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Original

Profiling of differentially expressed genes in human gingival epithelial cells and fibroblasts by DNA microarray

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Abstract: Gingival epithelial cells and fibroblasts play important roles and have a harmonious relationship under normal and disease conditions, but the precise differences between theses cells remain unknown. To study the differences in gene expression between human gingival epithelial cells (HGE) and human gingival fibroblasts (HGF), mRNA was recovered from primary cultured cells and analyzed using cDNA microarray technology. The cDNA retro-transcribed from equal quantities of mRNA was labeled with the fluorescent dyes Cy5 and Cy3. The mixed probes were then hybridized with 7276 genes on the DNA microarray, after which fluorescence signals were scanned and further analyzed uisng GeneSpring software. Of the 7276 genes screened, 469 showed expression levels that were more than 2-fold greater in HGE than in HGF, while 293 showed expression levels that were more than 2-fold greater in HGF than in HGE. To confirm the reliability of the microarray results, keratin K5 and desmocolin, and vimentin and gp130, which showed higher mRNA levels in HGE and HGF, respectively, were selected and their mRNA levels were further analyzed by RT-PCR. The results of RT-PCR correlated well with those of microarray analysis. The present findings using a 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. (J. Oral Sci. 46, 19-24, 2004)

Key words: DNA microarray; gingival; epithelial cell; fibroblast; gene expression.

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Introduction

Gingiva is covered by stratified squamous epithelium with architectural characteristics unique to dental areas. The gingival oral epithelium faces the oral cavity and extends from the gingival margin to the mucogingival junction, and the vast majority of cells that compose it are keratinocytes, characterized by their ability to produce cytoplasmic keratin. These epithelial keratinization processes appear to be prompted by the underlying connective tissue. Several cell types have been identified within gingival connective tissue, and gingival fibroblasts, which account for most connective tissue cells, are likely to be responsible for the constant function adaptation of gingival connective tissue (1). Fibroblasts also play a major role in normal connective tissue turnover, as well as in wound healing repair and regeneration (2).

The structural composition of the epithelial-connective tissue interface is influenced by interactions between the cells of 2 tissue types, epithelial cells and fibroblasts. Although each plays important roles and have a harmonious relationship under normal and diseases conditions, the precise differences between them remain largely unknown.

Differentially expressed genes in different specimens have recently been detected by parallel analysis using gene microarray, which has greatly improved on traditional experimental methods because expression of one or more

genes can be observed in each test (3).

In the present study, primary cultured human gingival epithelial cells (HGE) and gingival fibroblasts (HGF) from human normal gingival tissue were subjected to a gene microarray technique in order to analyze the different gene expression patterns by HGE and HGF.

Materials and Methods

Cell Culture

HGE were isolated and cultured according to a method previously reported. (4) Briefly, healthy human gingival tissues were obtained from tissues overlying the impacted third molar teeth of adult humans during the course of surgery. Informed consent was obtained before beginning the present study. The explants were treated with 6 mg/ml of dispase (Sigma Chemical, MO, USA) in HEPES-buffered saline at 4°C to separate the epithelium from the underlying fibrous connective tissue. The epithelium was then removed and incubated at 37°C in trypsin-EDTA (0.05% trypsin, 0.53 mM EDTA) for 10 min, and repeatedly pipetted to prepare a single-cell suspension. The cell pellets were collected and resuspended in a serum free keratinocyte growth medium (Clonetics Corporation, CA, USA) supplemented with human recombinant epidermal growth factor, hydrocortisone, bovine insulin, bovine pituitary extract, gentamicin sulfate, amphotericin B, and 0.15 mM of $CaCl_2$.

