The expression of four transcription factors (OCT3/4, SOX2, KLF4, and MYC) can reprogram mouse as well as human somatic cells to induced pluripotent stem (iPS) cells. We generated iPS cells from mesenchymal stromal cells (MSCs) derived from human third molars (wisdom teeth) by retroviral transduction of OCT3/4, SOX2, and KLF4 without MYC, which is considered as oncogene. Interestingly, some of the clonally expanded MSCs could be used for iPS cell generation with 30–100-fold higher efficiency when compared with that of other clonally expanded MSCs and human dermal fibroblasts. Global gene expression profiles demonstrated some up-regulated genes regarding DNA repair/histone conformational change in the efficient clones, suggesting that the processes of chromatin remodeling have important roles in the cascade of iPS cells generation. The generated iPS cells resembled human embryonic stem (ES) cells in many aspects, including morphology, ES marker expression, global gene expression, epigenetic states, and the ability to differentiate into the three germ layers in vitro and in vivo. Because human third molars are discarded as clinical waste, our data indicate that clonally expanded MSCs derived from human third molars are a valuable cell source for the generation of iPS cells.

In 2006, groundbreaking research demonstrated that mouse somatic cells could be reprogrammed to a pluripotent state by transduction of four transcription factors (Oct3/4, Sox2, Klf4, and Myc) (1). Furthermore, the researchers reported that the same four factors are also effective in reprogramming human somatic cells (2). Another group reported that a different set of four factors (OCT3/4, SOX2, NANOG, and LIN28) could reprogram human cells (3). The reprogrammed cells, named induced pluripotent stem (iPS) cells, closely resembled human embryonic stem (ES) cells in many aspects including morphology, gene expression, surface markers expression, epigenetic states, and the ability to differentiate into the three germ layers (endoderm, mesoderm, and ectoderm) in vitro and in vivo (1–12). Generation of iPS cells opened up a new avenue for the generation of patient-specific pluripotent stem cells, which are useful for drug screening, understanding the mechanisms of pathogenesis, and cell transplantation therapies (13–16).

However, three major concerns exist in the current reprogramming strategies for clinical applications: (i) the low reprogramming efficiency of human somatic cells makes it difficult to generate patient-specific iPS cells, especially using a small amount of the tissue of the patient; (ii) genomic integration of retro- or lentiviral fragments might cause carcinogenesis (17–19); and (iii) reactivation of MYC might also cause malignant tumor formation (9). Although iPS cells can be generated by three transcription factors (OCT3/4, SOX2, and KLF4) without MYC, reprogramming efficiency was significantly reduced (20).

Recent studies have demonstrated human iPS cell generation from various cell origins, such as neonatal or adult dermal fibroblasts (2, 4, 5, 7, 20), foreskin fibroblasts (2–4, 7, 8), lung fibroblasts (3, 4), fibroblast-like synoviocytes (2), and keratinocytes (6). Human dental fibroblasts (HDFs) are easily obtained by biopsy. However, cellular senescence and/or low proliferation capability have caused failure of iPS generation (4). One ideal cell source for iPS cell generation is discarded tissue, which contains cells with high proliferation capability with less incidence of cellular senescence. We and other groups previously reported that mesenchymal stromal cells (MSCs) from teeth or third molars (wisdom teeth), which were usually discarded as clinical waste, showed high proliferation when compared with MSCs from bone marrow (21–23).

Quite recently, another group reported that mesenchymal-like stem/progenitor cells from dental tissue could be reprogrammed by four factors transduction of OCT4/SOX2/KLF4/MYC or OCT4/SOX2/NANOG/LIN28 (24). However, the MYC is an oncogene, and overexpression of LIN28 might promote carcinogenesis via repression of let-7 and following derepressing the targets genes involved in MYC (25). Here, we show that various MSCs from human third molars could be reprogrammed to a pluripotent state by retroviral transduction with OCT3/4, SOX2, and KLF4 without MYC or LIN28. In particular, some clonally expanded MSCs showed extremely higher reprogramming efficiency than that of HDFs. We also
addressed the reprogramming efficiency from global gene expression profiles.

