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# Characterization of human dental pulp-derived cell lines

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## Abstract

**Suguro H, Asano M, Kaneko Y, Omagari D, Ogiso B, Moro I, Komiyama K.** Characterization of human dental pulp-derived cell lines. *International Endodontic Journal,* **41**, 609–616, 2008.

**Aim** To establish and characterize different types of fibroblastic cell lines derived from dental pulp tissue.

**Methodology** Human dental pulp tissue-derived cells were transfected with SV40 large T antigen by Lipofectamine transfection method. Geneticin (G418)-resistant cells were selected and different cell lines were established by a limiting dilution method. To characterize the lineages of cells, each clone was immunofluorescently stained by anti-fibroblast, anti-vimentin, anti-collagen type I and type III antibodies. Total RNA was extracted from each clone and subjected to a differential display experiment.

**Results** By transfecting SV40 large T antigen, nine different cell clones were obtained. All these cell clones were positively stained by anti-fibroblast, anti-vimentin, anti-collagen type I and type III antibodies. With differential display experiment, eight different genes, the expression levels of these genes were varied amongst each cell clone, were detected. After sequencing and database search, one gene was revealed to be identical to T-cell marker, Thy-1. Thy-1 expression in dental pulp tissue was confirmed by immunohistochemical staining.

**Conclusion** Fibroblastic cell lines derived from human dental pulp tissue possessed different gene expression profiles suggesting the existence of subpopulations.

**Keywords:** differential display, human dental pulp cell, SV40 large T antigen, Thy-1.

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## Introduction

The functional coupling of dental pulp and dentine is fundamentally relevant for many aspects of normal tooth function, and these two tissues are referred to as the pulpo-dentine complex (Hargreaves & Goodis 2002). The dental pulp is composed of several different cell types, including odontoblasts, fibroblasts and undifferentiated mesenchymal cells amongst others (Hargreaves & Goodis 2002). Recently, dental pulp-derived stem cells have become a central interest for many researchers, and the clinical application of these cells is an important potential research focus (Nakashima & Akamine 2005, Murray *et al.* 2007).

Immortalized dental papilla-derived cell lines have been established in several different species (MacDougall *et al.* 1995, Hanks *et al.* 1998, Thonemann & Schmalz 2000a,b, Galler *et al.* 2006). The mouse-derived cell line, MO6-G3, shows constitutive expression of dentine phosphoprotein, type I collagen and alkaline phosphatase and is considered to be a murine odontoblast cell (MacDougall *et al.* 1995). Bovine

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dental pulp-derived cells were immortalized by transfecting viral oncogenes, such as HPV18 E6/E7 or simian virus 40 large T antigen, and these cell lines have been shown to exhibit odontoblastic or tissue-specific properties (Thonemann & Schmalz 2000a,b). These cells are useful for many different experimental purposes such as cytotoxicity testing of dental filling materials (Schmalz *et al.* 1998, Schuster *et al.* 2001). The fact that normal diploid cells reach permanent growth arrest, resulting in replicative senescence, has prevented the establishment of different kinds of dental pulp-derived cell lines.

The purpose of this study was to establish several different cell lines derived from human dental pulp tissue and to investigate the properties of these subpopulations. These cell lines may contribute to the elucidation of many functional properties of dental pulp cells, for example, cytokine production or signalling cascades in response to extracellular stimuli such as bacterial or viral infections. Immortalization of human pulp-derived cells by transfecting the SV40 large T antigen has been reported by Galler *et al.* (2006). Utilizing the same viral oncogene, nine different fibroblastic cell clones were established. The gene expression profiles of these cells were examined using a differential display method, which yielded eight different genes. The expression of these genes has not previously been demonstrated in dental pulp tissue.

## Materials and methods

### Cells

This study was approved by the ethics committee at Nihon University School of Dentistry. Human dental pulp cells were isolated from a mandibular right third molar extracted at Nihon University Dental Hospital with the donor's informed consent (21-year-old female). Immediately after extraction, the tooth was rinsed in phosphate-buffered saline (PBS), and the crown and root portions were separated. The pulp tissue was obtained and minced in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, San Diego, CA, USA) supplemented with 10% heat-inactivated foetal calf serum (FCS), L-glutamine (50 μg $mL^{-1}$) (Sigma, St Louis, MO, UK) and 1% penicillin/streptomycin solution (Sigma) (10% FCS-DMEM) with scissors on a 10-cm culture dish. The cells were maintained at 37 °C in a 5% $CO_2$ incubator until they reached confluence.

