EARLY AND PROLONGED MESENCHYMAL INDUCTION FOR CHONDROGENIC DIFFERENTIATION OF HUMAN DERMAL FIBROBLAST-DERIVED INDUCED PLURIPOTENT STEM CELLS

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Induced pluripotent stem cells (iPSCs) hold a great promise in regenerative medicine due to large self-renewal capacity, no ethical controversies and possibility to rise every cell type of the body. Defined three germ layers formation of mesoderm, ectoderm and endoderm during iPSCs differentiation is a crucial step for the further wanted cell differentiation. So far, several early lineage commitment protocols have been developed for mesodermal induction of iPSCs in monolayer or embryoid body formation with defined growth factors Activin A, Wnt3, bFGF (and others) alone or in combinations. Second step is the prolonged mesodermal differentiation of early induced mesenchymal cells. However, current methods of mesenchymal induction are not finally understood and developed, for this reason effective and eligible differentiation may not be reached.

For this study, we derived hiPSCs from several human dermal fibroblasts, which showed normal karyotype and endogenous expression of pluripotent markers like OCT4, SOX2, NANOG (by RT-PCR), TRA-1-60, SSEA4 (by Flow cytometry) and TRA-1-60, ALP (by ICF) were detected. Chondrogenic differentiation was induced through early and prolonged mesenchymal differentiation in monolayer or pellets cultures with diverse combination of growth factors. Morphological and cell growth changes were typical for the stage of differentiation. Expression of pluripotency or differentiation related genes, including OCT4, SOX2, ATF3, TGIF1, CAV1.2, COL1, COL2, ACAN, SOX9 were evaluated in iPSCs differentiation. Dynamical changes in expression of those genes demonstrated the mesenchymal induction and efficient chondrogenic differentiation as determined on days 3, 14 and 21. Histochemical staining with Safranin-O and immunohistochemical Collagen type II detection confirmed chondrogenic differentiation.

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