Original Article

Induction of dental pulp-derived induced pluripotent stem cells in the absence of c-Myc for differentiation into neuron-like cells

Yun-Ching Chang [a,b], Wan-Chun Li [c], Nae-Fang Twu [b,d], Hsin-Yang Li [b,d], Wen-Liang Lo [c,e], Yuh-Lih Chang [b,f], Yi-Yen Lee [b,g], Chun-Fu Lin [b,g], Yang-Hsin Shih [b,g], Ming-Teh Chen [b,g,*]

[a] Department of Medical Research and Education, Taipei Veterans General Hospital, Taipei, Taiwan, ROC
[b] National Yang-Ming University School of Medicine, Taipei, Taiwan, ROC
[c] School of Dentistry, National Yang-Ming University, Taipei, Taiwan, ROC
[d] Department of Obstetrics and Gynecology, Taipei Veterans General Hospital, Taipei, Taiwan, ROC
[e] Division of Oral and Maxillofacial Surgery, Taipei Veterans General Hospital, Taipei, Taiwan, ROC
[f] Institute of Pharmacology, National Yang-Ming University, Taipei, Taiwan, ROC
[g] Department of Neurosurgery, Taipei Veterans General Hospital, Taipei, Taiwan, ROC

Abstract

Background: A recent research breakthrough has demonstrated that the ectopic expression of four genes is sufficient to reprogram human fibroblasts into inducible pluripotent stem cells (iPSCs). However, whether human dental pulp cells (DPCs) could be reprogrammed into iPSCs remains an open question. In this study, we demonstrated that DPCs from deciduous and permanent teeth can be reprogrammed into iPSCs without c-Myc and had the capacity to differentiate into neuron-like cells.
Methods: DPCs were obtained from donors and reprogrammed into iPSCs using retroviral transduction with SOX2, OCT4, and KLF4. Then, these iPSCs were differentiated into neuron-like cells. Microarray and bioinformatics were used to compare the gene expression profile among these iPSCs and iPSC-derived neuron-like cells.
Results: The DPCs displayed a high vitality and capability to quickly restart proliferation and expressed elevated pluripotency similar to mesenchymal stem cells. According to our results, DPC-derived iPSC colonies that could be subcultured and propagated were established as early as 10 days after transduction, in comparison with the skin fibroblast (DPC-derived iPSCs) without c-Myc presented embryonic stem cell-like properties and the pluripotent potential to differentiate into neuron-like cells, which resemble neurons both morphologically and functionally.
Conclusion: The human DPCs from deciduous and permanent teeth can undergo reprogramming to establish pluripotent stem cell lines without c-Myc. These surgical residues, usually regarded as medical waste, can be used as an alternative source of pluripotent stem cells for personalized medicine.
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Keywords: dental pulp; differentiation; inducible pluripotent stem cells; neural cell

1. Introduction

Induced pluripotent stem cells (iPSCs) are novel stem-cell populations induced from adult somatic cells through reprogramming by transduction of defined transcription factors.[1,2] Cumulative evidence has shown that iPSCs are indistinguishable from embryonic stem cells (ESCs) in terms of

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morphology and gene expression, and are capable of self-renewal and differentiation into three germ layers.[1,3] Thus, iPSCs offer a means to study disease-specific cells from living patients, and serve as a bridge between clinical and bench research.[4] Furthermore, these patient-specific iPSCs bearing specific mutations have great potential for modeling disease phenotypes, screening candidate drugs, and cell replacement therapy provided that the underlying disease-causing mutation can be corrected.[5,6] To date, iPSCs have been derived from many different species, such as humans, rhesus monkeys, mice, rats, marmosets, pigs, and rabbits. The cell types that have been successfully reprogrammed include hepatocytes, gastric epithelial cells, keratinocytes, stomach cells, mesenchymal cells, neural stem cells (NSCs), pancreatic cells, B and T lymphocytes, cord blood cells, and peripheral blood cells, among others. Generally, human iPSCs are normally derived from dermal fibroblasts because of their accessibility and relatively high reprogramming efficiency. However, prior to using dermal fibroblasts, skin biopsy and a prolonged period of cell culture expansion are required. During the skin biopsy, the exposure of the dermis to ultraviolet light might increase the risk for chromosomal aberrations. In addition, patients would experience pain and risk the possibility of infection during the process of obtaining dermal fibroblasts. These issues limit the wide application of iPSCs.

