대한치주과학회지 : Vol. 36, No. 3, 2006

Profiling of genes in healthy hGF, aging hGF, healthy hPDLF and inflammatory hPDLF by DNA microarray

Sang-Jun Yun[1], Byung-Ock Kim[1,2], Jeong-Hun Yun[2],
Dong-Wan Kang[2], Hyun-Seon Jang[1,2,*]

1. Department of Periodontology, College of Dentistry, Chosun University
2. Oral Biology Research Institute, College of Dentistry, Chosun University

I. INTRODUCTION

One of the main goals of periodontal treatment is to regenerate tissue from the periodontal ligament (PDL). Ideal periodontal healing is achieved by the promotion of PDL cells[1-4]. PDL cells have a potential to promote tissue regeneration through their various activities, such as migration, proliferation, osteoblast-like differentiation, cementoblast-like differentiation, and periodontal ligament fibroblasts. The main purpose in periodontal regeneration therapy is to regenerate periodontal tissue with human periodontal ligament fibroblast (hPDLF) being essential in this process.

Melcher et al[5]. reported that the phenotypes of cells re-collected from the root surface (such as gingival epithelium, gingival lamina propria, periodontal ligament, cementum, and alveoloar bone) determine the characteristics and quality of regeneration. This theory forms the biological basis of guided tissue regeneration (GTR). For periodontal regeneration, the ability of hPDLF cells to divide into various cells is important. Comparing the expression of hPDLF in the presence of a periodontal infection is essential for determining if the functions and roles of hPDLF cells can be used in periodontal regeneration therapy.

The most abundant cell in periodontal connective tissues is the gingival fibroblast. Periodontal ligament fibroblasts (PDLF) and gingival fibroblasts (GF) have distinct functional activities in the regeneration and repair of periodontal tissues as well as in inflammatory periodontal diseases[6-10]. Generally, the teeth extracted from severe periodontitis patients have no PDL. It should be noted that hGF can be

"이 논문은 2006년도 조선대학교 치과병원 학술연구비에 의하여 연구되었음"

* Corresponding author: Hyun-Seon Jang, Department of Periodontology, College of Dentistry, Chosun University, 421 Seosuk-dong, Dong-ku, Gwang-ju, 501-825, South Korea (E-mail:periojang@chosun.ac.kr)

used as a hPDF for periodontal tissue engineering. Han et al.[11] reported that PDLF and GF appear to have different gene expression patterns, which might reflect the intrinsic functional differences in the two cell populations and may well coordinate with their tissue-specific activities.

Studies have been carried out on hPDLF obtained and cultured from healthy individuals. However, few studies have been carried out in hPDLF in periodontitis patients. However, with the aging population, considerable attention has been drawn to the periodontal disease that is mainly encountered in adults. Shelton et al.[12] reported that in dermal fibroblasts, the senescent state mimics inflammatory wound repair processes. Hence, senescent cells may contribute to the pathology of chronic wounds. Rather than treating periodontal disease once it develops, prevention is considered better for maintaining a healthy periodontium.

The process of cellular aging includes the altered expression of pH-dependent b-galatosidase activity and the cell size[13]. Limited replicative capacity is a defining characteristic of most normal human cells, which culminates in senescence and an arrested state, where the cells remain viable but show altered gene and protein expression[14-16]. Recently, Kwak et al.[17] reported that the accumulation of nuclear actin was a much more sensitive and earlier event than the well-known, senescence-associated beta-galatosidase activity.

Studies of the phenotypes in mice and cell lines defective in the recA/RAD51 family genes show that these genes are essential for development and cell proliferation in mammals[18]. In particular, DMC1, a part of the recA-like family of genes, is a meiosis-specific gene. However, the expression of genes in healthy hGF, healthy hPDLF, inflammatory hPDLF, and aging hGF is not completely understood.

A specific marker of the genes in aging and cell-specificity (hPDLF or hGF) is available in the diagnosis and treatment of periodontitis. This study used DNA microarray analysis to screening the genes expressed in healthy hPDLF, inflammatory hPDLF, healthy hGF, and aging hGF .

II. MATERIALS AND METHODS

1. Cell culture

The healthy periodontal ligament tissue (20th decade, probably male) was obtained from periodontally healthy and non-carious human teeth that had been extracted for orthodontic reasons at the Hospital of Dentistry, Chosun University with the donors' informed consent.

The healthy human gingival tissue (40 decade, female) was obtained from periodontally healthy tissue, which had been removed for the secondary surgery of dental implantation at the Hospital of Dentistry, Chosun University.

Aging hGF cells were produced by the replicative senescence of healthy hGF. After the initial culturing, the replicative senescence of hGF was carried out serially. Cellular apoptosis was observed in the 18th generation. These generations were determined to be the final generations. The 2, 4, 8, 15, 16 generation of cells stored at -198(C were used in this study.

Inflammatory hPDLF (40 decade, male) was obtained from periodontitis teeth that had been extracted for periodontal reasons at the Chosun University Hospital of Dentistry.

The hGF and hPDLF cells were cultured in a medium containing Dulbecco's modified Eagle medium (DMEM; DMEM, Gibco BRL, USA) supplemented with 10% fetal bovine serum (FBS, Gibco BRL, USA) at 37(C in humidified air containing 5% CO2.

2. Total RNA extraction and microarray analysis

The total RNA was extracted using Trizol Reagent (Invitrogen, USA) and the mRNA expression level was analyzed for 18SRNA and 28SRNA. The genes expressed in healthy hGF, aging hGF, healthy hPDLF, and inflammatory hPDLF were screened using a DNA microarray. The genes were analyzed according to the level of aging, inflammation, and cell-specificity.

