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-MSC) have the ability to differentiate, lack of ethical concerns, no teratoma potential, easily cultured and expanded ex vivo, and are immunomodulatory via paracrine effects. Thus, BM-MSCs have been actively used as a therapeutic agent in a variety of clinical trials to treat diverse diseases including neurological diseases, cardiovascular diseases, hepatic diseases, lung injury, renal failure, cancers, wound healing and infections. However, concerns have been raised related to high cell population heterogeneity, poorly understood homing 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 cell surface markers, and 3) ability to differentiate into adipocytes, chondrocytes, and osteoblasts. 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

iPSC line
The iPSC line derived from skin fibroblasts of a healthy individual (MC0192) was used in this study. The pluripotency was validated by three germ layer differentiation(endoderm, mesoderm and ectoderm) and pluripotency assay through immunostaining. The mRNA expressions of stem cell markers were also confirmed by RT-qPCR (**Figure 1**).

Conversion of iPSCs to iMSCs
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 verified by flow cytometry for cell surface marker expression with BM-MSC used as a positive control. Briefly, cells were expanded to passage 3 then stained with antibodies for the MSC positive cell surface markers CD105, CD90 and CD73. Expression of the cell surface markers was verified using an Attune NxT flow cytometer.

Multilineage differentiation of iMSCs
The iMSCs were differentiated into chondrocytes, adipocytes and osteocytes using commercially available medium. For osteogenesis and adipogenesis, cells were grown for 12-14 days in specific differentiation medium and then fixed in 4% paraformaldehyde. Adipocyte differentiation was confirmed using BODIPY 493/503, a fluorescent stain that indicates the presence of lipids. Osteocytes were confirmed using Alizarin Red S, a stain that detects extracellular calcium deposits. For chondrocyte differentiation, cells were grown for 21 days under specific differentiation medium and then fixed with 4% paraformaldehyde. Cells were then stained with Alcian Blue, a stain that detects cartilage extracellular matrix.

Figure 1

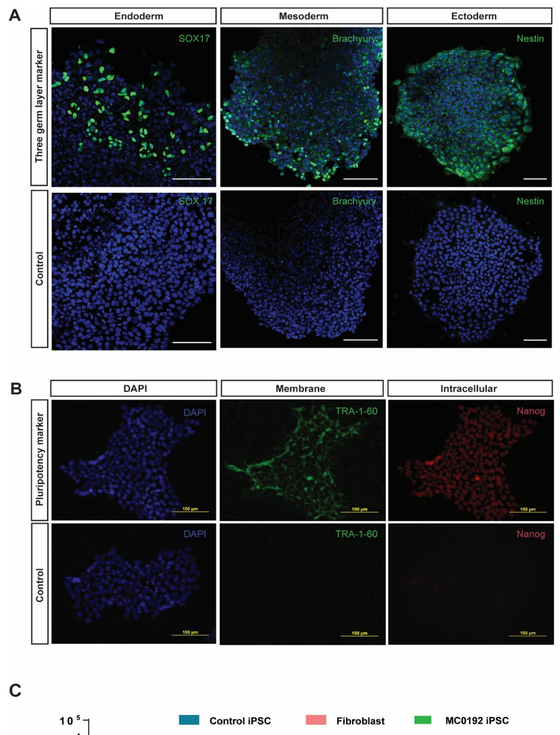


Figure 1. Characterization of human iPSC line (MC0192). (A) *In vitro* differentiation of the control iPSC line into all three primary germ layers. Cells were immunostained for SOX17 (endoderm), Brachyury (mesoderm), Nestin (ectoderm), and DAPI following fixation. Scale bar: 100 μm. Magnification: 20X. **(B)** Immunostaining of pluripotency markers (TRA-1-60 and Nanog), and DAPI (blue) Scale bar: 100μm. Magnification: 20X. **(C)** qRT-PCR measurements of mRNA levels of the reprogramming factors in a commercial iPSC and a control iPSC line (MC0192), which was quantified in relation to the skin fibroblast line.

