Establishment of periodontal ligament cell lines from temperature-sensitive simian virus 40 large T-antigen transgenic rats

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Abstract

Orthodontic tooth movement is controlled by various cell types in the periodontal ligament (PDL). Mechanical stresses, such as orthodontic force, are thought to induce differentiation of the mesenchymal cells in the PDL into osteoblasts and cementoblasts. The details of the process of differentiation, however, are not known, in part because adequate *in vitro* systems for their study do not yet exist. The purpose of this study was to establish and characterize immortalized PDL cell lines derived from the PDL of transgenic rats harboring the temperature-sensitive simian virus 40 T-antigen gene (TG rats). The PDL was removed from the molar roots of TG rats and incubated in tissue culture. Outgrowth cells from the PDL explant were passaged and cloned, depending on the shape of the colonies formed. The cell lines thus established were analyzed by reverse transcription–polymerase chain reaction for expression of type-I collagen, osteopontin, fibronectin, alkaline phosphatase (bone type), bone sialoprotein, the receptor activator of NF- κ B ligand, and osteoprotegerin. In addition, the capacity for formation of mineralized nodules was assessed by incubating cells in calcification-promoting medium at 37 °C. A total of 15 stable cell lines were successfully established and characterized. These cell lines were classified into six groups based on their pattern of gene expression at 33 °C. Moreover, three of these clones were capable of forming calcified nodules. In conclusion, differential gene expression was demonstrated in 15 established PDL cell lines. Some cells had the potential to differentiate into cell types found in mineralized tissues, such as osteoblasts and cementoblasts, as well as cells expressing molecules that regulate osteoclast differentiation.

Abbreviations: ALP – Alkaline phosphatase; BSP – Bone sialoprotein; COLL1 – Type-I collagen; DEX – Dexamethasone; FN – Fibronectin; OPG – Osteoprotegerin; OPN – Osteopontin; PDL – Periodontal ligament; RANKL – Receptor activator of NF- κ B ligand; TG rats – Transgenic rats harboring the temperature-sensitive simian virus 40 T-antigen gene

Introduction

The periodontal ligament (PDL) is a small connective tissue found between the alveolar bone

and the tooth that has an important role in the process of orthodontic tooth movement. The phenomenon of tooth movement is the result of bone remodeling, involving both resorption and

formation of alveolar bone, processes that are thought to be controlled by cells in the PDL (Yee 1979; Roberts and Chase 1981). This remodeling is exquisitely controlled, with the width of the PDL remaining constant despite the involvement of various cell systems, such as osteoclasts and osteoblasts, in the regulation of bone resorption and formation. The regulatory systems for these two processes are not yet well understood. In addition, the PDL is essential not only for orthodontic tooth movement, but also for normal tooth function.

Although the PDL is important for healthy tooth function, research on PDL cells has been hampered by the fact that the PDL consists of heterogeneous cell populations with complex interactions. These complications have made it difficult to analyze the characteristics of individual PDL cell types. Previously, these cells have been studied using primary cultures of mixed populations of cells. Several problems limit the utility of this approach, including (1) induction of cell transformation by repeated passage; (2) the difficulty of obtaining the same cell population, even when the ligament samples are extracted from the same tooth root; (3) the difficulty of maintaining cells that have low proliferative activity; and (4) the uncertainty involved in comparing data derived from experiments that used different populations of primary PDL cells.

To analyze the characteristics of PDL cells *in vitro*, cells that have stable characteristics over a long period of time are needed. Immortalization of PDL cells using transformation with the simian virus 40 (SV40) large T-antigen gene has been reported previously (D'Errico et al. 1999; Parkar et al. 1999). SV40-transformed cells have greater utility than primary PDL cells, because they maintain consistent characteristics over repeated passages. Research using these cell lines revealed the character of the PDL as a whole, reflecting the interactions among the diverse PDL cell types. Nonetheless, the details of these interactions remained unclear due to the mixed cell population, the difficulty in identifying individual cell subpopulations, and the lack of PDL-specific markers. Recently, cloned PDL cells from human cementifying fibroma was reported (Kudo et al. 2002). However, there are limitations in the research done with PDL cells, because there is no evidence that these cells of tumor origin have normal PDL cells characteristics.

