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Side population cells expressing ABCG2 in human adult dental pulp tissue

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Abstract

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Aim To investigate the presence of side population (SP) cells by the Hoechst exclusion method in human adult dental pulp tissue.

Methodology Human adult dental pulp-derived cells were generated from third molar teeth. The cells were stained with Hoechst 33342 and sorted into SP cells or non-SP cells [main population (MP) cells]. Both cell types were compared with cell growth and RT–PCR analyses.

Results SP cells that express ABCG2, Nestin, Notch-1 and α-smooth muscle actin were found at frequencies ranging from 0.67% to 1.02%. This SP profile disappeared in the presence of verapamil. These SP cells expressed dentine sialophosphoprotein and dentine matrix protein-1 when cultured in osteogenic medium.

Conclusion Human adult dental pulp tissue contains SP cells that differentiate into odontoblast-like cells.

Keywords: ABCG2, dental stem cells, Hoechst 33342, human dental pulp, odontoblast differentiation, side population.

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Introduction

During tooth development, dental pulp cells located close to the inner enamel epithelial cells gradually differentiate into odontoblasts and secrete the dentine matrix. It has been demonstrated that human dental pulp stem cells (DPSCs) injected subcutaneously into nude mice have the capacity to regenerate dentine (Gronthos *et al.* 2000). Previous studies have reported tissue engineering of a complex tooth structure using porcine tooth bud cells seeded onto scaffold (Young *et al.* 2002, Honda *et al.* 2005, 2006a). These results suggest that progenitor or stem cells located in dental pulp preserve the potential for dentinogenesis. Despite the large number of *in vitro* studies conducted in this area, the unequivocal identification of DPSCs has remained elusive, because of the lack of biological markers specific for these cell types (Kasugai *et al.* 1993, Buchaille *et al.* 2000, Gronthos *et al.* 2000).

Side population (SP) cells in the haematopoietic system have been identified on the basis of exclusion of the dye Hoechst 33342 (Goodell *et al.* 1996). SP cells give a characteristic profile distinct from the main cell population on fluorescence-activated cell sorting (FACS) analysis and can be visualized as negatively staining cells on a density dot plot. SP cells in bone marrow express the stem cell markers Sca-1 (stem cell antigen) and c-kit but lack expression of mature haematopoietic cell markers (Goodell *et al.* 1997; Scharenberg *et al.* 2002). SP cells from bone marrow

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are enriched for haematopoietic stem cell activity such that only 200 SP cells are required to fully reconstitute the bone marrow of a lethally irradiated mouse (Goodell *et al.* 1996). Recently, SP cells have been identified in solid tissues, including skeletal muscle, testes, lung and mammary glands (Asakura *et al.* 2002, McKinney-Freeman *et al.* 2002, Welm *et al.* 2002, Kubota *et al.* 2003, Summer *et al.* 2003). More recently, SP cells have been identified in human periodontal ligament cells (Kawanabe *et al.* 2006) and porcine dental pulp tissues (Iohara *et al.* 2006). SP cells are highly enriched for stem cell activity (Zhou *et al.* 2001, Matsuzaki *et al.* 2004). However, there are no data of SP cells in human adult dental pulp tissue.

The efficacy of Hoechst 33342 to identify stem cells resides in the capacity of stem cells to exclude the dye by membrane efflux pumps of ATP-binding cassette transporters, including BCRP1/ABCG2 (Zhou *et al.* 2001). Verapamil blocks the activity of these pumps and leads to the loss of the SP fraction when combined with the Hoechst dye. Hoechst efflux has proved to be a highly useful primary purification strategy for isolating stem cells from dental tissue in the absence of cell-surface markers (Iohara *et al.* 2006).

In the first part of this study, cultured human adult dental pulp-derived cells were generated then tested for the presence of typical SP cells by the Hoechst exclusion method in three different donor-derived dental pulp-derived cell lines. Dental pulp tissue was shown to contain SP cells, referred to as dental pulp SP cells. Gene expression analysis was used to assess the progenitor cell identity of isolated dental pulp SP cells.