### Immortalization of dental pulp-derived cells

The confluent cells were tripsinized and freshly plated on a 10-cm culture dish on the day before transfection. The cells were washed with OPTI-MEM (Invitrogen) three times and then transfected with 1 μg of SV40-large T antigen-encoded plasmid (pSV40) using the Lipofectamine Plus transfection method (Invitrogen) according to the manufacturer's instructions. Briefly, 1 μg of pSV40 plasmid DNA was mixed in 100 μL of OPTI-MEM with 6 μL of Plus reagent. At the same time, 6 μL of Lipofectamine was mixed with 100 μL of OPTI-MEM. Fifteen minutes later, the medium containing DNA was mixed with the Lipofectamine medium and incubated at room temperature for 15 min. The transfection media were then applied to the cells and incubated for 24 h at 37 °C in a 5% $CO_2$ incubator. The transfection media were replaced with fresh 10% FCS-DMEM and further cultured for 24 h. The cells were replated and cultured with 10% FCS-DMEM supplemented with 400 μg $mL^{-1}$ of geneticin (G418) (Sigma, Tokyo, Japan) to select transfectants. After 7 days, the surviving cells were collected with a cloning ring (Iwaki, Tokyo, Japan). Each cell was grown and subcloned by limited dilution.

### Detection of differentially expressed genes

To identify the genes expressed differentially amongst established cell lines, total RNA was extracted from each cell line and subjected to Gene Fishing differentially expressed gene (DEG) reactions (SeeGene, Seoul, Korea) (Kim *et al.* 2004). Total RNA was purified by using RNeasy mini kit (Qiagen, Tokyo, Japan). The first-strand cDNAs were synthesized as follows. Three micrograms of total RNA was mixed with 4 μL of 5× reaction buffer, 5 μL of dNTPs (each 2 mmol $L^{-1}$), 2 μL of 10 μmol $L^{-1}$ dT-ACP1 [5′-CTGTGAATGCTGCGACTACGAT(18)-3′], 0.5 μL of RNasin RNase inhibitor (40 U $\mu L^{-1}$; Promega, Tokyo, Japan) and 1 μL of Moloney murine leukemia virus reverse transcriptase (200 U $\mu L^{-1}$; Promega). First-strand cDNAs were diluted by the addition of 80 μL of ultra-purified water for the GeneFishing™ PCR, and stored at −20 °C until use.

Differential display was performed by using annealing control primers (ACP primers). First-strand cDNA (3–7 μL) was mixed with 2 μL of arbitrary ACP primer (5 μmol $L^{-1}$), 1 μL of dT-ACP2 (10 μmol $L^{-1}$) and 10 μL of 2× SeeAmp ACP Master Mix, and filled up to 20 μL with distilled water. For PCR, an initial 5-min hot start at 94 °C was followed by 50 °C for 3 min, and

another 3 min at 72 °C, followed by 40 cycles at 94 °C for 40 s, 65 °C for 40 s, 72 °C for 40 s and a final 72 °C, 5 min extension. Aliquots of the differential PCR were examined on a 2% agarose gel stained with ethidium bromide.

### Cloning and sequencing

The differentially expressed bands were extracted from the gel by using the GENCLEAN II Kit (Q-Biogene, Carlsbad, CA, USA), and directly cloned into a TOPO TA cloning vector (Invitrogen) according to the manufacturer's instructions. The cloned plasmids were sequenced with ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) using M13 forward primer (5′-CGCCAGGGTTTTCCCAGTCACGA-3′) or M13 reverse primer (5′-AGCGGATAACAATTTCACACAGGA-3′). The obtained sequences were checked against the database to identify the corresponding genes.

### Primer design and RT-PCR

Gene expression level of each gene was compared between nine cell clones by RT-PCR using sequence-specific primers listed in Table 1. The primers were designed according to the sequences from each gene.