III. RESULTS

1. Pattern of genes expressed between healthy hGF and aging hGF by DNA microarray

There was a difference in the genes expressed between the healthy hGF and aging hGF(Figure 1). The control was P2 (Passage No 2 of hGF). The experimental groups were P4, P8, P15, and P16 (Passage No 4, 8, 15, 16 of hGF). The red color means more up-regulation of the genes in the experimental group(P4, P8, P15, P16) than in the control group(P2). The green color means more up-regulation of the genes in the control group(P2) than in the experimental group(P4, P8, P15, P16). The black color means that there was no difference between the control and experimental groups.

The expression levels of the genes expressed in the healthy hGF and aging hGF from the control (P2) and experimental groups (P16) were compared (Table 1). Actin showed the highest level of change in the aging hGF(P16) compared with in the healthy hGF(P2). Keratin and CD 36 antigen also showed a higher level of change in the aging hGF(P16) than in the healthy hGF(P2). In contrast, the DMC1 dosage suppressor of the mck1 homolog, meiosis-specific homologous recombination showed the most significant change in the healthy hGF(P2) compared with the aging hGF(P16). The platelet derived growth factor D, tenascin XB, and the zinc finger protein 521 showed a higher level of change in the healthy hGF(P2) than in the aging hGF(P16).

Table 2 shows the pattern of the genes showing changes in expression more than 4 fold in the healthy hFG(P2) and aging hGF(P16) ac-

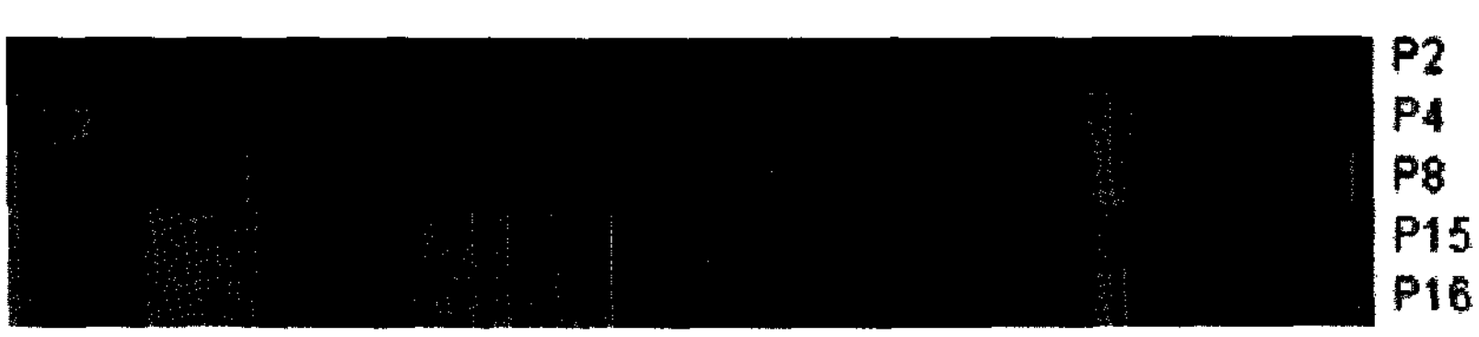

Figure 1. Gene profile of human gingival fibroblast according replicative senescence.

Control group : P2 (Passage No 2 of human gingival fibroblast),

Experimental groups: P4, P8, P15, P16 (Passage No 4, 8, 15, 16 of human gingival fibroblast)

red color: Experimental group > control group

green color: Experimental group < control group

black color: No difference between the control and experimental groups

Table 1. The level of change of genes expressed in the healthy hGF and aging hGF

Gene Symbol	Gene Name	Fold Change
ACTG2	actin, gamma 2, smooth muscle, enteric	(1247.6054110
BEX1	brain expressed, X-linked 1	(1056.4377170
ACTG2	actin, gamma 2, smooth muscle, enteric	(425.5639106
MYH11	myosin, heavy polypeptide 11, smooth muscle	(410.8206811
KCNMB1	potassium large conductance calcium-activated channel, subfamily M, beta member 1	(278.0641608
KRTHA4	Keratin, hair, acidic 4	(261.9745763
AGC1	aggrecan 1 (chondroitin sulfate proteoglycan 1, large aggregating proteoglycan, antigen identified by monoclonal antibody A0122)	(239.6763088
PPP1R14A	protein phosphatase 1, regulatory (inhibitor) subunit 14A	(202.7258568
HDAC1	histone deacetylase 1	(185.1964970
SERPINB2	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 2	(169.8130473
CD36	CD36 antigen (collagen type I receptor, thrombospondin receptor)	(137.1394375
	Omission	
PDGFD	platelet derived growth factor D	(46.7970473
ADH1B	alcohol dehydrogenase IB (class I), beta polypeptide	(49.2562639
TNXB	tenascin XB	(51.2683477
DHRS3	dehydrogenase/reductase (SDR family) member 3	(64.2873064
STATIP1	signal transducer and activator of transcription 3 interacting protein 1	(65.9251023
ADH1B	alcohol dehydrogenase IB (class I), beta polypeptide	(69.1263094
TRPA1	transient receptor potential cation channel, subfamily A, member 1	(71.4034709
ADH1C	alcohol dehydrogenase 1C (class I), gamma polypeptide	(71.8217300
ZNF521	zinc finger protein 521	(160.4035766
COLEC12	collectin sub-family member 12	(191.5237374
DMC1	DMC1 dosage suppressor of mck1 homolog, meiosis-specific homologous recombination (yeast)	(228.7121185

+: over expression in P16(Passage No. 16 of human gingival fibroblast, aging hGF), −: over expression in P2(Passage No. 2 of human gingival fibroblast, healthy hGF)

cording to gene function. 14500 genes were analyzed in the healthy hFG(P2) and aging hGF(P16). 826 (5.6%) genes were over-expressed in the P16 cells compared with in the P2 cells. 492 (3.3%) genes were over-expressed in the P2 cells compared with the P16 cells.