Dental pulp is a specific tissue originating from the cranial neural crest and is enclosed into a dental cavity surrounded by mineralized dentin. The dental pulp tissue can be isolated from human exfoliated deciduous and permanent teeth, which are usually regarded as medical waste.[7] Because of its differentiation potential and accessibility, interest in dental pulp in regenerative medicine is gradually increasing.[8,9] Previous studies have shown that the dental pulp cells (DPCs), including dental-pulp stem cells (DPSCs), express elevated levels of pluripotent factors, resulting in significantly more efficient and accelerated generation of iPSCs compared with conventional somatic cells. The c-Myc oncogene may contribute to tumorigenesis by overstimulating cell growth and metabolism and/or by causing genomic instability. Deregulated expression of c-Myc occurs in a wide range of human cancers and is often associated with poor prognosis, indicating that this oncogene plays a key role in tumor progression. The ability of *in vivo* teratoma formation has been used as a landmark for evaluating the pluripotency of iPSCs.[10] Previous studies have successfully generated iPSCs from mouse and human fibroblasts using only three transcription factors: OCT4/SOX2/KLF4 (without c-Myc), and mice derived from these iPSCs without c-Myc did not develop tumors during the study period.[11,12] However, whether reprogramming human DPCs from exfoliated deciduous and permanent teeth into iPSCs in the absence of c-Myc can be successfully generated and present the ability to differentiate is still unclear.

In this study, we took advantage of the remarkable capacity of the human DPCs, and reprogrammed the DPCs to establish pluripotent stem-cell lines from exfoliated deciduous and permanent teeth via retroviral transduction of OCT4, SOX2,

and KLF4. After receiving proper differentiation stimuli, these DPC-derived iPSCs (DPC-iPSCs) differentiated into functional neuron-like cells that expressed various neuronal markers. Our findings may provide an alternative cell source and strategy for iPSCs without c-Myc-based personalized therapies for human disease.

2. Methods

2.1. Isolation of human DPCs

Teeth were obtained from one child undergoing deciduous tooth extraction and two adult donors undergoing third molar extraction. All patients provided informed consent according to the guidelines of the Ethical Committee of the Taipei Veterans General Hospital (IRB No. 2012-08-009A). The extraction procedure was performed under standard conditions for local anesthesia. The extracted teeth were treated with disinfecting solution and—together with the pulp—transported in Hank's balanced salt solution (Invitrogen, Carlsbad, CA, USA) to the tissue cultures laboratory. Extracted dental pulp tissue was then enzymatically treated with 3 mg/mL collagenase type I and 4 mg/mL dispase to completely digest the pulp tissue. After centrifugation, the cell pellet was obtained and the supernatant aspirated. Human DPCs were maintained in Dulbeccos's modified Eagle's medium (DMEM) supplemented with 20% fetal bovine serum, nonessential amino acid, L-glutamine and penicillin/streptomycin at 37°C with 5% CO_2.

2.2. Human iPSC generation and culture

iPSCs were reprogrammed via the transduction of pMXs vectors encoding the transcription factors OCT4, SOX2, and KLF4. The Plat-A cells used for plasmid transfection were incubated overnight at a density of 2.5×10^6 cells/100-mm dish. The next day, 10 mg of pMXs-containing cDNA was transfected into the Plat-A cells with 10 mL of fresh DMEM using TransIT-LT1 (Mirus, Madison, WI, USA). Forty-eight hours after transfection, the virus-containing medium was collected for target cell infection. In preparation for viral infection, 5×10^4 target cells were seeded per well into six-well plates 1 day prior to transduction. Supernatants containing equal amounts of each of the four retroviruses were filtered through a 0.45-mm filter and supplemented with 10 mg/mL polybrene (Sigma, St. Louis, MO, USA), and the medium in the six-well plates was replaced with the virus-containing medium. The six-well plates were centrifuged at 2250 rpm for 1 hour, and then the medium was replaced. At Days 10−14 after infection, target cells were passaged onto mitotically inactivated mouse embryonic fibroblast (MEF) feeder layers and cultured using the human ESC medium. The drugs SB431542 (2 mM), PD0325901 (0.5 mM; Stemgent), and Thiazovivin (STEMCELL Technologies; Vancouver, BC, Canada) (0.5 mM) were added to the culture medium to aid colony formation. The drug-containing medium was replaced daily until iPSC colonies were detected. Undifferentiated

iPSCs were maintained on mitotically inactivated MEFs (50,000 cells/cm^2) in human ESC medium [DMEM/F12; Gibco (Grand Island, NY, USA)] supplemented with 20% KnockOut serum replacement (KSR; Invitrogen), 0.1 mM nonessential amino acids (Invitrogen), 1 mM L-glutamine, 0.1 mM β-mercaptoethanol, 10 ng/mL recombinant human basic fibroblast growth factor, and antibiotics (Gibco). To prevent MEF contamination, human iPSCs cells were transferred to a feeder-free/serum-free culture system in CSTI-8 medium (Cell Science & Technology Institute Inc., Sendai, Japan) without KSR supplementation.

2.3. In vitro differentiation

For in vitro differentiation, iPSCs were dispersed into small clumps using dispase (Sigma-Aldrich, St. Louis, MO, USA; 1 mg/mL for 30 minutes) and transferred onto ultralow attachment plates (Corning Inc., Corning, NY, USA) for Embryoid Body (EB) formation. The medium was changed daily for 3 days, and the medium used was the same type of medium as that used for routine hESC culture. EBs were then transferred onto 0.1% gelatin-coated culture dishes with the fetal bovine serum-containing medium. The medium was changed every 2 days for 20 days.