To address these problems, we used PDL cells from a transgenic rat harboring the temperature-sensitive SV40 T-antigen gene (TG rat) that had been transduced with a temperature-sensitive SV40 large T-antigen (SV40 tsA58) gene. Previously, it has been shown that cells extracted from various tissues of TG rats were immortalized and retained temperature sensitivity (Autillo-Touati et al. 1986; Paul et al. 1988; Yanai et al. 1991; Kameoka et al. 1995; Okuyama et al. 1995). Cell lines established from TG rats typically proliferate strongly when grown at 33 °C and differentiate when the temperature is shifted to 39 °C (Yanai et al. 1991; Jat and Sharp 1989). As cells extracted from TG rats are known to maintain stable characteristics, it was thought that it should be possible to separate mixed PDL cell populations into individual cell types by cloning. Such cloned PDL cell lines would then permit us to study several aspects of PDL physiology, including (1) the composition of the heterogeneous PDL cell lineages; (2) the identification of differing stages of cell differentiation, such as stem cells and mature cells of same mesenchymal cell lineage; (3) the characterization and comparison of the two hard tissue-forming cells, cementoblasts and osteoblasts, which differ significantly despite the shared trait of secretion of an extracellular matrix; and (4) the interactions between the various PDL cell subpopulations.

To characterize the functions of each cell subpopulation in the PDL, we established 15 different PDL cell lines from PDL explanted from TG rats, including fibroblast-like, osteoblast-like, or cementoblast-like cells. As the lack of PDL-specific markers makes it difficult to distinguish and classify the cell types in the PDL, we analyzed the gene expression profile for extracellular matrix component markers, including type-I collagen (COLL1), bone sialoprotein (BSP), osteopontin (OPN), fibronectin (FN), and alkaline phosphatase (bone type, ALP). In particular, we examined the existence and characteristics of hard tissue-forming cells.

Materials and methods

PDL cell cloning from TG rats

TG rats were obtained from YS New Technology Laboratory (Tochigi, Japan). Mandibles and

maxillae were removed from 6-week-old TG rats, and washed twice in phosphate-buffered saline (PBS); (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The first and second molars were extracted from each mandible and maxilla, along with adherent PDLs from the surrounding alveolar bone. The molars of 6-week-old rats could be extracted easily from the alveolar bone, without any contaminating pieces of the bone, because the roots of molars have no undercut at this growth stage. The central one-third of each root was cut and placed in a 35-mm tissue culture dish (Becton Dickinson Labware, NJ), then immersed in alpha-minimum essential medium (α -MEM; Biocell Laboratories, Inc., CA), supplemented with 10% fetal bovine serum (FBS), 100 U ml⁻¹ of penicillin-G and 100 μ g ml⁻¹ streptomycin sulfate (Wako). The cultures were incubated in a humidified atmosphere of 5% CO₂ in air, at the permissive temperature (33 °C) for SV40 ts T-antigen expression. When the outgrowth cells derived from the tissue explants reached confluency, they were detached with 0.05% trypsin (Sigma, St. Louis, MO) and 0.025% EDTA (Wako) in PBS and sub-cultured in culture flasks. The cultures were incubated until confluent monolayers appeared. The cell monolayers were detached by treatment with trypsin-EDTA and passaged. Cell Cloning were carried out according to the methods described previously (Yanai et al. 1991; Kameoka et al. 1995; Okuyama et al. 1995). Briefly, the cells were cloned by seeding at 10 cells in 35 mm dishes (primaria; Becton Dickinson labware): 1–2 colonies were developed, then cells in one colony were collected using a cloning cylinder. The clones were sub-cultured in α -MEM supplemented with 10% FBS in 35-mm tissue culture dishes (Becton Dickinson Labware). A total of 15 clones were thus successfully established from the PDLs of TG rats and their characteristics were analyzed.

SV40 tsA58 protein expression

Western immunoblot analysis was performed to confirm SV40 tsA58 protein expression. Cell lysates were separated by SDS-PAGE, transferred onto nitrocellulose membranes (pore size, 0.45 μ m) (Schleicher and Schuell, Dassel, Germany), and immunoblotting was performed using the ECL[®] Western Blotting System (Amersham Life Science, Oakville, Ontario, Canada). Non-specific binding sites were blocked

with 5% non-fat dry milk in a solution of 0.1% Tween-20 in PBS (PBS-Tween) for 0.5 h. All antibodies were diluted in 0.01% Tween in PBS (PBS-T). The membranes were incubated with a 1 : 400 dilution of mouse anti-SV40 tsA58 monoclonal antibody (Amersham Life Science) for 1 h, and then washed extensively with PBS-T. A 1 : 2000 dilution of a horseradish peroxidase-conjugated sheep anti-mouse antibody (Oncogene Research Products, Boston, MA) was added and incubated for an additional 1 h, followed by PBS-T washes. Immunoreactive proteins were visualized by chemiluminescence detection according to the manufacturer's instructions. The blots were exposed on FP-3000B SS film (Fujifilm, Tokyo, Japan) using an ECLTM Mini-camera (Amersham Pharmacia Biotech, Chalfont, Buckinghamshire, UK).

