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Transcriptomes in Healthy and Diseased Gingival Tissues

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Abstract

Objectives—Clinical and radiographic measures are gold standards for diagnosing periodontitis but offer little information regarding the pathogenesis of the disease. We hypothesized that a comparison of gene expression signatures between healthy and diseased gingival tissues would provide novel insights in the pathobiology of periodontitis, and would inform the design of future studies.

Methods—Ninety systemically healthy non-smokers with moderate to advanced periodontitis (63 with chronic and 27 with aggressive periodontitis) each contributed with ≥2 "diseased" interproximal papillae [with bleeding on probing (BoP), pocket depth (PD) ≥4mm, and attachment loss (AL) ≥3mm)] and a "healthy" papilla, if available (no BoP, PD ≤4mm and AL ≤2mm). RNA was extracted, amplified, reverse-transcribed, labeled, and hybridized with AffymetrixU133Plus2.0 arrays. Differential expression was assayed in 247 individual tissue samples (183 from diseased and 64 from healthy sites) using a standard mixed-effects linear model approach, with patient effects considered random with a normal distribution, and gingival tissue status considered a two-level fixed effect. Gene ontology analysis summarized the expression patterns into biologically relevant categories.

Results—Transcriptome analysis revealed that a total of 12,744 probe sets were differentially expressed after adjusting for multiple comparisons ($p<9.15\times10^{-7}$). Of those, 5,295 were up-regulated and 7,449 down-regulated in disease when compared to health. Gene ontology analysis identified 61 differentially expressed groups (adjusted $p<0.05$) including apoptosis, antimicrobial humoral response, antigen presentation, regulation of metabolic processes, signal transduction, and angiogenesis.

Conclusions—Gingival tissue transcriptomes provide a valuable scientific tool for further hypothesis-driven studies of the pathobiology of periodontitis.

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The authors declare that they have no conflicts of interest.

Summary of key findings

Gene expression signatures differentiate between healthy and diseased gingival tissues and may provide novel insights in the pathobiology of periodontitis

Keywords

Periodontitis; genomics; infection; gene expression; microarray

INTRODUCTION

A distinction between states of periodontal health and disease is feasible using a variety of diagnostic approaches. Among the common clinical variables, bleeding on probing (BoP) is considered to best reflect presence of an inflammatory infiltrate adjacent to the ulcerated epithelium of the periodontal pocket. [1, 2]. Probing depth (PD) exceeding the typical depth of the healthy gingival crevice, in presence of BoP, is also used to signify periodontal pathology, while clinical attachment loss (AL) describes the cumulative exposure to destructive periodontitis. [3] Loss of periodontal tissue support can also be assessed radiographically. [4].

However, clinical and radiographic variables reflect poorly the underlying pathobiology of the various forms of periodontitis. The distinct histologic features of periodontal health and disease were first documented in a classic publication by Page and Schroeder. [5] These authors described the detailed morphologic characteristics of the gingival, sulcular, and pocket epithelium, the underlying connective tissue, and the types of resident and infiltrating blood cells in the initial, early, established and advanced periodontal lesion. In parallel, microbiologic approaches established common and distinct constituents of the periodontal microbiota in health and disease [6, 7] while biochemical approaches documented levels of cytokines, chemokines and other inflammatory mediators within the tissues and the gingival crevicular fluid. [8-10]

A genomic tool that may add to the armamentarium of approaches to study the pathobiology of periodontitis is gene expression profiling, i.e., the systematic cataloging of messenger RNA sequences in a cell population, organ or tissue sample. In general, transcriptomes are a powerful means of generating comprehensive genome-level data sets on complex diseases and have provided enormous insights mostly in cancer research [11, 12], but also in other conditions such as muscular dystrophy [13], Alzheimer's disease and dementia [14, 15], rheumatologic disorders [16, 17], and asthma. [18, 19]

To our knowledge, a systematic transcriptome-based approach has not been applied so far in the study of periodontitis. Our group has initiated a series of studies to explore whether the currently recognized forms of periodontitis are characterized by distinct gene expression profiles in affected gingival tissues. [20] Our further goal is to explore the feasibility of a novel classification based on similarities in transcriptional profiles. The aim of this first report is to present a comprehensive description of the periodontal transcriptome in healthy and diseased gingival tissues.

MATERIAL AND METHODS

The study was approved by the Columbia University Institutional Review Board.

Subjects

Ninety subjects with moderate to severe periodontitis (63 with chronic and 27 with aggressive periodontitis) were recruited among those referred to the Columbia University College of Dental Medicine between November 2004 and April 2007. Eligible patients were (i) >13 yrs old; (ii) had ≥ 24 teeth; (iii) had no history of systematic periodontal therapy other than occasional prophylaxis, (iv) had received no systemic antibiotics or anti-inflammatory drugs for ≥ 6 months, (v) harbored ≥4 teeth with radiographic bone loss, (vi) did not have diabetes

or any systemic condition that entails a diagnosis of "Periodontitis as a manifestation of systemic diseases" [21], (vii) were not pregnant, and (ix) were not current users of tobacco products or nicotine replacement medication. Signed informed consent was obtained prior to enrollment.

Clinical examination

All participants underwent a full-mouth examination of the periodontal tissues at six sites per tooth by a single, calibrated examiner. Variables recorded included presence/absence of visible dental plaque (PL), presence/absence of bleeding on probing (BoP), probing depth (PD), and attachment level (AL). Data were entered chair-side to a computer and stored at a central server.

Gingival tissue donor areas and tissue sample collection

Subsequently to clinical data entry, a specially developed software identified periodontally "diseased" and "healthy" tooth sites based on the clinical data. "Diseased" sites showed BoP, had interproximal PD>4mm, and concomitant AL≥3mm. "Healthy" sites showed no BoP, had PD≤4mm and AL≤2mm. Next, the software identified (i) maxillary "diseased" and "healthy" interdental papillae, based on the above criteria, and (ii) pairs of diseased interdental papillae with similar clinical presentation (PD and AL within 2mm of each other). A posterior maxillary sextant encompassing a pair of qualifying "diseased" interdental papillae was identified.

Periodontal surgery was performed at the identified sextant with no prior supra- or subgingival instrumentation. After local anesthesia, submarginal incisions were performed, mucoperiosteal flaps were reflected, and the portion of each interproximal gingival papilla that adhered to the root surface was carefully dissected. This section comprised the ulcerated epithelial lining of the interproximal periodontal pockets and the underlying connective tissue. After dissection, the gingival tissue specimens were thoroughly rinsed with sterile normal saline solution and transferred into Eppendorf tubes containing a liquid RNA stabilization reagent*. A minimum of 2 diseased papillae were harvested from each sextant and, whenever available, a healthy tissue specimen was obtained from an adjacent site. After collection of the specimens, pocket elimination/reduction periodontal surgery was completed according to standard procedures. All patients received additional periodontal therapy according to their individual needs.