2.4. Quantitative reverse transcription-polymerase chain reaction

SYBR green was used for real-time reverse transcription-polymerase chain reaction (RT-PCR) detection. Briefly, total RNA (1 μg) of each sample was reverse-transcribed in 20 μL using 0.5 μg of oligo-dT and 200 U Superscript II RT (Invitrogen). Amplification was carried out in a total volume of 20 μL containing 0.5 μM of each primer, 4 mM MgCl$_2$, 2 μL LightCycler−FastStart DNA Master SYBR green I (Roche Diagnostics GmbH, Mannheim, Germany), and 2 μL of 1:10 diluted cDNA. All reactions were performed in triplicate. The transcript levels of genes were standardized to the corresponding glyceraldehyde-3-phosphate dehydrogenase (GAPDH) level, and for each candidate gene, mRNA levels relative to the highest candidate gene level were estimated in percentages.

2.5. Induced differentiation of DP-iPSCs into neuron-like cells

There were 1 × 10^5 iPSCs at the 5th to 8th passages that were treated with a serum-free modified neurogenic selection medium for 2 weeks. The serum-free medium for the selection of neural precursor cells contained DMEM/F12 1:1 (Gibco BRL) and was supplemented with 0.6% glucose, 25 μg/mL insulin, 100 μg/mL transferrin, 20 nM progesterone, 60 μM putrescine, 30 nM selenium chloride, 2 mM glutamine, 3 mM sodium bicarbonate, 5 mM HEPES buffering agent, 2 μg/mL heparin, 20 ng/mL Epidermal growth factor (EGF), and 20 ng/mL basic fibroblast growth factor (all from Sigma). For further neural differentiation, EGF was removed from the medium, and the medium was supplemented with 20 ng/mL Sonic hedgehog (SHH) (R&D), NY, USA, 10 ng/mL Brain-derived neurotrophic factor (BDNF) (R&D), and all-trans retinoic acid (100 nM) for another 7 days.[13]

2.6. Immunofluorescence

The living cells and spheres were fixed in 4% paraformaldehyde, permeabilized in 0.1% Triton X-100, and blocked in 5% normal goat serum−phosphate buffered saline. Cells were incubated with primary antibodies, washed three times in phosphate buffered saline, and then incubated with goat antimouse or secondary antibodies conjugated with fluorescein isothiocyanate (green) or phycoerythrin (red). DAPI (4′,6-diamidino-2-phenylindole) was used as a nuclear stain (blue). Images were obtained using fluorescent microscopy and a digital camera. The antibodies that were used are listed in Table S1.

2.7. Microarray analysis and bioinformatics

Total RNA was extracted from cells using Trizol reagent (Life Technologies, Bethesda, MD, USA) and the Qiagen RNeasy (Qiagen, Valencia, CA, USA) column for purification. Total RNA was reverse-transcribed with Superscript II RNase H-reverse transcriptase (Gibco BRL) to generate Cy3- and Cy5-labeled (Amersham Biosciences Co., Piscataway, NJ, USA) cDNA probes for the control and treated samples, respectively. The labeled probes were hybridized to a cDNA microarray containing 10,000 immoblized gene clone cDNA fragments. Fluorescence intensities of Cy3 and Cy5 targets were measured and scanned separately using a GenePix 4000B Array Scanner (Axon Instruments, Burlingame, CA, USA). Data analysis was performed using GenePix Pro 3.0.5.56 (Axon Instruments) and GeneSpring GX 7.3.1 software (Agilent, Palo Alto, CA, USA). The average-linkage distance was used to assess the similarity between two groups of gene expression profiles as described below. The difference in distance between two groups of sample expression profiles to a third was assessed by comparing the corresponding average-linkage distances [the mean of all pairwise distances (linkages) between members of the two groups concerned]. The error of such a comparison was estimated by combining the standard errors (the standard deviation of pairwise linkages divided by the square root of the number of linkages) of the average-linkage distances involved. Classical multidimensional scaling was performed using the standard function of the R program to provide a visual impression of how the various sample groups are related.

2.8. Statistical analysis

The results are expressed as mean ± standard deviation. Statistical analyses were performed using the Student t test for comparing two groups, and one-way or two-way analysis of variance followed by Bonferroni's test was used to detect differences among three or more groups. Results were considered statistically significant at $p < 0.05$.

Table 1
Clinical characteristics of patients.

Case no.	Age (y)	Phase	Isolation of DPC	Reprogramming DPC-iPSCs
Case 1	23	Permanent teeth	Successful	Successful
Case 2	6	Deciduous teeth	Successful	Successful
Case 3	37	Permanent teeth	Successful	Successful

DPC = dental pulp cell; DPC-iPSCs = DPC-derived inducible pluripotent stem cells.

