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

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

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지도교수 장 현 선

이 논문을 치의학 석사학위신청 논문으로 제출함.

2006년 4월 일

조선대학교 대학원

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윤상준의 석사학위 논문을 인준함.

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목 차

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표 목 차

국문 초록

*DNA microarray*법을 이용하여 건강한 치은섬유모세포, 복제노화된 치은섬유모세포, 건강한 치주인대섬유모세포와 염증성치주인대섬유모세포에서 유전자 발현

윤 상 준

지도교수 장 현 선

조선대학교 대학원 치의학과

이 연구의 목적은 DNA microarray 분석법을 이용하여 건강한 사람치주인대섬유모세포, 건강한 사람치은섬유모세포, 복제노화된 사람치은섬유모세포, 염증성 사람치주인대섬유모세포의 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 치주인대섬유모세포를 비교시, Regucalcin은 염증성 치주인대섬유모세포에서 가장 높게 나타났고, Vascular cell adhesion molecule 1도 두 번째로 높게 나타났다. 건강한 치주인대섬유모세포와 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.

Ⅰ. Introduction

Goal of periodontal treatment is regeneration of tissue from the periodontal ligament (PDL). Ideal periodontal healing is achieved by promotion of PDL cells[1-4]. PDL cells have a several potential, such as migration, proliferation, differentiation osteoblast-like, cementoblast-like, & periodontal ligament fibroblasts, achieve the promotion of tissue regeneration. The main purpose in periodontal regeneration therapy is in regenerating periodontal tissue. For this regeneration, human periodontal ligament fibroblast (hPDLF) is crucial.

Melcher et al.[5] stated that the phenotypes of cells re-collected in the root surface (such as gingival epithelium, gingival lamina propria, periodontal ligament, cementum, and alveoloar bone) would determine the adhesion, regeneration characteristics, and quality. This theory is the biological basis of guided tissue regeneration (GTR). For periodontal regeneration, hPDLF cell having the potential to divide into various cells is important. Comparing the expressions of hPDLF in the presence of periodontal infection is crucial in determining whether the functions and roles of hPDLF cell can be applied in periodontal regeneration therapy.

The most abundant cell in periodontal connective tissue is the gingival fibroblast. Periodontal ligament fibroblasts (PDLF) and gigival fibroblasts (GF) display distinct functional activities in the regeneration and repair of the periodontal tissues as well as during inflammatory periodontal diseases[6-10]. Generally, severe periodontitis patients expected tooth extration have no PDL. It will be worth that hGF can be used as a hPDF for the periodontal tissue engineering. Han et al[11] reported that PDLF and GF appear to display different gene expression patterns that may reflect intrinsic functional differences of the two cell populations and may well coordinate with their tissue-specific activities.

Studies have been done on hPDLF obtained and cultured from healthy individuals. However, not many studies have been done in hPDLF in patients with periodontitis.

Attention is drawn to periodontal disease seen mainly in adults with aging population. Shelton et al.[12] reported that in dermal fibroblasts, the senescent state mimics inflammatory wound repair processes and, as such, senescent cells may contribute to chronic wound pathologies. Rather than treating periodontal disease once it develops, prevention is better by maintaining a healthy periodontium.

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

Studies of phenotypes in mice and cell lines defective in the recA/RAD51 family genes show that the genes are essential for development and cell proliferation in mammals[18]. Expecially, DMC1, a part of recA-like genes, is well known as a meiosis-specific gene. However, the expression of genes in healthy hGF, healthy hPDLF, inflammatory hPDLF, and aging hGF is unclear.

A specific marker of genes in aging and cell-specificity (hPDLF or hGF) will be available in diagnosis and treatment of periodontits. The purpose of this study was to screening genes expressed in healthy hPDLF, inflammatory hPDLF, healthy hGF, and aging hGF by the DNA microarray analysis.

Ⅱ. Material and Methods

1. Cell culture

The healthy periodontal ligament tissue (20 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 that had been removed for second surgery of dental implant at the Hospital of Dentistry, Chosun University .

