Generation of mesenchymal stem cells from induced pluripotent stem cells for regenerative medicine

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Abstract

Adult stem cells represent self-renewing, multipotent cells that are capable of producing mature cells of another tissue through differentiation. Stem cells can be sourced from a variety of tissues such as bone marrow, adipose tissue, and cord blood. Bone marrow derived mesenchymal stem cells (BM-MSCs) have the ability to differentiate, lack of ethical concern, no teratoma potential, easily cultured and expanded in vitro, and are immunomodulatory via paracrine effects. Thus, BM-MSCs have been actively utilized as a therapeutic agent in a variety of clinical trials to treat diverse diseases including neurologic diseases, cardiovascular diseases, hepatic diseases, lung injury, renal failure, cancer, wound healing and infections. However, concerns have been raised relative to high-cell population heterogeneity, poorly understood immune capabilities, and a tendency to lose biological functions in vitro with each passage. Recently, induced pluripotent stem cells (iPSCs) have emerged as a new class of stem cells with greater versatility. iPSC technology allows for potential treatment of any degenerative diseases or injury due to the pluripotent potentials and unlimited manufacturing. In this study, we established a method to generate iPSC-derived MSCs (iMSCs). The International Society for Cellular Therapy (ISCT) have imposed several criteria for MSCs, which include 1) plastic adherence, 2) stable expression of CD90, CD105, and CD73, and 3) ability to differentiate into adipocytes, chondrocytes, and osteocytes. Our results demonstrated that iMSCS have similar property to that of BM-MSCs by satisfying the criteria. Future directions include creating clinical grade iMSCs which can be used for future regenerative therapies.

Methods

iPSCs line

The iPSCs line was derived from skin fibroblasts of a healthy individual (M03192) was used in this study. The pluripotency was validated by cell-gene expression for pluripotent and onchogene marker analyses, which includes OCT4, NANOG, and DAPI (blue) Scale bar: 100 μm. Magnification: 20X.

Figure 1. Visualization of the experimental design. The iPSCs were differentiated to mesenchymal stem cells (MSCs) through the use of induction media and supplements over ~2 weeks. To generate iMSCs, the iPSCs from human fibroblasts were converted to iPSCs by defined factors. iPSCs were expanded to passage 3. The iPSCs were stained for OCT4, NANOG, and DAPI. In controls, iPSCs, iPSCs were expanded to passage 3 and stained for OCT4, NANOG, and DAPI.

Conversion of iPSCs to MSCs

The iPSCs were differentiated to MSCS through published protocols with minor modification. Embryonic bodies (EBs) were formed from iPSCs by culturing in a round bottom dish. The EBs were further differentiated into MSCs on a gelatin-coated dish in a TGF-β containing medium (Figure 2).

Immunophenotypic analysis for iMSCs

The iMSCs were confirmed to express cell surface marker expression with BM-MSC used as a positive control. Briefly, cells were expanded to passage 3 then washed with antibodies for the iMSC positive cell surface marker CD105, CD90, and CD73. Expression of the cell surface markers was verified using an Alexa Fluor flow cytometer.

Multilineage differentiation of iPSCs

The iPSCs were differentiated into chondrocytes, adipocytes and osteocytes using commonly available medium. For osteogenic and adipogenic differentiation, the iMSCs were grown for 12-14 days in specific differentiation medium and then fixed with 4% paraformaldehyde. Adipocyte differentiation was confirmed using BODIPY 493/503, a fluorescent stain that indicates the presence of lipids. Osteoblast differentiation was confirmed using Alizarin Red S staining (endoderm, mesoderm and ectoderm) and pluripotency assay through fluorescent and immunohistological assays after 14-21 days in appropriate culture medium. Experiments are currently planned to test the effectiveness of the iMSCs in T-cell proliferation. We also plan to conduct animal models to investigate the therapeutic effects of iMSCs on sepsis and radiation induced brain injury.

Results

In this study, we have established a method to generate mesenchymal stem cells (iMSCs) from induced pluripotent stem cells (iPSCs). We have demonstrated that our induced MSCs (iMSCs) meet the criteria set by the International Society for Cellular Therapy (ISCT). First, plastic adherence was evident during the culturing of the iMSCs. Second, the majority of the iMSC population expressed the positive cell surface markers CD105+, CD90+, and CD73+; while showing less than 1% of the negative cell surface markers. Third, pluripotency was validated by three germ layer stained with Alcian Blue, a stain that detects cartilage extracellular matrix.

Discussion & Future Directions

Our ultimate goal is to generate iMSCs from GMP-grade iPSCs, and develop new regenerative medicine to treat diverse diseases including but not limited to neurological diseases, cardiovascular diseases, hepatic diseases, lung injury, renal failure, cancer, wound healing and infections.

References


Figure 3. Flow cytometry data for induced mesenchymal stem cells. iMSCs (passage 3) were stained with antibodies specific to cell surface markers, CD105, CD90, and CD73, and used as positive controls. The data showed that iMSCs meet the criteria set by the ISCT

Figure 4. Immunophenotypic analysis for iMSCs. The iMSCs were differentiated into MSCs (MSCs), chondrocytes (Chondro), adipocytes (Adipocytes), and osteocytes (Osteocytes) using specific culture conditions for each differentiation. The cell surface markers were analyzed by FACS.
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