3. Results

3.1. Isolation and characterization of DPCs from teeth

As shown in Table 1, teeth were obtained from one child undergoing deciduous tooth extraction (Case 2) and from two adult donors undergoing third molar extraction (Cases 1 and 3). We separated the teeth from the crown using Luer forceps, and we used sharp needles or excavators to release dental pulp from the pulp chamber, after wide access was achieved (Fig. 1A). A sparse number of DPCs attached to the culture dish and the majority of cells displayed a fibroblast-like, spindle-shaped morphology during the early days of incubation (Fig. 1B, upper). After plating for 7−10 days, these primary cells reached 80% confluence during their 1st passage (Fig. 1B, lower). Moreover, cells that were stored long term at −80°C demonstrated high vitality and capability to quickly restart proliferation. We used some markers to define our cultured cells. Our data indicated that DPCs expressed CD44, CD90, and CD105, but not CD34 and CD45. These findings are consistent with the undifferentiated state of DPCs and are similar to that of mesenchymal stem cells (MSCs) (Fig. 1C).

3.2. Characterization of human DPC-iPSCs

The isolation and cultivation of DPSCs have been previously reported.[14−16] However, it remains to be determined whether DPCs from exfoliated deciduous teeth and permanent teeth can provide alternative cell sources to be reprogrammed into iPSCs. In the present study, we isolated DPCs from human dental pulp and reprogrammed these cells into iPSCs via transfection with retroviral vectors encoding the transcription factors OCT4, SOX2, and KLF4. As the reprogramming progressed, these cells gradually formed colonies of
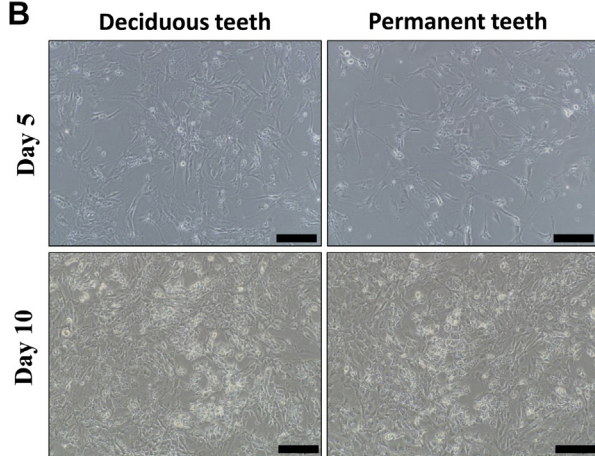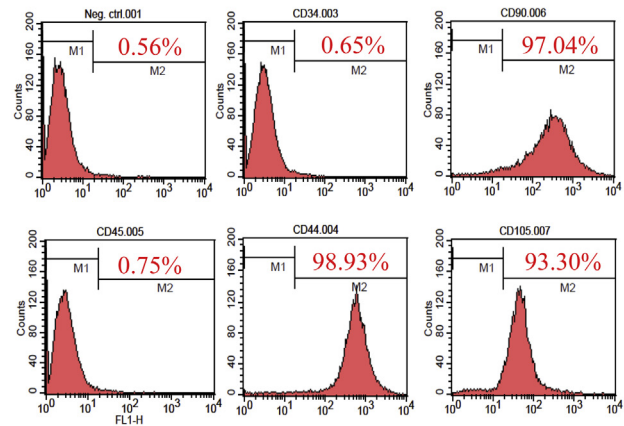

Fig. 1. Isolation and characterization of dental pulp cells (DPCs). (A) Extracted dental pulp tissue from exfoliated permanent teeth. (B) After 5 days and 10 days in expansion/selection medium, isolated DPCs were morphologically uniform and highly proliferative. Phase contrast microscopy; magnification, × 200. (C) Representative flow cytometer results of DPCs from permanent teeth on Day 14 after cell culturing with antibodies reactive to the stem cell-related surface molecules. Gate was defined considering the isotype control IgG-stained sample versus specific antibody staining profile.

increasing sizes. These colonies were cultivated on the MEF feeder cells in KSR-based media and stained positive for alkaline phosphate and exhibited a morphology indistinguishable from that of fibroblast-derived iPSCs and human ESCs (Fig. 2A). The RT-PCR results showed that the 20th DPC-iPSCs expressed the genes *OCT4*, *SOX2*, *REX1*, *GDF3*, *Nanog*, *KLF4*, and *DPPA2*, all of which are also expressed in the human ESCs (Fig. 2B). Immunofluorescence results indicated that DPC-iPSCs from the 20th passage showed a strong expression of Oct4, Nanog, SSEA4, Tra-1-81, and Tra-1-60 (Fig. 2C). Using differentiation protocols for tridermal lineages, the 20th DPC-iPSC-derived embryonic bodies could also be induced to differentiate into neuron-like cells, smooth muscle cells, and hepatocyte-like cells (ectoderm, mesoderm, and endoderm). Immunofluorescence analysis demonstrated the expression of markers specific to each lineage, i.e., Nestin, SMA, and AFP, respectively (Fig. 2D). Taken together, our data demonstrated that human DPCs can be reprogrammed, and DPC-iPSCs present ESC-like properties and are capable of multilineage differentiation.