Aging hGF cells were used by replicative senescence of healthy hGF. After initial culturing, replicative senescence of hGF was done serially. Cellular apoptosis was observed in the 18th generation. These generation were determined to be the last generations. The 2, 4, 8, 15, 16 generation cells storaged in the -198℃ were used in this study.

Inflammatory hPDLF (40 decade, male) was obtained from periodontitis teeth that had been extracted for periodontic 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℃ in humidified air with 5% CO_2.

2. Total RNA extraction and Microarray analysis

Total RNA was extracted using Trizol Reagent (Invitrogen, USA) and mRNA

expression was analyzed for 18SRNA and 28SRNA. For the screening of genes expressed in healthy hGF, aging hGF, healthy hPDLF, and inflammatory hPDLF, DNA microarray was performed. According to the aging, inflammation, and cell-specificity, the genes was analyzed.

Ⅲ. Results

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

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

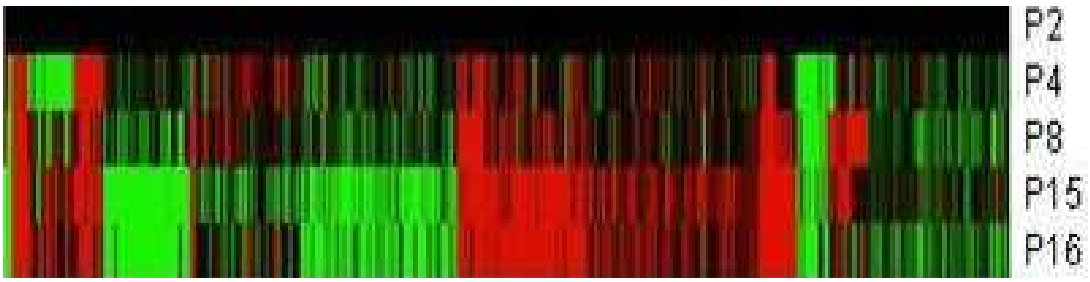

Fig. 1 Gene profile of human gingival fibroblast according replicative senescence.
Contro group : P2 (Passage No 2 of human gingival fibroblast),
Experimental groups : P4, P8, P15, P16 (Passage No 4, 8, 15, 16 of human gingival fibroblast)
red color: Experimental group > control group
Green color: Experimental group < control group
Black color: No difference between control group and experimental groups

For the detail comparison of genes expressed between healthy hGF and aging hGF, we analyzed the fold change between control group (P2) and experimenal group(P16)(Table 1).

Actin is showed best fold change in aging hGF(P16) than healthy hGF(P2). Keratin and CD 36 antigen were also showed higher fold change in aging hGF(P16) than healthy hGF(P2).

Whereas, DMC1 dosage suppressor of mck1 homolog, meiosis-specific homologous recombination was showed best fold change in healthy hGF(P2) than aging hGF(P16). Platelet derived growth factor D, tenascin XB, and zinc finger protein 521 were also expressed higher fold change in healthy hGF(P2) than aging hGF(P16).