### Immunofluorescence and immunohistochemical staining

For immunofluorescence cell staining, the cells were plated on the cover slips and cultured for 18 h. The cells were washed in PBS and fixed in 2% formaldehyde (Polysciences, Warrington, PA, USA) in PBS for 15 min at room temperature. After fixation, the cells were washed in PBS and permeabilized with 0.05% saponin (Sigma) in PBS for 10 min at room temperature. Permeabilized cells were washed twice with PBS and nonspecific staining was blocked by incubating the cells with 5% normal rabbit serum in PBS for 30 min at room temperature. The cells were then incubated with primary antibodies for 1 h at room temperature. Mouse anti-human fibroblast antibody (50×) (DBS), mouse anti-swine vimentin antibody (100×) (Dako Cytomation, Tokyo, Japan), rabbit anti-human type I collagen (80×) (Novotec, Lyon, France) and rabbit anti-human collagen type III (10×) (Monosan, Udon, the Netherlands) were used as first antibodies. After rinsing the cells three times with PBS, the cells were incubated with fluorescein-5-isothiocyanate (FITC)-conjugated goat anti-mouse antibody or FITC-conjugated goat anti-rabbit antibody (Cappel, Aurora, OH, USA) diluted in 5% normal rabbit serum-PBS. The cells were washed and cover slips were mounted in Aqua-Polymount (Polysciences). The images were viewed and photographed with a LSM510 confocal laser microscope (Carl Zeiss, Heidelberg, Germany).

For immunohistochemical study, the dental pulp tissues from the extracted teeth were carefully removed and fixed in 4% paraformaldehyde in PBS overnight. The fixed tissues were then dehydrated in a graded ethanol series, and embedded in paraffin according to standard histological procedures (Suzuki *et al.* 2007). Then 6-μm sections were cut and mounted onto poly-L-lysine coated slides (Muto Pure Chemical Co., Tokyo, Japan) and stored at room temperature. After deparaffinization, the sections were incubated with methanol containing 0.3% hydrogen peroxide for 15 min to block endogenous peroxidase activity, and with 1% bovine serum albumin–PBS (1% BSA–PBS) for 30 min to block nonspecific immunoreactive sites. The sections were then incubated with a 100× dilution of polyclonal rabbit anti-human Thy-1 antibody (Santa Cruz Biotech, Santa Cruz, CA, USA) in 1% BSA–PBS for 1 h. After washing in PBS three times, the sections were incubated with a 1000× dilution of peroxidase-conjugated goat anti-rabbit IgG (H + L) antibody (Jackson Immunoresearch, West Grove, PA, USA) for 1 h. The sections were thoroughly washed with PBS and the peroxidase reaction was developed in 0.05 mol $L^{-1}$ Tris–HCl buffer, pH 7.6, containing 0.05% 3,3′-diaminobenzidine tetrahydrochloride (DAB) (Sigma) and 0.05% hydrogen peroxide for 5 min. Specimens were

**Table 1** Sequence-specific primers used for RT-PCR

| Gene | Sequences |
|---|---|
| DEG 1 | |
| Forward primer | TAGCTCCTTTAAGAAAATGC |
| Reverse primer | GAACAGGTAAAGAGGAAACA |
| DEG 2 | |
| Forward primer | ACTTTATTGAATGACACTGT |
| Reverse primer | AAGTCTAGGGACAGGAAGAT |
| DEG 3 | |
| Forward primer | AATTCGTGGACATCATCAAT |
| Reverse primer | ATGGGTGGTTCACATACACA |
| DEG 7 (clone A) | |
| Forward primer | AACCAAGTGCAGACATCAGT |
| Reverse primer | TGAAGAATAGAATTGGCCAG |
| DEG 7 (clone B) | |
| Forward primer | AGGATTCAGGTTTCAAACAA |
| Reverse primer | ATGCTCTATTCCTTCTTCAT |
| DEG 11 | |
| Forward primer | ATGCAGGTTTGACCAGGAAA |
| Reverse primer | AGAGGCTTGGTTTTATTGTG |

counterstained with Mayer's haematoxylin. Negative control sections were incubated with a 100× dilution of normal rabbit serum. The images were viewed and photographed using a light microscope (Olympus, Tokyo, Japan).