RNA extraction, reverse transcription, *in vitro* cRNA synthesis

The tissue specimens were stored in a liquid RNA stabilization reagent* overnight at 4°C, snap-frozen and stored in liquid nitrogen. All further processing occurred simultaneously for gingival biopsies originating from the same donor. Specimens were homogenized in a liquid buffer†. After incubation with chloroform and centrifugation at 12,000g, RNA collected in the upper aqueous phase was precipitated by mixing with isopropyl-alcohol and additional centrifugation and washed in 75% ethanol. The extracted RNA was purified using a total RNA isolation kit‡, quantitated spectrophotometrically, and 7.5 micrograms of total RNA was reverse-transcribed using a one-cycle cDNA synthesis kit§. Synthesis of biotin-Labeled cRNA was performed using appropriate amplification reagents for in vitro transcription‖. The cRNA yield was determined spectrophotometrically at 260 nm. The cRNA was fragmented by incubation in fragmentation buffer at 94°C for 35min and stored at -80°C until hybridizations.

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*(RNA*later*, Ambion, Austin, TX)
*RNA*later*, Ambion, Austin, TX, USA
†Trizol; Invitrogen Life Technologies, Carlsbad, CA, USA
‡RNeasy; Qiagen, Valencia, CA, USA
§GeneChip Expression 3′ amplification one-cycle cDNA synthesis kit; Affymetrix, Santa Clara, CA, USA
‖GeneChip Expression 3′-Amplification Reagents for IVT labeling kit; Affymetrix

Gene Chip hybridizations

Human Genome arrays[¶] were used including 54,675 probe sets to analyze more than 47,000 transcripts including 38,500 well-characterized human genes. Hybridizations, probe array scanning and gene expression analysis were performed at the Gene Chip Core Facility, Columbia University Genome Center. Each sample was hybridized once and each person contributed with 2 to 4 (median 3) samples.

Data analysis

Two statistical analyses packages were used throughout[*†]. Expression data were normalized and summarized using the log scale robust multi-array analysis (RMA) [22] with default settings. Differential expression was assayed using a standard mixed-effects linear model approach, with patient effects considered random with a normal distribution, and gingival tissue status considered a two-level fixed effect ("healthy" vs. "diseased"). Statistical significance for each probe set was determined using both the Bonferroni criterion and q-value. [23] For each probe set, a fold-change was computed by dividing the raw expression values among "diseased" tissue samples by the raw expression values among "healthy" samples. Therefore, fold-change values represent relative RNA levels in "disease" vs. "health".

Gene Ontology analysis was performed using ermineJ [24] with the Gene Score Resampling method. P-values were used as input to identify biologically-relevant groups of genes showing differential expression in health and disease. Gene symbols and descriptions were derived from the Gemma System (HG-U133_Plus_2_NoParents.an.zip) and downloaded from: http://www.bioinformatics.ubc.ca/microannots/.

Additional ontology analysis of all genes with a q-value of <0.05 was carried out using Pathway Express [25] in which the differentially expressed genes were mapped to the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (http://www.genome.jp/kegg/).

Experimental details and results following the MIAME standards [26] are available at the Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE 10334.

RESULTS

The mean age of the patients was 42 years (range 13-76; Table 1). Based on self-reported race/ethnicity, 37% of the patients were White, 21% Black, 32% of mixed race, and 76% Hispanic. According to the 1999 International Workshop for the Classification of Periodontal Diseases and Conditions criteria [27], 70% of the patients had chronic and 30% aggressive periodontitis. On average, study participants had 28 teeth present, 57 sites with PD≥5 mm, 54 sites with AL≥5 mm and 71% BoP. Among the 247 harvested gingival tissue samples (183 from diseased and 64 from healthy sites), 67% had PD≥5 mm and 62% AL≥5 mm (Table 2). No healthy gingival tissues samples were available from 26 subjects.

Transcriptome analysis revealed that 32,598 probes sets were differentially expressed between healthy and diseased tissue samples at q<0.05. Of those, 51% were up-regulated and 49% down-regulated in disease when compared to health. Applying the Bonferroni correction for 54,675 comparisons, a total of 12,744 probe sets were differentially regulated ($p<9.15\times10^{-7}$; 5,295 up-regulated and 7,449 down-regulated in disease when compared to health). The

[¶]Human Genome U-133 Plus 2.0 arrays; Affymetrix
[*]R version 2.3.1 for Linux OS
[†]SAS for PC version 9.1; SAS Institute, Cary, NC, USA

complete list of differentially regulated probe sets can be viewed on Online (Supplemental Table 1).

Fold-changes in expression ranged between 5.73 and 3.89 (all p-values<1.1×10^{-16}) for the top 50 probe sets with increased expression in diseased relative to healthy tissue samples (Fig. 1A), and between 4.35 and 2.13 (all p-values<1.1×10^{-16}) for the top 50 probe sets with decreased expression in diseased samples [inverse values of the strongest (0.23) and weakest (0.47) fold change values quoted; Fig. 1B].

Gene ontology analysis identified 61 differentially expressed groups at $p<0.05$ including apoptosis, antimicrobial humoral response, antigen presentation, regulation of metabolic processes, signal transduction, and angiogenesis (Table 3). Four selected differentially regulated pathways by Pathway Express analysis are illustrated in Fig. 2 (MAPK signaling pathway, Fig. 2A; cytokine-cytokine receptor interaction, Fig. 2B; cell adhesion molecules, Fig. 2C; and apoptosis, Fig. 2D). The top 50 pathways identified by this analysis are listed in Table 4.

DISCUSSION

To the best of our knowledge, this is the first study to systematically describe the transcriptomes of healthy and diseased gingival tissues in patients with destructive periodontal diseases. The primary aim of this report is to provide a comprehensive description that will serve as an information resource for investigators interested in the pathobiology of periodontitis. Clearly, the presented gene expression data need to be subjected to additional verification steps before their exact biological significance is fully appreciated. These may include confirmation by independent techniques on the mRNA level such as real time RT-PCR, and by proteomic analyses. Therefore, at this point, the presented data are not meant to provide unequivocal evidence for the involvement of any particular gene in the disease process, but rather to identify broad consortia of genes and pathways that are likely differentially expressed in states of gingival health and disease.

Our study has several strengths relevant to its ambition to serve as a high quality research resource. First, we have involved a relatively large sample of well-characterized, patients with periodontitis that were free of confounding exposures such as systemic disease, medications and smoking. Second, our gene expression data are generated by a large number of arrays representing strictly defined clinical conditions and multiple sites per subject. Third, our gingival tissue samples were obtained prior to any therapeutic manipulation of the gingival tissues. Lastly, by allowing direct access to our raw data, we enable independent investigators to conduct focused analyses targeting individual genes and pathways of particular interest to them.

