions.

Current methods used for the study of MSCs heterogeneity

The single-cell RNA-sequencing technology was regarded as one of the most powerful tools to investigate cell heterogeneity under various conditions (85). Hou et al. found that MSCs derived from the four tissues (BM, UC, adipose tissue, and synovial tissue) possessed different trilineage differentiation potentials through single-cell RNA-sequencing analysis (86). They found that UC-MSCs exhibited the highest immunosuppression. The differentiation potentials of MSC subsets showed significant differences in each other, and are strongly associated with their subtypes and tissue sources. Huang et al. analyzed the 361 single-cell transcriptomes of MSCs derived from two samples harvested at different passages and stimulated with or without inflammatory cytokines. The single-cell RNA-sequencing revealed the existence of subsets in hUC-MSCs, and these subsets were conserved independent of donors and passages. In addition, the expression of key cytokines and chemokines for MSCs-mediated immune modulation showed a similar expression pattern at different passages (p0, p2, p5) at the persistence of inflammatory factors (87).

Efforts have been made to explore the characteristics and differences of AD-MSCs and BM-MSCs at the single- and bulk-cell assays and further study the clinical effects of two types of cells in treating osteoarthritis. Single-cell RNA sequencing of AD-MSCs and BM-MSCs showed that the transcriptional heterogeneity of AD-MSCs cell population is lower than that of BM-MSCs. Furthermore, compared with BM-MSCs, AD-MSCs were less dependent on mitochondrial respiratory energy supply. Furthermore, AD-MSCs had a lower HLA I antigen expression level and higher immunosuppressive ability. Meta-analysis of current clinical trials using BMSCs to treat osteoarthritis showed that the therapeutic effect of AD-MSCs is more stable than that of BM-MSCs. AD-MSCs may be a more controllable source of stem cells, more suitable for survival in hypoxic joint cavities, and may be more advantageous in regulating inflammation (88).

Besides the single-cell sequencing, other technologies based on single-cell or colony assays were generated for the research field. It was reported that a single-cell FTIR microspectroscopy-based method was developed for the quantitative analysis of cellular heterogeneity by quantitatively calculating the cell-to-cell Euclidean distance. The result showed that the size, shape, and chemical component spatial distribution patterns exhibited remarkable heterogeneity among the different cell populations (89). Russell et al. developed a high-capacity assay to quantify the clonal heterogeneity of MSCs. The MSCs were classified according to colony-forming efficiency and quantified the trilineage differentiation potentials of MSC clones. The authors reported that the heterogeneity in the trilineage potential of normal BM-MSCs is more complex than previously reported (90). Rennerfeldt et al. quantified the heterogeneity that emerged over multiple cell divisions by observing BM-MSC colony formation in real time using time-lapsed optical imaging and analysis. They found that the cells in the subpopulation are pure initially but became functionally heterogeneous after cultured (91).

Proteomic methods have been extensively used to analyze MSC heterogeneity on the expression of proteins on cell surface that may be changed due to the passages (92). For example, FACSCAP Lyoplate proteomic analysis system has been used to research the expression changes of cell surface proteins in BM-MSCs through different culture passages (93).

Furthermore, besides the methods above mentioned, microfluidic technologies were used for the study of MSC heterogeneity (94, 95). It is well known that MSC

morphology and size may indicate differentiation potential of MSCs. Liu et al. developed a high-throughput microfluidic platform to analyze the MSC heterogeneity according to the cell size (96). They found that larger MSCs with slow proliferation displayed a senescent phenotype.

The new efforts for resolving the heterogeneity of MSCs

Mesenchymal stem cell heterogeneity is one of the greatest challenges for cell therapy and regenerative medicine. A lack of technologies or methods to dissect and resolve heterogeneity of MSCs greatly impeded the application of MSCs in the clinic and their standardized production. Although many efforts have been made to study the heterogeneity of MSCs, most works just focused on discovering the existence of heterogeneity and understanding how heterogeneous the MSCs are. However, many attempts have been made to establish the evaluation standard through bioinformatics analysis by mining the mass data of cell sequencing. However, these works were largely confined to existing biological knowledge. Controlling or eliminating the inherent heterogeneity of MSCs is the ultimate aim of this research field, is a crucial requirement to achieve the curative effect of MSC-based therapies, and is a premise of the industrialization of MSCs. Recently, new efforts have been made in this respect.

The idea of induced pluripotent stem cell (iPSC) generation have been used to produce highly uniform MSCs. The iPSCs may have the potential to overcome heterogeneity due to their capacity for infinite proliferation and multilineage differentiation. Recently, a kind of iPSC-derived MSCs from a single donor was produced by Ctmerus™ for the therapy of steroid-resistant acute graft versus host disease (SR-aGvHD) (97). The iPSC-MSC potency is consistent with MSCs of earlier generations undergoing the $CD4^+$ T-cell potency assay (98). Although iPSC-derived MSCs took a big step forward for the manufacturing of MSCs, they still cannot be a substitute for the genuine MSCs. Moreover, transgene technologies used for the generation of iPSC-derived MSCs caused new problems such as off-target effects and high cytotoxicity (99–101).

In our recent study, a novel approach was developed to produce single-cell-derived sphere (SCDS) that can reflect the potential of stem cells *in vitro* (102). SCDSs can be formed due to the self-renewal ability of single cells. To high throughput produce hUC-MSC SCDSs, special cell chips were fabricated for single hUC-MSCs two-dimensional (2D) patterning and 3D culturing for several days. Some hUC-MSCs formed SCDSs for their higher self-renewal ability. Compared with 2D cultured hUC-MSCs, 3D SCDSs had an enhanced self-renewal ability, multi-directional differentiation potential, anti-aging, anti-stress, migration ability, survival ability, and paracrine effects *in vitro*. The SCDS cultured hUC-MSCs remarkably promoted angiogenesis *in vivo* and displayed greater therapeutic potential on acute liver failure (ALF) in mice. This method based on cell chip screening provided a tool to purify the hUC-MSCs according to the self-renewal ability and also may offer a possibility to eliminate the inherent heterogeneity of MSCs.

Perspective

Although many preclinical and clinical studies have demonstrated the great potential of MSCs for cell therapy of various diseases, inherent heterogeneity is the main obstacle to impede MSCs' clinical application and industrialization. High-throughput sequencing (HTS), especially single-cell based HTS, offers a powerful tool to discover and evaluate the heterogeneity of MSCs. However, controlling and eliminating heterogeneity are the ultimate goals of the research field. In future, more technologies regarding the functional or phenotypic purification of MSCs need to be developed. Through purification, the functional or phenotypic heterogeneity of MSCs may be efficiently eliminated, and the quintessential and high-quality MSCs with higher self-renewal, differentiation potential, and paracrine abilities may be screened out from the bulk of MSCs. Integrating single-cell-based HTS, cell function assays, animal tissue repair experiments and clinical evaluation, the quality assessment standard of MSCs and indication selection criteria of cell therapy for different MSCs can be well established. Relying on these criteria, the standardized production of high-quality MSCs can be carried out in industrialization, and different sources of MSCs can be classified to meet different indications in clinic.

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Conflict of interest and funding

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