Calcified nodule formation

The cloned cell lines were seeded separately at an initial density of 5×10^4 cells cm⁻² in 35-mm tissue culture dishes. The cells were grown in calcification-promoting medium (Nohutcu et al. 1997), comprising α -MEM, supplemented with 10% FBS, 100 U ml⁻¹ penicillin-G, 100 μ g ml⁻¹ streptomycin sulfate, 50 μ g ml⁻¹ ascorbic acid (Sigma), 10 mM beta-glycerophosphate (Sigma) and 100 nM dexamethasone (Wako). The cultures were incubated in a humidified atmosphere of 5% CO₂ in air at 37 °C, and the medium was changed every third day. The alizarin red staining method was used to detect the formation of calcified nodules on days 15 and 30 after the cells were seeded. The incubated cells were rinsed with PBS and fixed in 95% ethyl alcohol for 5 min. Fixed cells were rinsed with water thoroughly and stained with 40 mM alizarin red-S (Wako) in distilled water at room temperature for 5 min. The cells were washed thoroughly with water.

Reverse transcription-polymerase chain reaction

The expression of the genes for glyceraldehyde 3-phosphate dehydrogenase (GAPDH), OPN, ALP, BSP, COL1, FN, the receptor activator of NF- κ B ligand (RANKL) and osteoprotegerin (OPG) in PDL cells was assayed by RT-PCR. 5×10^4 PDL cells cm⁻² were incubated at 33 °C in α -MEM, supplemented with 10% FBS for 3 days, total RNA was isolated using the QuickPrep Total RNA

Table 1. Primers used for RT-PCR

Gene	Oligonucleotides	Fragment size (bp)	Reference
G3PDH	5'-CATGTAGGCCATGAGGTCCACCAC 3'-TGAAGGTCGGTGTCAACGGATTTGGC	983	Tso et al. (1985)
FN	5'-TAACAAATCTCCTGCCTGG 3'-GTCATTCTGTTTGATCTGGA	820	MacLeod et al. (1996)
OPN	5'-TGGCTTACGGACTGAGGTCA 3'-GACCTCAGAAGATGAACCTCT	486	Zohar et al. (1997)
Collagen	5'-AACGATGGTGCCAAGGGTGAT 3'-ATTCTTGCCAGCAGGACCAAC	1077	Kataoka et al. (2000)
ALP	5'-GACTGGTACTCGGATAACGAGATGC 3'-TGCGGTTCCAGACATAGTGG	265	Ikegame et al. (2001)
BSP	5'-AACAATCCGTGCCACTCA 3'-GGAGGGGGCTTCACTGAT	1048	Chen et al. (1999)
OPG	5'-TGGCACACGAGTGATGAATGCG 3'-GCTGGAAAGTTTGCTCTTGCG	538	Myers et al. (1999)
RANKL	5'-ACGCAGATTTGCAGGACTCGAC 3'-TTCGTGCTCCCTCCTTTCATC	493	Myers et al. (1999)

Extraction Kit (Amersham Pharmacia Biotech, Inc., Piscataway, NJ) according to the manufacturer's protocol. The first-strand cDNA synthesis was performed using Ready-To-Go You Prime First, Strand Beads (Amersham Pharmacia Biotech) and an oligo(dT)₁₅ primer (Promega, Madison, WI).

PCR amplification was performed using KOD Dash DNA polymerase kit (Toyobo Co., Ltd., Osaka, Japan). The 25 μ l reaction mixture contained 2 μ l of 2 mM of deoxynucleoside triphosphate (dNTP), 2.5 μ l of 10 $\times$ PCR buffer, 10 pmol each of the sense and antisense PCR primers, 100 ng of each cDNA, and 0.25 U of KOD dash DNA polymerase. Each cycle consisted of a heat-denaturation step at 94 °C for 30 s, an annealing step at a temperature optimized for each primer pair (Table 1) for 2 s and an extension step at 74 °C for 30 s. The PCR products were electrophoresed and visualized by ethidium bromide staining with UV light illumination (Gel Doc 2000; Bio-Rad, Hercules, CA).