HGF were prepared according to the method of Somerman et al. (5). Normal human gingival tissue specimens were obtained during premolar extraction performed as part of orthodontic treatment. Informed consent was obtained before beginning the present study. Gingival epithelium was removed and the explant was dissected into sections approximately 1-2 mm^3 in size, which were placed in a 35-mm tissue culture dish and covered with a sterilized glass coverslip. The culture was performed in α-MEM (GIBCO, NY, USA) supplemented with 100 μg/ml of penicillin G (Banyu Pharmaceutical, Tokyo, Japan), 50 μg/ml of gentamycin sulfate, 250 ng/ml of amphotericin B, 5 mM of HEPES buffer (pH 7.2), and 10% fetal bovine serum. When the cells that grew out from the explants had reached confluence, they were detached with 0.025% (w/v) trypsin in PBS for 10 min and subcultured in flasks. Cells that remained attached to the bottom of the flask were discarded in order to avoid contamination by epithelial cells, which are not as easily detached as fibroblasts. Both HGE and HGF were cultured in a humidified incubator (Forma CO_2 incubator MIP-3326, Sanyo Electric Medica System, Tokyo, Japan) in the presence of 95% air and 5% CO_2.

Gene microarray analysis

Total RNAs were extracted from both HGE and HGF at confluence by homogenizing with Trizol Reagent (Gibco BRL, Life Technologies, NY, USA) (6) using a FastPrep machine (FP120; BIO 101, Vista, CA, USA) at a speed setting of 6.0 for 30 s. The probes from HGE and HGF were used to synthesize cDNA labeled with Cy5-deoxyuridine triphosphate (dUTP) and Cy3-dUTP, respectively. These two labeled cDNA probes were then mixed and simultaneously hybridized to a UniGEM V human cDNA microarray bearing 7276 different genes (Incyte Genomics, MO, USA), where they competitively reacted with the arrayed cDNA copies. The global changes in gene expression profiles were reflected in the balanced differential expression (7). Hybridization signals were analyzed by computer to derive the relative expression levels of the genes present in the cDNA microarray representing 7276 human genes.

A differentially expressed gene was defined as that with a variation in gene expression of more than 2-fold. Gene ontology was classified according to the Gene Ontology consortium using GeneSpring software (Silicon Genetics, CA).

RT-PCR analysis

Synthesis of cDNA and amplification by reverse transcription-polymerase chain reaction (RT-PCR) were carried out using a GeneAmp RNA PCR kit (Perkin-Elmer, CT, USA). Briefly, cDNA synthesis was carried out at 42°C for 15 min in a final volume of 20 μl containing 4 μl of $MgCl_2$ solution (25 mM), 2 μl of 10 × PCR Buffer II (500 mM KCl, 100 mM tris-HCl, pH 8.3), 2 μl of dNTP (10 mM each), 1 μl of RNase inhibitor (20 U/μl), 1 μl of random hexamers (25 μM), 1 μl of Oligo d(T)16, 1 μl of MuLV reverse transcriptase (25 U/μl), and 2 μl of total RNA (1 μg/μl). The PCR mixture, containing 20 μl of the cDNA solution, 4 μl of 25 mM $MgCl_2$, 8 μl of 10 × Buffer II, 1 μl of forward primer, 1 μl of reverse primer, 65.5 μl of H_2O, and 0.5 μl of AmpliTaq DNA polymerase, was subjected to amplification using a Gene Amp PCR system 9600 (Perkin-Elmer, CT, USA) set at 94°C for 1 min, 55°C for 2 min, and 72°C for 2 min. The primers for keratin K5 and desmocolin, vimentin and gp130, and glyceraldhyde-3-phosphate dehydrogenase (GAPDH) were designed using Primer 3 software (http://wwwgenome.wi.mit.edu/cgibin /primer/primer3_www.cgi). The primers used were as follows: 5'-CCCAGTATGAGGAGATTGCCAACC-3' (forward primer for keratin K5); 5'-TATCCAGAGGA-AACACTGCTTGTG-3' (reverse primer for keratin K5, expected size = 475 bp); 5'-GGCTGTTGTCATTA-TACCCAAATG-3' (forward primer for desmocolin); 5'-