Table 1. Fold change of genes expressed between healthy hGF and aging hGF

Gene Symbol	Gene Name	Fold change	GENE ID
ACTG2	actin, gamma 2, smooth muscle, enteric	1247.605411	hCG40742
BEX1	brain expressed, X-linked 1	1056.437717	hCG17759
ACTG2	actin, gamma 2, smooth muscle, enteric	425.5639106	hCG40742
MYH11	myosin, heavy polypeptide 11, smooth muscle	410.8206811	hCG19652
KCNMB1	potassium large conductance calcium-activated channel, subfamily M, beta member 1	278.0641608	hCG36798
KRTHA4	keratin, hair, acidic, 4	261.9745763	hCG1641096
AGC1	aggrecan 1 (chondroitin sulfate proteoglycan 1, large aggregating proteoglycan, antigen identified by monoclonal antibody A0122)	239.6763088	hCG28649
PPP1R14A	protein phosphatase 1, regulatory (inhibitor) subunit 14A	202.7258568	hCG42867
HDAC1	histone deacetylase 1	185.196497	hCG41610
SERPINB2	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 2	169.8130473	hCG33721
CD36	CD36 antigen (collagen type I receptor, thrombospondin receptor)	137.1394375	hCG17062
OASL	2′-5′-oligoadenylate synthetase-like	135.388088	hCG27362
NEF3	neurofilament 3 (150kDa medium)	127.1976847	hCG16610
RODH	3-hydroxysteroid epimerase	122.6991547	hCG20393
FLJ23514	hypothetical protein FLJ23514	104.9754891	hCG1730055
OAS1	2′,5′-oligoadenylate synthetase 1, 40/46kDa	93.36862939	hCG40366
MX1	myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)	91.81096754	hCG401239
		89.55710577	hCG1749898
NRXN3	neurexin 3	88.7718895	hCG1811459
FKHL18	forkhead-like 18 (Drosophila)	86.62098063	hCG2019197
EFHD1	EF hand domain containing 1	81.8079645	hCG33453
		81.09994326	hCG2042143
MYOCD	myocardin	65.99233682	hCG1811056
HSPB7	heat shock 27kDa protein family, member 7 (cardiovascular)	52.95538892	hCG23506
ITIH3	inter-alpha (globulin) inhibitor H3	51.80340417	hCG17557
RaLP	rai-like protein	50.78402013	hCG2002255
OAS1	2′,5′-oligoadenylate synthetase 1, 40/46kDa	47.23772892	hCG40366
		46.58281761	hCG15194
C1QTNF7	C1q and tumor necrosis factor related protein 7	46.04572455	hCG40569
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PDGFD	platelet derived growth factor D	-46.79704732	hCG40536
ADH1B	alcohol dehydrogenase IB (class I), beta polypeptide	-49.25626397	hCG41484
TNXB	tenascin XB	-51.26834779	hCG2001565
DHRS3	dehydrogenase/reductase (SDR family) member 3	-64.28730645	hCG1738619
STATIP1	signal transducer and activator of transcription 3 interacting protein 1	-65.92510231	hCG23805
ADH1B	alcohol dehydrogenase IB (class I), beta polypeptide	-69.12630949	hCG41484
TRPA1	transient receptor potential cation channel, subfamily A, member 1	-71.40347091	hCG17382
ADH1C	alcohol dehydrogenase 1C (class I), gamma polypeptide	-71.82173004	hCG1778210
ZNF521	zinc finger protein 521	-160.4035766	hCG1811237
COLEC12	collectin sub-family member 12	-191.5237374	hCG38030
DMC1	DMC1 dosage suppressor of mck1 homolog, meiosis-specific homologous recombination (yeast)	-228.7121185	hCG2014045

+: 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)

Table 2 showed pattern of genes over 4 fold between healthy hFG(P2) and aging hGF(P16) according to gene fuction. 14500 genes was analyzed between healthy hFG(P2) and aging hGF(P16). 826 (5.6%) genes were over-expressed in P16 than P2. 492 (3.3%)genes were over-expressed in P2 than P16. According to the signal transduction of gene function, 183 genes were over-expressed in P16 than P2, and 118 genes over-expressed in P2 than P16.

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

Fuction of 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 genes expressed between healthy hPDLF and inflammatory hPDL by DNA microarray

The pattern of genes was differentially expressed between healthy hPDLF and inflammatory hPDLF(Fig. 2). The control was healthy hPDLF. The experimental group were inflammatory hPDLF.

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

Fig. 2 Gene profile between healthy hPDLF and inflammatory hPDLF.
red color: hPDLF involved periodontitis > healthy hPDLF
Green color: hPDLF involved periodontitis < healthy hPDLF
Black color: No difference between hPDLF involved periodontitis and healthy hPDLF

For the detail comparison of genes expressed according to the inflammation, we analyzed the fold change between control group (healthy hPDLF) and experimental group(inflammatory hDPLF)(Table 3).