We would like to draw attention to a number of points that will facilitate a correct interpretation of our findings: it must be realized that the reported transcriptomes represent the composite gene expression of a variety of cells that constitute and populate the healthy and diseased gingival tissues, including epithelial cells, connective tissue fibroblasts and infiltrating cells. Although the assayed tissue samples were deemed to be “diseased” or “healthy” based on accepted clinical signs of gingival inflammation the extent of the inflammatory infiltrate, the degree of vascularization and the epithelial/connective tissue content of each gingival tissue sample were unknown and likely variable. In future studies, use of cell-capture techniques may facilitate the study of homogeneous cell subpopulations, and may generate data that can be directly comparable to those stemming from well-defined *in vitro* systems, such as the recently reported transcriptional profiles of cultured oral epithelial cells challenged by specific periodontal pathogens and commensals [28-30], or the *in vivo* regulation of specific proteins in

rodent junctional and pocket epithelia. [31] With respect to the clinical status of the obtained gingival tissue samples, it must be noted that the transcriptomes of healthy and intact gingival tissues of periodontitis patients may not necessarily be identical to those of healthy sites in subjects that have not experienced destructive periodontitis. Consequently, since our data are based exclusively on a cohort of patients with periodontitis, our findings cannot identify "susceptibility genes". Furthermore, the observed heterogeneity in expression among diseased tissue samples even for genes that were, on average, undisputedly differentially regulated between health and disease may reflect varying states of disease activity among clinically homogeneous sites. There are additional potential explanations for this heterogeneity, such as differential bacterial colonization patterns across diseased sites. Future analyses from our group will incorporate data on bacterial colonization patterns and will be informative in this regard. Lastly, while a potential effect of infiltration anesthesia on gene expression is conceivable, there is little reason to expect differential anesthesia-mediated effects in diseased versus healthy samples, and thus a systematic bias in the reported comparisons.

In this first report, we will not proceed with an in-depth discussion of specific differentially regulated pathways in health and disease but will rather provide examples that underscore the utility of the expression data. At first glance, one can view the transcriptome findings as largely confirmatory of anticipated differences based on earlier histologic or biochemical analyses. For example, the vast majority of the top genes with increased expression in disease as compared to health are indeed immunoglobulin-related genes. However, genes far less readily associated with periodontitis were also observed to be least expressed in disease or, alternatively, most expressed in health (e.g. desmocollin 1, arylacetamide deacetylase-like 2, guanylate cyclase C). Likewise, the most expressed chemokine in disease (by 3.85-fold, $p<10^{-18}$) was CXCL6 (granulocyte chemoattractant protein 2, GCP-2), a molecule known to be involved in inflammatory bowel diseases [32] but not earlier associated with gingival inflammation. Lastly, confirming and extending recent preliminary findings [33], our data showed that matrix metalloproteinases 7, 13, 3, 1, 9, 14, 2 and 28 and their inhibitors TIMP-3 and TIMP-2 are significantly up-regulated in diseased tissues. The above examples illustrate the utility of transcriptional data in guiding future focused studies of the pathobiology of periodontitis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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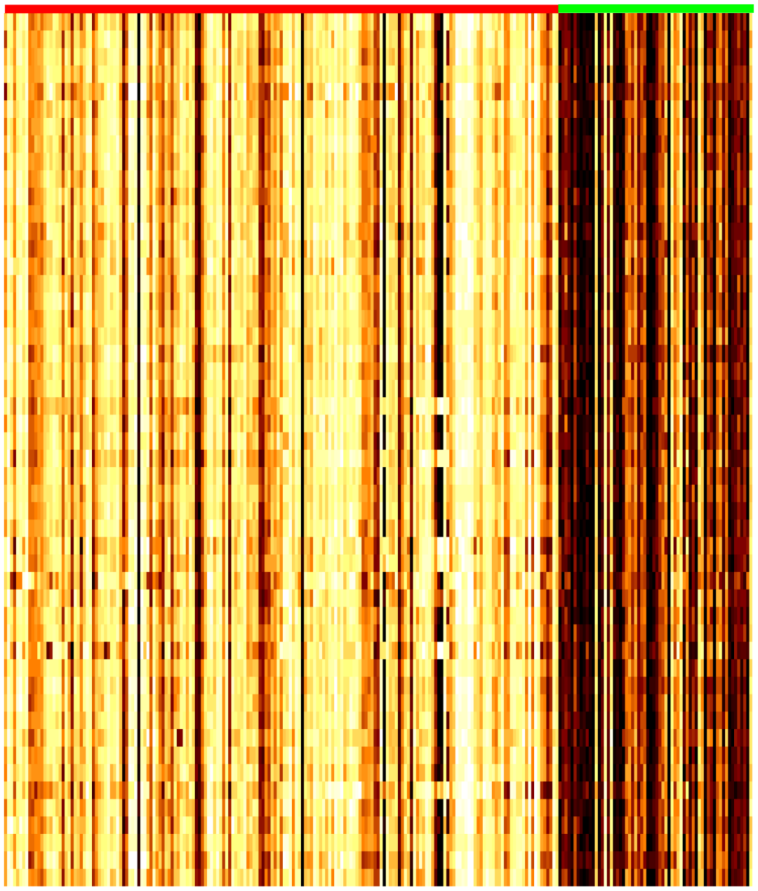
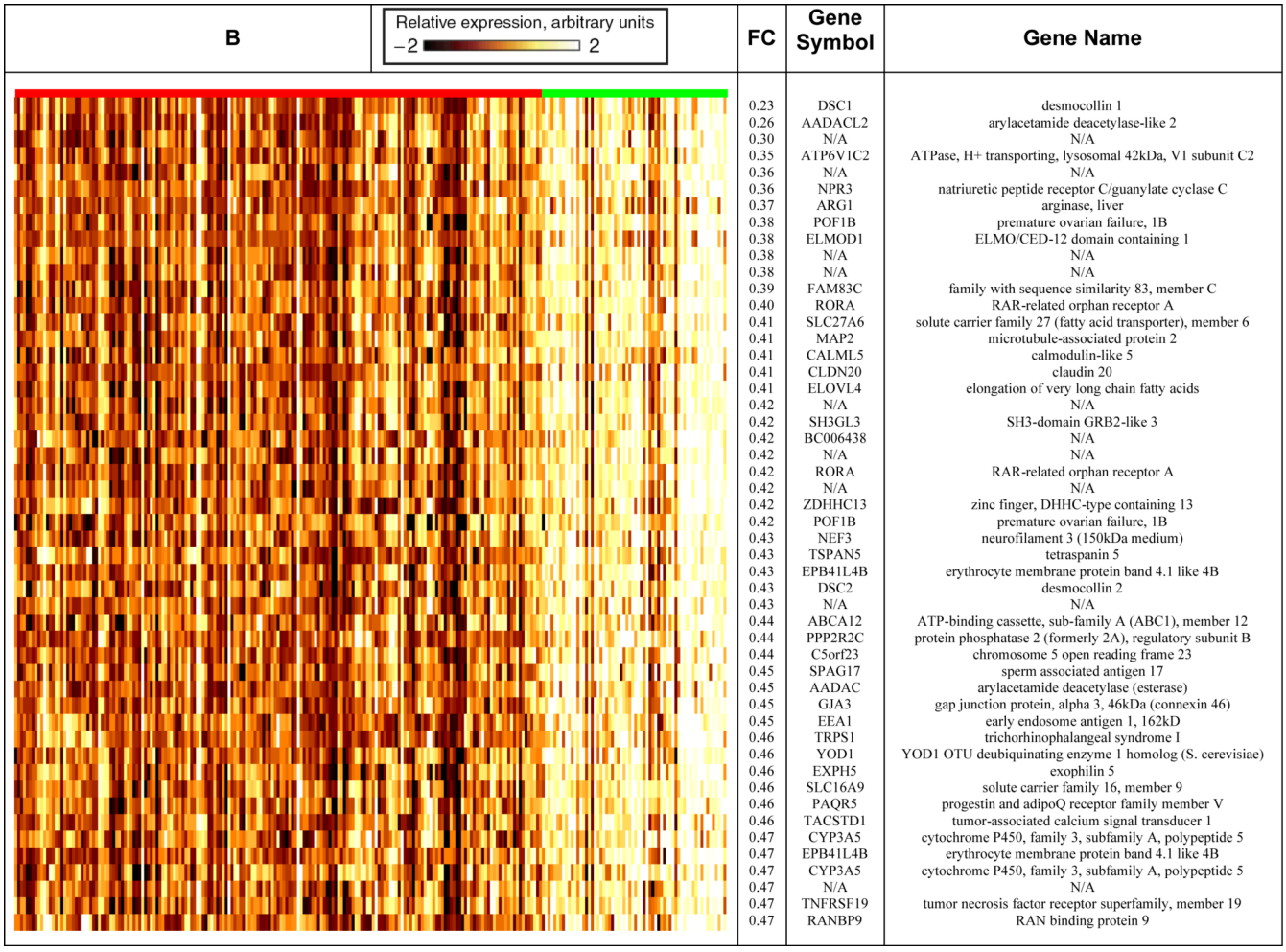