**EXPERIMENTAL PROCEDURES**

*Plasmid Construction*—Reading frame cassette A (Invitrogen) was introduced into the EcoRI site of the pMXs retroviral vector (26). The open reading frames of human OCT3/4 (POLUI/P1 isofrom-1), SOX2, and KLF4 were amplified by reverse transcription-polymerase chain reaction (RT-PCR) and cloned into pENTR-D/TOPO (Invitrogen). To evaluate the viral infection efficiency, the open reading frame of DsRed-Express from pIRES2-DsRed-Express (Clontech) was amplified by PCR and cloned into pENTR-D/TOPO (Invitrogen). All of the genes were transferred to the pMXs retroviral vector (kindly donated by Dr. Kitamura) by Gateway Technology (Invitrogen), according to the manufacturer’s instructions.

*Cell Culture*—This study was approved by the ethics committee of the National Institute of Advanced Industrial Science and Technology. Isolation of third molars and culture expansion of MSCs from the molars were carried out from three donors (10, 16, and 13 years old) after informed consent was obtained. The cultured method of these MSCs was described in our previous report (23). The frozen stocked MSCs were thawed and used for the generation of iPSC cells. HDFs were purchased from Cell Applications. Platinum-A (Plat-A) cells were purchased from Cell Biolabs (27). SNL76/7 feeder cells were purchased from the European Collection of Cell Cultures. MSCs were maintained in minimum essential medium α (Invitrogen) containing 15% fetal bovine serum (FBS; Invitrogen), 100 units/ml penicillin, and 100 μg/ml streptomycin (Invitrogen). HDF, Plat-A, and SNL feeder cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM; Invitrogen) containing 10% FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin. The iPSCs were generated and maintained in human ES cell medium (DMEM/F-12 with GlutaMAX-I (Invitrogen), 10 mM CaCl2 (WAKO), and 20% knock-out serum replacement (Invitrogen), 0.1 mM non-essential amino acids (Invitrogen), 0.1 mM 2-mercaptoethanol (Invitrogen), 100 units/ml penicillin, and 100 μg/ml streptomycin) supplemented with 5 ng/ml recombinant human basic fibroblast growth factor (basic FGF; WAKO). For passaging, MSCs, HDFs, Plat-A, and SNL76/7 feeder cells were trypsinized with 0.05% trypsin/0.53 mM EDTA (Invitrogen) at room temperature. Primary antibodies included stage-specific embryonic antigen (SSEA)-3 (1:100, MA54303, Millipore), SSEA-4 (1:100, MA54304, Millipore), OCT3/4 (prediluted, N1584, Dako), III-tubulin (1:200, CBL412, Millipore), and tyrosine hydroxylase (1:200, AB152, Millipore). Secondary antibodies used were Alexa Fluor 488 donkey anti-goat IgG (1:200, A11034), Alexa Fluor 488 donkey anti-mouse IgG (1:200, A21202), Alexa Fluor 488 conjugated goat anti-mouse IgG (1:200, A11031), Alexa Fluor 568 goat anti-mouse IgM (1:200, A21043), Alexa Fluor 488 goat anti-mouse IgM (1:200, A21042), Alexa Fluor 568 goat anti-rabbit IgG (1:200, A11011), Alexa Fluor 488 goat anti-rabbit IgG (1:200, A11034), Alexa Fluor 488 donkey anti-goat IgG (1:200, A11055),

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and Alexa Fluor 488 goat anti-rat IgM (1:200, A21212) (all from Molecular Probes). Nuclei were stained with 0.2 μg/ml Hoechst 33342 (Molecular Probes).

**Bisulfite Sequencing**—One microgram of genomic DNA was treated with the EpiTect bisulfite kit (Qiagen), according to the manufacturer’s recommendations. The promoter regions of the human OCT3/4 and NANOG genes were amplified by PCR. The PCR products were subcloned into pCR2.1-TOPO (Invitrogen). Ten clones of each sample were verified by sequencing with the M13 universal primer. Primer sequences used for PCR amplification are shown in supplemental Table S1.