Regucalcin was showed best fold change in inflammatory hPDLF than healthy hPDLF. Vascular cell adhesion molecule 1 was also showed second higher fold change in inflammatory hPDLF than healthy hPDLF. Whereas, hypothetical protein FLJ36701 was showed best fold change in healthy hPDLF than inflammatory hPDLF.

Table 3. Fold change of genes expressed between healthy hPDLF and inflammatory hPDLF

Gene Symbol	Gene Name	Fold change	GENE ID
RGN	regucalcin (senescence marker protein-30)	51.17863272	hCG1791812
VCAM1	vascular cell adhesion molecule 1	48.57224622	hCG32384
		48.12644656	hCG1641027
T1A-2	lung type-I cell membrane-associated glycoprotein	47.19191138	hCG15341
CST6	cystatin E/M	34.09668082	hCG23285
KRTAP1-1	keratin associated protein 1-1	33.44716437	hCG1752441
		31.65636281	hCG15194
BNC1	basonuclin 1	27.33958995	hCG27264
BEX1	brain expressed, X-linked 1	26.35244651	hCG17759
CCL7	chemokine (C-C motif) ligand 7	26.34021835	hCG29304
MEST	mesoderm specific transcript homolog (mouse)	25.791243	hCG18967
CGI-125	CGI-125 protein	23.59435781	hCG32816
MLPH	melanophilin	23.57406751	hCG23030
ANGPTL4	angiopoietin-like 4	22.80561704	hCG23958
C17	cytokine-like protein C17	22.7452439	hCG39126
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		-21.53981569	hCG1647787
ADH1C	alcohol dehydrogenase 1C (class I), gamma polypeptide	-21.89036796	hCG1778210
		-21.96421449	hCG1731588
		-22.96055007	hCG1991523
		-24.87614454	hCG41017
FLJ35773	hypothetical protein FLJ35773	-26.93598654	hCG1985344
		-27.89016182	hCG1994767
		-33.04804444	hCG2040728
FAM13C1	family with sequence similarity 13, member C1	-50.94160819	hCG41127
MN1	meningioma (disrupted in balanced translocation) 1	-51.50347961	hCG40087
EPB41L3	erythrocyte membrane protein band 4.1-like 3	-58.06202565	hCG37215
EPB41L3	erythrocyte membrane protein band 4.1-like 3	-76.12861888	hCG37215
FLJ36701	hypothetical protein FLJ36701	-223.3455964	hCG2036980

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

Table 4 showed pattern of genes over 4 fold between healthy hPDLF and inflammatory hDPLF according to gene fuction. 12901 genes was analyzed between healthy hPDLF and inflammatory hDPLF.

330 (2.5%) genes were over-expressed in inflammatory hPDLF than healthy hPDLF. 264 (2.0 %)genes were over-expressed in healthy hPDLF than inflammatory hPDLF.

According to the signal transduction of gene function, 65 genes were over-expressed in inflammatory hPDLF than healthy PDLF, and 60 genes over-expressed in healthy PDLF than inflammatory hPDLF.

Table 4. Genes expressed between healthy hPDLF and inflammatory hPDLF according to the gene funtion

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

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

For the detail comparison of genes expressed according to the cell specificity, we analyzed the fold change between control group (healthy hGF) and experimental group(healthy inflammatory hDPLF)(Table 5).

Ribosomal protein S4, Y-linked 1 was showed best fold change in healthy hPDLF than healthy hGF. Hypothetical protein FLJ36701, Interleukin 11 were also showed higher fold change in healthy hPDLF than healthy hGF..

Whereas, FLJ45224 protein (Prostaglandin D2 synthase 21kDa) was showed best fold change in healthy hGF than healthy hPDLF. Thioredoxin interacting protein and regucalcin were also showed higher fold change in healthy hGF than healthy hPDLF.