According to the signal transduction of the gene function, 183 genes were over-expressed in the P16 cells compared with the P2 cells, and 118 genes were over-expressed in the P2 cells compared with the P16 cells.

Table 2. Pattern of the genes expressed between the healthy hGF and aging hGF according the gene function

Function of the genes	No. of genes up-regulated over 4-fold (%)		Total
	P16	P2	
Amino acid metabolism	8(4.0)	8(4.0)	197
Apoptosis	14(3.8)	11(3.0)	366
Carbohydrate metabolism	14(3.5)	0(0)	396
Cell cycle	51(7.2)	15(2.1)	705
Cell proliferation and differentiation	38(6.4)	28(4.7)	585
Developmental processes	121(8.2)	71(4.8)	1475
Immunity and defense	87(9.4)	36(3.9)	923
Intracellular protein traffic	23(3.2)	7(0.9)	709
Lipid, fatty acid and steroid metabolism	20(3.8)	28(5.4)	513
Nucleoside, nucleotide and nucleic acid metabolism	110(4.1)	57(2.1)	2681
Oncogenesis	25(5.6)	15(3.3)	443
Protein metabolism and modification	75(3.5)	73(3.4)	2113
Signal transduction	183(7.3)	118(4.7)	2477
Transport	57(6.2)	25(2.7)	917
Total	826(5.6)	492(3.3)	14500

P16: Passage No. 16 of human gingival fibroblast(aging hGF), P2: Passage No. 2 of human gingival fibroblast(healthy hGF).

2. Pattern of the genes expressed in the healthy hPDLF and inflammatory hPDL by DNA microarray

Figure 2 shows the pattern of the genes differentially expressed in the healthy hPDLF and inflammatory hPDLF. Healthy hPDLF was used as the control. Inflammatory hPDLF was used in the experimental group .

The red color means more Up-regulation of the genes in the experimental group (inflammatory hPDLF) than in the control group (healthy hPDLF). The green color means more up-regulation of the genes in the control group (healthy hPDLF) than in the experimental group (inflammatory hPDLF). The black color means there is no difference between the control and experimental groups.

Figure 2. Gene profile of the healthy hPDLF and inflammatory hPDLF.

red color: hPDLF involved periodontitis 〉 the healthy hPDLF

green color: hPDLF involved periodontitis 〈 the healthy hPDLF

black color: No difference between the hPDLF involved periodontitis and healthy hPDLF

Table 3. Change in the genes expressed in the healthy hPDLF and inflammatory hPDLF

Gene Symbol	Gene Name	Fold Change
RGN	regucalcin (senescence marker protein-30)	(51.17863272
VCAM1	vascular cell adhesion molecule 1	(48.57224622
T1A-2	lung type-I cell membrane-associated glycoprotein	(47.19191138
CST6	cystatin E/M	(34.09668082
KRTAP1-1	keratin associated protein 1-1	(33.44716437
BNC1	basonuclin 1	(27.33958995
BEX1	brain expressed, X-linked 1	(26.35244651
CCL7	chemokine (C-C motif) ligand 7	(26.34021835
MEST	mesoderm specific transcript homolog (mouse)	(25.79124300
CGI-125	CGI-125 protein	(23.59435781
MLPH	melanophilin	(23.57406751
ANGPTL4	angiopoietin-like 4	(22.80561704
C17	cytokine-like protein C17	(22.74524390
	Omission	
ADH1C	alcohol dehydrogenase 1C (class I), gamma polypeptide	(21.89036796
FLJ35773	hypothetical protein FLJ35773	(26.93598654
FAM13C1	family with sequence similarity 13, member C1	(50.94160819
MN1	meningioma (disrupted in balanced translocation) 1	(51.50347961
EPB41L3	erythrocyte membrane protein band 4.1-like 3	(58.06202565
EPB41L3	erythrocyte membrane protein band 4.1-like 3	(76.12861888
FLJ36701	hypothetical protein FLJ36701	(223.34559640

+: over expression in inflammatory hPDLF, -: over expression in healthy hPDLF

The level of gene expression expressed in the control (healthy hPDLF) and experimental groups (inflammatory hDPLF) was analyzed according to the level of inflammation (Table 3). Regucalcin showed the highest level of change in the inflammatory hPDLF compared with the healthy hPDLF. Vascular cell adhesion molecule 1 also showed a significantly higher level of change in the inflammatory hPDLF than in the healthy hPDLF. In contrast, the hypothetical protein FLJ36701 showed the highest level of change in the healthy hPDLF compared with the inflammatory hPDLF.

Table 4 shows the pattern of the genes with changes in expression more than 4 fold in the healthy hPDLF and inflammatory hDPLF according to the gene function. 12901 genes in the healthy hPDLF and inflammatory hDPLF were analyzed. 330 (2.5%) genes were over-expressed in the inflammatory hPDLF compared with the healthy hPDLF. 264 (2.0 %) genes were over-expressed in the healthy hPDLF compared with the inflammatory hPDLF.

