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Original Article

Proteomic analysis of saliva obtained from patients with chronic periodontitis

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Abstract: Background: Chronic periodontitis (CP) is a global chronic disease that threatens oral health and is the leading cause of adult tooth loss. In addition, the relationship between periodontitis and systemic diseases is intriguing because increasing evidence shows that chronic periodontitis is related to cardiovascular disease, diabetes, rheumatoid arthritis, premature birth, and low birth weight in infants. Quantitative proteomics based on mass spectrometry is a powerful tool to discover new biological pathways and search for new therapeutic targets. Materials and methods: LC-MS/MS was performed to analyze the proteomic expression profiles of saliva obtained from CP patients and to compare them with those from healthy control subjects. The protein concentrations of saliva samples from healthy controls and patients with chronic periodontitis were measured using the Bradford method. Results: We identified the top 10 protein enrichments involved in biological processes, molecular functions, and cellular components, as well as the top 10 enrichments in the KEGG pathway maps. Our data indicated 133 proteins with significant differences ($P < 0.001$). Among these differentially expressed proteins, in the saliva from CP patients, 85 proteins were increased by more than two-fold ($P < 0.001$) and 41 proteins showed a greater than two-fold decrease ($P < 0.001$). In addition, ELISA confirmed that resistin and MMP-8 were significantly increased in the saliva of CP patients compared with the control group. Meanwhile, the production of galectin-3 was markedly reduced in CP patients. Conclusion: The present study revealed the enriched proteins and biological pathways that might be activated during the occurrence of CP. Among the differentially expressed proteins, resistin, galectin-3, and MMP-8 might be potential biomarkers for CP patients.

Keywords: Chronic periodontitis, quantitative proteomics, biological pathway, enriched proteins, saliva

Introduction

Periodontitis cases are classified into three types by the American Academy of Periodontology: chronic periodontitis (CP), aggressive periodontitis, and periodontitis associated with systemic disease. CP is a multifactorial disease and is known to be caused by bacterial infection and associated with a variety of infectious diseases characterized by complicated host-microbial interactions in the periodontium [1]. CP is a serious threat to oral health, and treatment failure usually results in the loss of teeth or adjacent supporting bones as well as the destruction of periodontal ligaments [2]. In addition, there is increasing evidence that shows that CP is related to various diseases, such as diabetes, rheumatoid arthritis, cardiovascular disease, premature birth, and low birth weight in infants [3]. However, the internal mechanism and biological pathways involved are still poorly understood due to the complex symptoms of CP.

Quantitative proteomics based on high-resolution mass spectrometry has been shown to have great potential in biological science, especially for comparing protein expression differences and discovering new cell signaling pathways [3]. The technique can be divided into two categories: stable isotope labeled quantitative proteomics (e.g., SILAC and iTRAQ) and label-free quantitative proteomics (e.g., proteomic analysis based on MaxQuant software) [4].

Label-free quantitative proteomics is now widely used to identify protein posttranscriptional modifications (PTMs) and activated signal transduction pathways under physiological or pathological conditions [5].

Saliva has long been used in internal medicine for the clinical diagnosis of diseases because it is an important component of the human oral immune defense system; it might also be an effective source for CP biomarkers because it reflects changes in oral and systemic health [6]. Comparing the protein expression differences between CP and control groups via western blot analyses, enzyme-linked immunosorbent assays (ELISAs), or quantitative reverse transcription polymerase chain reaction (RT-QPCR) methods can help identify unknown biological pathways or new biomarkers involved in this oral disease [7].

In this study, we performed the labeling-free quantitative proteomic analysis of saliva samples from CP patients and healthy subjects. The samples were analyzed by LC-MS/MS using a Q-Exactive mass spectrometer (Thermo Finnigan), and the data were collected. The level 2 functional classification of biological processes, molecular functions, and cellular components was performed, and the GOA GO slim statistics were calculated; in addition, a functional analysis of the whole human genome was performed as a control. The KEGG (Kyoto Encyclopedia of Genes and Genomes) database was also used for the data file analysis [8]. With this approach, we identified the top 10 protein enrichments separately involved in biological processes, molecular functions, and cellular components, as well as the top 10 enrichments in the KEGG pathway maps [9].

Methods

Ethics statement

This study was approved by the Biomedical Ethics Committee of the School & Hospital of Stomatology, Tongji University. Adult subjects and the parents of pediatric subjects signed an informed consent form before the start of the study.