Table 5. Fold change of genes expressed between healthy hGF and healthy hPDLF

Gene Symbol	Gene Name	Fold change	GENE ID
RPS4Y1	ribosomal protein S4, Y-linked 1	3033.242233	hCG1988058
FLJ36701	hypothetical protein FLJ36701	588.1788101	hCG2036980
RPS4Y2	ribosomal protein S4, Y-linked 2	205.3615102	hCG38986
IL11	interleukin 11	157.3461425	hCG37996
NR4A3	nuclear receptor subfamily 4, group A, member 3	110.1860672	hCG28754
		65.47108579	hCG2040553
		50.3255455	hCG21763
		48.53135716	hCG2042143
FAM13C1	family with sequence similarity 13, member C1	45.85162629	hCG41127
KRTHA4	keratin, hair, acidic, 4	43.53770332	hCG1641096
DTR	diphtheria toxin receptor (heparin-binding epidermal growth factor-like growth factor)	42.92587687	hCG45297
APXL	apical protein-like (Xenopus laevis)	42.88163473	hCG401216
CYorf15B	chromosome Y open reading frame 15B	41.48556415	hCG1987333
SLCO4A1	solute carrier organic anion transporter family, member 4A1	40.0671892	hCG1748044
		38.72914374	hCG2004844
		38.46682896	hCG1647787
ACTG2	actin, gamma 2, smooth muscle, enteric	36.9299243	hCG40742
		35.83674499	hCG2011180
ENO1	enolase 1, (alpha)	35.70719744	hCG22399
THBD	thrombomodulin	35.07888533	hCG1643886
TRPC6	transient receptor potential cation channel, subfamily C, member 6	33.32682557	hCG40899
		33.15810377	hCG1817350
PENK	proenkephalin	32.50835164	hCG40756
IL8	interleukin 8	30.49058658	hCG16372
		30.08998537	hCG1991170
-----	----------Omission----------	------	-----
PSG4	pregnancy specific beta-1-glycoprotein 4	-30.25296	hCG1730647
		-30.80912918	hCG2007896
CYP1B1	cytochrome P450, family 1, subfamily B, polypeptide 1	-31.19897225	hCG14819
MYCT1	myc target 1	-37.53315079	hCG22051
KIAA0746	KIAA0746 protein	-43.0888294	hCG1811766
PSG1	pregnancy specific beta-1-glycoprotein 1	-44.83333763	hCG1995688
		-48.98187176	hCG1818437
		-53.3050865	hCG1820421
C10orf10	chromosome 10 open reading frame 10	-56.03040439	hCG23312
		-71.61460828	hCG37212
RGN	regucalcin (senescence marker protein-30)	-77.3327172	hCG1791812
TXNIP	thioredoxin interacting protein	-131.2158329	hCG37372
FLJ45224:PTGDS	FLJ45224 protein;prostaglandin D2 synthase 21kDa (brain)	-185.2741762	hCG1780827

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

Table 6 showed pattern of genes over 4 fold between healthy hPDLF and healthy hGF according to gene fuction. 13336 genes was analyzed between healthy hPDLF and healthy hGF.

557 (4.1 %) genes were over-expressed in healthy hPDLF than healthy hGF. 237 (1.7 %) genes were over-expressed in healthy hGF than healthy hPDLF.

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

Table 6. Genes expressed between healthy hPDLF and healthy hGF according to the gene funtion

Fuction of Genes	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

4. Pattern of genes expressed between inflammatory hPDLF and aging hGF by DNA microarray

For the detail comparison of genes expressed between aging and inflammation, we analyzed the fold change between control group (inflammatory hPDLF) and experimental group(aging hGF). 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 showed pattern of genes over 4 fold between inflammatory hPDLF and aging hGF according to gene fuction. 14401 genes was analyzed between inflammatory hPDLF and aging hGF.

208 (1.4 %) genes were showed similar amount of genes expressed between inflammatory hPDLF and aging hGF since P8 (Passage No 8 of human gingival fibroblast). 149 (1.0%) genes were showed similar amount of genes expressed between inflammatory hPDLF and aging hGF since P15 (Passage No 15 of human gingival fibroblast).