3.3. Differentiation of human dental pulp-derived iPSCs into neural progenitor cells

The protocol for differentiating iPSCs into neural progenitors followed previously established methods, and both the iPSCs and ESCs were separated from the feeder fibroblasts prior to differentiation.[17] Four weeks after the neural induction, DPC-iPSC-derived neurons formed extensive networks and stained positively for Nestin and the mature neuronal marker MAP2 (Fig. 3A). Using microarray analysis, we subsequently compared the gene expression profile among hippocampus, SH-SY5Y cell line (neuroblast from neural tissue), ESC-derived neuron-like cells (ESC-Neus), and DPC-iPSC-derived neuron-like cells (DPC-iPSC-Neus) (Fig. 3B). The profile of differentially expressed genes of DPC-iPSC-Neus was similar to those of ESC-Neus, hippocampus and SH-SY5Y cell line, but different from those of NSCs, ESCs, or DPC-iPSCs. Multidimensional scaling (Fig. 4A) and average distance analysis (Fig. 4B) further showed that the gene expression pattern of DPC-iPSC-Neus was closer to the gene
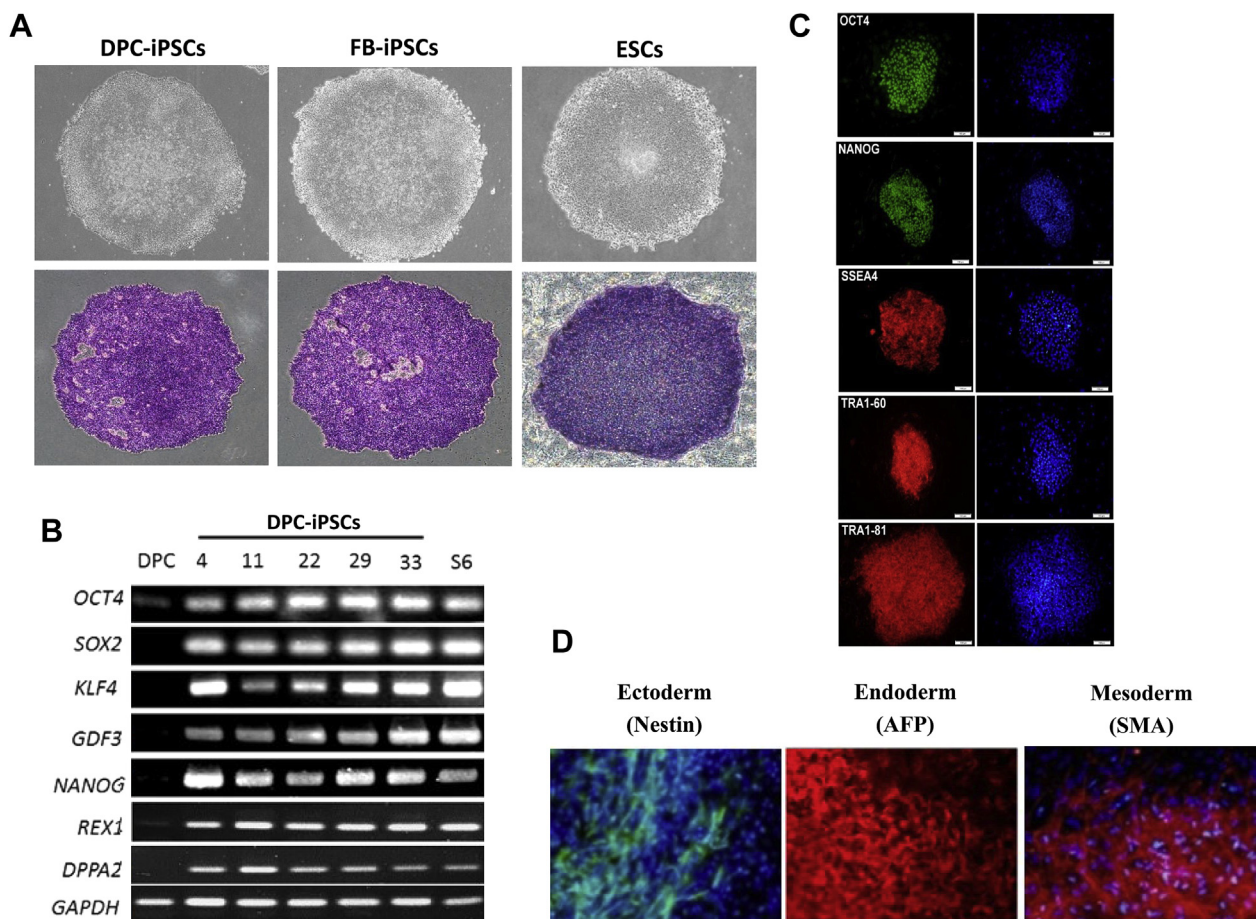

