

Generation of induced pluripotent stem cells from normal human dermal fibroblast using non-integrative Sendai virus

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Abstract

Induced pluripotent stem cells (iPSC) exhibited embryonic-like properties with unlimited self-renewal and multilineage differentiation properties, which is a potential cell source in regenerative medicine and transplantation therapy. Although retroviral and lentiviral transduction methods to generate iPSC are well established, the risk of mutagenesis limited the use of the cells in therapeutic applications. This study aims to generate iPSC from normal human dermal fibroblast (NHDF) cell line via non-integrative Sendai virus (SeV) transduction. When NHDF reached 70-80% confluency, SeV vectors expressing Yamanaka factors (Oct4, Sox2, Klf4 and c-Myc) were added and incubated for 24 hours. On day 6, the transduced cells were re-plated on a vitronectin-coated plate and daily medium change was performed. On day 18-26, colonies with embryonic stem cell (ESC)-like cellular morphology were observed and transferred to a new vitronectin-coated plate. After Passage 10, the iPSC generated were free of SeV as confirmed with RT-PCR. NHDF-derived iPSCs expressed multiple pluripotency markers by immunofluorescence staining and qRT-PCR as compared to parental NHDF. Following suspension culture in low attachment plate for 8-10 days, iPSC formed embryoid body-like spheres, similar to ESC. NHDF-derived iPSC also demonstrated the ability to undergo directed differentiation into cells of different germ layers. Taken together, NHDF were successfully reprogrammed into iPSC using non-integrating SeV. Further characterisation, such as teratoma formation and genome-wide sequencing, are required to elucidate the molecular profile and function of these cells.

Keywords: Induced pluripotent stem cells, human dermal fibroblast, Sendai virus

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