Subjects

Patients were diagnosed with CP according to the classification criteria for periodontal diseases established at the international symposium held in 1999: (1) periodontal pocket > 6 mm, attachment loss > 5 mm, alveolar bone resorption > 50% of root length, presence of furcation involvement, and increased tooth mobility and (2) patients had more than 30% of sites with attachment loss and bone resorption and obvious inflammation. Subjects with CP (n = 5) were randomly selected from the outpatients at the Department of Periodontology, School & Hospital of Stomatology, Tongji University. Three of the subjects who participated in this study were male, and two were female, and their average age was 45.9 ± 3.83 years. All patients met the revised criteria for diagnosis by the American Academy of Periodontology [1]. Healthy subjects (n = 5) were recruited from the students of the School of Stomatology, Shanghai Tongji University. These subjects had < 10% of sites with bleeding on probing (BOP), a probing depth < 3 mm, < 1% of sites with attachment loss > 2 mm, and no alveolar resorption. The control subjects included three male subjects and two female subjects with an average age of 28.4 ± 0.71 years. The common inclusion criteria for subjects with CP and healthy subjects were no systemic diseases, females who were not pregnant or nursing, no periodontal basic therapy within 6 months, no antibiotics within 3 months, and no caries.

Sample collection

Whole unstimulated saliva was subjected to mild centrifugation (700 ×g for 15 min at 4°C) to obtain a cell pellet without lysis. The supernatant was collected and centrifuged under stronger conditions (12,000 ×g for 10 min at 4°C) to remove all suspended insoluble debris. All samples from each subject were collected in the same session between 7 and 9 a.m. and stored at -80°C until use.

Protein cleavage and quantification

First, 200 μL SDT lysis buffer (4% SDS, 150 mM Tris, pH 8) was added to each sample, and the samples were then boiled in a water bath for 15 min. After ultrasonic degradation, the samples were placed in a boiling water bath for 5 min, and the supernatant was collected after a 15-min centrifugation at 14,000 ×g. The protein concentrations were determined using the BCA method [10].

SDS-polyacrylamide gel electrophoresis (PAGE) analysis

After adding 5 × protein loading buffer, aliquots containing 20 μg of proteins from each sample were loaded on a gel and separated by 12% SDS-PAGE under a voltage of 90 V for 30 min and then 120 V for another 60 min. The gel was stained by Coomassie Brilliant Blue (R-350).

FASP enzymatic hydrolysis

DL-dithiothreitol (DTT) was added to each 150-μg sample to a final concentration of 100 mM. After boiling in a water bath for 5 min, 200 μL UA buffer was added to each sample followed by centrifuging for 15 min at 14000 ×*g*. Then, 100 μL IAA (50 mM IAA in UA) was added to the precipitation residue, and the samples were shaken at 600 rpm for 1 min and placed at room temperature for 30 min before a 10-min centrifugation at 14,000 ×*g*. Next, 100 μL buffer was added before a 10-min centrifugation at 14,000 ×*g*, and this was repeated twice. After this, 100 μL NH_4HCO_3 (25 mM) was added, and the mixture was centrifuged for 10 min at 14,000 ×*g*, repeating twice. Finally, 40 μL trypsin buffer (2 μg trypsin in 25 mM NH_4HCO_3) was added to each sample, and the samples were shaken at 600 rpm for 1 min and placed at 37°C for 16-18 h. After a 10-min centrifugation at 14,000 ×*g*, the OD 280 of the peptides was measured [11].

LC-MS/MS analysis

A 2-μg enzymatic hydrolysate was used for the LC-MS/MS analysis. An EASY-nLC1000 HPLC was used to separate the proteins with an A phase (2% CH_3CN solution with 0.1% formic acid) and a B phase (84% CH_3CN solution with 0.1% formic acid). A Thermo EASY column SC200 (150 μm × 100 mm, RP-C_{18}) was balanced with 100% A phase, and the samples were then loaded on Thermo EASY column SC001 traps (150 μm × 20 mm, RP-C_{18}) (Thermo) for separation at a speed of 400 nL/min. The sequence was set up as B phase from 0% to 45% for 100 min, B phase from 45% to 100% for another 8 min, and B phase at 100% for the next 12 min. After separation by capillary high-performance liquid chromatography, the samples were analyzed using a Q-Exactive mass spectrometer (Thermo Finnigan) for 120 min. The positive ion mode was used, and the mass range was set up as 300-1800 m/z. The mass electron ratios of polypeptides and peptide fragments were collected according to the following methods: after each full scan, 10 MS^2 scans (HCD) were collected. The MS^1 resolution of M/Z 200 was 70,000 and the MS^2 resolution of M/Z 200 was 17,500 [12].