Figure 2

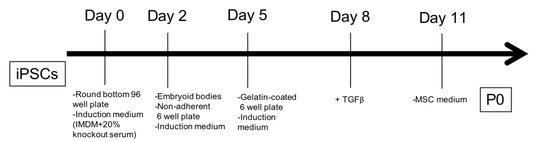


Figure 2. Visualization of the experimental design. The iPSCs from human fibroblasts were converted to mesenchymal stem cells (MSCs) through the use of induction media and supplements over ~2 weeks. To confirm the conversion, induced MSC should display plastic adherence, have positive expression of specific cell surface markers and be capable of differentiating into chondrocytes, adipocytes and osteocytes.

Figure 3

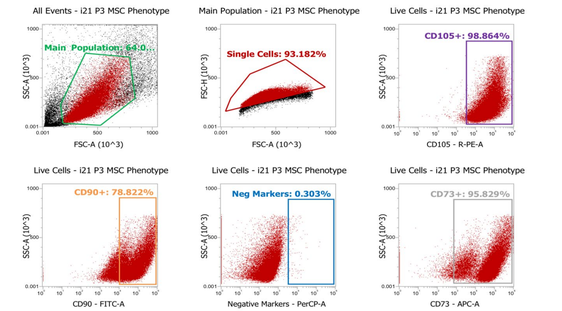


Figure 3. Flow cytometry data for induced mesenchymal stem cells. iMSCs at passage 3 were stained with antibodies specific to cell surfaces markers CD105+, CD90+ and CD73+ and a cocktail of negative control markers. The iMSC population was over 95% for the cell surface markers CD105+ and CD73+ and ~79% for CD90+ when analyzed by FACS.

Figure 4

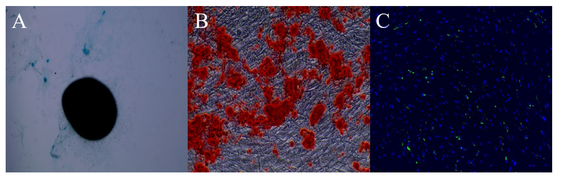


Figure 4. Representative images of the differentiated induced mesenchymal stem cells. (A) Chondrogenesis staining after 21 days by Alcian Blue. **(B)** Osteogenesis staining after 14 days by Alizarin Red S. **(C)** Adipogenesis immunostaining after 14 days with BODIPY and DAPI.

Results

Immunophenotypic analysis

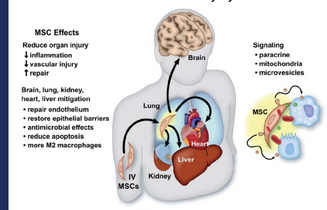
- The iMSC population had greater than 95% expression of the positive cell surface markers CD105+ and CD73+ and ~79% expression for the positive cell surface marker CD90+. Expression of negative cell surfaces markers was less than 1% (**Figure 3**).

Multilineage differentiation

- Successful multilineage differentiation of the iMSCs was observed (**Figure 4**). Positive staining with Alcian Blue, Alizarin Red S and BODIBY indicate chondrogenesis, osteogenesis and adipogenesis occurred in the iMSCs, respectively.

Discussion & Future Directions

In this study, we have established a method to generate mesenchymal stem cells (MSCs) from induced pluripotent stem cells (iPSCs). We have demonstrated that our induced MSCs (iMSCs) meet the criteria set by the International Society of 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 providing evidence that the iPSC cells have been differentiated into MSC. Last, multilineage differentiation of the iMSCs was confirmed 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.



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Our ultimate goal is to generate iMSCs from GMP-grade iPSCs, and develop new regenerative medicine to treat diverse diseases including neurological diseases, cardiovascular diseases, hepatic diseases, lung injury, renal failure, cancers, wound healing and infections.

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