In the second part of this study, the capacity of SP cells to differentiate into odontoblast-like cells was investigated. The influence of dexamethasone on the differentiation of SP cells into odontoblast-like cells was assessed by analysing the level of dentine sialophosphoprotein (DSPP) mRNA (Gronthos *et al.* 2000, Alliot-Licht *et al.* 2005) and dentine matrix protein 1 (DMP-1) mRNA. Expression of DSPP and DMP-1 is an important characteristic of odontoblasts (Bleicher *et al.* 2001; Feng *et al.* 2003). DMP-1 is a major acidic phosphoprotein that was first cloned from the mineralized dentine matrix (George *et al.* 1993) and later from bone matrix (MacDougall *et al.* 1998), it has been postulated to play an important role in mineralized tissue formation (George *et al.* 1993). The DSPP gene, which encodes the two dentine proteins, dentine sialoprotein and dentine phosphoprotein, is considered as a major odontoblastic marker (D'Souza *et al.* 1997, Buchaille *et al.* 2000).

Materials and methods

The procedures used to acquire human adult dental pulp tissue from the surgically extracted teeth conformed to the tenets of the Declaration of Helsinki. This project was approved by the local ethical committee of the Institutional Animal Care and Use Committees (IACUC) at the Institute of Medical Science, the University of Tokyo and the Ethical Review Committee of the Tokushima University Hospital.

Isolation of human adult dental pulp-derived cells

Human adult dental pulp-derived cells were obtained from impacted mandibular third molars extracted from three patients (between 14- and 17-year olds) during orthodontic treatment. Informed consent was obtained for the use of tooth germs. The central portion of the dental pulp tissue was sectioned into small pieces with a scalpel and placed in a culture flask. The tissue was then cultured in alpha modification of Eagle medium (α-MEM; Sigma-Aldrich Co., St Louis, MO, USA) containing 10% foetal bovine serum (FBS; Sigma-Aldrich), 1 × Glutamax (Invitrogen Corp., Carlsbad, CA, USA), 50 μg mL^{-1} L-ascorbic acid phosphate magnesium salt n-hydrate (Wako Pure Chemical Industries Ltd, Osaka, Japan), 100 units mL^{-1} penicillin and 100 mg mL^{-1} streptomycin (Invitrogen). The culture was maintained in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. The cells migrating from the dental pulp tissue were collected by treating the culture with 0.05% trypsin/0.53 mmol L^{-1} EDTA (Invitrogen) for 20 min. Cells were maintained as a monolayer culture under the same conditions [heterogeneous dental pulp cell (HPC)] until the third passage. The medium was changed every 3 days. Three populations of the dental pulp cells derived from different donors were generated for FACS and after a sufficient number of the cells for the following analysis were obtained, the cells were kept in cold storage at −80 °C. At the same time, after thawing in a hot bath, three different derived-dental pulp cells were analysed by FACS flow cytometry. After sorting, the cells were cultured in the same culture conditions, and the subcultured cells were analysed for the presence of SP fractions by flow cytometry.

For cellular differentiation and the induction of calcified matrix deposition as reported previously (Gronthos *et al.* 2000, Alliot-Licht *et al.* 2005),

dexamethasone (Dex, Sigma-Aldrich) at a final concentration of 10^{-8} mol L^{-1} was added to the experimental cultures. The culture medium was composed of α-MEM containing 10% FBS. Cells were cultured for 3 weeks for RT-PCR analysis. The data presented are representative of reproducible results from three different donors.