Data analysis with MaxQuant software

All six LC-MS/MS original files were imported into MaxQuant software (version number 1.3.0.5) for the database searching procedure. iBAQ labeling-free quantitative analyses were performed (uniprot_Human_133549_20130303.fasta). The files from the database search using MaxQuant were analyzed using Perseus software (version number 1.3.0.4) [13].

Cytokine quantification by ELISA

The production of resistin, galectin-3, and matrix metalloproteinases-8 (MMP-8) was measured using an ELISA Kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's recommendations. The experiments were repeated three times.

Statistical analyses

The amounts of protein are expressed as the mean ± SD. The difference in the amount of protein between the control group and the CP group was evaluated using Student's paired t-test for three representative proteins, i.e., resistin, galectin-3, and MMP-8. All statistics were two-tailed and performed using SAS software (version 9.1.3; SAS Inc., Cary, NC, USA). The significance level was defined as $P < 0.05$ [14].

Results and discussion

The protein concentrations of saliva samples from healthy controls and patients with chronic periodontitis were measured using the Bradford method [15] (Bio-Rad, Hercules, CA, USA) with bovine c-globulin as an internal control (Sigma-Aldrich), and the OD280 values of the peptides within each sample were also measured. All samples were also subjected to SDS-PAGE and stained by Coomassie Brilliant Blue [16] (R-350). All samples from the control and experimental groups were separately analyzed using a Q-Exactive mass spectrometer (Thermo

Table 1. Cytokine determination for resistin, galectin-3, and MMP-8 in the saliva from the CP and control groups

	Control	CP	P
MMP-8 (ng/L)	361.7 ± 360.2	525.3 ± 556.1	< 0.05
Resistin (ng/L)	1.5 ± 0.6	3.6 ± 0.4	< 0.001
Galectin-3 (ng/L)	0.8 ± 0.3	0.5 ± 0.7	< 0.05

$$Z\text{-}score = \frac{r - n\frac{R}{N}}{\sqrt{n\left(\frac{R}{N}\right)\left(1-\frac{R}{N}\right)\left(1-\frac{n-1}{N-1}\right)}}$$

Where:

N - total number of proteins in reference dataset
R - number of the proteins in test dataset
n - total number of proteins in function or pathway from reference dataset
r - number of the proteins in function or pathway from test dataset

Figure 1. Z-score formula.

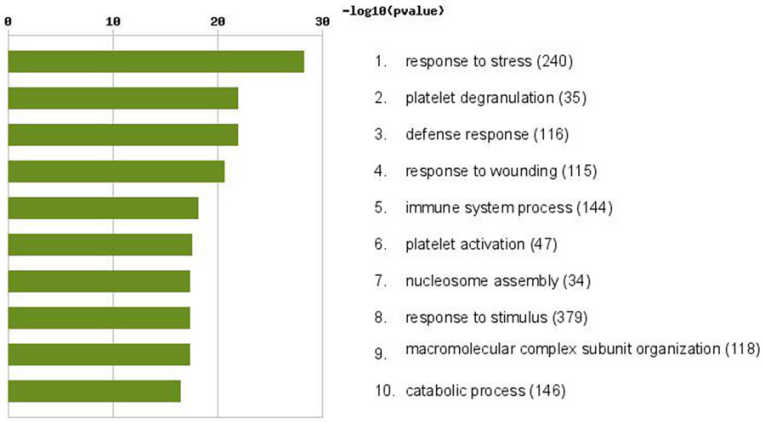

Figure 2. Top 10 enrichments in biological processes.

Finnigan). All of the original files from the LC-MS/MS analysis were processed with MaxQuant software (version number 1.3.0.5) for a database search. We performed iBAQ labeling-free Quantitative analysis and identified 767 protein groups as well as 4584 unique peptides [17].

The samples from healthy controls and CP patients were each combined to obtain a C group and a CP group. Then, the C group and CP group samples were detected by LC-MS three times, and the collected results were analyzed with t-tests (both side). iBAQ analysis of all the proteins in the two groups indicated 133 proteins with significant differences in their expression level ($P < 0.001$) [18]. Among these differentially expressed proteins, 85 of 133 proteins were elevated by more than two-fold ($P < 0.001$), suggesting that these proteins were increased in the saliva from CP patients (Supplementary Table 1). Meanwhile, 41 proteins exhibited more than a two-fold decrease ($P < 0.001$), indicating that these proteins were reduced in the saliva from CP patients (Supplementary Table 2).