According to the signal transduction of gene

Table 4. Genes expressed in the healthy hPDLF and inflammatory hPDLF according to the gene function

Fuction of genes	No. of genes up-regulated over 4-fold (%)		Total
	healthy hPDLF	inflammatory hPDLF	
Amino acid metabolism	2(1.1)	4(2.2)	174
Apoptosis	5(1.4)	5(1.4)	334
Carbohydrate metabolism	8(2.2)	8(2.2)	361
Cell cycle	7(1.0)	32(4.8)	664
Cell proliferation and differentiation	13(2.4)	21(3.8)	541
Developmental processes	41(3.2)	36(2.8)	1265
Immunity and defense	20(2.6)	31(4.0)	764
Intracellular protein traffic	7(1.0)	9(1.3)	657
Lipid, fatty acid and steroid metabolism	20(4.4)	8(1.7)	448
Nucleoside, nucleotide and nucleic acid metabolism	29(1.1)	45(1.8)	2479
Oncogenesis	10(2.4)	14(3.4)	403
Protein metabolism and modification	27(1.3)	40(2.0)	1947
Signal transduction	60(2.8)	65(3.1)	2071
Transport	15(1.8)	12(1.5)	793
Total	264(2.0)	330(2.5)	12901

function, 65 genes were over-expressed in the inflammatory hPDLF compared with the healthy PDLF, and 60 genes over-expressed in the healthy PDLF compared with the inflammatory hPDLF.

3. Pattern of genes expressed in the healthy hGF and healthy hPDLF by DNA microarray

The level of change in the genes expressed in the control (healthy hGF) and experimental group (healthy inflammatory hDPLF) was analyzed according to the cell specificity (Table 5).

Ribosomal protein S4 and Y-linked 1 were showed the highest level of change in the healthy hPDLF compared with the healthy hGF. Hypothetical protein FLJ36701 and Interleukin 11 also showed a higher level of change in the healthy hPDLF than in the healthy hGF.

In contrast, the FLJ45224 protein (Prostaglandin D2 synthase 21kDa) showed the highest level of change in the healthy hGF compared with the healthy hPDLF. Thioredoxin interacting protein and regucalcin also showed a higher level of change in the healthy hGF than in the healthy hPDLF.

Table 6 shows the pattern of the genes showing a change in expression of more than 4 fold in the healthy hPDLF and healthy hGF according to the gene function. 13336 genes in the healthy hPDLF and healthy hGF were analyzed. 557 (4.1 %) genes were over-expressed in the healthy hPDLF compared with

Table 5. Level of change in the genes expressed in the healthy hGF and healthy hPDLF

Gene Symbol	Gene Name	Fold Change
RPS4Y1	ribosomal protein S4, Y-linked 1	(3033.2422330
FLJ36701	hypothetical protein FLJ36701	(588.1788101
RPS4Y2	ribosomal protein S4, Y-linked 2	(205.3615102
IL11	interleukin 11	(157.3461425
NR4A3	nuclear receptor subfamily 4, group A, member 3	(110.1860672
FAM13C1	family with sequence similarity 13, member C1	(45.8516262
KRTHA4	keratin, hair, acidic, 4	(43.5377033
DTR	diphtheria toxin receptor (heparin-binding epidermal growth factor-like growth factor)	(42.9258768
APXL	apical protein-like (Xenopus laevis)	(42.8816347
CYorf15B	chromosome Y open reading frame 15B	(41.4855641
SLCO4A1	solute carrier organic anion transporter family, member 4A1	(40.0671892
ACTG2	actin, gamma 2, smooth muscle, enteric	(36.9299243
	Omission	
PSG4	pregnancy specific beta-1-glycoprotein 4	(30.2529600
CYP1B1	cytochrome P450, family 1, subfamily B, polypeptide 1	(31.1989722
MYCT1	myc target 1	(37.5331507
KIAA0746	KIAA0746 protein	(43.0888294
PSG1	pregnancy specific beta-1-glycoprotein 1	(44.8333376
C10orf10	chromosome 10 open reading frame 10	(56.0304043
RGN	regucalcin (senescence marker protein-30)	(77.3327172
TXNIP	thioredoxin interacting protein	(131.2158329
FLJ45224; PTGDS	FLJ45224 protein;prostaglandin D2 synthase 21kDa (brain)	(185.2741762

+: over expression in healthy hPDLF, -: over expression in healthy hGF

the healthy hGF. 237 (1.7 %) genes were over-expressed in the healthy hGF compared with the healthy hPDLF.

According to the signal transduction of the gene function, 133 genes were over-expressed in the healthy hPDLF compared with the healthy hGF, and 61 genes over-expressed in the healthy HGF compared with the healthy hPDLF.

4. Pattern of the genes expressed in th inflammatory hPDLF and aging hGF by DNA microarray

The level of change in the genes expressed in the control (inflammatory hPDLF) and experimental groups (aging hGF) were analyzed according to the level of aging and inflammation. P8 (Passage No. 8 of human gingival fibroblast) and P15 (Passage No. 15 of human gingival fibroblast) were used as the aging hGF.

Table 7 shows pattern of the genes showing

Table 6. Genes expressed in the healthy hPDLF and healthy hGF according to the gene function

Genes Function	No. of genes up-regulated over 4-fold (%)		Total
	healthy hPDLF	healthy hGF	
Amino acid metabolism	3(1.6)	4(2.2)	178
Apoptosis	14(3.9)	3(0.8)	352
Carbohydrate metabolism	10(2.7)	6(1.6)	363
Cell cycle	31(4.6)	8(1.2)	662
Cell proliferation and differentiation	34(6.1)	13(2.3)	552
Developmental processes	82(6.1)	34(2.5)	1337
Immunity and defense	48(5.9)	18(2.2)	804
Intracellular protein traffic	16(2.3)	4(0.5)	670
Lipid, fatty acid and steroid metabolism	21(4.5)	8(1.7)	462
Nucleoside, nucleotide and nucleic acid metabolism	66(2.6)	33(1.3)	2535
Oncogenesis	20(4.7)	8(1.8)	422
Protein metabolism and modification	42(2.1)	23(1.1)	1984
Signal transduction	133(6.0)	61(2.7)	2186
Transport	37(4.4)	14(1.6)	829
Total	557(4.1)	237(1.7)	13336