Staining of human adult dental pulp-derived cells with Hoechst 33342

Staining of three kinds of human adult dental pulp-derived cells with Hoechst 33342 was performed according to the procedure developed by Goodell *et al.* (1996). Collected pulp cells were resuspended at a concentration of 1×10^6 cells mL^{-1}. No more than 10 million cells per 50 mL tube were used. Cells were stained in Hoechst 33342 (5 μg mL^{-1}; Sigma-Aldrich) at 37 °C for 90 min in α-MEM supplemented with 2% FCS, 1 mmol L^{-1} HEPES, and penicillin/streptomycin. Cells were then placed immediately on ice. Control cells were exposed to verapamil (Sigma-Aldrich; 50 mmol L^{-1} stock dissolved in 95% ethanol) at a final concentration of 0.2 mmol L^{-1} to block the cell membrane pumps that exclude Hoechst 33342. After staining, cells were resuspended in cold Hanks' balanced salt solution supplemented with 2% FCS at 1×10^6 cells per millilitre. Cell analysis and sorting were performed using FACS VantageSE [three lasers, Becton Dickinson Immunocytometry System (BDIS), Mountain View, CA, USA]. Hoechst 33342 was excited at 350 nm, and fluorescence emission was detected using 405/BP30 and 585/BP20 optical filters against Hoechst Blue and Hoechst Red, respectively. A 555-nm-long pass dichroic mirror (Omega Optical Inc., Brattelboro, VT, USA) was used to separate the emission wavelengths of Hoechst Blue and Hoechst Red, and live cells were collected after cells positive for propidium iodide staining (2 μg mL^{-1}) (Sigma-Aldrich) were excluded according to the configuration of Hoechst Blue/Hoechst Red emission.

Cell proliferation analysis

Sorted SP, non-SP (MP: G0–G1) cells which originated from sorted cells and HPCs obtained prior to sorting were plated at low density (1000 cells per well) onto 100 mm petri dishes coated with collagen type I (Col-1, Asahi Technoglass Corp. Tokyo, Japan). Cell proliferation was measured using a WST-8 kit (Wako, Tokyo, Japan) according to the manufacturer's protocol. The cells were cultured as described above. Cell proliferation was assessed after 1, 4 and 7 days of culture in triplicate. Absorbance was measured at 450 nm using a microtitre plate reader (SmartSpeck™ 3000, Bio-Rad, Tokyo, Japan). The experimental value was given as mean + SD. Student's *t*-test was used to analyse statistically significant differences using EXCEL 7.0 software. Morphology of the cultured SP and MP cells was observed using a phase-contrast microscope.

Semiquantitative RT-PCR analysis

RNA extracted using TRIZOL reagent (Invitrogen) from SP and MP cells obtained from three different dental pulp-derived cell lines, and SP and MP cells differentiated in osteoinductive medium, was used to generate cDNA. Total RNA was reverse transcribed using the SuperScript™ First-Strand Synthesis System (Invitrogen) for RT-PCR. PCR SuperMix (Invitrogen) was used in all PCR reactions. One microgram of RNA was used per reaction. Typical amplification conditions were 95 °C for 30 s, 51–62 °C for 30 s and 72 °C for 30 s. RT-PCR products were separated by electrophoresis on a 2% agarose gel and stained with ethidium bromide. Primary sequences and cycling conditions are shown in Table 1. The housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), was used as control. The PCR was performed thrice using RNAs obtained from each SP and MP cell fraction. In addition, the percentage of mRNA expression in SP and MP cells from *in vitro* gene expression analysis was measured using SCION IMAGE picture-imaging software (Scion Corp., Frederick, MD, USA).

Results

SP cells were detected in human adult dental pulp-derived cells

Initial studies on human adult dental pulp-derived cells after freezing and re-thawing established the optimal conditions for the isolation of SP cells. The optimal Hoechst concentration was 2.5 μmol L^{-1} and incubations were performed for 90 min. The FACS analysis of three kinds of dental pulp cells revealed that 0.6–1.02% (average 0.79%) of the total cell population were a distinct and reproducible SP cell population (Fig. 1a). Verapamil, a powerful pan-ABC transporter (Zhou *et al.* 2001), completely abolished

Table 1 Primer sequences used for human gene expression analysis by the reverse transcription/polymerase chain reaction