Fig. 2. Establishment and characterization of inducible pluripotent stem cells derived from dental pulp cells (DPC-iPSCs). (A) Representative phase-contrast photomicrograph and alkaline phosphatase activity of DPC-iPSCs and fibroblast (FB)-iPSCs; magnification, × 40. (B) The reverse transcription-polymerase chain reaction (RT-PCR) results indicated an embryonic stem cell (ESC)-like gene expression pattern in representative colonies of DPC-iPSCs. DPCs were used as a negative control, and human ES S6 cells were used as a positive control. (C) Immunofluorescence staining demonstrated the expression of pluripotency markers in undifferentiated DPC-iPSCs. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI; blue); magnification, × 100. (D) Immunofluorescence staining demonstrated that markers for the three germ layers were expressed in spontaneously differentiated iPSCs. Nuclei were counterstained with DAPI (blue); magnification, × 100. Data shown here are derived from permanent teeth, representatively.
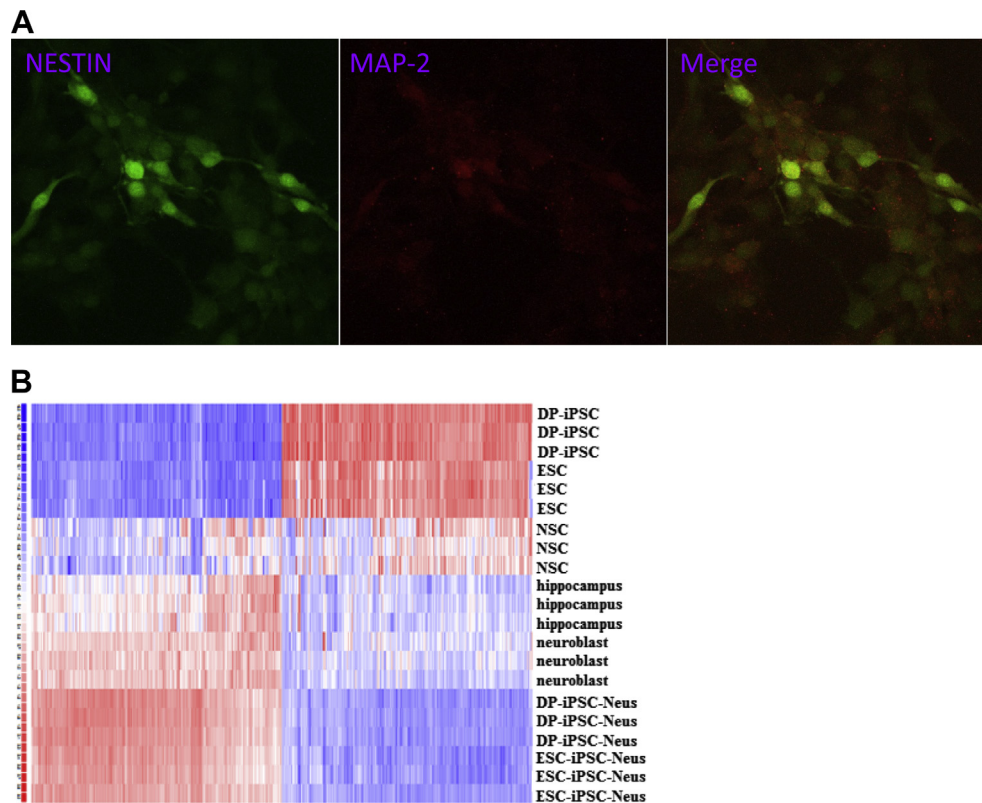
Fig. 3. Differentiation of three-gene DPC-iPSCs into neural cells. (A) Immunofluorescence staining for neuronal marker Nestin and MAP2 demonstrated morphology and neurons formed extensive networks in DPC-iPSC-Neus. Nuclei were counterstained with DAPI (blue); magnification, × 200. (B) Gene expression microarray analysis showing genes that were differentially expressed in DPC-iPSCs, ESCs, NSCs, hippocampus, neuroblast (SH-SY5Y), ESC-Neus, and DPC-iPSC-Neus using a hierarchy heat map. Data shown here are derived from permanent teeth, representatively. DAPI = 4′,6-diamidino-2-phenylindole; DPC = dental pulp cell; DPC-iPSCs = DPC-derived iPSCs; DPC-iPSC-Neus = DPC-iPSC-derived neuron-like cells; ESCs = embryonic stem cells; ESC-Neus = ESC-derived neuron-like cells; iPSCs = inducible pluripotent stem cells; NSCs = neural stem cells.

signature of ESC-Neus, but was dissimilar to DPCs and DPC-iPSCs. Quantitative real-time RT-PCR analysis revealed that the expression of neural-specific markers, including Nestin, GFAP, MAP2, and NCAM1, were initially upregulated in DPC-iPSC-Neus after the induction of neural differentiation for 7 days, 14 days, and 21 days (Fig. 4C). The mRNA expressions of these neural-specific markers were statistically increased during the course of differentiation. Taken together, these data supported the view that DPC-iPSCs without c-Myc present the pluripotent potential to differentiate into neuron-like cells, which resemble neurons both morphologically and functionally.