**Detection of Telomerase Activity**—Telomerase activity was verified with a TRAPEze telomerase detection kit (Millipore) according to the manufacturer’s instructions. Each sample was separated by Tris-borate-EDTA-based 10% polyacrylamide gel electrophoresis. The gel was stained with ethidium bromide.

**Teratoma Formation**—One well of a 6-well plate of iPS cells cultured on SNL76/7 feeder cells was harvested by dissociation solution treatment and transferred to a low attachment culture dish (PrimeSurface; Sumitomo Bakelite) in human ES cell medium without basic FGF. The medium was changed every other day. Eight days after floating culture, the EBs were transferred onto gelatin-coated plates and cultured in the same medium for another 8 days. PA6 cells were used for differentiation into dopaminergic neurons. The PA6 cells were seeded on gelatin-coated plates and cultured for 4 days. Small clumps of iPS cells were seeded on the PA6 cells in Glasgow minimum essential medium (Invitrogen) containing 10% knock-out serum replacement, 0.1 mM non-essential amino acids, 0.1 mM 2-mercaptoethanol, 100 units/ml penicillin, and 100 μg/ml streptomycin.

**Karyotype Analysis**—Chromosomal G-band analyses and multicolor FISH were performed at the Nihon Gene Research Laboratories (Sendai, Japan).

**DNA Microarray**—The microarray study was carried out using GeneChip Human Genome U133 Plus 2.0 gene expression arrays (Affymetrix). The analyses were performed according to Affymetrix technical protocols. Data from these experiments and the GEO data base were analyzed with GeneSpring GX10 software. For the cluster analyses, our data from the GeneChip Human Genome U133 Plus 2.0 gene expression arrays (archived at GEO as accession number GSE16963) were compared with DNA microarray data for the human ES cell lines BG01 and H9 retrieved from GEO datasets (GSM367061, GSM367062) as being representative of human ES cells for comparison purposes.

**Statistical Analysis**—Differences between the two data sets from DNA microarray were evaluated by the Student’s t test for the expression of each gene. Then, the false discovery rate was estimated using the Benjamini-Hochberg procedure (52). Differentially expressed genes were selected if they satisfied false discovery rate <0.05 in the average values for each set being compared.

**Real-time PCR**—PCR reactions were carried out in a StepOnePlus real-time PCR system (Applied Biosystems) using TaqMan gene expression master mix (Applied Biosystems) according to the manufacturer’s instructions. The following TaqMan primers and probes (TaqMan gene expression assays; Applied Biosystems) were used: β-actin (Hs99999903_m1), OCT3/4 (Hs030051111_g1), SOX2 (Hs01053049_s1), NANOG (Hs02387400_g1), KLF4 (Hs00358836_m1), and P53 (Hs9999147_m1). The expression of genes of interest was normalized to that of β-actin in all samples. Relative expression of genes of interest was estimated using the relative standard curve method.

**RESULTS**

**Generation of iPS Cells from Culture-expanded MSCs**—We have previously reported that culture-expanded MSCs from human third molars show high proliferation as well as differentiation capability into mesenchymal lineages (28). The MSCs from three donors (10F, 10-year-old female; 16F, 16-year-old female; and 13M, 13-year-old male) were seeded at a cell density of 5 × 10^5 cells/100-mm dish the day before retrovirus infection (day 0). We introduced the pMXs retrovirus vectors containing human OCT3/4, SOX2, and KLF4 into PLAT-A packaging cells (26, 27). Viral supernatants were collected at 48 and 72 h after transduction and infected into MSCs (day 1 and day 2). At day 4, viral-infected cells were trypsinized and seeded onto SNL76/7 feeder cells (5 × 10^5 viral-infected cells/100-mm dish) and then cultured in human ES cell medium supplemented with basic FGF. At ~25 days, we detected several colonies displaying human ES cell-like morphology (supplemental Fig. S1). At day 30, the reprogramming efficiencies of MSCs from 10F, 16F, and 13M were 0.0302, 0.0042, and 0.0026%, respectively (n = 10). Thus, the 10F cells showed higher reprogramming efficiency when compared with the other donors (Fig. 1A).