Gene	Primer set (5′–3′)	Products	Accession # or reference
GAPDH	S-CGTCTTCACCACCATGGAGA	300	M33197
	A-CGGCCATCACGCCACAGTTT		
ABCG2	S-ATGGATTTACGGCTTTGCAG	344	XM032424
	A-GATGGCAAGGGAACAGAAAA		
Nestin	S-GCCCTGACCACTCCAGTTTA	200	BC051373
	A-GGAGTCCTGGATTTCCTTCC		
Notch-1	S-GCACTGCGAGGTCAACAC	177	AF308602
	A-AGGCACTTGGCACCATTC		
α-SMA	S-AGGAAGGACCTCTATGCTAACAAT	355	Jester *et al.* (2003)
	A-AACACATAGGTAACGAGTCAGAGC		
Col-1	S-AGGCCCTCAAGGTTTCCAAGG	233	Y00724
	A-CCAGACCATTGTGTCCCCTAA		
DSPP	S-CCTAAAGAAAATGAAGATAATT	293	Alliot-Licht *et al.* (2005)
	A-TAGAAAAACTCTTCCCTCCTAC		
DMP-1	S-GACTCTACAGAAAACAGCAACT	522	NM004407
	A-GTTGCTATCTTCTTTGGATCTG		
AMBL	S-GCTAAAACACTTATTACCCTT	262	Buchaille *et al.* (2000)
	A-AATAGTGTCATGCTGGTAAGAG		

S, sense; A, antisense; ABCG2, ATP-binding cassette transporter ABCG2(BCRP1); α-SMA, α-smooth muscle actin; Col-1, collagen type I; DSPP, dentine sialophosphoprotein; DMP-1, dentine matrix protein 1; AMBL, ameloblastin.

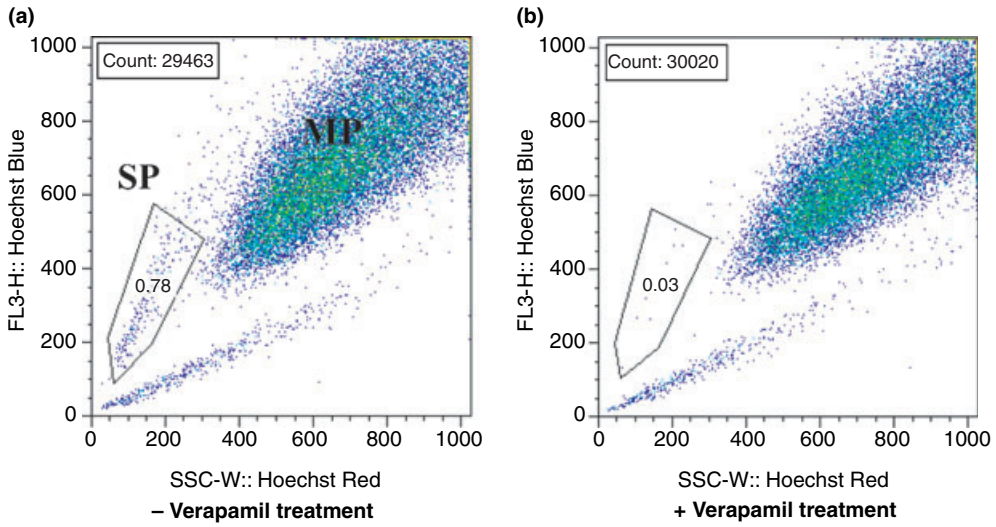

Figure 1 Detection of SP cells in human adult dental pulp. Hoechst 33342 staining of dental pulp-derived cells revealed that (a) approximately 0.78% of total dental pulp-derived cells showed the SP cell staining pattern that disappeared with (b) verapamil treatment. The gated region is indicative of SP and MP cells.

these SP cells (Fig. 1b). All results of the analysis using verapamil obtained from three different donors were consistent. Although the thawed human adult dental pulp cells contained SP cell fractions, the SP fractions disappeared after the dental pulp SP cells were subcultured for all three different donor-derived dental pulp cells.