Table 7. Genes expressed in the inflammatory hPDLF and aging hGF according to the gene function

Gene Function	No of genes over 4-fold (%)		Total
	No difference since P8	No difference since P15	
Amino acid metabolism	1(0.5)	1(0.5)	194
Apoptosis	6(1.6)	3(0.8)	358
Carbohydrate metabolism	5(1.2)	3(0.7)	396
Cell cycle	15(2.1)	30(4.2)	707
Cell proliferation and differentiation	9(1.5)	14(2.4)	582
Developmental processes	22(1.5)	16(1.1)	1449
Immunity and defense	17(1.8)	10(1.0)	916
Intracellular protein traffic	10(1.4)	2(0.2)	700
Lipid, fatty acid and steroid metabolism	5(0.9)	0(0)	505
Nucleoside, nucleotide and nucleic acid metabolism	41(1.5)	29(1.0)	2676
Oncogenesis	8(1.8)	8(1.8)	443
Protein metabolism and modification	17(0.8)	12(0.5)	2109
Signal transduction	40(1.6)	21(0.8)	2449
Transport	12(1.3)	0(0)	917
Total	208(1.4)	149(1.0)	14401

P8: Passage No. 8 of human gingival fibroblast, P15: Passage No. 15 of human gingival fibroblast (healthy hGF). No difference since P8: similar amount of genes expressed between inflammatory hPDLF and hGF since P8, No difference since P15: similar amount of genes expressed between inflammatory hPDLF and hGF since P15.

a change in expression of more than 4 fold in the inflammatory hPDLF and aging hGF according to the gene function. 14401 genes were analyzed in the inflammatory hPDLF and aging hGF.

208 (1.4 %) genes showed a similar level of expression in the inflammatory hPDLF and aging P8 hGF (Passage No. 8 of human gingival fibroblast). 149 (1.0%) genes showed a similar level of expression in the inflammatory hPDLF and aging P15 hGF (Passage No. 15 of human gingival fibroblast).

IV. DISCUSSIONS

The ultimate aim of periodontal regeneration therapy is the regenerate destroyed tissues including the alveoloar bone, cementum, and periodontal ligament. Tissue engineering is used to overcome the limited tissue regeneration using the factors that stimulate the regeneration of alveoloar bone and periodontal attachment. Human periodontal ligament fibroblasts (hPDLF) can be differentiated and proliferated into osteoblast-like and cementoblast-like cells, and play a key role in periodontal regeneration. People are requiring their teeth for longer with the increasing life expectancy, and wish to maintain healthy periodontal tissue from an esthetic point of view.

The causes of periodontitis are aging, infections, and mechanical stress. Chronic periodontitis is a common in adults. Generally, there is a loss of inflammatory PDL or PDL in severe periodontitis patients. Inhibiting the aging of periodontal cells is essential for both preventing and regenerating the periodontal tissue in periodontitis patients. The use of the hGF as a hPDLF has potential.

Despite their similar spindle-shaped appearance, the PDLF and GF appear to show different gene expression patterns, which might reflect the intrinsic functional differences in the two cell populations and may well coordinate with their tissue-specific activities[11]. Wang et al.[19] reported that the DNA microarray of the mRNA levels of eight genes in human gingival fibroblasts (HGFs) showed differences in gene expression between the healthy and inflammatory gingival tissues. Abiko et al.[20] reported that the DNA microarray detected differences in the gene expression profiles of HGE and HGF, which might be beneficial for a genetic diagnosis of the periodontal tissue metabolism and periodontal diseases. However, there have been few genetic specific marker studies on aging, inflammation and cell-specificity between hGF and hPDLF using DNA microarray analysis. This study used DNA microarray technology to determine the pattern of gene expression in healthy hPDLF, healthy hGF, aging hGF, and inflammatory hPDLF.

In this study, approximately 4.1 % (557 genes) were found to be more abundant in the healthy hPDLF, whereas 1. 7 % (237 genes) were expressed at higher levels, more than four-fold, in the healthy GF.

Periostin is preferentially expressed in the periosteum and periodontal ligament, indicating its tissue specificity and a potential role in the maintenance of tissue structure[11,21]. In this study, the expression level of the periostin gene was in the hPDLF was 4.46 time, higher than in the hGF.

Interleukin (IL)-11 is a pleiotropic cytokine with effects on many cell types. Yashiro et

al.[22] reported that IL-11 mRNA expression and production of IL-11 were augmented by transforming growth factor(TGF)-betaδin both PDL and hGF, with higher values in the PDL. In this study, the expression level of the IL-11 gene was 157 times higher in the hPDLF than in the hGF.

Han et al.[11] reported that the expression level of IL-8 mRNA was 85.1 times higher in the hGF than in the PDLF. However, in this study, the expression level of the IL-8 gene was was 30.4 times higher in the hPDLF than in the hGF. Further studies will be needed to determine the role of IL-8 in hGF and hPDLF

Mammalian thioredoxin is known to be a direct inhibitor of the apoptosis signal- regulating kinase (ASK) 1[23]. Thioredoxin interacting protein (Txnip) gene is a candidate of tumor suppressor genes in vivo. Sheth et al.24) reported that the microarray analyses of tumors, non-tumor adjacent, and normal tissue of Txnip-deficient mice. This highlighted the genetic differences leading to the predisposition and onset of hepatocellular carcinoma (HCC), and a deficiency in the thioredoxin interacting protein (Txnip) is sufficient to initiate HCC. In this study, the Txnip gene showed a higher level of change in the healthy hGF than in the healthy hPDLF.