Table 7. Genes expressed between inflammatory hPDLF and aging hGF according to the gene funtion

Fuction of Genes	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

Ⅳ. Discussions

The ultimate purpose of periodontal regeneration therapy is the regeneration of destroyed tissues including the alveoloar bone, cementum, and periodontal ligament. Tissue engineering is applied to overcome limited tissue regeneration using the factors that would stimulate the regeneration of alveoloar bone and periodontal attachment. Human periodontal ligament fibroblast (hPDLF) can be differentiated and proliferated into osteoblast-like cell and cementoblast-like cell, playing a central role in periodontal regeneration. People need their teeth longer as the life expectancy increased and want to prepare for healthy older years by maintaining healthy periodontal tissue in shape-wise and from esthetic point of view.

The causes of periodontitis is known aging, infection, and mechanical stress. Chronic periodontitis is a common in adult people. Generally, severe periodontitis patients have a inflammatory PDL or PDL loss. For the prevention and regeneration in periodontitis patients, inhibition of aging in periodontal cells is essential, and it will be worth using the hGF as a hPDLF.

Dispite their similar spindle-shaped appearance, PDLF and GF appear to display different gene expression patterns that may reflect intrinsic functional differences of the two cell populations and may well coordinate with their tissue-specific activities[11]. Wang et al.[19] reported that DNA microarray of the mRNA levels of eight genes in human gingival fibroblasts (HGFs) detect differences in gene expression between healthy and inflammatory gingival tissues. Abiko et al.[20] reported that the DNA microarray to detect differences in the gene expression profiles of HGE and HGF may be beneficial for genetic diagnosis of periodontal tissue metabolism and periodontal diseases. However, a genetic specific marker study is rare in aging, inflammation and cell-specificity between hGF and hPDLF by DNA microarray analysis. In the present study, we used DNA microarray technology to determine the pattern of gene expression of healthy hPDLF, healthy

hGF, aging hGF, inflammatory hPDLF.

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

Periostin was well known to be preferentially expressed in the periosteum and periodontal ligament, indicating its tissue specificity and a potential role in maintenance of tissue structure[11,21]. In this study, periostin gene was also found to be highly expressed in hPDLF compared with hGF, with a differential expression of 4.46-fold.

Interleukin (IL)-11 is a pleiotropic cytokine with effects on multiple cell types. Yashiro et al.[22] reported that IL-11 mRNA expression and IL-11 production were augmented by TGF-beta in both PDL and hGF, with higher values in PDL. In our study, IL-11 gene was also showed to be highly expressed in hPDLF compared with hGF, with a differential expression of 157-fold.

Han et al.[11] reported that IL-8 mRNA was found to be highly expressed in hGF compared with PDLF, with a differential expression of 85.1-fold. However, in our study, IL-8 gene was showed to be highly expressed in hPDLF compared with hGF, with a differential expression of 30.4-fold. Further studies are arranted to elucidate the present role of IL-8 in hGF and hPDLF

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

White et al.[25] had cloned and characterized the human gene for the 21-kDa brain form of prostaglandin D2 synthase. Yamashima et al.[26] reported because human

arachnoid and meningioma cells exclusively express prostaglandin D synthase (PGDS), it can be considered their specific cell marker. In this study, Prostaglandin D2 synthase 21kDa was showed best fold change in healthy hGF than healthy hPDLF. Further studies are required to elucidate 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 the RT-PCR.. However, for total gene analysis, the method is restricted compared to microarry analysis

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

Thus, this study is especially important in that cultured hPDLF obtained in the periodontitis patient were compared genetically with healthy hPDLF. In this study, we compared to gene profile between healthy PDLF and inflammatory hPDLF. About 2.0 %(264 genes) were found to be comparatively more abudant in healthy hPDLF, whereas 2.5 % (330genes) were expressed at higher leves in inflammatory hPDLF by more than four-fold.