Results

Establishment of periodontal ligament cell lines

We established 15 cloned cell lines from the PDL of TG rats: i.e., RPD-L-A, C, D, E, F, G, H, J, L, M, N,

O, P, Q, and R. Cells were selected and isolated by microscopy based on the shape of the cells. Although the general morphology of all clones was spindle-shaped and fibroblastic, each cell line differed in cell size and the degree of elongation of the spindle shape (Figure 1). The observation of the expression of SV40 large T-antigen, as determined by Western immunoblotting (Figure 2). We also examined the calcification potential and the gene expression patterns in the cloned cell lines.

Calcification potential

When the cloned cell lines were grown in calcification-promoting medium at the differentiated temperature for the tsA58 SV40 large T-antigen (37 °C), calcified nodules were detected clearly in three clones, RPD-L-D, H, and P, after 15 days of culture. At 30 days of culture, these three cell lines remained the only clones showing signs of calcification (Figure 3).

Gene expression analysis

GAPDH, FN, OPN, COL1, ALP, BSP, and OPG gene expression at 33 °C was analyzed using RT-PCR. The expression of all genes was detected in a mixed PDL cell population from which the

Figure 1. The cell shapes of the clones RPDL-A, C, D, E, F, G, H, J, L, M, N, O, P, Q, and R. Cells were selected and isolated based on the shape of the cells. Each cell line differed in cell size and the degree of elongation of the spindle shape. Bar: 200 μ m.

cloned cell lines were derived (Figure 4). *COLL1*, *OPN*, and *FN* gene expression was seen in all 15 cloned cell lines. *ALP* expression was detected in only nine clones, *OPG* expression was detected in eight, and *BSP* expression was detected in only one clone. Expression of the *RANKL* gene was not observed in any of the cloned cell lines. The gene expression profiles for all cloned cell lines are shown in Figure 5.

Discussion

This is the first report on the development of cloned PDL cell lines from SV40 tsA58 TG rats. Previously,

primary cultures of PDL cells have been reported to show characteristics of heterogeneous cell populations (Lekic and McCulloch 1996), including unstable proportions of the constituent cell types. These primary mixed cell cultures have yielded inconsistent experimental results. Moreover, it is difficult to analyze the characteristics of individual cells in primary PDL cell cultures, and to analyze the interactions between each cell. Before cloning, all gene markers in the cells that were cultured in mixed populations were expressed, except *RANKL* (data not shown). The gene expression pattern in such mixed populations might be the sum of the gene expression patterns of the various PDL cell lines. This bears out the importance of

Figure 2. Western blot analysis showing the expression of SV40 tsA58 protein in all clones. All cell lines were shown to have immortalized characteristics.

Figure 3. Alizarin red staining were performed to analyze calcified potential. The incubated cells were rinsed with PBS and fixed in 95% ethyl alcohol for 5 min. Fixed cells were rinsed with water thoroughly and stained with 40 mM alizarin red-S in distilled water at room temperature for 5 min. The cells were washed thoroughly with water. Calcified nodules formed by RPD-L-H (a), D (b) and P (c) cells after 15 days incubation. Definite calcified nodule formations are shown. No calcified nodules were visible in any of the other clones after 15 days of incubation. (d) The picture of RPD-L-N after 30 days of incubation. The cell line shown stands for the cell lines, which did not have the potential of calcification nodule formation. Calcified nodules formation was not seen in any clones except RPD-L-D, H, and P after 30 days of incubation. Bar: 50 μ m.

cloning PDL cell lines to study their individual properties and interactions.

All cells derived from TG rats in this study harbored the tsA58 SV40 T-antigen gene (Western blot shown in Figure 2), and consequently were immortalized and showed stabilized cell-type-specific functions. Several immortalized cell lines derived from TG rats have been established in previous studies, including hepatocyte cells (Yanai et al. 1991), gastric surface mucous cells (Sugiyama et al. 1993), and retinal pigment epithelium-derived cells (Kato et al. 1996). As the expression of the transduced gene is temperature sensitive, the expression of the SV40 T-antigen can be manipulated by using a temperature shift: these cells proliferate at 33 °C, and differentiate at 37–39 °C, when the expression of the SV40 T-antigen, and hence proliferation, is suppressed. These properties

of TG rat cells make them useful for analyzing cell lines in both the undifferentiated and differentiated states, permitting classification according to their characteristics under the two conditions.