## Results

### Establishment of human dental pulp-derived cell lines

The outgrowing cells created a sheet-like structure on the culture dish. At this time point, cells were replated and transfected with SV40 large T antigen. After selection of SV40 large T antigen transfectants with 400 μg $mL^{-1}$ of G418, more than 30 cell lines were observed. Although some cells ceased replication and died after approximately 20 passages, nine different cell lines were maintained and to date these cells have been growing actively for more than 2 years. The growth curves of the representative clones A10 and A17 are shown in Fig. 1(c). When the cells were plated at a density of $1 \times 10^4$ per dish, they grew until they reached confluence on day 5 and decreased in number thereafter. These cells were fibroblastic in shape as shown in Fig. 1(a). To determine the cell lineages of established cell lines, immunofluorescent cell staining was first performed. Anti-fibroblast, anti-vimentin and anti-collagen type I and type III antibodies were used. All established cells showed positive staining for these antibodies (Fig. 1(b)), indicating that these cell lines had fibroblastic lineages (representative A17 clone was shown).

### Detection of differentially expressed genes

Although the established cell lines had fibroblastic characteristics, their gene expression patterns could have been different. To identify the genes which are not expressed in dental pulp tissue, we performed differential display using a Gene Fishing DEG kit. After total RNA extraction and first-strand cDNA synthesis, differential display was performed by using 20 arbitrary PCR primers. The PCR products were loaded on 2% agarose gels and upregulated genes were identified. Figure 2 shows the results of the first screening, which revealed seven different genes (indicated by arrows). The DEGs were observed when arbitrary primers 1–7 (indicated on the top) were used (each gene was designated as DEG 1, 2, 3, 7A, 7B and 11 respectively). These genes were purified, subcloned into TOPO vector and sequenced by M13 forward and reverse primers.

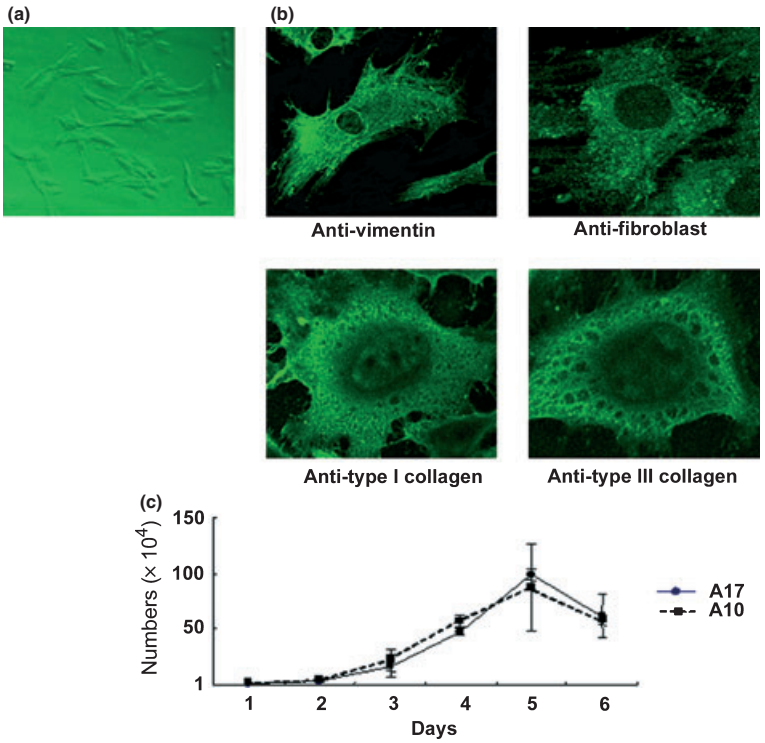


**Figure 1** Established human dental pulp-derived cells showed fibroblastic characters. (a) Cells are spindle shaped with ovoid nuclei. (b) The established cells were stained with anti-fibroblast (upper left panel), anti-vimentin (upper right panel) and anti-collagen type I (lower left panel) and type III (lower right panel) antibodies followed by FITC-conjugated goat anti-mouse IgG (H + L) or FITC-conjugated goat anti-rabbit IgG (H + L) antibodies. (c) The growth rates of representative cell lines (A10 and A17) are shown.