White et al.[25] cloned and characterized the human gene for the 21-kDa brain form of prostaglandin D2 synthase. Yamashima et al.[26] reported that prostaglandin D synthase (PGDS) might be a specific cell marker because it is expressed exclusively in human arachnoid and meningioma cells. In this study, prostaglandin D2 synthase 21kDa showed the highest level of change in the healthy hGF than in the healthy hPDLF. Further studies will be needed to determine the cell-specific role of Txnip and PGDS in hGF and hPDLF.

Kim et al.[27] reported that PDLs22, Type 1 collagen, Fibronectin, MMP-1 and TIMP-1 mRNA in hPDLF showed age dependent expression patterns using RT-PCR. However, for total gene analysis, the method is restricted compared with microarray analysis.

The microarray technique has recently been used successfully to identify the host molecular pathways by comparative analysis of the host transcriptional response to an infection, thereby providing insights into the mechanism that control the life span and age related phenotypes[28-29].

Therefore, this study is particularly important in that the cultured hPDLF obtained in periodontitis patient were compared genetically with healthy hPDLF. This study compared the gene profile between healthy PDLF and inflammatory hPDLF. Approximately, 2.0 % (264 genes) of genes were found to be comparatively more abundant in the healthy hPDLF compared with the inflammatory hPDLF. Whereas, 2.5 % (330genes) of genes were expressed at higher levels (more than four-fold) in the inflammatory hPDLF compared with the healthy hPDLF.

Senescence marker protein-30 (SMP30) is a calcium binding protein that is also called regucalcin (RC). The SMP30 gene is expressed mainly in the liver and protects cells against various injuries by stimulating the membrane calcium pump activity[30]. Maruyama et al.[31] reported that SMP30 has an antiapoptotic function and SMP30-KO mice are highly susceptible to various harmful reagents. SMP30 might be a useful tool for aging and biological monitoring.

Nakagawa et al.[32] reported that the over-expression of regucalcin (SMP30) suppresses apoptotic cell death in cloned normal rat kidney proximal tubular epithelial NRK52E cells.

Cell adhesion molecules (CAMs) are cell surface proteins that are involved in the binding of cells to each other, to endothelial cells, or to the extracellular matrix. The soluble forms of CAMs (sCAMS) are believed to be produced by proteolytic cleavage from the cell surface and are shed into the gingival crevicular fluid (GCF). Hannigan et al.[33] reported statistically significant differences between the levels of sVCAM-1 in the periodontal health and disease using the GCF. However, there are no reports of genetic analysis on cultured hPDLF using microarray analysis.

In this study, the SMP30 gene showed the highest level of change in the inflammatory hPDLF compared with the healthy hPDLF. This means that the expression of SMP30, an anti-apoptotic gene, might increase according to the level of inflammation. Vascular cell adhesion molecule (VCAM)-1 also showed a large increase in expression in the inflammatory hPDLF compared with healthy hPDLF. This suggests that the SMP30 gene and VCAM-1 might be an available marker for periodontitis, and further research will be required.

In this study, approximately 5.6% (826 genes) of all genes were found to be up-regulated more than four-fold, whereas 3.3% (492 genes) of the genes were down-regulated more than 75% according to the replicative senescence of the hGF.

There are two RecA-like recombinases, Rad51 and Dmc1, in eukaryotes. While, Rad51 is essential for both mitotic and meiotic recombination events, the function of Dmc1 is restricted to meiosis. Sehorn et al.34) reported that the DNA strand exchange activity of hDmc1 is probably indispensable for repairing the double-strand breaks of DNA during meiosis and for maintaining the ploidy of the meiotic chromosomes.

In this study, actin showed the highest level of change in the aging hGF(P16) compared with the healthy hGF(P2). While the DMC1 dosage suppressed the mck1 homolog, the meiosis-specific homologous recombination showed highest level of change in the healthy hGF(P2) compared with the aging hGF(P16). This suggests that the actin gene might be a useful marker for aging in hGF, whereas the DMC1 gene might be a marker for meiosis in hGF.

The change in the expression level of the genes associated with aging and inflammation in the control (inflammatory hPDLF) and experimental groups (aging hGF) were analyzed. 149 (1.0%) genes showed a similar level of gene expression in the inflammatory hPDLF and aging P15 hGF (Passage No 15 of human gingival fibroblast). This suggests that aging is also related with inflammation.

These results suggest that the genes expressed in the healthy hPDLF, healthy GF, aging GF, inflammatory hPDLF show different gene expression patterns. In particular, Actin, DMC1, SMP30, VCAD-1, Periostin, IL-11, and Thioredoxin interacting protein genes might be useful markers of aging, inflammation, or cell-specificity in hGF and hPDLF. However, further research will be needed.

V. CONCLUSIONS

This study compared total gene expression of healthy human gingival fibrobast(hGF), aging hGF, healthy human periodontal ligament fibroblast(hPDLF) and inflammatory hPDLF using DNA microarray analysis.