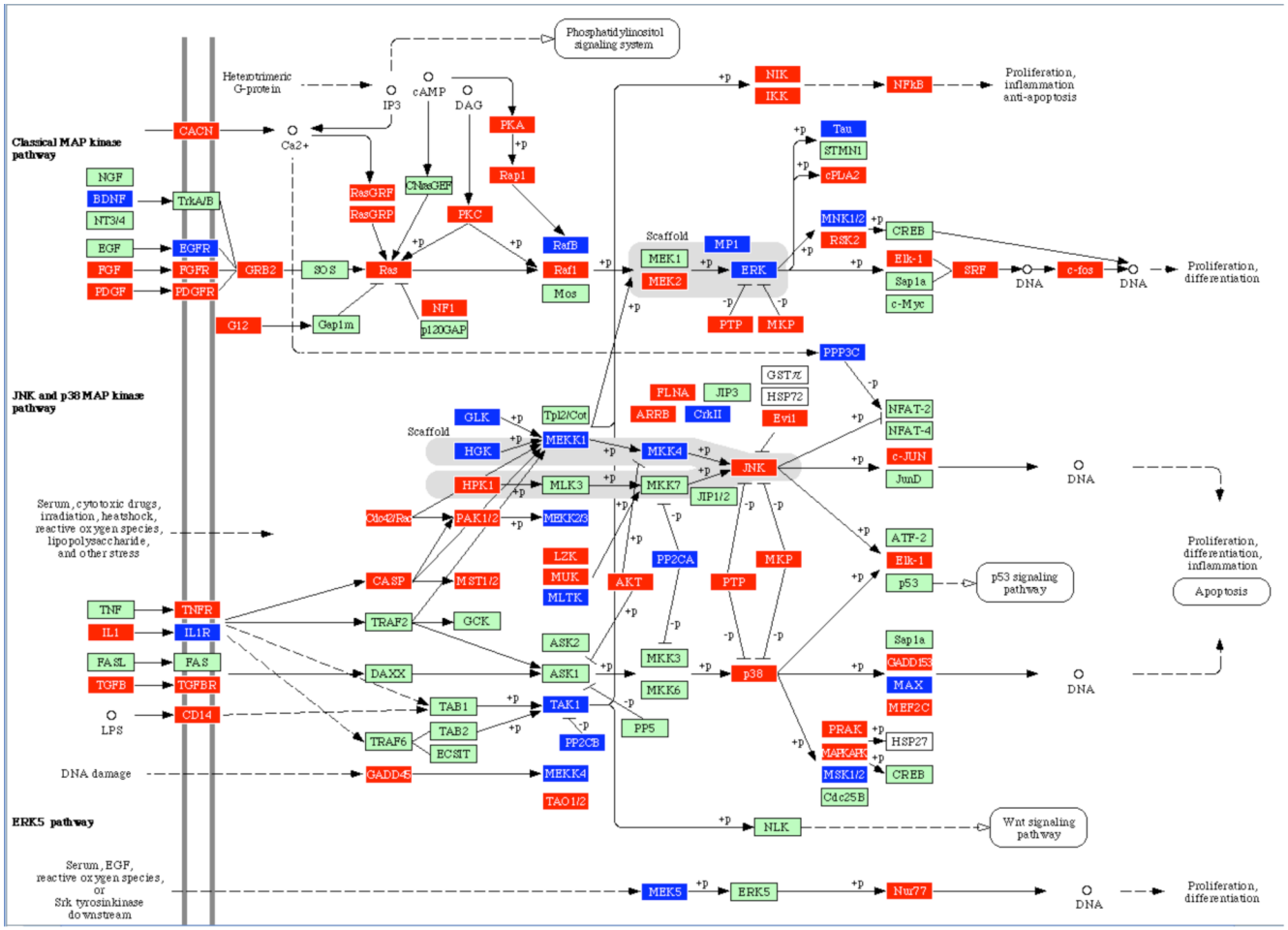
FC	Gene Symbol	Gene Name
5.73	MGC29506	hypothetical protein MGC29506
5.49	IFI6	interferon, alpha-inducible protein 6
5.4	IGHA1	immunoglobulin heavy constant alpha 1
5.24	IGKC	immunoglobulin kappa constant
5.14	N/A	N/A
5.1	IGKC	immunoglobulin kappa constant
5.04	IFI6	interferon, alpha-inducible protein 6
5.04	IFI6	interferon, alpha-inducible protein 6
5.02	IFI6	interferon, alpha-inducible protein 6
4.98	N/A	N/A
4.95	IGL@	immunoglobulin lambda locus
4.86	IFI6	interferon, alpha-inducible protein 6
4.79	IGL@	immunoglobulin lambda locus
4.75	IGL@	immunoglobulin lambda locus
4.74	IGHV1-69	immunoglobulin heavy variable 1-69
4.73	IGLC1	immunoglobulin lambda constant 1 (Mcg marker)
4.71	IGL@	immunoglobulin lambda locus
4.68	IGL@	immunoglobulin lambda locus
4.63	IGKC	immunoglobulin kappa constant
4.63	IGL@	immunoglobulin lambda locus
4.63	IGKC	immunoglobulin kappa constant
4.63	N/A	N/A
4.62	POU2AF1	POU domain, class 2, associating factor 1
4.61	IGL@	immunoglobulin lambda locus
4.59	IGL@	immunoglobulin lambda locus
4.5	TNFRSF17	tumor necrosis factor receptor superfamily, member 17
4.44	IGL@	immunoglobulin lambda locus
4.43	IGKC	immunoglobulin kappa constant
4.43	N/A	N/A
4.41	IFI6	interferon, alpha-inducible protein 6
4.4	N/A	N/A
4.4	FAM46C	family with sequence similarity 46, member C
4.36	IFI6	interferon, alpha-inducible protein 6
4.25	IGKC	immunoglobulin kappa constant
4.22	IFI6	interferon, alpha-inducible protein 6
4.19	IGKC	immunoglobulin kappa constant
4.16	FCRL5	Fc receptor-like 5
4.15	IGL@	immunoglobulin lambda locus
4.14	IGL@	immunoglobulin lambda locus
4.1	IGL@	immunoglobulin lambda locus
4.09	IGHA1	immunoglobulin heavy constant alpha 1
4.07	IGL@	immunoglobulin lambda locus
4.06	IFI6	interferon, alpha-inducible protein 6
4.04	IFI6	interferon, alpha-inducible protein 6
4.04	KIAA0125	KIAA0125
3.97	IFI6	interferon, alpha-inducible protein 6
3.96	IGHV1-69	immunoglobulin heavy variable 1-69
3.89	IGKC	immunoglobulin kappa constant
3.89	IGKC	immunoglobulin kappa constant
3.89	N/A	N/A