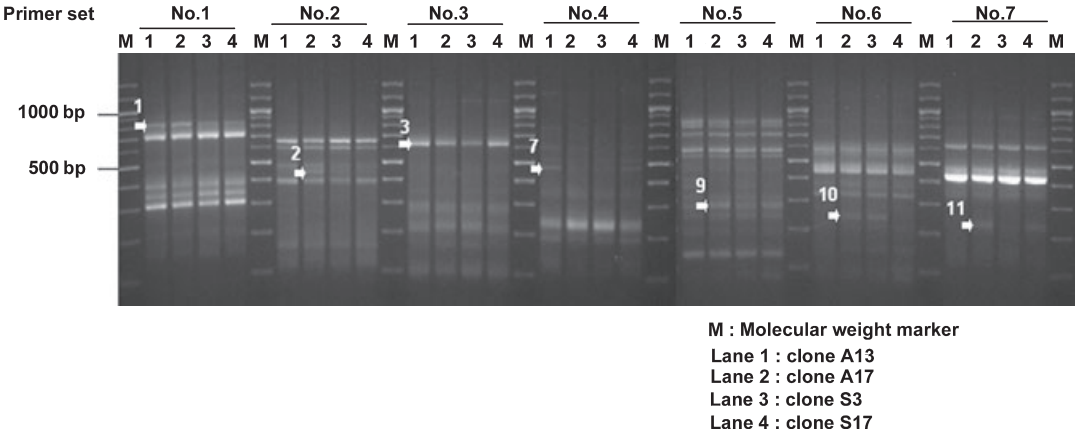


**Figure 2** Detection of differentially expressed genes. Total RNA was extracted from four different cell lines and subjected to differential display experiments. Twenty arbitrary primers were used to isolate the differentially expressed genes. The PCR products were loaded on a 2% agarose gels. Differential expression patterns were observed when the indicated primer sets 1–7 were used (indicated at the top of each photo). The arrows on the left-hand side indicate the differentially expressed genes. Each number corresponds to a DEG gene in Table 2.

The obtained sequences were database searched. Table 2 shows the results of the search. Subcloning of product NO. 7 yielded two different clones designated DEG 7A and 7B. A total of eight different genes were obtained, two of which (DEG 9 and 10) did not show any similarity to other known sequences.

### Different expression patterns of DEGs in pulp cell lines

Based on the DEG sequences, sequence-specific primers for DEG 1, 2, 3, 7A, 7B and 11 (Table 1) was designed and RT-PCR was performed to confirm the differential gene expression of the DEGs. As shown in Fig. 3, all cell lines showed variable expression patterns for these DEGs and overall expression patterns were consistent with the data obtained in the differential display experiments.

The cell lines were roughly classified into two groups according to the gene expression profiles. In one group, A7, A10 and A18 did not express the DEGs examined in Fig. 3. In the second group, A 13, A17, S3, S7, S10 and S17 expressed all these DEGs.

**Table 2** The results of database search

| Gene | |
|---|---|
| DEG1 | Homo sapiens complement component 1, subcomponent, transcript variant 2 |
| DEG2 | Homo sapiens interferon-induced transmembrane protein 1 (9–27) |
| DEG3 | Homo sapiens triosephosphate isomerase 1 (TPI1) |
| DEG7(clone A) | Homo sapiens alkB, alkylation repair homolog 5 (*Escherichia coli*) |
| DEG7 (clone B) | Homo sapiens ubiquitin-activating enzyme E1 |
| DEG9 | Unknown sequence |
| DEG10 | Unknown sequence |
| DEG11 | Homo sapiens Thy-1 cell surface antigen (Thy-1) |

### Detection of Thy-1$^+$ cells in dental pulp tissue

Amongst the identified DEGs, the DEG 11 gene was focused upon. DNA sequence analysis revealed that this gene was identical to Thy-1. Thy-1 is known as a T-cell marker, but it was not known whether it existed in dental pulp tissue. To examine the expression of Thy-1, immunohistochemical staining was conducted using anti-Thy-1 antibody. As shown in Fig. 4, Thy-1$^+$ cells were sparsely observed in the pulp tissue. They had ovoid nuclei and a fibroblastic shape. These positive reactions were specific, because no positive staining was detected in the negative controls. Not all cells with fibroblastic shape had Thy-1 antigen on their surface (red arrows in Fig. 4).