Furthermore, we validated three cytokines among these differentially expressed proteins (resistin, galectin-3, and MMP-8) in 27 CP patients and 27 healthy controls. Consistent with the data from the proteomic analysis, the ELISA results indicated that the production of resistin and MMP-8 was significantly increased in the saliva of CP patients compared with that in the control group. Meanwhile, we found that galectin-3 secretion was markedly reduced in CP patients (**Table 1**). Any imbalance in MMP secretion initiates the destruction of collagen in gum tissue, resulting in the pathogenesis of chronic periodontitis. The amount of MMP-8 in gingival crevicular fluid has been reported to be higher in CP patients than in healthy subjects [19, 20]. Resistin levels are increased in the gingival crevicular fluid of CP patients, suggesting that resistin may be considered a potential inflammatory marker of periodontitis [21]. Galectin-3, a member of the β-galactoside-binding lectin family, is involved in broad biological functions, including cell adhesion, growth, and apoptosis, and inflammation [22]. It has been widely reported that galectin-3 promotes the inflammatory response [23, 24]. However, another study demonstrated that galectin-3 suppresses mucosal inflammation and reduces disease severity in experimental colitis [25]. In our study, we first identified that galectin-3 was

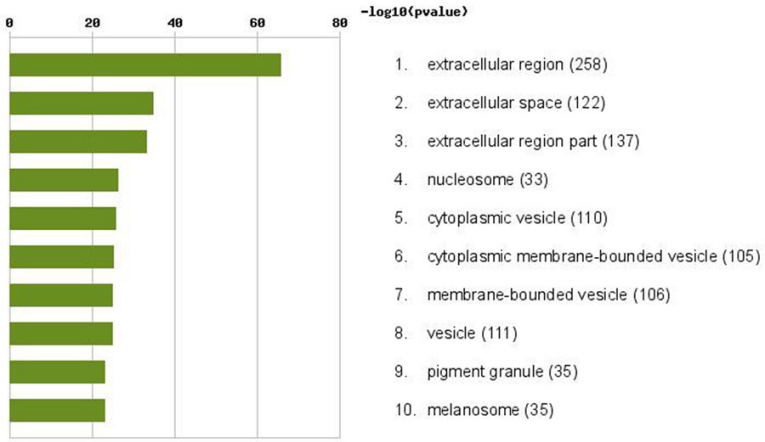

Figure 3. Top 10 enrichments in cellular components.

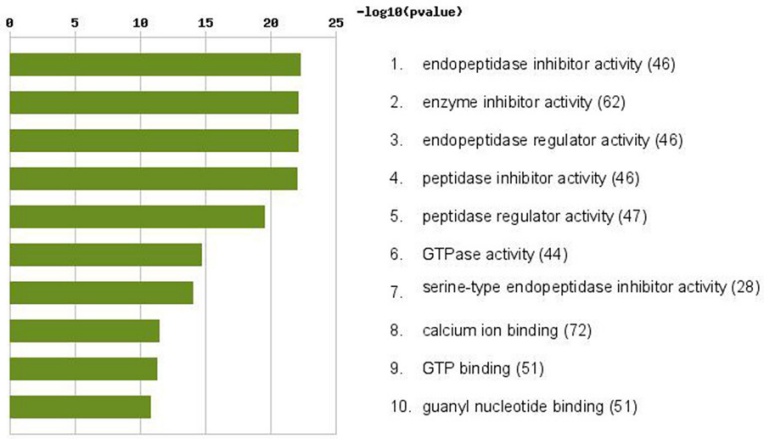

Figure 4. Top 10 enrichments in molecular functions.

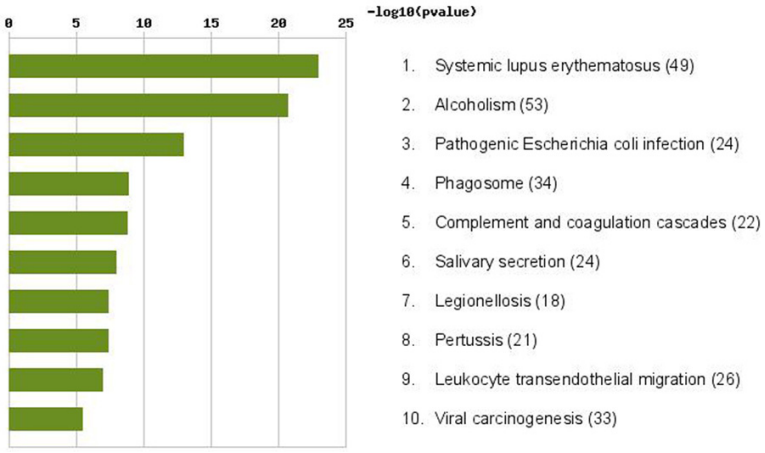

Figure 5. Top 10 enrichments in the KEGG pathway maps.

decreased in the saliva of CP patients, but its specific roles in CP remain to be determined.