ATAACTATGAAGGAAGAGGATCGG-3' (reverse primer for desmocolin, expected size = 423 bp); 5'-CAGCAATATGAAAGTGTGGCTGCC-3' (forward primer for vimentin); 5'-GGAAGAGGCAGAGAAATC-CTGCTC-3' (reverse primer for vimentin, expected size = 428 bp); 5'-TCAAAGAGTCATATTGCCCAGTGG-3' (forward primer for gp130); 5'-CTGGACTGGATTCA-TGCTGACTGC-3' (reverse primer for gp130, expected size = 505 bp); 5'-ATCACCATCTTCCAGGAG-3' (forward primer for GAPDH); and 5'-ATGGACTGTGG-TCATGAG-3' (reverse primer for GAPDH, expected size = 318 bp). PCR fragments were electrophoresed on a 1.5% agarose gel, stained with ethidium bromide and visualized under UV light.

Results

As shown in Fig. 1, the scatter plot of mRNA levels based on fluorescent signal values in HGE and HGF exhibited a dispersed distribution pattern. In the scatter plot of the genes, 469 genes were expressed at levels more than 2-fold higher in HGE than in HGF, while 193 genes were expressed at levels higher in HGF than in HGE. Table 1 shows the gene descriptions based on gene ontology classification, and annotations of 10 selected genes from both HGE than HGF are shown in Table 2.

In order to confirm the microarray analysis results, we selected the keratin K5 and desmocolin genes, which were expressed at higher levels in HGE than in HGF, and the vimentin and gp130 genes, which were expressed at higher levels in HGF than in HGE. We then further analyzed the mRNA levels of these genes by RT-PCR, with the results shown in Fig. 2. The RT-PCR results for each gene agreed with the microarray data.