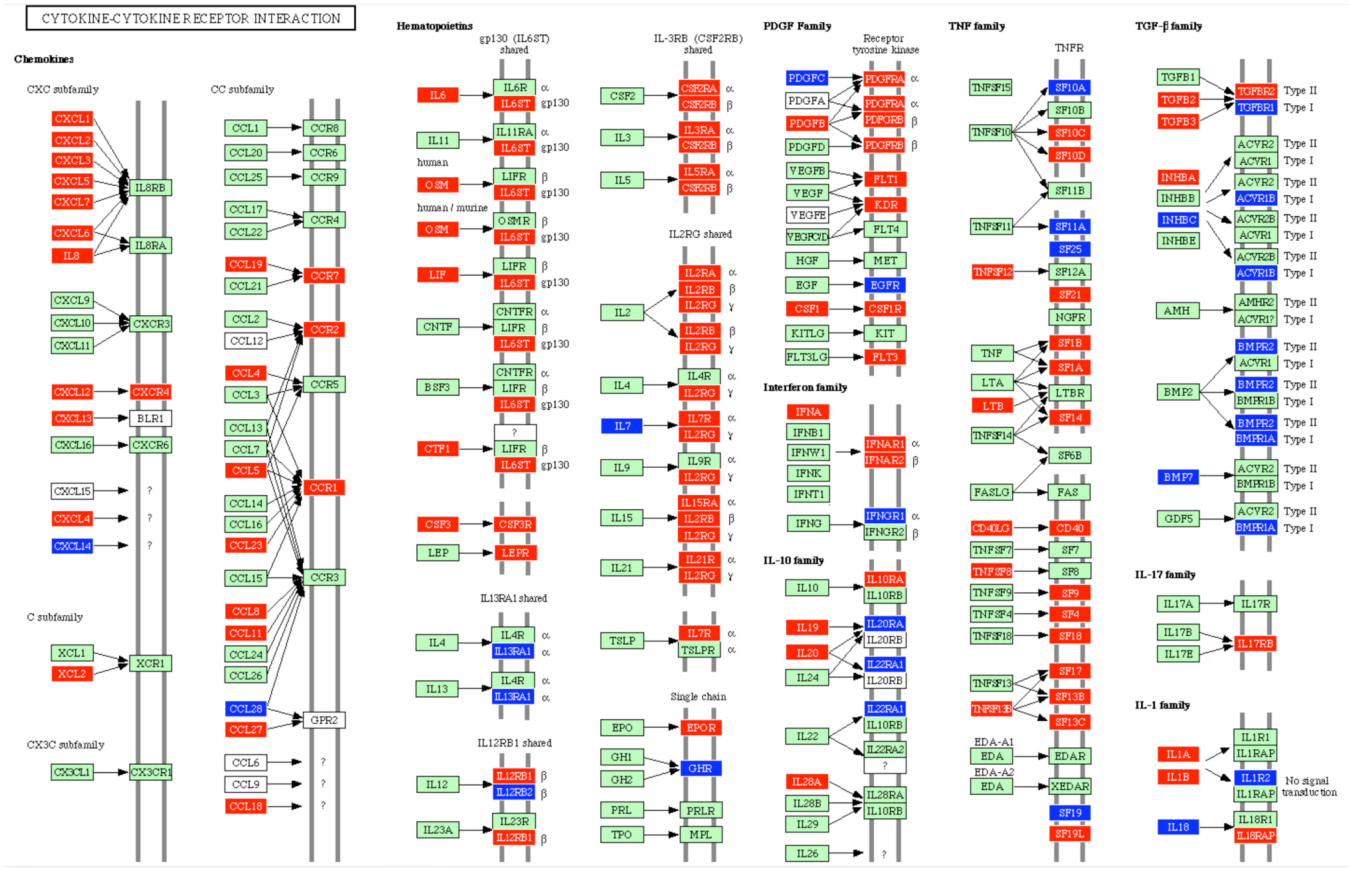
FC	Gene Symbol	Gene Name
0.23	DSC1	desmocollin 1
0.26	AADACL2	arylacetamide deacetylase-like 2
0.30	N/A	N/A
0.35	ATP6V1C2	ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C2
0.36	N/A	N/A
0.36	NPR3	natriuretic peptide receptor C/guanylate cyclase C
0.37	ARG1	arginase, liver
0.38	POF1B	premature ovarian failure, 1B
0.38	ELMOD1	ELMO/CED-12 domain containing 1
0.38	N/A	N/A
0.38	N/A	N/A
0.39	FAM83C	family with sequence similarity 83, member C
0.40	RORA	RAR-related orphan receptor A
0.41	SLC27A6	solute carrier family 27 (fatty acid transporter), member 6
0.41	MAP2	microtubule-associated protein 2
0.41	CALML5	calmodulin-like 5
0.41	CLDN20	claudin 20
0.41	ELOVL4	elongation of very long chain fatty acids
0.42	N/A	N/A
0.42	SH3GL3	SH3-domain GRB2-like 3
0.42	BC006438	N/A
0.42	N/A	N/A
0.42	RORA	RAR-related orphan receptor A
0.42	N/A	N/A
0.42	ZDHHC13	zinc finger, DHHC-type containing 13
0.42	POF1B	premature ovarian failure, 1B
0.43	NEF3	neurofilament 3 (150kDa medium)
0.43	TSPAN5	tetraspanin 5
0.43	EPB41L4B	erythrocyte membrane protein band 4.1 like 4B
0.43	DSC2	desmocollin 2
0.43	N/A	N/A
0.44	ABCA12	ATP-binding cassette, sub-family A (ABC1), member 12
0.44	PPP2R2C	protein phosphatase 2 (formerly 2A), regulatory subunit B
0.44	C5orf23	chromosome 5 open reading frame 23
0.45	SPAG17	sperm associated antigen 17
0.45	AADAC	arylacetamide deacetylase (esterase)
0.45	GJA3	gap junction protein, alpha 3, 46kDa (connexin 46)
0.45	EEA1	early endosome antigen 1, 162kD
0.46	TRPS1	trichorhinophalangeal syndrome I
0.46	YOD1	YOD1 OTU deubiquinating enzyme 1 homolog (S. cerevisiae)
0.46	EXPH5	exophilin 5
0.46	SLC16A9	solute carrier family 16, member 9
0.46	PAQR5	progestin and adipoQ receptor family member V
0.46	TACSTD1	tumor-associated calcium signal transducer 1
0.47	CYP3A5	cytochrome P450, family 3, subfamily A, polypeptide 5
0.47	EPB41L4B	erythrocyte membrane protein band 4.1 like 4B
0.47	CYP3A5	cytochrome P450, family 3, subfamily A, polypeptide 5
0.47	N/A	N/A
0.47	TNFRSF19	tumor necrosis factor receptor superfamily, member 19
0.47	RANBP9	RAN binding protein 9

