Single OCT4 gene introduced into human bone-marrow derived mesenchymal stromal cells can generate putative iPS cells with the potential to differentiate into CD34+ hematopoietic progenitor cells ex vivo

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Abstract

Background: The ex vivo production of CD34+ hematopoietic progenitor cells from human bone-marrow mesenchymal stromal cells derived induced pluipotent stem cells (iPSCs) could serve as a feasible way to study patient-specific hematological disease from the perspective of hematopoietic differentiation. Different studies using virus-based or virus-free methods to reprogramming somatic cells into iPSCs by using fewer than four transcription factors, of which have the potential to differentiation in CD34+ hematopoietic progenitor cells. In this study, we demonstrate the generation of putative iPS cells from BMSCs with single OCT4 by plasmid transfection, which can differentiate into hematopoietic progenitor cells in defined culture system.

Objective: To generate induced pluripotent stem cells (iPSCs) from bone marrow stromal cells (BMSCs) using a plasmid pcDNA3.1 constructed with a single transcription factor gene OCT4 (pcDNA3.1-OCT4) and to evaluate the hematopoietic differentiation potential of the putative BMSCs-iPSCs.

Methods: BMSCs with ectopic high expression of OCT4 (BMSCs-OCT4) previously established by our group were cultured in traditional human ESC medium. Colonies with characteristic embryonic stem (ES) cell morphologies were selected and expanded in vitro. The undifferentiated status of putative BMSCs-iPSCs was confirmed by alkaline phosphatase (ALP) staining, telomerase activity assay, pluripotent marker expression and differentiation in vitro to form EBs and in vivo teratoma formation. The expression of pluripotent markers and ES markers were verified by RT-PCR, flow cytometry (FCM) and cellular immunofluorescence assay (CIFA). The hematopoietic differentiation potential into CD34+ progenitor cells by exposure to a defined culture system supplemented with a cocktail of hematopoietic growth factors was evaluated, of which the expression was confirmed by RT-PCR and FCM.

Results: BMSCs were successfully reprogrammed into pluripotent stem cells resembling ESCs by introduction single transcription factor OCT4 gene constructed into the eukaryogenic plasmid pcDNA3.1. The putative BMSC-iPSCs were positive for ALP and telomerase activity, as well as the pluripotent stem cell markers including TRA-1-60, SSEA4, TRA-1-81, SOX2 and NANOG as detected by

FCM and CIFA. Moreover, the above MSCs-OCT4 could form EBs ex vivo and express ectoderm (TUBB3+, WNT1+), mesoderm (Brachyury+, TBX20+), and endoderm (SPARC+) genes. By treatment with a cocktail containing BMP4 (50ng/ml), IL-3 (10ng/ml), IL-6 (10ng/ml), Flt-3 Ligand (300ng/ml), SCF (300ng/ml) and G-CSF (50ng/ml), the proportion of CD34+ progenitor cells increased from $0.93 \pm 0.46\%$ in untransfected parental BMSCs and $1.58 \pm 1.29\%$ in undifferentiated BMSC-iPS cells to $16.16 \pm 1.27\%$ and $25.40 \pm 3.08\%$ in day 14 and 21 differentiated BMSC-iPS cells, respectively. Moreover, the proportion of CD34+ progenitor cells were higher in the group with diverse concentration of growth factor cocktail induction, the proportion of CD34+ cells reached $31.39 \pm 3.60\%$ and $73.68 \pm 6.63\%$ in day 14 and 21 differentiated BMSC-iPS cells, respectively.

Conclusion: In this study, we have clearly demonstrated the generation of putative iPS cells (or partly reprogrammed iPSCs from BMSCs with ectopic high expression of OCT4 by plasmid transfection). The BMSCs-derived iPSCs display the typical morphology and growth pattern as iPS cells when they are maintained in undifferentiated pluripotent state. Moreover, the putative BMSCs-derived iPSCs can differentiate into hematopoietic progenitor cells in defined culture system containing a cocktail of six or seven growth factors. Our findings provide a feasible way to generate hematopoietic progenitor cells using patient-specific iPSCs generated by plasmid transfection for hematological disease modeling.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed.

However, the manuscript can be downloaded and accessed as a PDF.

Tables

Table 1 Primers for TFs and ectoderm, endoderm, mesoderm Genes by RT-PCR

Genes	Primer Sequences (5'-3')	Products (bp)
<i>GAPDH</i>	For: GAAGGTGAAGGTCGGAGTC Rev: GAAGATGGTGATGGGATTTC	226
<i>OCT4-I</i>	For: CGGGCTGATGGGCAAGTT Rev: GGGCAGGAAGGATGGGTAA	247
<i>OCT4-II</i>	For: CGCAAGCCCTCATTTAC Rev: GGCAGGCACCTCAGTTTG	1 143
<i>NANOG</i>	For: CAAAGGCAAACAACCCACTT Rev: CTGGATGTTCTGG GTCTGGT	426
<i>SPARC</i>	For: CCTGATGAGACAGAGGTGGTGG Rev: GCTTGTGGCCCTTCTTGGTG	349
<i>TBX20</i>	For: AAGGAGGCGACGGAGAACA Rev: TCCTGCCCCGACTTGGTGAT	289
<i>TUBB3</i>	For: AGCTCAACGCTGACCTGC Rev: GCCTCGGTGAACTCCATCT	499
<i>WNT1</i>	For: CCGATGGTGGGGTATTGTG Rev: TCATGAGGAAGCGCAGGTC	500
<i>Brachyury</i>	For: AAGAACGGCAGGAGGATG Rev: TCTCAGGGAAGCAGTGGC	373
<i>C-MYC</i>	For: CAGCCCACTGGTCCTCAA Rev: TTTCCGCAACAAGTCCTC	391
<i>KLF4</i>	For: CGGGCTGATGGGCAAGTT Rev: GGGCAGGAAGGATGGGTAA	391
<i>LIN28</i>	For: GTTCGGCTTCCTGTCCAT Rev: ACTCCACTGCCTCACCT	397
<i>SOX2</i>	For: GAATTCATGTACAACATGATGGAGACGG Rev: CTCGAGTCACATGTGTGAGAGGGGCGAGTG	966

Figures

Figure 1

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Figure 2

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Figure 3

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