**Generation of iPS Cells from Clonally Expanded MSCs**—One of the possibilities was that the 10F cells might contain tissue-specific stem or progenitor cells, such as dental pulp stem cells (21) or stem cells from human exfoliated deciduous teeth (22). We also reported that tooth germ progenitor cells, which were clonally expanded mesenchymal cells from human third molars, differentiated into osteoblasts, neural cells, and hematocytes (23). Consequently, we examined whether the high reprogramming efficiency of the 10F cells was due to the presence of stem/progenitor cells. For this purpose, we clonally expanded the MSCs from the 10F cells and selected five clones, named 10F-5, 10F-8, 10F-15, 10F-101, and 10F-107. The 10F-5, 10F-8, and 10F-15 clones could differentiate into osteoblasts under osteogenic culture conditions; however, the 10F-101 and 10F-107 clones could not (data not shown). We found that 10F-5, 10F-8, and 10F-15 exhibited rapid proliferation when compared with 10F-101, 10F-107, or HDF (Fig. 1B).

Using the methods described above, we seeded these five clonal cells and HDFs and then transduced human OCT3/4, SOX2, and KLF4 with retrovirus. At day 30, we counted the
number of colonies that resembled the ES cell-like morphology and performed alkaline phosphatase (ALP) staining. Numerous ALP-positive colonies were seen in 10F-5, 10F-8, and 10F-15; however, a few colonies were seen in 10F-101 and HDF. The colonies were not seen in 10F-107. Therefore, the rapid proliferation clones were efficiently reprogrammed (Fig. 1, B, C, and D). To check the viral transduction efficiency, we introduced pMXs retrovirus vector containing DsRed-Express into each parental cell. There was no significant difference of viral transduction efficiency among these cells (Fig. 1E). These colonies were picked and expanded for several passages and then checked for ALP activity. All the colonies showed high ALP activity (Fig. 1F). The data in the following sections were obtained from the putative iPS colonies derived from the clonally expanded MSCs. Particularly, clones of 10F-15 and 10F-101 were used. Corresponding parental cells and HDF were also used as controls.

Characterization of iPS Cells from Clonally Expanded MSCs—To confirm that the cells from the putative colonies were iPS cells, we examined the human ES cell markers by immunocytochemistry, DNA methylation states, RT-PCR, and telomerase activity. Immunocytochemistry showed that the iPS cells expressed human ES cell-specific surface antigens, such as SSEA-3, SSEA-4, TRA-1–60, and TRA-1–81. These cells also expressed the ES cell-specific transcription factors, OCT3/4, and NANOG (Fig. 2A and supplemental Fig. S2).

The DNA methylation states of CpG dinucleotides in the OCT3/4 and NANOG promoter regions were evaluated by bisulfite genomic sequencing analysis. All The parental cells were highly (OCT3/4) or partially (NANOG) methylated, whereas all of the iPS cells were highly unmethylated (Fig. 2B). These data suggested that these promoters were active in these iPS cells. All of the iPS cells expressed undifferentiated ES cell maker genes, such as OCT3/4, SOX2, NANOG, reduced expression 1 (REX1), undifferentiated embryonic cell transcription factor 1 (UTF1), growth and differentiation factor 3 (GDF3), developmental pluripotency-associated 2 (DPPA2), DPPA4, and DPPA5. However, the cells did not express retroviral transgenes (Tgs), such as Tg-OCT3/4, Tg-SOX2, and Tg-KLF4, indicating retroviral silencing. The cells before retroviral transduction (parental cell) expressed KLF4 and MYC but did not express other markers (Fig. 2C).