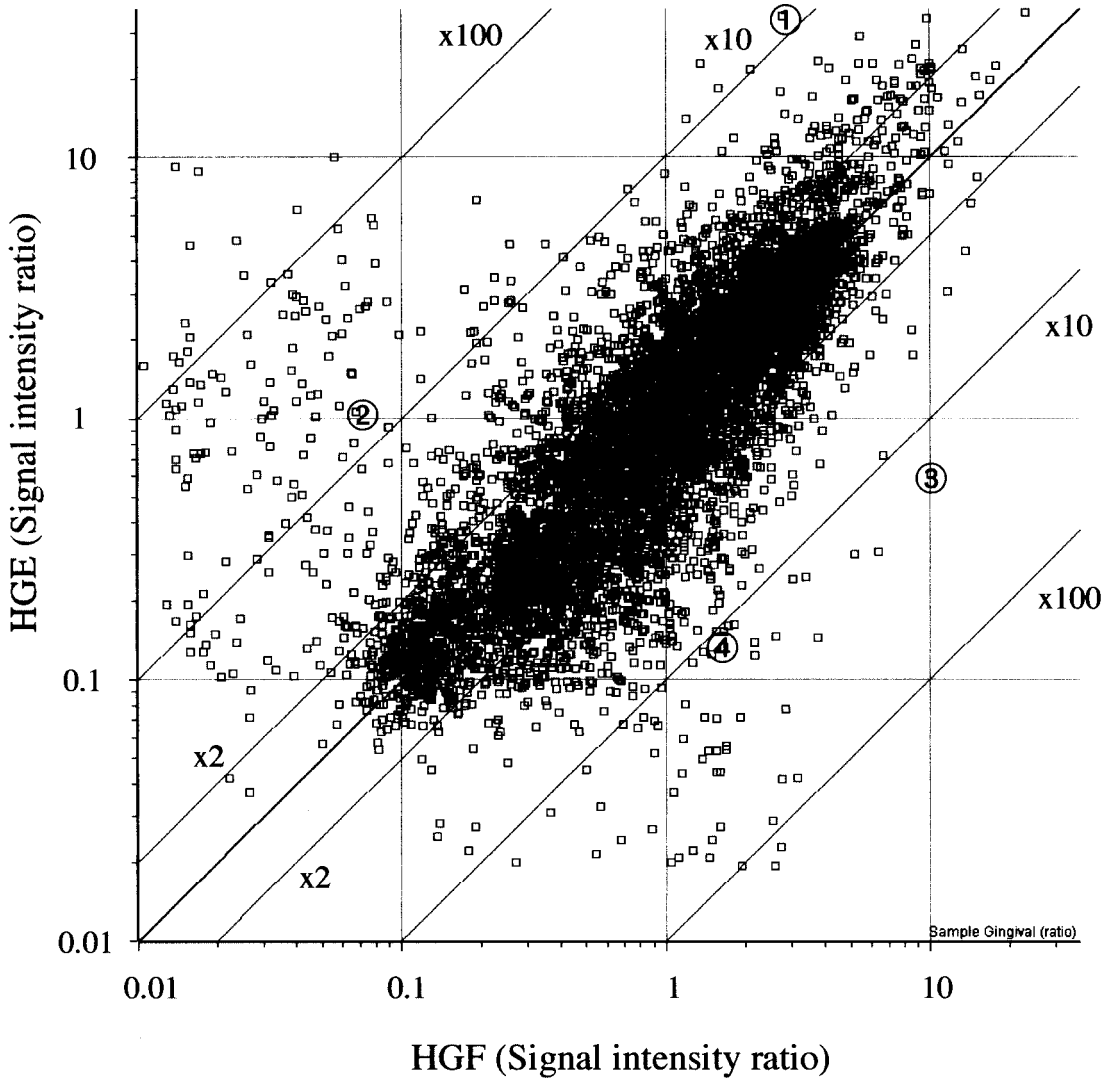

Fig. 1 Scatter plots of hybridization signals pattern in HGE and HGF. Square, 7276 genes on microarray. ①keratin K5; ②desmocolin; ③vimentin; ④gp130.

Table 1 Gene ontology

Gene Ontology	hGE>hGF		hGF>hGE	
	No.	(%)	No.	(%)
Cell Growth	54	12	34	12
Cell Cycle	22	5	14	5
Cell Motility	9	2	6	2
Cell Metabolism	42	9	28	10
Cell Adhesion	34	7	32	11
Cell Death	19	4	4	1
Transport	59	13	44	15
Cell Maintenance	15	3	13	4
Cell Surface Signalling	54	12	30	10
Intracellular Signalling	27	6	18	6
Developmental processes	89	19	48	16
Physiological Processes	45	10	22	8
Total	469	100	293	100

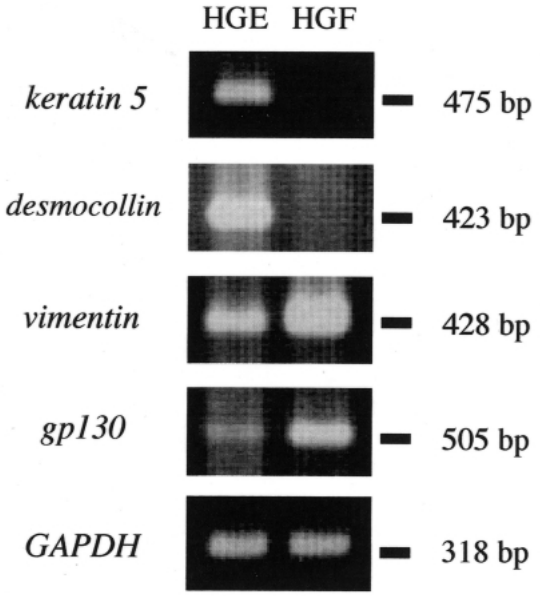

Fig. 2 RT-PCR analysis of mRNA levels.

