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Extended Abstract
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Stem Cell Congress 2019-Robust, Efficient and Pure Induced Mesenchymal Stem Cells Generation from mRNA Induced Pluripotent Stem Cells in Suspension- Rajneesh Verma- Stem Cells Co. Ltd

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Introduction

Induced Pluripotent Stem Cells (iPSCs) were first established in 2006. Later, human iPSCs were successfully derived by Thompson and many other groups . In past years, integrative method was initially employed to deliver reprogramming factors for iPSC generation . These methods had the potential to produce tumorigenic insertional mutations and residual or reactivation of transgene expression during iPSC differentiation . To overcome these problems, various methods were explored to derive transgene-free iPSCs, such as plasmid vectors, minicircle DNA vectors, piggyBac, mRNA, adenovirus, Sendai virus, proteins, small molecules and episomal. Expression of mRNA reprogramming factor provides another way of making transgenefree iPSCs. It has been shown that mRNAs transcribed in vitro can express reprograming factors efficiently when transfected into human fibroblasts. The mRNA is immediately converted into proteins by ribosomes after the delivery of synthetic mRNA into the cytosol and no entry into the nucleus is needed. Induced MSCs (iMSCs) derivation and characterization from iPSCs are on the rise. When applied to a range of animal models, iMSCs have been shown to promote regeneration and healing; multiple sclerosis, limb ischemia, arthritis, liver damage, bone defects, wound healing, and brain hypoxia. The primary human bone marrow Mesenchymal Stem Cells (MSCs) comprise a sub-population of multipotent stem cells that hold the capacity for osteogenic, chondrogenic and adipogenic differentiation. Such multipotent MSCs are isolated from fetal femur in addition to adult sources. MSCs offer significant benefits because of their highly proliferative, immune-modulatory properties and paracrine orchestration is therapeutic potential for an increasing aging demographics. MSCs distinct from iPSCs (iMSCs) is a cell type derived from iPSCs that are of primary interest to bypass shortcomings associated with primary MSCs. It has already been shown the similarity of iMSCs to primary MSCs and their in vivo regenerative capacity. In addition, donor age expression in iMSCs has been shown to be restored to a younger state and expressed in iMSCs from patients with early-onset aging syndromes. iPSC-derived iMSCs are identified as a potential source of transplantable donor cells for regenerative therapies clinic and subsequently differentiated to pure iMSCs without iPSC in culture using a suspension process. Such iMSCs are contrasted with UC-MSC in cells based markers, cell cycle, senescence tests, and in-vitro differentiation.

Material and Methods

Ethical approval:

Skin samples were obtained from a healthy volunteer donor (Full genetic test was conducted prior to the biopsy) from 38 yrs old male (human), with written permission from the Stem Cell 21 Ethics Committee, Bangkok (Thailand).

Fibroblasts culture

Fibroblasts have been isolated from 4 mm Healthy donor skin biopsy, which has been fully screened for any genetic mutations or abnormalities. The skin was chopped using sterile surgical instruments and plated in six-well dishes with a medium xenofree fibroblast plate (FP) (Fibro-life Cat. No: LM-0001) and was grown for 14 days in a 5% CO2 incubator at 37°C [4].

Reprogramming fibroblasts by mRNA

Skin fibroblasts of 60000 cells were reprogrammed using mRNA 3rd generation Reprogramming Kit (Stemgent Cat. No: 00-0076) according to manufacturer instructions. iPSC, were expanded under xeno-free conditions on iMatrix (Reprocell Cat. No: NP892-011) with Nutristem medium (Reprocell Cat. No: 01-0005) 5% CO2 at 37°C as described previously

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[6]. Alkaline phosphatase staining Putative iPSC colonies were tested for alkaline phosphatase (AP) using a diagnostic AP substrate kit according to the manufacturer's specification (Stemgent Cat. No: 00-0055).

Results

Human foreskin fibroblasts were expanded at low passages and reprogramed using mRNA Reprogramming Kit (Reprocell). Individual colonies were picked and subcultured into individual cell lines after 10-15 days and analyzed at cellular and genetic level to confirm successful reprogramming. After 25 days generated colonies displayed a typical human Embryonic Stem Cell (hESC) colony-like morphology with refractive edges as seen by bright field (BF) and phase-contrast (PC) microscopy and the cells had high nuclear/cytoplasmic ratio. Once infected four times fibroblasts with mRNA, the spindlelike morphology changed to small compact cells similar to pluripotent cells Figure 2. A red stain assay called alkaline phosphatase has confirmed the pluripotent reprogramming of fibroblasts using the mRNA method.

Conclusion

Takahashi and Yamanaka's discovery of iPSCs is really a decadelong breakthrough in stem cell science. The last decade has seen tremendous improvement in our understanding of induced pluripotency molecular mechanisms, and in 2014 we moved from the "bench to bedside." The latest long-term study involving the application of dopaminergic neurons in primate derived from human iPSC at the Center for iPS Cell Research and Development, Kyoto University, Japan, primate Parkinson's disease (PD) models shows that human iPSCs are medically relevant to the care of PD patients. The cell therapy based on the iPSC is still in its infant stage. The remaining obstacles that block the path to effective clinical therapy implementation of this technology must be resolved. The footprint-free iPSCs obtained through mRNA-based reprogramming are promising cells for clinical application to generate desired cell types. Highly efficient footprint-free iPSC generation and effective differentiation into iMSC will increase this technology's potential in translational research, therapy, and disease modeling.

References

1. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131:861-872.

2. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. Science. 1998;282:1145-1147 .

3. Bellin M, Marchetto MC, Gage FH, Mummery CL. Induced pluripotent stem cells: the new patient? Nat Rev Mol Cell Biol. 2012;13:713-726.

4. Zhou H, Wu S, Joo JY, Zhu S, Han DW, Lin T, et al. Generation of induced pluripotent stem cells using recombinant proteins. Cell Stem Cell. 2009;4:381-384.

5. Warren L, Manos PD, Ahfeldt T, Loh YH, Li H, Lau F, et al. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. Cell Stem Cell. 2010;7:618-630.

6. Yakubov E, Rechavi G, Rozenblatt S, Givol D. Reprogramming of human fibroblasts to pluripotent stem cells using mRNA of four transcription factors. Biochem Biophys Res Commun. 2010;394:189-193.

7. Stadtfeld M, Nagaya M, Utikal J, Weir G, Hochedlinger K. Induced pluripotent stem cells generated without viral integration. Science. 2008;322:945-949.

8. Teramura T, Onodera Y, Mihara T, Hosoi Y, Hamanishi C, Fukuda K. Induction of mesenchymal progenitor cells with chondrogenic property from mouse-induced pluripotent stem cells. Cell Reprogram. 2010;12:249-261. 9. Villa-Diaz LG, Brown SE, Liu Y, Ross AM, Lahann J, Parent JM, et al. Derivation of mesenchymal stem cells from human induced pluripotent stem cells cultured on synthetic substrates. Stem Cells. 2012;30:1174-1181.

10. Bilousova G, Jun du H, King KB, De Langhe S, Chick WS, Torchia EC, et al. Osteoblasts derived from induced pluripotent stem cells form calcified structures in scaffolds both in vitro and in vivo. Stem Cells. 2011;29:206-216

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