We also examined telomerase activity, which is known to be highly activated in cancer cells and ES cells. Each iPS cell showed high telomerase activity, whereas parental cells as well as heat-treated cells did not (Fig. 2D).
**Generation of iPS Cells from Wisdom Teeth**

**FIGURE 2.** Characterization of iPS cells from clonally expanded MSCs and HDF. 
(A) Immunocytochemistry of SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, OCT3/4, and NANOG for iPS cells. Scale bars = 100 μm. 
(B) DNA methylation states of the OCT3/4 and NANOG promoter regions. Open circles indicate unmethylated CpGs, and closed circles indicate methylated CpGs. 
(C) RT-PCR analysis of ES cell marker genes and retroviral Tgs for iPS cells from different colonies (cl.8/cl.15 derived from 10F-15 clonal cells, and cl.1/cl.8 derived from 10F-101 clonal cells). D, telomerase activity detected by TRAP assay. +, heat-treated; −, non-treated samples; P, parental cell. IC, internal control.

**Embryoid Body Formation and in Vitro Differentiation**—To examine the differentiation potential of iPS cells, we performed floating culture to demonstrate embryoid body formation. After 8 days of floating culture, iPS cells formed embryoid bodies seen as sphere structures (Fig. 3A). These embryoid bodies were transferred onto gelatin-coated plates for another 8 days to induce spontaneous differentiation. The cells attached to the culture dish showed various types of cell morphologies; immunochemistry demonstrated that the cells were positive for SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, OCT3/4, and NANOG for iPS cells (scale bars = 100 μm, B). DNA methylation states of the OCT3/4 and NANOG promoter regions. Open circles indicate unmethylated CpGs, and closed circles indicate methylated CpGs. C, RT-PCR analysis of ES cell marker genes and retroviral Tgs for iPS cells from different colonies (cl.8/cl.15 derived from 10F-15 clonal cells, and cl.1/cl.8 derived from 10F-101 clonal cells). D, telomerase activity detected by TRAP assay. +, heat-treated; −, non-treated samples; P, parental cell. IC, internal control.

**Teratoma Formation**—To test for pluripotency, we injected iPS cells into the testis of severe combined immunodeficient mice. Nine weeks after injection, all showed tumor formation. Histological examination of the tumor demonstrated representative tissues of three embryonic germ layers, such as gut-like epithelium (endoderm), cartilage (mesoderm), and neuroepithelial rosettes (ectoderm) (Fig. 4, A–I). These findings indicated that the tumor was teratoma. Thus, like ES cells, iPS cells derived from clonally expanded MSCs have in vivo capability of differentiation into the three embryonic germ layers.

**Karyotype Analysis**—We also performed karyotype investigations by chromosomal G-band analysis (supplemental Fig. S3) and multicolor FISH analysis (data not shown). Two lines of iPS cells from 10F-101 showed an additional change in chromosome 16. However, the corresponding parental cell also showed the addition at the same locus, suggesting that the chromosomal aberration was already present in the parental cell (data not shown). Other iPS cells (10F-15 and HDF) showed normal karyotypes (supplemental Fig. S3).

**Global Gene Expression Profiles (Parental Cells versus iPS Cells)**—We explored global gene expression profiles of the parental cells (10F-15, 10F-101, and HDF) and iPS cells. Previous studies reported that expression of reprogramming factors (OCT3/4, SOX2, KLF4, MYC, NANOG, LIN28, and UTF1) and inhibition of p53 expression were effective for the generation of iPS cells (2, 3, 8). Microarray analysis showed that the mRNA levels of reprogramming factors, including OCT3/4, SOX2, NANOG, LIN28, and UTF1, were highly expressed in iPS cells when compared with those of the parental cells (Fig. 5A). The expression levels of MYC and p53 were similar in each parental cell and iPS cells. However, KLF4 expression was significantly lower (0.1-fold) in 10F-15 parental cells when compared with that of other parental cells and iPS cells. In addition, KLF5 expression was slightly higher (3-fold) when compared with that of the other cells (Fig. 5A).