Table 2 Selected genes expressed in HGE and HGF

Ratio (hGE / hGF)	GeneBank ID	Annotation
46.2	U46572	small inducible cytokine subfamily A (Cys-Cys), member 11 (eotaxin)
38.1	U41517	aquaporin 1 (channel-forming integral protein, 28kD)
35.6	J00124	keratin 14 (epidermolysis bullosa simplex)
29.1	M93426	protein tyrosine phosphatase, receptor-type, zeta polypeptide 1
21.6	AI830036	Small proline-rich protein SPRK, human, odontogenic keratocysts
15.8	AI394286	desmocollin 2
12.4	M19723	keratin 5 (epidermolysis bullosa simplex)
11.6	L42611	keratin 6B
10.9	X16662	annexin VIII
6.8	U69611	a disintegrin and metalloproteinase domain 17
Ratio (hGF / hGE)	**GeneBank ID**	**Annotation**
66.8	AF029674	basic leucine zipper protein
52.1	AI093287	caveolin 2
32.9	AA923547	annexin IV
27.4	Y11395	G protein-coupled receptor 69A
26.1	W49820	hyaluronan synthase 2
17.3	U09413	zinc finger protein 135
16.5	M25246	vimentin
10.8	M57230	gp130, oncostatin M receptor
5.7	AF017178	collagen, type I, alpha 1
5.5	Y00651	membrane cofactor protein, CD46

Discussion

The periodontium functions as a single unit, even though each of its components has a distinct composition and connective tissue architecture. It has been revealed that matrix constituents of a single periodontal structure can influence the cellular activities of adjacent structures. However, there is little information on the molecular-based characteristics of the unique interactions between gingival HGE and HGF, and no reports to date have described their gene expression profiles. We believed that use of DNA microarray technology for studying transcriptomes in HGE and HGF offered an attractive approach for such studies.

In the present study, a gene microarray technique was employed to analyze the differences between the gene expression patterns of HGE and HGF. cDNA probes from HGE labeled with Cy5 and those from HGF labeled with Cy3 were hybridized with a microarray of 7276 genes. This microarray analysis using mRNA isolated from HGE and HGF revealed numerous differences in gene expression.

In wound healing and in many pathologic conditions, keratinocytes are activated to become migratory and hyperproliferative cells that produce and secrete extracellular matrix components and signaling polypeptides. At the same time, their cytoskeletons are altered by the production of specific keratin proteins. TGF-β, which induces expression of keratin 5, reverts keratinocytes to the healthy basal phenotype and thus completes the activation cycle (8).

Desmosomes contain 2 types of cadherin; desmocollin and desmoglein. Desmocollin interacts with components of keratinocyte adherens junctions (9) and mediates adhesion in desmosomes, one of the principal types of intercellular junction in epithelia. Desmocollin comprises 3 genetic isoforms that are positionally associated with cell proliferation and the early stages of differentiation in epithelia (10). Our DNA microarray results demonstrated that keratin 5 and desmocollin genes were expressed at higher levels in HGE, and RT-PCR analysis confirmed these mRNA levels. The cytoskeleton, an important complex and dynamic cell component, is composed of 3 networks; microtubules, microfilaments, and intermediate filaments. The intermediate filaments comprise a heterogeneous family of different proteins including vimentin, which is often utilized as a marker for fibroblasts (11). Healthy HGF contain trace amounts of mRNA for the IL-6 receptor, but high levels of mRNA for gp130 (12). Furthermore, the inflammatory cytokine IL-6 was shown to down-regulate α5β1 integrin and β-actin expression through phosphorylation of gp130 and impaired HGF adherence (13).

Periodontal disease is a chronic inflammatory condition that is associated with Gram-negative pathogenic bacterial infection and a concomitant increase of proinflammatory cytokine expression in periodontal tissue. One aspect of early periodontal disease onset is significant epithelial cell proliferation and migration, while proliferation and invasion of junctional and sulcular epithelia, as well as connective tissue, begins early in the disease process, and may ultimately result in periodontal pocket formation. Regulation of this proliferation is poorly understood, but it is most likely controlled by locally expressed factors, and it has been shown that dense infiltration of lymphocytes and mononuclear cells, pathologic alteration of fibroblasts and continuing loss of connective tissue occur in inflamed gingival tissue. (14) The present study results illustrate that utilization of DNA microarray techniques to detect differences the gene expression profiles of HGE and HGF may also aid genetic diagnosis of periodontal tissue metabolism and periodontal diseases.

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