In this study, we established 15 PDL cell lines from SV40 tsA58 TG rats. Cloned cell lines were originally selected using the shapes of cells in the colonies (Figure 1). To classify these cell lines, the potential to differentiate into cells capable of forming calcified tissues in calcification-promoting medium, such as osteoblasts and cementoblasts, was examined. Furthermore, the gene expression patterns in the undifferentiated stage at 33 °C were analyzed.

For osteoblasts, the differentiation of osteoprogenitor cells into osteoblasts has been subdivided into three stages based on bone nodule formation

Figure 4. Patterns of marker gene expression detected in a mixed population of PDL cells. A total of 5×10^4 cells cm^{-2} of PDL cells before cloning were incubated at 33 °C for 3 days, then total RNA was isolated. GAPDH, FN, OPN, COLL1, ALP, BSP, and OPG gene expression was analyzed, and the expression of all genes was detected in a mixed PDL cell population.

in vitro: (1) proliferation; (2) extracellular matrix development and maturation, and (3) mineralization; with characteristic changes in gene expression at each stage (Aronow et al. 1990). Aubin and Triffitt (2002) categorized osteoblast differentiation into seven transitional stages using the simultaneous expression patterns of up to 12 markers. The expression of the most frequently assayed osteoblast-associated genes (COLL1, ALP, OPN, OCN, and BSP) is upregulated asynchronously, acquired, or lost as the progenitor cells differentiate and the matrix matures and mineralizes. In general, ALP increases and then decreases when mineralization has well progressed. OPN peaks twice, once during proliferation and again later, but before BSP and OCN; BSP is expressed transiently very early and is then upregulated again in differentiated osteoblasts forming bone; and OCN is expressed concomitantly with mineralization. All these osteoblast-associated markers, except OCN, which is upregulated only in post-proliferative osteoblasts, are upregulated before the cessation of proliferation in osteoblast precursors. This suggests that differentiation progresses when the osteoblast precursors are still in the proliferative cycle. Accordingly, we analyzed the PDL gene expression pattern in the proliferation-permissive condition at 33 °C.

All 15 PDL cell lines expressed the FN, COLL1, and OPN genes. FN and COLL1 are components of the extracellular matrix (ECM) in the PDL (Lukinmaa et al. 1991), and are considered markers for fibroblastic cells (Hou and Yaeger 1993). It has been reported that FN increases the proliferation rate of PDL cells (Kapila et al. 1998), which led to the suggestion that FN is associated with PDL regeneration, although the functions of FN in the PDL are virtually unknown. OPN exists in many tissues, and is a component of the bone ECM. OPN has many functions, including cell adhesion, migration, survival, intracellular signaling, mediating mechanical stress (Noda and Denhardt 2002), bone and cementum formation (Bosshardt et al. 1998), bone resorption (Ikeda et al. 1992), and the inhibition of hydroxyapatite formation (Hunter et al. 1996). It is produced by both osteoblasts and osteoclasts (Merry et al. 1993), but its functions in the PDL are little known. All cell lines tested expressed the OPN gene, suggesting that it has an important role in PDL functions.

The PDL cell lines could be divided into six groups (Groups I–VI), according to their capacity to form calcified nodules (Table 2) and the expression of ALP, BSP, and OPG. The expression of ALP and of BSP were regarded as markers for

Figure 5. Patterns of marker gene expression detected in cloned PDL cell lines. A total of 5×10^4 cells cm^{-2} of cloned PDL cells were incubated at 33°C for 3 days, then total RNA was isolated. COLL1, OPN, and FN gene expression was seen in all 15 cloned cell lines. ALP expression was detected in nine clones, OPG expression was detected in eight, and BSP expression was detected in only one clone.

Table 2. Classification of established cell lines.