Scatter plot analysis showed that ES cell marker genes, such as OCT3/4, NARNOG, LIN28, sal-like 4 (SALL4), and teratocarcinoma-derived growth factor 1 (TDGF1), were highly expressed in iPS cells (Fig. 5B). Heat map analysis showed that gene expression profiles of each iPS cell were similar but different from the corresponding parental cell. These profiles of iPS cells were also similar to those of BG01 and H9 human ES cells (Fig. 5C).

**Global Gene Expression Profiles (High Reprogramming Cells versus Low Reprogramming Cells)**—To explore the reprogramming mechanism(s) whose pathways and genes are significantly different between parental cells of high and low reprogramming cells, we examined global gene expression profiles of microarray data using z-score transformation (30). First, we analyzed previously reported reprogramming factors (2, 3, 8, 20, 31–35). Although expressions of KLF4, KLF2, KLF5, MYC, TP53, TBX3, and CHD1 were detected in each parental cell, it is hard to show statistical differences of these gene expressions between the high and low reprogramming cells (supplemental Table S2). Expressions of other reprogramming factors (OCT3/4, SOX family, KLF1, MYC family, NANOG, LIN28, UTF1, ESR2B, SALL4, and NR5A2) were not detected in each parental cell (supplemental Table S2). Although significant difference was not found, the KLF4 gene expressions...
Generation of iPS Cells from Wisdom Teeth

DISCUSSION

In our preliminary experiments, we examined whether or not MSCs from the human third molars could be reprogrammed by transduction of three transcription factors, OCT3/4, SOX2, and KLF4, using commercially available lentivirus. We generated iPS cells using the lentivirus; however, the efficiency was extremely low (data not shown). Therefore, we used pMXs retrovirus in this study. We transduced these three transcription factors using the retrovirus into culture-expanded but not clonally expanded MSCs from third molars of 10-, 13-, and 16-year-old donors. We detected the different efficiency of iPS generation. The most effective MSCs were from the 10-year-old donor (Fig. 1A). Because the MSCs were a heterogeneous cell population, we then clonally expanded the MSCs from the donor and selected five clones of 10F-5, 10F-8, 10F-15, 10F-101, and 10F-107. We found that proliferation ability was positively correlated with reprogramming efficiency (Fig. 1, B, C, and D), whereas viral transduction efficiency was similar (Fig. 1E). The clonally expanded MSCs of 10F-5, 10F-8, and 10F-15 could be efficiently reprogrammed at a rate that is ~30–100-fold higher than that of 10F-101, 10F-107, and HDF (Fig. 1C). In addition, 10F-15 reprogrammed 7-fold higher efficiency when compared with the non-cloned parental MSCs (Fig. 1, A and C). These results indicated that the parental MSCs from the 10-year-old donor contained more susceptible cells for iPS generation than other parental MSCs.

We tried to explore the difference among 10F-15, 10F-101, and HDF to determine the factor(s) affecting reprogramming promotion. One of the possibilities is that 10F-15 might have highly expressed reprogramming factor(s) in the same way as neural stem cells, which have highly expressed endogenous Sox2, can be easily reprogrammed (36). Furthermore, the family genes of Sox2 (Sox1, Sox3, Sox15, and Sox18), Klf4 (Klf1, Klf2, and Klf5), and Myc (L-Myc and N-Myc) can reprogram mouse embryonic fibroblasts to iPS cells (20). Recently, transduction of UTF1 and/or introduction of p53-siRNA improved the efficiency of human iPS generation (8). Therefore, we analyzed these mRNA expression levels by DNA microarrays. The reprogramming factors, including OCT3/4, SOX2, MYC, NANO.G, LIN28, UTF1, and p53, were not different in each parental cell (Fig. 5A). However, the expression level of KLF4 was significantly lower (0.1-fold), and KLF5 was slightly higher (3-fold) when compared with parental cells of 10F-101 and HDF (Fig. 5A). Other SOX, KLF, and MYC family genes did not differ in each parental cell (data not shown).