Characterization of isolated SP and MP cells by gene expression analysis

Semiquantitative RT-PCR was used to analyse gene expression in SP and MP cells. To determine if the dental pulp SP cells had characteristics typical of SP cells, we tested whether the ABCG2 was expressed or

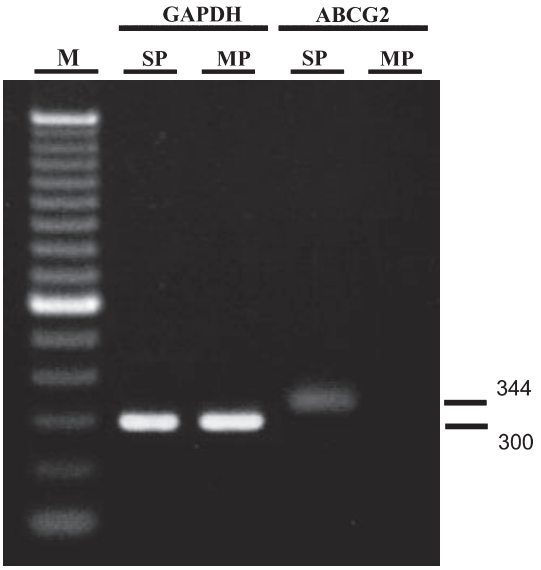

Figure 2 RT-PCR demonstrates the expression of ABCG2 mRNA in SP cells, but not in MP cells. The 300-bp GAPDH product, which serves as a control, was expressed in both cells. M, 100-bp DNA ladder.

not by RT-PCR. The ABCG2 was expressed in the SP cell fraction, but not in the MP cell fraction (Fig. 2). The results were consistent in all three types of SP and MP cell fractions. Next, whether both SP and MP cells expressed stem cell and odontoblast-related marker genes were tested. The expressions of Nestin, Notch-1, α-smooth muscle actin (α-SMA), Col-1, DMP-1, DSPP and ameloblastin (AMBL) were also investigated in the SP and MP cell fractions. Significantly higher expression of Nestin and α-SMA mRNAs were found in SP cells compared with MP cells (Fig. 3a). The expression level in SP cells was 2.5-fold higher for Nestin and 3.8-fold higher for α-SMA mRNAs than in MP cells as determined by SCION IMAGE analysis. Notch-1 and Col-1 were expressed at a similar level in SP and MP cells (Fig. 3b). In contrast, transcripts for DSPP, DMP-1 as a marker for odontoblasts, and AMBL were not detected (Fig. 3b). AMBL is one of the markers for ameloblasts derived from dental epithelium (Krebsbach *et al.* 1996).

Cell proliferation analysis

The proliferative capacity of SP cells was assessed by comparing the rate of cell growth of SP cells with that of MP cells although the evaluated number of SP and MP cell fractions was low ($n = 3$). Isolated SP cells grew significantly more rapidly than MP cells over 7 days of culture (Fig. 4a). SP cells generated from all populations proliferated, whilst MP cells generated from 1 of 3 populations ceased dividing once seeded on the culture dish. The proliferation in heterogeneous cells was slightly slower than in SP cells; however, there was no significant difference.

Cells appeared after 1 day in culture [Fig. 4b(i, ii)] and both SP and MP cells gradually became multilayered after 7 days in culture [Fig. 4b(iii, iv)]. The morphology of SP and MP cells was similar in the culture dish.

Expression of DSPP and DMP-1 in differentiating SP cells

Heterogeneous human dental pulp cells cultured without Dex were negative for DSPP and DMP-1 mRNA expressions, indicating an undifferentiated phenotype (Fig. 5). At the beginning of the culture prior to Dex treatment, neither SP nor MP cells expressed DSPP or