Figure 1.

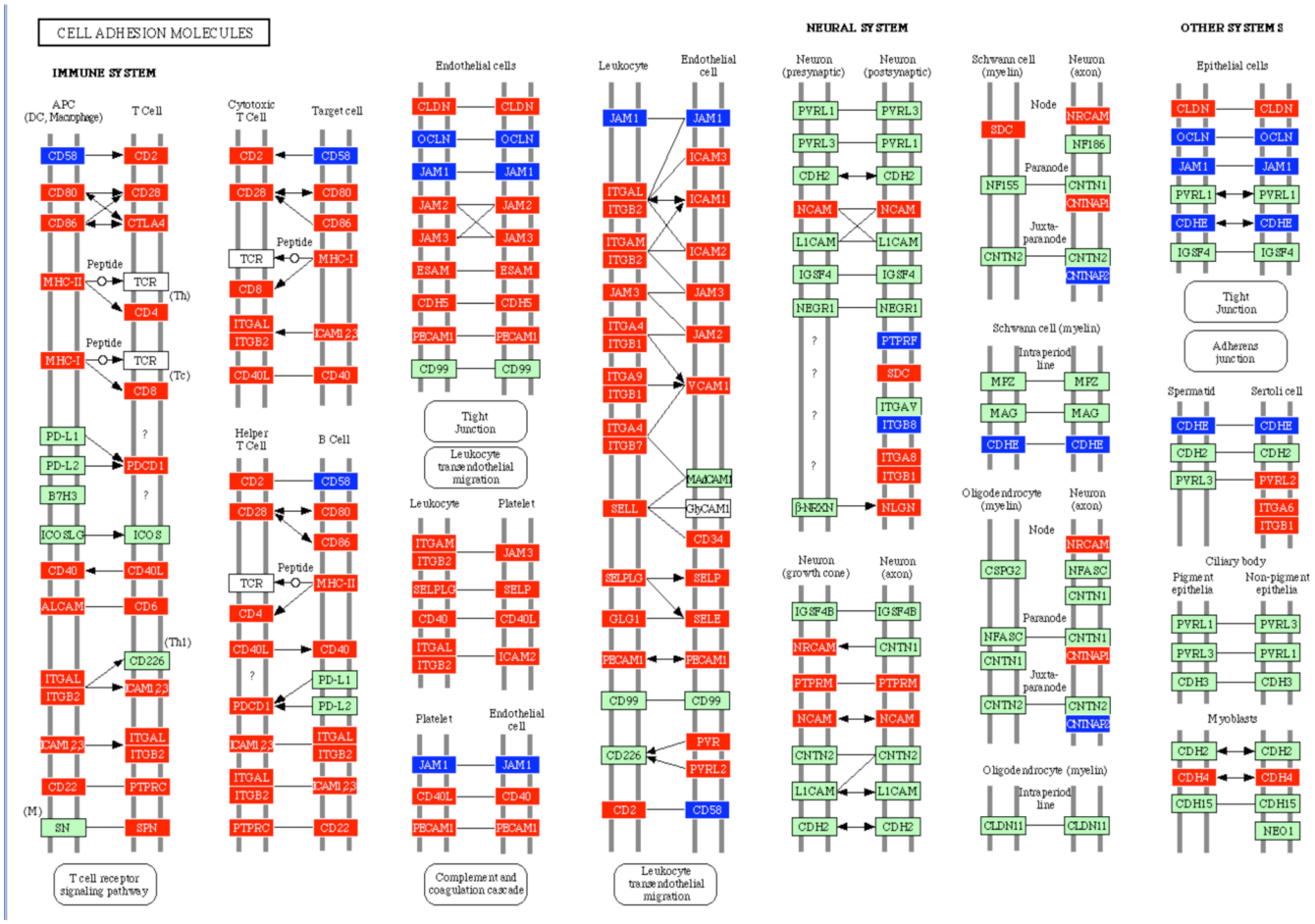
Visualization of the top 50 probe sets with increased expression in diseased, relative to healthy gingival tissue (A) and of the top 50 probe sets with decreased expression in diseased, relative to healthy gingival tissue (B). Gingival tissue samples are grouped according to clinical periodontal status with diseased tissues on the left (red horizontal bar) and healthy tissues on the right (green bar). The color of each pixel represents gene expression level with darker colors indicating lower relative expression values. Columns correspond to individual tissue samples and rows correspond to probe sets. Fold change (FC) describes the ratio of mean expression in diseased tissue over the mean expression in healthy tissue. Note that multiple probe sets map to a single gene. Due to space limitations, only one gene symbol and gene name per probe set are identified. A complete list of gene symbols and names per probe set is provided in the Online Supplement Table 1.