4. Discussion

DPSCs were first isolated by Gronthos and coworkers in 2000.[14] They described the identification of DPSCs by virtue of their clonogenic abilities, rapid proliferative rates, and capacity to form mineralized tissues both *in vitro* and *in vivo*. Compared with other stem cells such as MSCs from the bone marrow and NSCs from cadavers, DPSCs are more easily isolated.[18] There are several published data reporting the successful isolation of a stem cell population from human exfoliated deciduous teeth, third molars, or wisdom teeth.[14−16]

In addition, extraction of DPSCs presents no loss to the individual and can be expanded with ease or stored without detriment.[19] This feature of DPSCs opens up the potential for autologous transplantation and removes the need for immunosuppressive therapy. However, preliminary studies have shown that the stem cell or potential progenitor cell population in dental pulp comprises <1% of the total cells.[20,21] Histomorphometric analyses of pulpal cell populations imply that age-related reductions in pulpal cell numbers occur.[22] Therefore, the efficiency of DPSCs isolated from exfoliated permanent teeth and the future clinical applications of DPSCs remain an open question.

iPSCs provide a unique platform for the investigation of disease mechanisms, drug discovery, and personalized treatments.[4] The accumulated research on this topic has demonstrated that human iPSCs can be generated from human adult cells via transfection with the transcription factors OCT4, SOX2, KLF4, and c-Myc.[1,2,23] Nevertheless, information about the potential of DPC-reprogrammed iPSCs is still lacking. The use of DPCs might bring advantages for iPSCs reprogrammed over the use of other somatic cells as follows: DPCs were reported to have a higher proliferation rate and increase cell population doublings; DPCs are retrieved from a tissue that is "disposable" and readily accessible in young
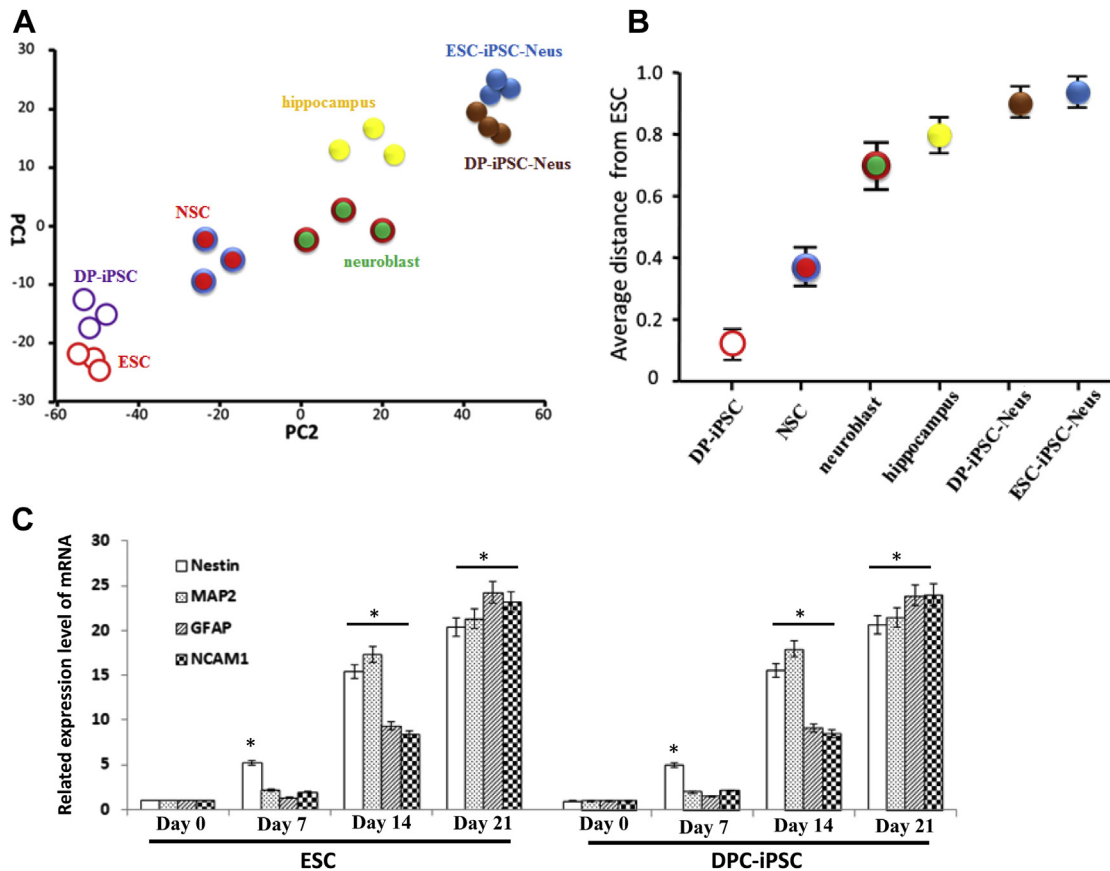
Fig. 4. Characterization of three-gene DPC-iPSC-Neus. (A) Multidimensional scaling and (B) average distance analysis show that the expression pattern of DPC-iPSC-Neus was closer to the gene signature of ESC-Neus. Principal component analysis (PC1: vertical axis; PC2: horizontal axis; Unit: D-chip unit) is used to measure the expression profiling using the bioinformatics methods. (C) Expression of neural-specific genes during the differentiation course of iPSC-Neus for 7 days, 14 days, and 21 days. Data shown here are the means ± standard deviation of three independent experiments. *$p < 0.05$ versus D0. Data shown here are derived from permanent teeth, representatively. DPC-iPSC = dental pulp cell-derived inducible pluripotent stem cell; DPC-iPSC-Neus = DPC-iPSC-derived neuron-like cells; ESC-Neus = ESC-derived neuron-like cells; iPSC-Neus = iPSC-derived neuron-like cells.