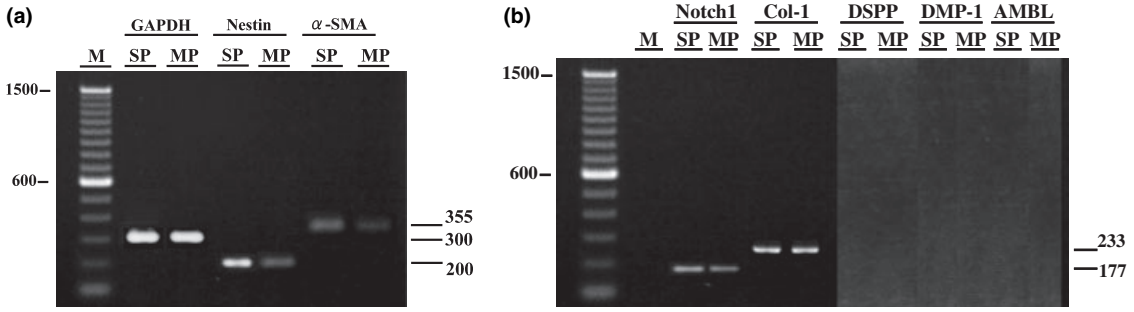

Figure 3 Gene expression profiles of SP and MP cells. (a) SP cells isolated by FACS express Nestin and α-SMA. Nestin and α-SMA expression profiles are distinct. The 300-bp GAPDH product, which serves as a control, was expressed at the same level. (b) Notch-1 and Col-1 show expression at similar levels in SP and MP cells. DSPP, DMP-1 and ameloblastin expressions were not detected in both SP and MP cells. M, 100-bp DNA ladder.

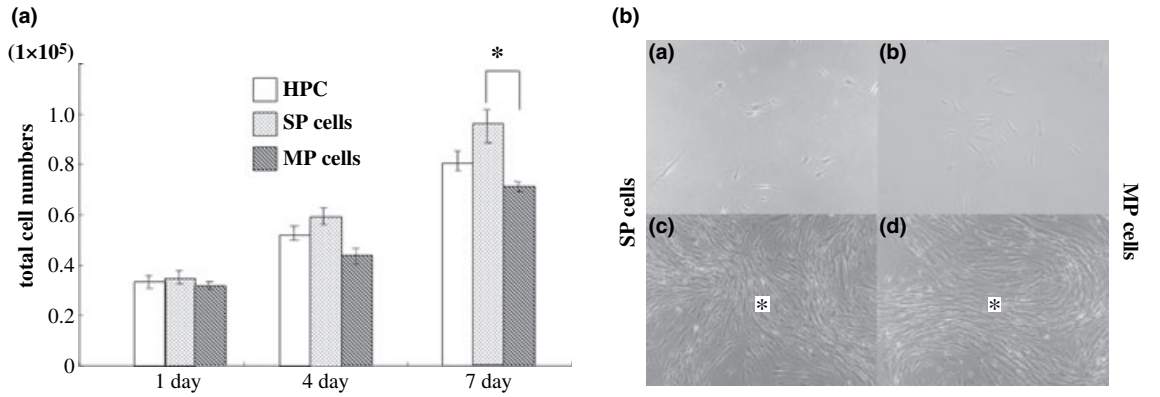

Figure 4 Proliferation potential of SP cells and analysis of cell morphology. (a) Cell proliferation of SP cells (n = 3), MP cells (n = 3) and heterogeneous pulp cells (n = 3). SP cells proliferated at a greater rate than MP cells. (b) Standard phase-contrast photomicrographs of SP (a, c) and MP cell (b, d) cultures after 1 day (a, b) and 10 days (c, d) showing spindle shape morphology*. There is no remarkable difference.

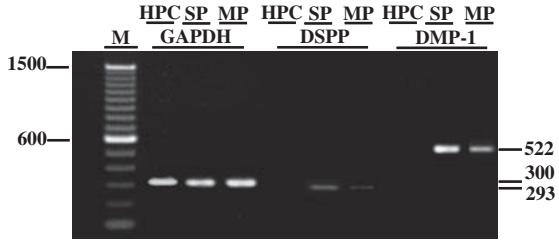

Figure 5 Effects of Dex on the expression of DSPP and DMP-1 in dental pulp SP cells. DSPP and DMP-1 mRNA were expressed in both SP and MP cells exposed to osteoinductive medium (SP), but not in primary heterogeneous human dental pulp cells without Dex. The 300-bp GAPDH product, which serves as a control, was expressed at the same level. M, 100-bp DNA ladder.

