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SNL fibroblast feeder layers support derivation and maintenance of human induced pluripotent stem cells

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Abstract

Induced pluripotent stem (iPS) cells can be derived from human somatic cells by cellular reprogramming. This technology provides a potential source of non-controversial therapeutic cells for tissue repair, drug discovery, and opportunities for studying the molecular basis of human disease. Normally, mouse embryonic fibroblasts (MEFs) are used as feeder layers in the initial derivation of iPS lines. The purpose of this study was to determine whether SNL fibroblasts can be used to support the growth of human iPS cells reprogrammed from somatic cells using lentiviral expressed reprogramming factors. In our study, iPS cells expressed common pluripotency markers, displayed human embryonic stem cells (hESCs) morphology and unmethylated promoters of *NANOG* and *OCT4*. These data demonstrate that SNL feeder cells can support the derivation and maintenance of human iPS cells.

Keywords: induced pluripotent stem cells; derivation; maintenance; SNL; feeder cells

Introduction

Human embryonic stem cells (hESCs) are regarded as pluripotent cells since they have the ability to differentiate into virtually all cell types. hESCs hold great promise for regenerative medicine and have become a powerful tool for basic research (Thomson et al., 1998; Reubinoff et al., 2000). There are, however, several factors which limit the clinical applications of hESCs. One challenge is immunological incompatibility causing rejection and necessitating the use of immune-suppressing agents (Cabrera et al., 2006). In addition, the generation and use of hESCs is restricted in several countries due to legal and ethical considerations.

To overcome these limitations, a new research field of cell reprogramming has emerged based on the over-expression of selected transcription factors relevant to the pluripotent phenotype. This technology represents a breakthrough in regenerative medicine. One benefit of these induced pluripotent stem (iPS) cells is that since they can be derived from the patient, the problem of immunological incompatibility can be eliminated. Another benefit is that they largely remove the ethical concerns that restrict the use of hESCs.

iPS research is now in its infancy, and there are still many questions that need to be clarified. The process of derivation and maintenance of iPS cells needs to become more efficient and less complicated. Since the presence of

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feeder cells is required for self-renewal of hESCs and iPS cells (Nichols et al., 1998), investigators that have established such lines have generally used mouse embryonic fibroblasts (MEFs) as feeder cells (Thomson et al., 1998; Yu et al., 2007). As proliferation of primary MEFs is limited, it is necessary to isolate MEFs from mouse fetuses repeatedly to supply feeders for ES cells or iPS cells culture. MEFs also tend to lose the capacity to support proliferation of ES cells or iPS cells with increasing passages.

The SNL cell line is an immortalized subclone of the STO line manipulated to stably express the neomycin resistance and leukaemia inhibitory factor (LIF) genes (McMahon and Bradley, 1990). The STO cell line itself was derived from mouse SIM embryonic fibroblasts, and is resistant to 6-thioguanine and ouabain, sensitive to HAT selection (hypoxanthine, aminoprotein and thymidine) and negative for HPRT (hypoxanthine guanine phosphoribosyl transferase). As SNL cells are immortalized, they are easier to maintain than MEFs for the preparation of feeder layers. However, few groups have used it as a feeder cell line for iPS cell derivation. Shinya Yamanaka and his group utilized SNL feeder cells for the induction of mouse and human iPS cells by retrovirally delivered factors (*OCT4*, *SOX2*, *KLF4* and *C-MYC*) (Takahashi and Yamanaka, 2006; Takahashi et al., 2007). In this study, we provide evidence that SNL cell line can be used for the establishment and maintenance of human iPS cells induced by lentiviral reprogramming factors (*LIN28*, *NANOG*, *OCT4* and *SOX2*).

Materials and methods

Cell culture

Human fibroblasts cells (IMR90: CCL-186, ATCC, USA) were cultured in Dulbecco's Modified Eagle Medium (DMEM) (HyClone Laboratories, UT, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS, HyClone Laboratories), 2 mmol/L L-glutamine, 50 U/mL penicillin and 50 mg/mL streptomycin (HyClone Laboratories). Approximately 2×10^5 IMR90 cells were seeded in 6-well gelatin-coated plates the day before transduction. The cells were transduced using all four reprogramming factors in the presence of polybrene at 6.0 μg/mL final concentration in complete culture medium. Two rounds of overnight infection were performed. The medium was changed daily for 2 days. Cells were split 1 to 2 onto a layer of inactivated SNL cells and the media changed to serum-free human ES culture medium (DMEM/F12 supplemented with 20% KnockOut Serum Replacement (Invitrogen, Carlsbad, CA, USA), 0.1 mmol/L MEM non-essential amino acids (Sigma, USA), 1 mmol/L L-glutamine (HyClone Laboratories), 0.1 mmol/L β-mercaptoethanol (Sigma) and 5 ng/mL basic recombinant human fibroblast growth factor (bFGF; Invitrogen, Camarillo, CA, USA)).

At approximately 3 weeks, colonies with hESCs morphology (iPS colonies) were isolated mechanically for expansion. After the first five passages, an enzymatic method using collagenase IV (1 mg/mL) was used. Human iPS colonies were passaged every 4 to 6 days.

SNL cells were cultured in DMEM containing 2 mmol/L L-glutamine, 50 U/mL penicillin and 50 mg/mL streptomycin (HyClone Laboratories), 0.1 mmol/L MEM non-essential amino acids (Sigma), 0.1 mmol/L β-mercaptoethanol (Sigma) and 10% FBS (HyClone laboratories).

Human ES cell line H1 (WA01) and human iPS cells derived from foreskin (iPS(Foreskin)-1-DL-1) (purchased from WiCell, USA) were cultured on MEFs in serum-free human ES culture medium.

Mitotic inactivation of SNL cells

Cells were treated with 10 mg/mL Mitomycin C (Sigma) for 1.5 h. The Mitomycin C treated SNL cells were washed extensively in PBS and replated on 0.1% gelatin coated tissue culture dishes at a density of 3.5×10^4 cells/cm^2. Feeder cell dishes were used within a week of plating. The original culture medium was changed to stem cell medium just before iPS cells were added.

Lentiviral production

Expression lentiviral vectors of human *OCT4*, *SOX2*, *NANOG* and *LIN28* genes were obtained from Addgene (Yu et al., 2007). Viral particles were produced in the 293FT cell line using the psPAX2 packaging plasmid, pMD2.G envelope plasmid and FuGENE HD transfection reagent (Roche, USA). Virus was harvested at 36 h post-transfection for 3 days and concentrated 30-fold by ultracentrifugation. Viral stocks were stored at –80°C.