Actin showed the highest level of change in aging hGF(P16) compared with the healthy hGF(P2). While the DMC1 dosage suppressed the mck1 homolog, the meiosis-specific homologous recombination showed highest level of change in the healthy hGF(P2) compared with the aging hGF(P16). Regucalcin showed the highest level of change in the inflammatory hPDLF compared with the healthy hPDLF. In addition, VCAM-1 also showed a higher level of change in the inflammatory hPDLF than the healthy hPDLF. IL-11 and periostin showed a higher level of change in the healthy hPDLF than in healthy hGF. In particular, prostaglandin D2 synthase 21kDa showed the highest level of change in the healthy hGF compared with the healthy hPDLF. The thioredoxin interacting protein also showed a higher level of change in the healthy hGF than in the healthy hPDLF. 149 genes showed similar level of gene expression in the inflammatory hPDLF and aging P15 hGF (Passage No 15 of hGF).

The DNA microarray showed that the genes in the healthy hGF, aging hGF, healthy hPDLF and inflammatory hPDLF were differentially expressed. This study suggests that the expression of genes can differ according to the aging, inflammation or cell type. Further research will be needed to test the possibility of using gene marker or gene therapy for aging, inflammation and cell-specificity in periodontitis patients.

VI. REFERENCES

1. Polson AM, Caton J. Factors influencing periodontal repair and regeneration. J Periodontol 1982;53:617-625.
2. Kawanami M, Sugaya T, Gama H, et al. Periodontal healing after replantation of intentially rotated teeth with healthy and denuded root surfaces. Dent Traumatol 2001;17:127-133.
3. Shimono M, Ishikawa T, Ishikawa H, et al. Regulatory mechanisms of periodontal regeneration. Microsc Res Tech 2003;60: 491-502.
4. Silva TA, Rosa AL, Lara VS. Dentin matrix proteins and soluble factors: intrinsic regulatory signals for healing and resoption of dental and periodontal tissues. Oral Diseases 2004;10:63-74.
5. Melcher AH. On the repair potential of periodontal tissues. JP. 1976;47:256-260.
6. Park JC, Kim HJ, Jang HS, et al.. Isolation and characterization of cultured human periodontal ligament fibroblast-specific cDNAs. Biochem Biophys Res Commun 2001;282:1145-1153.
7. Kasasa SC, Soory M. The effect of interleukin-1 (IL-1) on androgen metabolism in human gingival tissue (HGT) and periodontal ligament (PDL). J Clin Periodontol 1996;23:419-424.
8. Lekic PC, Pender N, McCulloch CA. Is fibroblast heterogeneity relevant to the health, disease, and treatments of periodontal tissues? Crit Rev Oral Biol Med 1997;8:253-268.
9. Nishimura F, Terranova VP. Comparative study of the chemotatic responses of periodontal ligament cells and gingival fibro-

blasts to polypeptide growth factors. J Dent Res 1996;75:986-992.
10. Oates TW, Mumford JH, Carnes DL, Cochran DL. Characterization of proliferation and cellular wound fill in periodontal cells using an in vitro wound model. J Periodontol 2001;72: 324-330.
11. Han X and Amar S. Identification of genes differentially expressed in cultured human periodontal ligament fibroblasts vs. human gingival fibroblasts by DNA Microarray Analysis. J Dent Res 2002;81:399-405.
12. Shelton DN, Chang E, Whittier PS, Choi D, Funk WD. Microarray analysis of replicative senescence. Current biology 1999;9: 939-945.
13. Dimri GP, Lee S, Basile G, et al. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc. Natl. Acad. Sci. USA. 1995;92:9363-9367.
14. Sawa Y, Phillips A, Hollard J, et al. The in vitro life-span of human periodontal ligament fibroblasts. Tissue & Cell 2000;32: 163-170.
15. Nishimura F, Terranova VP, Braithwaite N, et al. Comparison of in vitro proliferative capacity of human periodontal ligament cells in juvenile and aged donors. Oral Diseases 1997;3:162-166.
16. West MD, Pereira-Smith OM, Smith JR. Replicative senescence of human skin fibroblasts correlates with a loss of regulation and overexpression of collagenase activity. Exp Cell Res 1989;184:138-147.
17. Kwak IH, Kim HS, Choi OR, Ryu MS, Lim IK. Nuclear accumulation of globular actin as a cellular senescence marker. Cancer Res 2004;64:572-580.
18. Kawabata M, Kawabata T, Nishibori M. Role of recA/RAD51 family proteins in mammals. Acta Med Okayama 2005;59:1-9.
19. Wang PL, Ohura K, Fujii T, et al. DNA microarray analysis of human gingival fibroblasts from healthy and inflammatory gingival tissues. Biochem Biophys Res Commun 2003;305:970-973.
20. Abiko Y, Shimizu N, Yamaguchi M, Suzuki H, Takiguchi H. Effect of aging on functional changes of periodontal tissue cells. Ann Periodontol 1998;3: 350-369.
21. Horiuchi K, Amizuka N, Takeshita S, et al. Identification and characterization of a novel protein, periostin, with restricted expression to periosteum and periodontal ligament and increased expression by trnasforming growth factor beta. J Bone Miner Res 1999;14:1239-1249.
22. Yashiro R, Nagasawa T, Kiji M, et al. Transforming growth factor-beta stimulates interleukin-11 production by human periodontal ligament and gingival fibroblasts. J Clin Periodontol 2006;33:165-171.
23. Saitoh M, Nishitoh H, Fujii M, et al. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. EMBO J 1998;17:2596-2606.
24. Sheth SS, Bodnar JS, Ghazalpour A, et al. Hepatocellular carcinoma in Txnip-deficient mice. Oncogene 2006;
25. White DM, Mikol DD, Espinosa R, et al. Structure and chromosomal localization of the human gene for a brain form of prostaglandin D2 synthase. J Biol Chem 1992; 267:23202-23208.
26. Yamashima T, Sakuda K, Tohma Y, et al. Prostaglandin D synthase (beta-trace) in human arachnoid and meningioma cells: roles as a cell marker or in cerebrospinal fluid absorption, tumorigenesis, and calcifi-