The data of low expression of KLF4 in high reprogramming cells (10F-15, 10F-5, and 10F-8) (Fig. 5A and supplemental Fig. S4) was somehow confusing because the KLF4 is a well...
known factor to induce iPS cells. In this regard, it was reported that KLF4 is highly expressed in growth-arrested cells and terminally differentiated cells (37, 38). 10F-5, 10F-8, and 10F-15 parental cells could differentiate into osteoblasts when cultured in osteogenic culture medium containing dexamethasone, whereas 10F-101 and 10F-107 parental cells could not. The data might indicate that the existence of abundant competent stem cells with low KLF4 expression in the third molar is feasible for iPS generation.

We attempted to identify the unknown factor(s) that facilitate the reprogramming in high reprogramming cells (10F-15, 10F-5, and 10F-8). As seen in supplemental Table S2, among these genes, we are interested in PAXIP1 (also called PTIP) gene because Ptip1/H11002 mouse ES cells exhibited low expression of Oct3/4, whereas they exhibited high expression of trophectodermal marker Hand1 (39). The PAXIP1 gene was highly expressed in the high reprogramming cells detected by global gene expression profiles. PAXIP1 acts as component of a histone H3 lysine four (H3K4) methyltransferase complex (40, 41). It is known that histone acetylation and H3K4 methylation promote activation of transcription. One of the histone deacetylase inhibitors, valproic acid, accelerates reprogramming efficiency in mouse and human fibroblasts (7, 42). Recently, it was reported that H3K4 methylation is associated with efficient reprogramming of pluripotent gene (Oct3/4, Sox2, and Sall4) expression by mouse somatic cell nuclei transplantation into amphibian oocytes (43). These results might imply that the high expression of PAXIP1 in the high reprogramming cells (10F-15, 10F-5, and 10F-8) enhanced the generation of iPS cells. Interestingly, PAXIP1 is also reported to have roles in DNA double-strand break repair (44). Similarly, INO80, SRCAP (also called SWR1), poly(ADP-ribose)polymerase (PARP), and so on are known as other factors involved in both histone modification and DNA repair (45–47). Among them, we are particularly focusing on the PARP family (47), especially the PARP-1 gene, because as seen in supplemental Table S2C, its expressions in all high reprogramming cells (10F-15, 10F-5, and 10F-8) were higher than those of low reprogramming cells.

As described, expressions of PAXIP1 gene in high reprogramming cells were high and about 30% more than low reprogramming cells. Furthermore, we also detected 3–4 times more expression of PAXIP1 in iPS cells when compared with each parental cell (Fig. 5A). This noticeable change (high expression after iPS cell induction) was also seen in PARP-1 expression after iPS cell induction. This was confirmed at both the gene and the protein expression levels.4 PARP-1 is the most abundant of PARP family members, accounting for >85% of nuclear PARP activity and modifying histone structure through NAD+-dependent "PARylation," i.e. poly(ADPribosyl)ation. We thus speculate that high iPS generation clones may be accessible for

4 Y. Yoshimura, Y. Oda, H. Ohnishi, M. Todokoro, Y. Katsube, M. Sasao, K. Hattori, S. Saito, K. Horimoto, S. Yuba, and H. Ohgushi, manuscript in preparation.
reprogramming factors due to feasible conformational change of chromatin by direct/indirect actions of chromatin modification proteins such as PAXIP1 and possibly PARP-1. Further extensive studies are needed to elucidate the mechanisms to promote reprogramming cascade.

In this study, we demonstrated that MSCs from human third molars can generate iPSCs by retroviral transduction of three transcription factors (OCT3/4, SOX2, and KLF4) without MYC or LIN28. The iPSCs were similar to human ES cells in many aspects, including morphology, surface markers and gene expression, DNA methylation states in the promoter regions, in vitro differentiation, and teratoma formation. Because human third molars are discarded as clinical waste and MSCs isolated from the molars could be frozen and stored for many years, MSCs are a useful cell source for the generation of iPSCs. Interestingly, even clonally expanded MSCs could be reprogrammed with high iPSC generation efficiency. Consequently, the cells might be available for iPSC generation by other methods using plasmids (48), chemicals, proteins (49, 50), and microRNAs (51) aiming for the clinical use of the iPSCs in regenerative medicine.

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