A



B



C

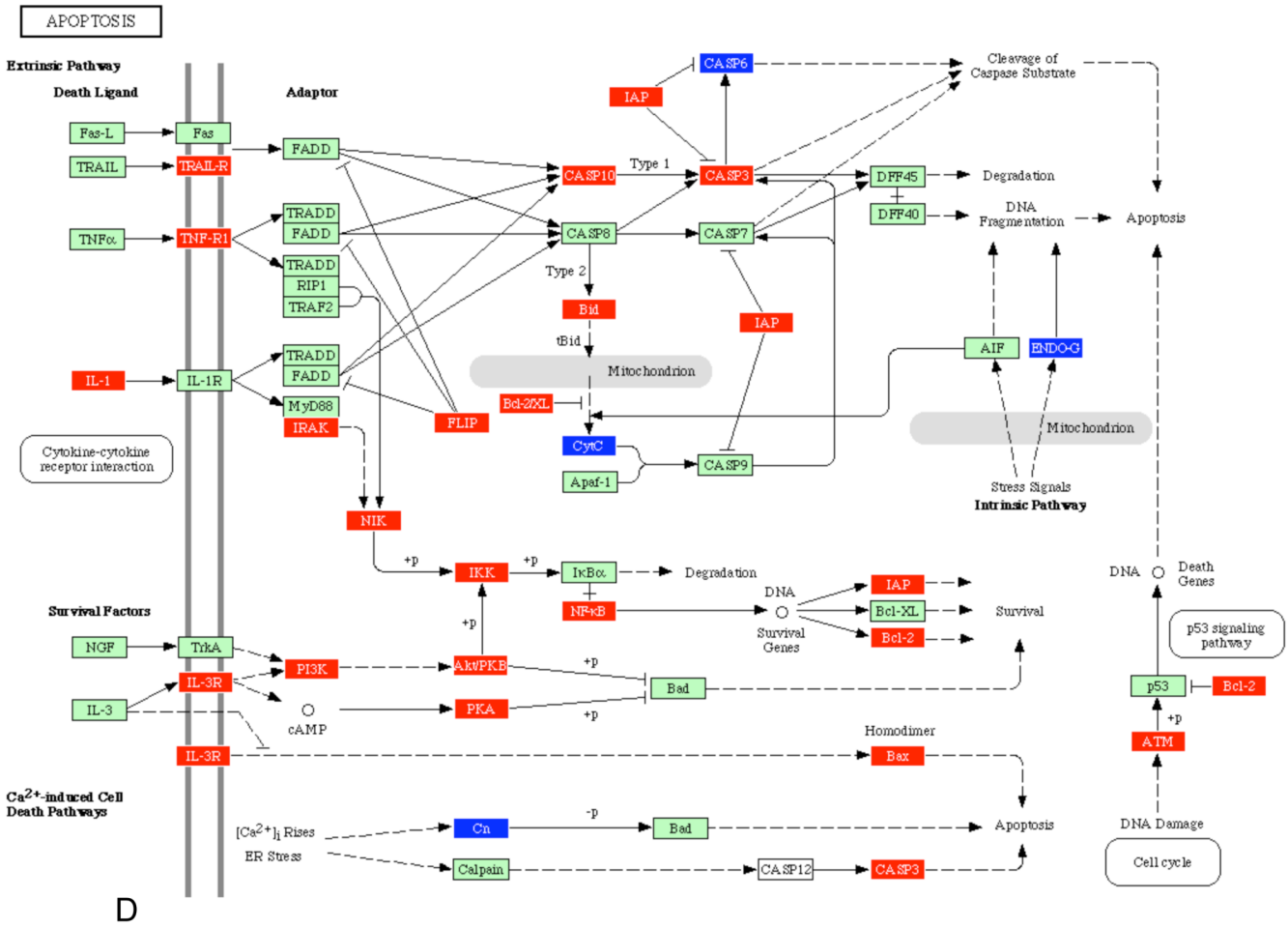


Figure 2.

Ontology analysis of selected pathways. (A) MAPK signaling pathway; (B) cytokine-cytokine receptor interaction; (C) cell adhesion molecules; (D) apoptosis. Genes shown in red are over-expressed and genes shown in blue under-expressed in diseased gingival tissues when compared to healthy tissues. Genes in green are unchanged at the $p<0.05$ significance level.

Table 1

General characteristics of the study participants (n=90)

Characteristic	(Mean±SD or %)	Range
Age (years)	42±13	13 - 76
Female	50%	
Race		
Black	21%	
White	37%	
Asian	1%	
Mixed	32%	
Other	5%	
Declined to report	4%	
Ethnicity		
Hispanic	76%	
Non-Hispanic	23%	
Declined to report:	1.1%	
Periodontal diagnosis		
Chronic periodontitis	70%	
Aggressive periodontitis	30%	
Clinical Periodontal Variables		
Number of teeth	28±3	22 - 32
Percent of sites with bleeding on probing (%)	71±0.2	24 - 100
Pocket depth (PD; mm)	3.9±0.7	2.9 - 6.5
Number of sites/subject with PD ≥ 5 mm	57±25	12 - 156
Clinical attachment level (AL; mm)	4.1±0.9	2.7 - 6.5
Number of sites/subject with AL≥ 5 mm	54±30	10 - 150

Table 2

Distribution of tissue samples according to pocket depth (PD) and clinical attachment levels (AL)

% of tissue samples in specified PD range		% of tissue samples in specified AL range	
1-2 mm	19%	1-2 mm	18%
3-4 mm	14%	3-4 mm	18%
5 mm	31%	5 mm	21%
≥6 mm	36%	≥6 mm	41%
		Non-readable	2%

Table 3

Gene Ontology groups differentially expressed in diseased and healthy gingival tissues at $p<0.05$

Group Name	ID	p-value	# of probes	# of genes
Induction of apoptosis	GO:0006917	1.26E-09	360	152
Negative regulation of cell proliferation	GO:0008285	1.76E-05	373	170
Protein metabolic process	GO:0019538	5.19E-05	441	180
Negative regulation of apoptosis	GO:0043066	1.00E-04	368	169
Regulation of cellular process	GO:0050794	1.36E-04	360	163
Antimicrobial humoral response (sensu Vertebrata)	GO:0019735	2.50E-04	145	87
Antimicrobial humoral response	GO:0019730	2.53E-04	142	85
Regulation of Ras protein signal transduction	GO:0046578	3.10E-04	275	99
Cell motility	GO:0006928	3.57E-04	382	180
Antigen processing and presentation of peptide antigen via MHC class I	GO:0002474	4.04E-04	163	52
Taxis	GO:0042330	5.74E-04	189	110
Lipid biosynthetic process	GO:0008610	6.29E-04	211	98
Positive regulation of apoptosis	GO:0043065	9.02E-04	321	138
Chemotaxis	GO:0006935	9.72E-04	194	114
Rho protein signal transduction	GO:0007266	9.92E-04	260	96
Protein kinase cascade	GO:0007243	1.11E-03	305	117
Enzyme linked receptor protein signaling pathway	GO:0007167	1.16E-03	250	90
Protein complex assembly	GO:0006461	1.18E-03	372	146
Regulation of growth	GO:0040008	1.53E-03	217	98
Rrna metabolic process	GO:0016072	2.16E-03	98	53
Induction of programmed cell death	GO:0012502	2.21E-03	263	114
Lymphocyte activation	GO:0046649	2.41E-03	103	52
Regulation of apoptosis	GO:0042981	2.63E-03	342	143
Anti-apoptosis	GO:0006916	2.75E-03	326	145
Localization of cell	GO:0051674	3.28E-03	267	130
MAPKKK cascade	GO:0000165	4.21E-03	170	72
Transmembrane receptor protein tyrosine kinase signaling pathway	GO:0007169	4.37E-03	338	127
Blood vessel morphogenesis	GO:0048514	4.57E-03	147	59
Cellular defense response	GO:0006968	4.70E-03	184	95
Angiogenesis	GO:0001525	5.68E-03	147	62
Antigen processing and presentation of peptide antigen	GO:0048002	5.72E-03	153	49
Tissue development	GO:0009888	5.74E-03	214	121
Dephosphorylation	GO:0016311	5.79E-03	377	164
Endocytosis	GO:0006897	6.32E-03	339	144
Response to DNA damage stimulus	GO:0006974	7.99E-03	388	182
Anatomical structure formation	GO:0048646	8.97E-03	139	56
Macromolecule complex assembly	GO:0065003	9.55E-03	333	127
Regulation of programmed cell death	GO:0043067	0.014246	239	99