cation process. J Neurosci 1997;17:2376-2382.
27. Kim BO, Cho IJ, Park JC, Kook JK, Kim HJ, Jang HS. Cellular study of replicative senescence in human periodontal ligament fibroblast using molecular biology. Kor Acad Periodontol 2005:35:623-634.
28. Detweiler CS, Cunanan DB, Falkow S. Host microarray analysis reveals a role for the Samonella response regulator phoP in human macrophage cell death. Proc Natl Acad Sci USA 2001;98:5850-5855.
29. Wan PL, Ohura K, Fujii T, et al. DNA microarray analysis of human gingival fibroblasts from healthy and inflammatory gingival tissues. Biochem Biophys Res Commun 2003;305:970-973.
30. Ishigami A, Handa S, Maruyama N, Supakar PL. Nuclear localization of senescence marker protein-30, SMP 30, in cultured mouse hepatocytes and its similarity to RNA polymerase. Biosci Biotechnol Biochem 2003;67:158-160.
31. Maruyama N, Ishigami A, Kuramoto M, et al. Senescence marker protein-30 knockout mouse as an aging model. Ann N Y Acad Sci 2004;1019:383-387.
32. Nakagawa Y, Yamaguchi M. Overexpression of regucalcin suppresses apoptotic cell death in cloned normal rat kidney proximal tubular epithelial NRK52E cells: Change in apoptosis-related gene expression. J Cell Biochem 2005;96:1274-1285.
33. Hannigam E, O'Connell DP, Hannigan A, Buckley LA. Soluble cell adhesion molecules in gingival crevicular fluid in periodontal health and disease. J Periodontol 2004;75: 546-550.
34. Sehorn MG, Sigurdsson S, Bussen W, Uager VM, Sung P. Human meiotic recombinase Dmc1 promotes ATP-dependent homologous DNA strand exchange. Nature 2004;429: 433-437.

- 국문초록 -

DNA microarray법을 이용하여 건강한 치은섬유모세포, 복제노화된 치은섬유모세포, 건강한 치주인대섬유모세포와 염증성치주인대섬유모세포에서 유전자 발현

윤상준[1], 김병옥[1,2], 윤정훈[2], 강동완[2], 장현선[1,2]

1. 조선대학교 치과대학 치주과학교실
2. 조선대학교 치과대학 구강생물학연구소

이 연구의 목적은 DNA microarray 분석법을 이용하여 건강한 사람치주인대섬유모세포, 건강한 사람치은섬유모세포, 복제노화된 사람치은섬유모세포, 염증성 사람치주인대 섬유모 세포의 유전자 발현 형태를 상호비교하고자 하였다. 환자의 동의하에 충치, 치주염이 없이 교정발치된 치아의 치주인대세포를 배양하여 건강한 치주인대섬유모세포로, 만성치주염으로 발거된 치아에서 채취하여 배양한 세포를 염증성 치주인대섬유모세포로 선정하였다. 구강에서 채취한 치은결체조직에서 배양한 사람치은섬유모세포를 일차 배양한 후 계대배양을 통해 복제 노화를 유도하였다. -198℃의 액화질소에 저장되어 있던 2, 4, 8, 15, 16세대 세포를 실험에 이용하였다. 위의 모든 세포들은 60 ㎜ 배양접시에서 세포들이 80-90%의 밀생이 될 때까지 5% CO_2, 37℃, 100% 습도의 배양기에서 2일 간격으로 10% FBS가 함유된 DMEM 세포 배양액을 교체하면서 세포를 배양하였다. Trizol Reagent (Invitrogen, USA)를 이용하여 제조회사의 지시에 따라 total RNA를 추출하였다. 18S RNA와 28S RNA를 확인한 후 DNA microarray 분석을 실시하였다. 4배수 이상의 변화양상을 비교시 상호 유전자 발현의 차이를 나타내었다. 건강한 사람치은섬유모세포(2세대)와 노화된 사람치은섬유모세포를 비교시(16세대), Actin은 노화된 치은섬유모세포에서 가장 높은 발현변화를 나타낸 반면, DMC1 dosage suppressor of mck1 homolog, meiosis-specific homologous recombination,은 건강한 치은섬유모세포에서 가장 높게 나타났다. 염증성 치주인대섬유모세포와 건강한 치주인대 섬유모세포를 비교시, Regucalcin은 염증성 치주인대섬유모세포에서 가장 높게 나타났고, Vascular cell adhesion molecule 1도 두 번째로 높게 나타났다. 건강한 치주인대섬유모 세포와 건강한 치은섬유모세포를 비교시, IL-11과 periostin이 치주인대섬유모세포에서 높은 발현을 나타낸 반면, Prostaglandin D2 synthase 21kDa과 Thioredoxin interacting protein은 치은섬유모세포에서 높은 발현을 나타내었다. 염증성 치주인대섬유모세포와 노화된 치은섬유모세포(15세대 이상)를 비교시 149개의 유전자가 유사한 발현 수준을 나타내었다. 이 연구는 노화, 염증, 세포 형태에 따라서 유전자 수준에서 가장 높거나 높은 수준 변화를 보이는 유전자가 다를 수 있음을 나타낸다. 향후, 치주염 환자들에서, 노화, 염증, 세포 특이성에 관한 유전자 표시자를 이용하여 진단하거나 치료에 응용하기 위해서는 더 많은 연구가 필요하리라 사료된다.

Key words : gene, DNA microarray, human periodontal ligament fibroblast (hPDLF), human gingival fibroblast, replicative senescence