## Discussion

The goal of this study was to establish different cell lines derived from human dental pulp tissue. For this

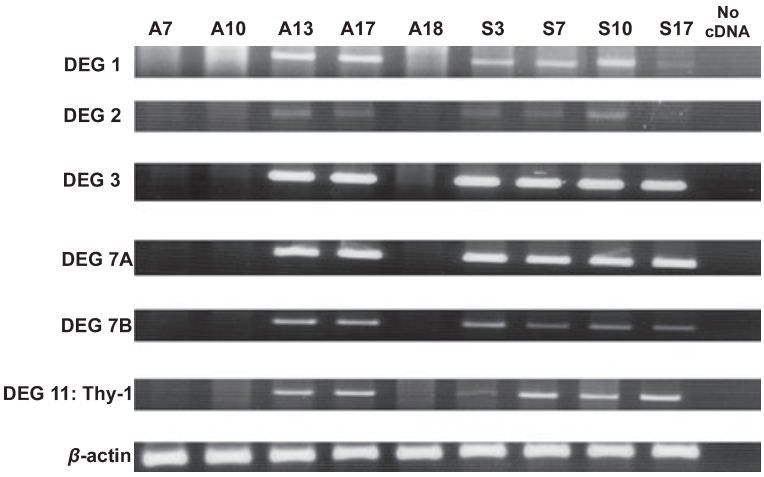


**Figure 3** Different expression levels of DEGs between established cells. The different expression patterns of each gene was confirmed by RT-PCR using sequence-specific primers. A7, A10 and A18 were negative for all DEGs. DEG 11 corresponds to Thy-1 (the lowest panel).

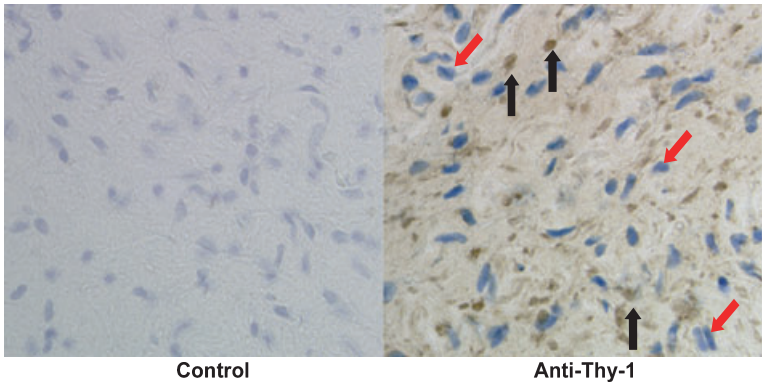


**Figure 4** Thy-1-positive fibroblastic cells in human dental pulp tissue. Immunohistochemical staining of the dental pulp tissue. The dental pulp tissue was fixed in 4% paraformaldehyde and embedded in paraffin. Six-micrometre sections were prepared and immunohistochemically stained with anti-Thy-1 antibody followed by HRP-conjugated goat anti-rabbit antibody. The color reactions were performed with DAB. Black and red arrows indicate the Thy-1-positive and-negative cells respectively.

purpose, SV40 large T antigen was transfected to dental pulp-derived cells. The establishment of human dental pulp-derived cell lines has been described by Galler *et al.* (2006). Although they obtained 12 cell clones, most of them ceased proliferation around passage 15, and finally only one clone (tHPC #V2) achieved continuous growth. Their method involved the use of electroporation to introduce the SV40 large T antigen. In this study, on the other hand, an attempt was made to introduce the same plasmid with the Lipofectamine method, which is much less harmful to the cells; nine different clones were obtained. Thonemann & Schmalz (2000a,b) also established bovine dental pulp cell lines, obtaining four different clones using the electroporation method. These results indicate that the efficiency of cell immortalization may vary with the transfection method used. More research is needed to identify the most efficient method of immortalizing dental pulp cells.

