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Original Article

Generation of high quality of hepatocyte-like cells from induced pluripotent stem cells with Parp1 but lacking c-Myc

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Abstract

Background: Induced pluripotent stem cells (iPSCs) have a great potential for application in patient-specific therapy. The reprogramming method that does not involve c-Myc reduces tumorigenic risk, but also largely reduces the efficiency of generation of iPSCs, especially for those reprogrammed from damaged cells. Poly(ADP-ribose) polymerase 1 (Parp1) catalyzes a reaction of poly(ADP-ribosylation) and has been reported to enhance cell reprogramming.
Methods: Using Oct-4/Sox2/Klf4/Parp1 (OSKP) reprogramming method, reprogramming factors plus Parp1 were capable of generation of iPSCs from adult fibroblasts and further toward to differentiate from iPSCs status into hepatocyte-like cells.
Results: Our results showed that Oct-4/Sox2/Klf4/Parp1 (OSKP)-derived iPSC exhibited regular pluripotent properties, long-term passages and more stable cellular-divided period. These OSKP-derived iPSCs can effectively differentiate into hepatocyte-like cells (OSKP-iPSC-Heps), and present high mRNA levels of Sox17, HNF3b, and HNF4a in OSKP-iPSC-Heps. The mature hepatic functions, including CYP3A4, LDL uptake, glycogen synthesis and urea secretion were analyzed and well detected in OSKP-iPSC-Heps on day 14 post-differentiation.
Conclusion: In conclusion, we demonstrated that Parp1 promoted reprogramming process to generate the high quality of iPSCs, which could be used as a high quality source of hepatocytes.

Keywords: Hepatocyte; Induced pluripotent stem cells; Poly(ADP-ribose) polymerase 1

1. Introduction

Orthotopic liver transplantation has shown efficacy in the treatment of end-stage liver failure, liver cirrohsis[1−3] and Non-alcoholic fatty liver disease (NAFLD).[4] However, high cost, donor organ shortage and life-long immunosuppressive medications limit the availability of such treatment.[5] Recently, transplantation of mesenchymal stem cells in mice resulted in functional engraftment[6] and remarkable therapeutic efficacy[7] in recipients affected by NASH, suggesting that cell-based therapy

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can be applied as a feasible alternative to treatment of this disease. However, the molecular and cellular mechanisms of NASH are still not fully understood. Therapeutic strategies that target specific pathways in the pathogenesis are urgently needed.

Recent progress in induced pluripotent stem cell (iPSC) study has demonstrated that somatic cells can be reprogrammed into a pluripotent state by forced expression of Yamanaka's four factors.[8−11] This novel technology has raised the possibility of personalized therapy, using patient-specific iPSCs. However, senescence is a critical barrier that may limit reprogramming. To overcome this obstacle, Lapasset et al. have reported a protocol using a combination of six factors, Oct4/Sox2/Klf4/c-Myc/Lin28/Nanog, to improve iPSC reprogramming efficiency of senescent fibroblasts derived from aged donors.[12] However, this protocol uses c-Myc, which is an proto-oncogene that has been linked to the risk of tumorigenesis, and therefore it may hinder its clinical application.[13] Although avoiding the use of c-Myc gene reduced the tumorigenic incidence, the reprogramming efficiency was concomitantly suppressed.[14,15] As can be expected, the use of such c-Myc-free protocol may have particularly low efficiency in reprogramming of senescent cells. Therefore, it remains an open question how to efficiently increase reprogramming rate and pluripotency of senile tissues/fibroblasts and simultaneously avoid the undesired effects induced by exogenous c-Myc transduction.

Poly(ADP-ribose) polymerase 1 (Parp1), enzyme that catalyzes PARylation, is a key effector involved in DNA repair, replication, transcription and genomic methylation.[16,17] It has been observed that hepatocytes from Parp1-deficient mice have DNA damage and decreased proliferative responses to mitogens.[18] Malfunction of Parp1 signaling may exacerbate diet-induced obesity and insulin insensitivity.[19] These findings suggested that Parp1 is a crucial factor in hepatic protection. Recently, in the work of Doege et al. and in our previous work, it was demonstrated that Parp1 can promote reprogramming,[20,21] particularly in the absence of c-Myc gene.[21] Hence, it will be important to investigate whether Parp1 could also enhance iPSC generation from senescent somatic cells. Whether such resultant iPSCs, generated by Parp1-mediated reprogramming, can be employed as a cell source of high qualities of hepatocyte generation, remains an open question.