DMP-1 (Fig. 3a). However, both SP and MP cells were induced to differentiate and expressed both DSPP and DMP-1 in the presence of Dex osteogenic medium (Fig. 5). The expression levels of DSPP and DMP-1 in SP cells were 2.7-fold and 1.5-fold higher, respectively, than those in MP cells as determined by SCION IMAGE analysis.

Discussion

SP cells were first discovered in the haematopoietic system (Goodell *et al.* 1996) and are now considered to be primitive stem cells within this system (Jackson *et al.* 1999, Zhou *et al.* 2001, Bunting 2002, Kim *et al.* 2002, Lechner *et al.* 2002, McKinney-Freeman *et al.* 2002, Welm *et al.* 2002, Summer *et al.* 2003, Umemoto *et al.* 2005). In terms of dental tissue, the lack of specific surface markers has hindered the isolation and subsequent biochemical characterization of DPSCs. Although the number of SP cell fractions that used to obtain from human adult dental pulp was low (n = 3), the present study has demonstrated that they are present in human adult dental pulp cells after both freezing and re-thawing. The SP cell ratio varies from 0.05% in human bone marrow, 0.1% in human foetal pancreas (Zhang *et al.* 2005), 0.64% in human limbal tissue (Budak *et al.* 2005) to 15.1% in murine brain (Goodell *et al.* 1997, Asakura *et al.* 2002). The present study has shown that human adult dental pulp-derived cells contain SP cells at a level of approximately 0.8%. Recently, Iohara *et al.* (2006) reported SP cell detection in porcine adult dental pulp tissue, and that the SP cell ratio was approximately 0.2% of the gated population of primary pulp cells. This ratio of SP cells is similar to the present study. In another study on dental tissues, the SP cell fractions in human periodontal tissue were demonstrated to be about 3.9% of total cells (Kawanabe *et al.* 2006). This ratio is much higher than in the present study. This discrepancy may be a tissue specific phenomenon. From these results, the range of SP cell ratios in each tissue may be broad. In the present study, the SP cell fraction disappeared after subculture, which was an unexpected finding. One reason may be that it is difficult to maintain the dental pulp SP cells in 2D culture. Another possible reason is that the culture method used may not be suitable for maintaining the dental pulp-derived SP cells although the culture

technique is standard. Further study is needed to explore suitable culture conditions for human adult dental pulp SP cells.

Several studies have demonstrated that ABCG2 is responsible for the Hoechst dye efflux pattern in SP cell fraction, and so ABCG2 is considered as a general stem cell marker (Zhou *et al.* 2001, Bunting 2002, Staud & Pavek 2005). Recently, SP cells derived from human pancreatic islets were shown to express ABCG2 (Lechner *et al.* 2002). In the present study, SP cells expressed ABCG2 mRNA, but MP cells did not. Therefore, it is likely that ABCG2 is involved in human dental pulp SP cells.

Nestin is a known marker of neural stem cells (Lendahl *et al.* 1990), and is expressed during odontoblast development in human and rodent tooth germ (Terling *et al.* 1995, About *et al.* 2000). Nestin is thought to play a major role in the differentiation of odontoblasts and the capacity to produce dentine. Moreover, E18 mouse tooth germ cells expressed Nestin with a specific antibody (Priam *et al.* 2005). Recently, Nestin and Notch-1 were detected in subcultured human dental follicle cells as markers for undifferentiated cells (Morsczeck *et al.* 2005). Dental follicle cells are known to harbour precursor cells for periodontium. In addition, Notch-1 plays a critical role in normal human mammary development, by acting on both stem cells and precursor cells, affecting self-renewal and lineage-specific differentiation (Dontu *et al.* 2004). Furthermore, Notch-1 is a well-recognized stem cell marker for haematopoietic (Stier *et al.* 2002), neural (Kumano *et al.* 2001) and dental tissue stem cells (Harada *et al.* 1999). Both Nestin and Notch-1 are now recognized as general markers of stem/progenitor cell. It has been demonstrated that Nestin and Notch-1 are expressed in both SP and MP cells from human adult dental pulp tissue although the expression levels are different. However, after SP and MP cells were subcultured, Nestin and Notch-1 could not be detected in either SP cells or MP cells by RT-PCR (data not shown). It may be that Nestin and Notch-1 are transiently expressed in undifferentiated human adult dental pulp cells. In addition, SP cells may be more immature than MP cells as the expression level of Nestin in SP cells is higher than in MP cells. In this study, it was shown that the SP cell markers, ABCG2, Nestin and Notch-1, are expressed in dental pulp derived SP cells, providing evidence of the stem/progenitor character of these cells. Further studies are needed to evaluate the function of Nestin and Notch-1 during tooth development.