All of the established cell lines stained positive for fibroblast-specific antigen, vimentin and collagen type I and type III, indicating that they originated as pulp fibroblasts. Recently, it was demonstrated that dental pulp tissue contains stem cells and that these cells could be cultured *in vitro* (Gronthos *et al.* 2000, Miura *et al.* 2003).

Characterization of these cells has been contributing to the understanding of many aspects of dental pulp tissue (Huang *et al.* 2006, Thibodeau *et al.* 2007, Wei *et al.* 2007). Clinical applications using these cells will shed new light on endodontic therapies (Nakashima & Akamine 2005, Murray *et al.* 2007). In addition to stem

cell therapy, there are other possible options for regeneration in endodontics, for instance, pulp implantation, scaffold implantation or gene therapy. Further understanding of the characteristics of fibroblast subpopulations in dental pulp tissue might confer some advantages in the regeneration therapy mentioned above.

The results of the differential display experiment clearly indicated that the gene expression pattern was not identical amongst the clones. These results were confirmed by RT-PCR with sequence-specific primers. In this study, only 20 arbitrary primers were used. It may be possible to identify more genes which are differentially expressed between established cell lines using other primers.

Amongst the genes identified, the focus was on Thy-1, which was thought to be a T-cell-specific antigen (Mansour Haeryfar & Hoskin 2004, Rege & Hagood 2006) and was not originally expected to be detected by RT-PCR. The intrinsic function of Thy-1 has never been fully elucidated. In an attempt to identify fibroblast-specific antigens, Saalbach *et al.* (1998) obtained the monoclonal antibody, AS02. Characterization of this antibody revealed that AS02 recognizes the membrane protein on fibroblasts, and this antigen was demonstrated to be Thy-1. Other cell types, such as ovarian cancer cells, endothelial cells, neurons or haematopoietic cells are known to express Thy-1 on their surfaces (Rege & Hagood 2006). Thy-1 contributes to many biological functions, such as cell adhesion, cell signaling, apoptosis or proliferation (Mansour Haeryfar & Hoskin 2004, Rege & Hagood 2006).

This is the first report to demonstrate the expression of Thy-1 in dental pulp tissue. Further experiments are needed to elucidate the function of Thy-1, and in particular, the function of Thy-1 on dental pulp-derived fibroblasts.

Nine fibroblastic cell clones were established, however, not all of these clones were positive for Thy-1. Based on their different gene expression profiles, the established cell lines were divided into two groups. As shown in Fig. 3, clones A7, A10 and A18 were negative for Thy-1 and DEG 2, 3, 7A and 7B. The results suggest that there are at least two different subpopulations of fibroblasts in dental pulp tissue. In fact, the heterogeneity of fibroblasts has been reported in lung, periodontal ligament and gingival fibroblasts (Fries *et al.* 1994). The functional differences of these two groups are not known; therefore, it is important to study their precise properties to achieve further understanding of the dental pulp-derived fibroblast.

DEG 9 and DEG 10 did not show any similarities with other genes. Attempts are being made to obtain their full-length clones, which may help to elucidate the unknown functions of dental pulp tissue.

## Conclusion

By transfecting SV40 large T antigen, nine different fibroblastic cell lines derived from dental pulp tissue were established. Differential display experiment revealed that eight different genes were expressed with various expression levels amongst these cell clones. Database search indicated that the Gene No. 11 was identical to T-cell marker, Thy-1. Although the expression of Thy-1 in dental pulp tissue was confirmed with immunohistochemical stainings, not all cells with fibroblastic shape were positive for Thy-1. These results indicated that there are some subpopulations in dental pulp-derived fibroblastic cells.

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## References

Fries KM, Blieden T, Looney RJ *et al.* (1994) Evidence of fibroblastic heterogeneity and the role of fibroblast subpopulations in fibrosis. *Clinical Immunology and Immunopathology* **72**, 283–92.