Group	Cell line	GAPDH	COLL 1	OPN	FN	ALP	BSP	OPG	RANKL	Calcification
I	RPDL-H	+	+	+	+	+	+	+	—	+
II	RPDL-D,P	+	+	+	+	—	—	+	—	+
III	RPDL-G,Q,R	+	+	+	+	+	—	+	—	—
IV	RPDL-A,C,E,L,M	+	+	+	+	+	—	—	—	—
V	RPDL-F,O	+	+	+	+	—	—	+	—	—
VI	RPDL-J,N	+	+	+	+	—	—	—	—	—

osteoblastic cells. BSP exists in the bone ECM and plays an important role in the initial stages of mineralization (MacNeil et al. 1996; Bosshardt et al. 1998; Satomura et al. 2000). It has been suggested

that cells expressing ALP and BSP have the characteristics of osteoblastic or pre-osteoblastic cells (Aubin and Triffitt 2002). Accordingly, the cell line in Group I (RPDL-H), which expresses ALP

and BSP, might be an osteoblastic cell line. Conversely, the cell lines in Groups V and VI, which do not express ALP or BSP, are considered more fibroblastic, and less likely to differentiate into osteoblastic cells.

The ability of the Group II cell lines to form calcified nodules in the calcification experiment despite the lack of detectable ALP or BSP expression in the RT-PCR experiment might have been due to differences in the experimental conditions. The ascorbic acid and beta-glycerophosphate in the calcification-promoting medium promote calcification in osteoblastic cells. Dexamethasone (DEX), which was also included in the calcification-promoting medium, induces osteoblastic cell differentiation in many cell systems (McCulloch and Tenenbaum 1986; Bellows et al. 1990). We speculate that the calcification-promoting medium and temperature shift promoted the Group II cell lines to differentiate to the osteoblastic stage. Alternatively, other molecules that were not examined in this experiment might explain this difference. Since osteoblast lineage cells are now known to produce many other molecules, with differentiation stage-specific changes in expression levels, they may produce unknown molecules with yet to be determined functions.

Although the gene expression patterns in Groups II and V were the same, only the cell lines in Group II formed calcified nodules in the calcification-promoting medium. Group II might consist of immature osteoprogenitor cells that are committed, and able to differentiate into osteoblastic cells with an adequate stimulus. Alternatively, since the expression of ALP disappears in the differentiated stage (Aubin 1998), the cell lines in Group II might differentiate beyond the transient status with the expression of ALP.

Groups III and IV both expressed ALP, but did not form calcified nodules. Although these cell lines appeared less sensitive to the calcification-promoting medium, factors other than the calcification-promoting medium should be examined, since transcription factors, hormones, cytokines, and growth factors may also regulate osteoblast differentiation (McCarthy et al. 2000). It will be necessary to characterize these cells in greater detail.

In this study, we could not clarify the relationship between the marker gene expression patterns and calcification potential, and more detailed analyses are needed to distinguish the functions of these cell

lines. Further research should be done to clarify the expressions of the gene; on a protein level in order to confirm their functions. Since the functions of the genes expressed in the PDL are poorly known, we need to analyze further the alteration of gene expression patterns in the calcification process in order to elucidate the complexity of PDL function.

In addition to the expression of genes for constituents of the extracellular matrix, the expression of two genes encoding factors associated with osteoclast differentiation was analyzed. The expression of OPG was detected in eight clones. By contrast, RANKL was not detected in any of the clones studied. Recent findings on osteoclastogenesis, combined with our results regarding gene expression patterns (Kanzaki et al. 2001), suggest that osteoclastogenesis is suppressed in the PDL. These observations raise the possibility that osteoclast differentiation requires a stimulus that induces bone remodeling, and that osteoclastogenesis is suppressed in the PDL when such stimuli are absent.

There might be heterogeneity among osteoblast lineage developmental pathways. Many unknowns remain. Must osteoprogenitor cells transit all stages or can they skip some? Is the diversity of marker expression in different osteoblasts non-reversible or reversible? Do the autocrine and paracrine cytokine systems or adhesion molecules affect the cell subdivisions differently? Which signals lead to the diversity of osteoblast gene expression profiles? Furthermore, the protein levels do not always match the mRNA levels. The biological or physiological consequences of the observed differences in gene expression are unknown, but they support the notion of heterogeneity of PDL.

In conclusion, we succeeded in establishing 15 individual cell lines from SV40 tsA58 TG rat PDLs. Based on their patterns of gene expression and calcification abilities, these cell lines were classified into six groups. These cell lines can now be used to conduct basic research on the physiological role of PDL cells in the processes of bone remodeling and tooth formation, or in the screening of drugs for administration to PDLs. These PDL cell lines can also be used in studies on orthodontic tooth movement, periodontal disease, and dental implants. The immortalized cell lines with stable characteristics described in this study will be valuable tools for future basic and clinical periodontal research.

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