Senescence marker protein-30 (SMP30), a calcium binding protein was also called regucalcin (RC). Senescence marker protein-30 (SMP30) gene, expressed mostly in the liver, protects cells against various injuries by stimulating 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 overexpression of regucalcin (SMP30) supresses apoptotic cell death in cloned normal rat kidney proximal tubular epithelial NRK52E cells.

Cell adhesion molecules (CAMs) are cell surface proteins involved in the binding of cells to each other, to endothelial cells, or to the extracellular matrix. The

soluble forms of CAMs (sCAMS) are thought to be produced by proteolytic cleavage from the cell surface and are shed into the gingival crevicular fluid (GCF). Hannigan et al.[33] reported that statistically significant differences were found between the levels of sVCAM-1 in periodontal health and disease using the GCF. However, no study including genetic analysis has been done on cultured hPDLF by microarray analysis.

In this study, SMP30 gene found to be the best fold-change in inflammatory hPDLF than healthy hPDLF. This means that SMP30, antiapoptotic gene, might be incresed according to the inflammation. Vascular cell adhesion molecule 1 was also showed second higher fold change in inflammatory hPDLF than healthy hPDLF. We suggest that SMP30 gene and VCAM-1 might be a available marker for periodontitis, and further research is required.

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

Two RecA-like recombinases, Rad51 and Dmc1, exist in eukaryotes. Whereas Rad51 is needed 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 repair of DNA double-strand breaks during meiosis and for maintaining the ploidy of meiotic chromosomes. (Nature, Sehorn)

In this study, Actin is showed best fold change in aging hGF(P16) than healthy hGF(P2). Whereas, DMC1 dosage suppressor of mck1 homolog, meiosis-specific homologous recombination was showed best fold change in healthy hGF(P2) than aging hGF(P16). We suggest that Actin gene might be a useful marker of aging in hGF, whereas DMC1 gene might be a available marker of meiosis in hGF.

For the detail comparison of genes expressed between aging and inflammation, we analyzed the fold change between control group (inflammatory hPDLF) and experimental group(aging hGF). 149 (1.0%) genes were showed similar amount of

genes expressed between inflammatory hPDLF and aging hGF since P15 (Passage No 15 of human gingival fibroblast). This means that aging can be co-related with inflammtion.

We suggest that genes expressed in healthy hPDLF, healthy GF, aging GF, inflammatory hPDLF appear to display different gene expression patterns. Expecially, Actin, DMC1, SMP30, VCAD-1, Periostin, IL-11, and Thioredoxin interacting protein genes might be a useful marker of aging, inflammation, or cell-specificity in hGF and hPDLF. Further research is required.

Ⅴ. Conclusions

The purpose of this study was to compare total gene expression of healthy human gingival fibrobast(hGF), aging hGF, healthy human periodontal ligament fibroblast(hPDLF) and inflammatory hPDLF by DNA microarray analysis.

The results is followed:

1. Actin is showed best fold change in aging hGF(P16) than healthy hGF(P2). Whereas, DMC1 dosage suppressor of mck1 homolog, meiosis-specific homologous recombination was showed best fold change in healthy hGF(P2) than aging hGF(P16).
2. Regucalcin was showed best fold change in inflammatory hPDLF than healthy hPDLF. And, Vascular cell adhesion molecule 1 was also showed second higher fold change in inflammatory hPDLF than healthy hPDLF.
3. IL-11 and periostin was showed higher fold change in healthy hPDLF than healthy hGF. Whereas, Prostaglandin D2 synthase 21kDa was showed best fold change in healthy hGF than healthy hPDLF. Thioredoxin interacting protein was also showed higher fold change in healthy hGF than healthy hPDLF.
4. 149 genes were showed similar amount of genes expressed between inflammatory hPDLF and aging hGF since P15 (Passage No 15 of hGF).

Consequently, the genes in healthy hGF, aging hGF, healthy hPDLF and inflammatory hPDLF were differentially expressed by DNA microarray. This study suggest that the best or hihger fold changed genes can be different according to the aging, inflammation or cell type. Further research is needed for possibility of gene marker or gene therapy for aging, inflammation and cell-specificity in periodontitis patients.

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