Galler KM, Schweikl H, Thonemann B, D'Souza RN, Schmalz G (2006) Human pulp-derived cells immortalized with simian virus 40 T-antigen. *European Journal of Oral Sciences* **114**, 138–46.

Gronthos S, Mankani M, Brahim J, Gehron Robey P, Shi S (2000) Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*. *Proceedings of the National Academy of Sciences of the United States of America* **97**, 13625–30.

Hanks CT, Sun ZL, Fang DN *et al.* (1998) Cloned 3T6 cell line from CD-1 mouse fetal molar dental papillae. *Connective Tissue Research* **37**, 233–49.

Hargreaves KM, Goodis HE (2002) *Seltzer and Bender's Dental Pulp*. Chicago, IL: Quintessence Publishing Co., pp. 95–122.

Huang GT, Shagramanova K, Chan SW (2006) Formation of odontoblast-like cells from cultured human dental pulp cells on dentin in vitro. *Journal of Endodontics* **32**, 1066–73.

Kim YJ, Kwak CI, Gu YY, Hwang IT, Chun JY (2004) Annealing control primer system for identification of differentially expressed genes on agarose gels. *BioTechniques* **36**, 424–6.

MacDougall M, Thiemann F, Ta H, Hsu P, Chen LS, Snead ML (1995) Temperature-sensitive simian virus 40 large T antigen immortalization of murine cell cultures: establishment of clonal odontoblast cell line. *Connective Tissue Research* **33**, 97–103.

Mansour Haeryfar SM, Hoskin DW (2004) Thy-1: more than a mouse pan-T cell marker. *The Journal of Immunology* **173**, 3581–8.

Miura M, Gronthos S, Zhao M *et al.* (2003) SHED: stem cells from human exfoliated deciduous teeth. *Proceedings of the National Academy of Sciences of the United States of America* **100**, 5807–12.

Murray PE, Garcia-Godoy F, Hargreaves KM (2007) Regenerative endodontics: a review of current status and a call for action. *Journal of Endodontics* **33**, 377–90.

Nakashima M, Akamine A (2005) The application of tissue engineering to regeneration of pulp and dentin in endodontics. *Journal of Endodontics* **31**, 711–8.

Rege TA, Hagood JS (2006) Thy-1, a versatile modulator of signaling affecting cellular adhesion, proliferation, survival, and cytokine/growth factor responses. *Biochimica et Biophysica Acta* **1763**, 991–9.

Saalbach A, Kraft R, Herrmann K, Haustein UF, Anderegg U (1998) The monoclonal antibody AS02 recognized a protein on human fibroblasts being highly homologous to Thy-1. *Archives of Dermatological Research* **290**, 360–6.

Schmalz G, Barth M, Schuster U, Thonemann B, Schweikl H (1998) Dentin barrier test using transfected bovine pulp derived cells. *Journal of Dental Research* **77**, 823.

Schuster U, Schmalz G, Thonemann B, Mendel N, Metzl C (2001) Cytotoxicity testing with three-dimensional cultures of transfected pulp-derived cells. *Journal of Endodontics* **27**, 259–65.

Suzuki I, Harada T, Asano M *et al.* (2007) Phosphorylation of ERK in trigeminal spinal nucleus neurons following passive jaw movement in rats with chronic temporomandibular joint inflammation. *Journal of Orofacial pain* **21**, 225–31.

Thibodeau B, Teixeira F, Yamauchi M, Caplan DJ, Trope M (2007) Pulp revascularization of immature dog teeth with apical periodontitis. *Journal of Endodontics* **33**, 680–9.

Thonemann B, Schmalz G (2000a) Immortalization of bovine dental papilla cells with simian virus 40 large t antigen. *Archives of Oral Biology* **45**, 857–69.

Thonemann B, Schmalz G (2000b) Bovine dental papilla-derived cells immortalized with HPV18 E6/E7. *European Journal of Oral Sciences* **108**, 432–41.

Wei X, Ling J, Wu L, Liu L, Xiao Y (2007) Expression of mineralization markers in dental pulp cells. *Journal of Endodontics* **33**, 703–8.