It is reported that pericytes are present in human pulp culture (Alliot-Licht *et al.* 2001). Most DPSCs present with a phenotype consistent with pericytes (Shi & Gronthos 2003) and express α-SMA (Brock *et al.* 2002, Alliot-Licht *et al.* 2005). Pericytes are usually postulated to give rise to odontoblasts and form reparative dentine. Pericytes are thought to be the multipotent progenitor cell/stem cell type with the capacity to regenerate odontoblasts (Alliot-Licht *et al.* 2001). To clarify this issue, SP cells expressing α-SMA mRNA were identified and it was shown that SP cells expressed α-SMA at higher levels than undifferentiated MP cells. The origin and precise location of dental pulp SP cells was unknown. However, like a number of different stem cell types, it is possible that they also arise from developing blood vessels (Bianco & Cossu 1999). The above results on the characteristics of dental pulp SP cells, including the expression of ABCGS, other stem cell related markers and proliferation activity, clearly show that dental pulp SP cells are similar to previously reported SP cells (Goodell *et al.* 1996, 1997, Jackson *et al.* 1999, Zhou *et al.* 2001, Bunting 2002, Kim *et al.* 2002, Lechner *et al.* 2002, McKinney-Freeman *et al.* 2002, Welm *et al.* 2002, Summer *et al.* 2003, Umemoto *et al.* 2005). However, further study is needed to identify the location of SP cells.

In the present study, the isolated and characterized SP cells were evaluated for their capacity to differentiate into odontoblasts. DSPP and DMP-1 are members of the small integrin-binding ligand N-linked glycoprotein (SIBLING) family. DMP-1 is critical for mineralization and tooth morphogenesis, and is expressed during odontogenesis (Narayanan *et al.* 2001, Lu *et al.* 2005). Although DSPP is expressed at a low level in bone (Qin *et al.* 2002), expression of DSPP is considered as a terminal phenotypic hallmark of mature odontoblasts (D'Souza *et al.* 1997, Buchaille *et al.* 2000). It is thought that progenitors within dental pulp tissue terminally differentiate into odontoblasts (Smith *et al.* 1995). *Ex vivo* expanded DPSCs have been shown not to express DSPP when cultured under noninductive conditions (Gronthos *et al.* 2000). Dex treatment results in an increase in DSPP mRNA in human dental pulp with α-SMA positive cells (Alliot-Licht *et al.* 2005). The possibility that Dex promotes the differentiation of a subpopulation of dental pulp SP cells towards an odontoblastic phenotype was investigated. Based on the effect of Dex on DSPP and DMP-1 expression, it was demonstrated that Dex stimulates the differentiation of both SP and MP cells into

odontoblast-like cells. In addition, the expression levels of DSPP and DMP-1 in SP cells were higher than those of MP cells. These results suggest that SP cells might have high level activity for differentiation into odontoblasts. Further study needs to clarify the activity for differentiation.

Dental pulp-derived SP cells investigated in this study do not represent a homogenous cell population, further investigations are required to determine multipotency and capacity to produce dentine of isolated SP cell clones.

Conclusion

This study provides evidence that SP cells isolated from dental pulp tissue have the capacity to differentiate into odontoblast-like cells and contributes significantly to the DPSC research. The isolation of pure populations of stem cells from dental pulp has great value in the field of tooth development and tissue engineering (Honda *et al.* 2006b, 2007).

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