In the present study, we demonstrated that Parp1 can enhance iPSC generation from somatic cells in the presence of three other reprogramming factors Oct4/Sox2/Klf4 (OSKP). After induction of hepatic differentiation into hepatocyte-like cells (iPSC-Heps), these OSKP-iPSC-Heps expressed liver-specific markers and characteristics, exhibited mature hepatocyte functions. Our data may facilitate development of safe protocol for the patient-specific cell therapy of elderly patients with liver failures.

2. Methods

2.1. Generation of iPSC cell lines and differentiation protocols

iPSCs were generated from skin fibroblasts derived from the normal donors by the transduction of retroviral vectors encoding four transcription factors (Oct-4/Sox2/Klf4/c-Myc; OSKM), three transcription factors (Oct-4/Sox2/Klf4; OSK), or three factors plus Parp1 (Oct-4/Sox2/Klf4/Parp1; OSKP), as described previously.[15] Total of 12 clones (Re-1 to Re-12; OSKM) were selected and established. Undifferentiated iPSCs were routinely cultured and expanded on mitotically-inactivated MEFs (50,000 cells/cm2) in six-well culture plates (BD Technology) in the presence of 0.3% leukemia inhibitory factor in an iPSC medium consisting of Dulbecco's Modified Eagle's Medium (DMEM; Sigma), supplemented with 15% fetal bovine serum (FBS; Invitrogen), 100 mM minimal essential medium (MEM) nonessential amino acids (Sigma), 0.55 mM 2-mercaptoethanol (Gibco), and antibiotics (Invitrogen), and 0.3% leukemia inhibitory factor. Every three to four days, colonies were detached with 0.2% collagenase IV (Invitrogen), dissociated into single cells with 0.025% trypsin (Sigma−Aldrich) and 0.1% chicken serum (Invitrogen) in PBS, and replated onto MEFs. For embryoid body (EB) formation, iPSCs were dissociated into a single cell suspension by 0.25% trypsin−EDTA and plated onto non-adherent culture dishes in DMEM with 15% FBS, 100 mM MEM nonessential amino acids, 0.55 mM 2-mercaptoethanol and antibiotics at a density of 2×10^6 cells/100 mm plate. After 4 days in floating culture, EBs were transferred onto gelatin-coated plates and maintained in the same medium for 24 h. EBs were then induced to differentiate into hepatocyte lineage by using a two-step procedure as previously described.[15] For endoderm induction, iPSCs were incubated for 24 h in RPMI 1640 medium (Invitrogen/Gibco, Rockville, MD, USA), supplemented with 100 ng/ml Activin A (Peprotech). D the following 2 days, 0.1 and then 1% insulin-transferrin-selenium (Invitrogen/Gibco) was added to this medium. Following Activin A treatment, the differentiated iPSCs were cultured in Hepatocyte Culture Medium (HCM) (Cambrex, Baltimore, MD, USA) containing 30 ng/ml FGF4 for 4 days. Then, the differentiated cells were incubated in HCM containing 20 ng/ml HGF for 6 days, in HCM containing 10 ng/ml oncostatin-M (R&D, Minneapolis, MN, USA) plus 0.1 mM dexamethasone (Sigma Aldrich) for 5 days.

2.2. Real-time reverse transcription-polymerase chain reaction (RT-PCR)

Real-time RT-PCR was performed as previously described.[22] For real-time RT-PCR analysis, the total RNA was extracted using the RNeasy kit (Qiagen, Valencia, CA). The total RNA (1 μg) from each sample was reverse transcribed, using 0.5 μg of oligo dT and 200 U Superscript II RT (Invitrogen, Carlsbad, CA). The amplification was carried out in a total volume of 20 μl, containing 0.5 μM of each primer, 4 mM $MgCl_2$, 2 ml LightCycler FastStart DNA Master SYBR green I (Roche Diagnostics, Pleasanton, CA) and 2 ml of 1:10 diluted cDNA. The quantification of the unknown samples was performed by LightCycler Relative Quantification Software, version 3.3 (Roche Diagnostics). In each experiment, the GAPDH housekeeping gene was amplified as a reference standard. PCR reactions were prepared and performed in

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