patients, i.e., exfoliated deciduous teeth. Some previous studies proposed that dental pulp tissue containing stem cells expresses elevated levels of pluripotent factors, is significantly more efficient, and exhibits an accelerated generation of iPSCs compared with conventional somatic cells.[14,24] The first aim of this study was to address whether deciduous and permanent teeth can be used to generate human iPSCs. Using the three-gene delivery method (OCT4/SOX2/KLF4), we successfully created iPSCs by dedifferentiating DPCs isolated from the clinical waste of donor teeth. Under a serum-free and feeder-free cultured system, these iPSCs remained stable through 30 passages while retaining ESC-like pluripotency. The EBs derived from these DPC-iPSCs could be differentiated into neuroectodermal lineage neuron-like cells.

The major advantage of iPSCs over ESCs cells is that iPSCs can be derived from a patient's own somatic cells, thereby avoiding immune rejection after transplantation and the ethical concerns regarding the use of ESCs. In addition, the development of the technology for derivation of iPSCs from human patients has opened up new pathways to the better understanding of many human diseases, and has created new opportunities for therapeutic approaches.[1,2] Among the four factors (OCT4, SOX2, KLF4, and c-Myc) for conventional

iPSCs reprogramming, the pro-oncogene c-Myc plays a critical role in influencing reprogramming efficiency.[12] However, a previous report suggests that the deregulated expression of c-Myc occurs in a wide range of human cancers, is often associated with poor prognosis, and may contribute to tumorigenesis by overstimulating cell growth and metabolism and/or by causing genomic instability.[12,25] Therefore, c-Myc might be a major obstacle in iPSC-based cell transplantation because of its tumorigenetic propensity and terotoma formation *in vivo*.[26] To eliminate teratoma formation, some alternative experimental approaches have been used in several studies. In one of our recent studies, we have shown that the oncogene c-Myc was expected to substantially contribute to tumor formation in Acute hepatic failure (AHF) recipients, but the tumor formation could be prevented in the livers of mice that received three-gene iPSCs and three-gene iPSC-Heps 6 months after transplantation.[11] In this study, three-gene iPSCs from DPCs were positive for OCT4, SSEA-1 (ESC markers), and alkaline phosphatase, were not affected by passage number, and were identical to that of four-gene iPSC colonies. Furthermore, the microarray gene expression between three-gene IPSCs, four-gene iPSCs, and ESCs were similar, and the three-gene iPSC-derived embryonic bodies could also be

differentiated into neuroectodermal lineage neuron-like cells. This molecular and differentiation evidence implies the niche of three-gene iPCs in future cell therapy. iPSCs without c-Myc, especially under differentiated states, represents a more feasible cell source for iPSC-based replacement therapy and *in vitro* screening.

Because viral integration into the host genome has been demonstrated to possibly increase the risk of tumorigenicity, the production of virus-free iPSCs addresses a critical safety concern for the potential use of iPSCs in regenerative medicine.[27] A large part of the tooth, including the dental pulp, is derived from the neural crest—the same neural crest that forms neurons during development.[28] Thus, DPCs and DPSCs are possibly more prone to forming neurons than other stem cells. Direct reprogramming of DPCs into neural combined with nonviral vector delivery should be encouraged in future studies. In conclusion, this study showed that with the iPSC-based technology, surgical residues usually regarded as medical waste such as DPCs can be used as an alternative source of pluripotent stem cells for personalized medicine.

Acknowledgments

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jcma.2014.08.009.

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