Group Name	ID	p-value	# of probes	# of genes
Actin filament-based process	GO:0030029	0.014402	272	99
Cell growth	GO:0016049	0.014682	281	124
Actin cytoskeleton organization and biogenesis	GO:0030036	0.01542	345	125
Negative regulation of signal transduction	GO:0009968	0.0155	168	65
Ectoderm development	GO:0007398	0.017542	137	78
RNA metabolic process	GO:0016070	0.017803	215	93
Ribosome biogenesis and assembly	GO:0042254	0.021545	110	59
Positive regulation of transcription from RNA polymerase II promoter	GO:0045944	0.021767	139	45
Phospholipid metabolic process	GO:0006644	0.02178	122	64
Positive regulation of transcription, DNA-dependent	GO:0045893	0.021971	309	113
R-rna processing	GO:0006364	0.021976	106	57
Cytoskeleton organization and biogenesis	GO:0007010	0.028137	258	96
Epidermis development	GO:0008544	0.030145	119	71
Cytokine and chemokine mediated signaling pathway	GO:0019221	0.034265	45	25
Regulation of cell growth	GO:0001558	0.03479	282	130
Cell migration	GO:0016477	0.036679	227	92
Carboxylic acid transport	GO:0046942	0.036845	76	37
Protein amino acid dephosphorylation	GO:0006470	0.037447	343	146
DNA replication	GO:0006260	0.037785	257	125
Cellular lipid metabolic process	GO:0044255	0.038116	313	144
Membrane invagination	GO:0010324	0.048082	206	85

Number of probe sets and number of genes refer to the number of probe sets and genes represented in each ontology group. Analysis was carried out using on ermineJ [23].

Table 4

Ontology analysis of the top 50 differentially expressed pathways in diseased and healthy gingival tissues

Impacted pathway [a]	Impact Factor [b]	Input genes / Pathway genes (%) [c]	p-value
Antigen processing and presentation	42.2	39.0	0.102874871
Cell adhesion molecules (CAMs)	40.5	53.0	6.38E-05
B cell receptor signaling pathway	16.1	68.3	8.03E-07
Adherens junction	14.0	63.6	3.30E-06
Leukocyte transendothelial migration	13.1	56.0	2.02E-05
Natural killer cell mediated cytotoxicity	11.9	51.9	5.12E-05
Circadian rhythm	11.8	41.7	0.402801166
Focal adhesion	10.8	50.8	9.47E-05
Renal cell carcinoma	10.6	60.9	7.58E-05
Regulation of actin cytoskeleton	10.4	49.0	1.61E-04
Phosphatidylinositol signaling system	10.0	35.1	0.689660919
Colorectal cancer	8.8	55.3	4.88E-04
T cell receptor signaling pathway	8.3	52.7	0.001630673
MAPK signaling pathway	8.1	47.3	9.90E-04
Tight junction	6.9	49.6	0.004079315
VEGF signaling pathway	6.5	52.9	0.004965142
GnRH signaling pathway	6.3	50.5	0.006525075
Fc epsilon RI signaling pathway	6.3	52.0	0.00785814
Cytokine-cytokine receptor interaction	6.2	43.4	0.008217367
Small cell lung cancer	6.1	51.2	0.007222158
Wnt signaling pathway	5.7	46.3	0.010884856
Chronic myeloid leukemia	5.7	51.3	0.010359917
Notch signaling pathway	5.7	53.2	0.010958518
Glioma	5.7	50.0	0.029273605
Alzheimer”s disease	5.6	63.6	0.011980061
Epithelial cell signaling in H. pylori infection	5.5	50.7	0.019515233
Insulin signaling pathway	5.4	47.4	0.012739089
Jak-STAT signaling pathway	5.3	45.1	0.021944981
Pancreatic cancer	5.1	49.3	0.027534648
Prostate cancer	4.8	48.8	0.022130731
ErbB signaling pathway	4.8	48.3	0.027500726
Toll-like receptor signaling pathway	4.7	46.7	0.041239323
Long-term depression	4.7	47.4	0.05266026
ECM-receptor interaction	4.2	46.0	0.057512646
Dorso-ventral axis formation	4.1	53.6	0.044417147
Melanogenesis	4.1	45.1	0.053503025
Endometrial cancer	4.1	50.0	0.046475053
TGF-beta signaling pathway	3.8	44.0	0.085106723
Axon guidance	3.7	43.8	0.09158817

Impacted pathway [a]	Impact Factor [b]	Input genes / Pathway genes (%) [c]	p-value
Ubiquitin mediated proteolysis	3.6	48.9	0.081701274
Type I diabetes mellitus	3.4	43.2	0.131289041
Adipocytokine signaling pathway	3.3	44.4	0.121801571
Apoptosis	3.3	42.9	0.190763082
Huntington”s disease	3.2	50.0	0.114527344
Neurodegenerative Disorders	3.1	50.0	0.114527344
Melanoma	2.9	43.7	0.177477033
Calcium signaling pathway	2.9	40.6	0.198877948
Gap junction	2.9	42.4	0.178991356
Thyroid cancer	2.9	41.9	0.374566966
Long-term potentiation	2.8	43.5	0.190074619

[a] Analysis carried out by means of Pathway Express [24] using all genes with FDR <0.05, mapped to Kyoto encyclopedia of Genes and Genomes (KEGG) pathways (http:www.genome.jp/kegg/) and ranked according to Impact Factor.

[b] The impact factor identifies the relatively most affected pathways by considering and integrating the proportion of differentially regulated genes, the perturbation factors of all pathway genes, as well as the consistency of the propagation of these perturbations throughout the pathway.

[c] Ratio of the number of regulated genes in the pathway over the total number of genes currently mapped to the pathway.