GO slims are cut-down versions of the GO ontologies containing a subset of the terms in the whole GO. They give a broad overview of the ontology content without the detail of the specific fine-grained terms. GO slims are particularly useful for giving a summary of the results of a GO annotation of a genome, microarray, or cDNA collection when the broad classification of gene product function is required [26]. $P < 0.05$ indicated significant screening, while a Z-score > 0 (**Figure 1**) indicated significant enrichment, and Z-score < 0 indicated a significant deficiency. We identified the top 10 protein enrichments within biological processes (**Figure 2**), cellular components (**Figure 3**), and molecular functions (**Figure 4**).

An in-house Perl script based on the KEGG API was used to map to the KEGG pathway [27]. To obtain enrichment pathway maps, the hypergeometric distribution with FDR correction and Z-score were calculated using R (www.r-project.org). The top 10 protein enrichments were identified (**Figure 5**).

Conclusions

In this study, we collected and prepared clinical samples from CP patients. The labeling-free quantitative analysis of the samples was performed, and we identified the top 10 protein enrichments involved in biological processes, molecular functions, and cellular components, as well as the top 10 enrichments in the KEGG pathway maps. Moreover, resistin, galectin-3, and MMP-8 might be potential bio-

markers for CP. These results will aid in the understanding of the pathogenesis of chronic periodontitis, as well as the discovery of new biomarkers for CP.

Acknowledgements

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Disclosure of conflict of interest

None.

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Supplementary Table 1. Up-regulated proteins in saliva obtained from CP patients

Protein IDs	Protein names	C/ iBAQaverage	CP/iBAQaverage	Ratio (CP/C)
P36222	Chitinase-3-like protein 1	0	610970	#DIV/0!
B7ZLE5	Fibronectin	47060.67	3527600	74.95856412
A2J1N9	Rheumatoid factor RF-ET12 (Fragment)	691546.7	19122666.67	27.65202638
Q6MZU6	Putative uncharacterized protein DKFZp686C15213	20995667	119030000	5.669265086
P31146	Coronin-1A	17723000	55747666.67	3.145498317
P02671	Fibrinogen alpha chain	3646833	31885333.33	8.743293268
P00738	Haptoglobin	13310000	228953333.3	17.2016028
P02746	Complement C1q subcomponent subunit B	0	777590	#DIV/0!
B2R9F2	Corticosteroid-binding globulin	0	498290	#DIV/0!
A6XGL1	Transthyretin	31143000	124100000	3.984844106
B4E1V0	cDNA FLJ54839	11447.33	6654500	581.3144255
P26583	High mobility group protein B2	0	6067600	#DIV/0!
P04003	C4b-binding protein alpha chain	163180	796286.6667	4.879805532
Q7Z6G4	HBA2 (Fragment)	0	2387900	#DIV/0!
D3DNU8	Kininogen-1	388713.3	5416933.333	13.93554805
Q15365	Poly(rC)-binding protein 1	381096.7	1212933.333	3.182744536
B4DE30	cDNA FLJ51711	0	109652.3333	#DIV/0!
H0YAC1	Plasma kallikrein	0	218250	#DIV/0!
Q5TEC6	Histone H3	1631267	5473233.333	3.355204545
Q9UK54	Hemoglobin beta subunit variant (Fragment)	0	2295466.667	#DIV/0!
Q13231	Chitotriosidase-1	0	567346.6667	#DIV/0!
P19652	Alpha-1-acid glycoprotein 2	6171067	22269333.33	3.60866841
P02675	Fibrinogen beta chain	14870667	159176666.7	10.70407065
Q6N093	Ig gamma-2 chain C region	43455000	191676666.7	4.410923177
P07195	L-lactate dehydrogenase B chain	448150	3039533.333	6.782401726
Q9HD89	Resistin	3283967	13137000	4.000345111
P01023	Alpha-2-macroglobulin	6403100	46550000	7.269916134
B5BU24	14-3-3 protein beta/alpha	4241100	10077933.33	2.376254588
Q53H26	Transferrin variant (Fragment)	43196000	235066666.7	5.441861901
D9ZGG2	Vitronectin	1706867	8069600	4.727727219
Q9P1C5	PRO2769	0	738436.6667	#DIV/0!
P22314	Ubiquitin-like modifier-activating enzyme 1	25334.67	417096.6667	16.46347561
P02760	Protein AMBP	51626.67	4496133.333	87.0893595
Q53FR4	Vacuolar protein sorting-associated protein 35	0	105222.3333	#DIV/0!
Q6ZW64	cDNA FLJ41552 fis, clone COLON2004478	3393600	58292333.33	17.17713736
B3KQL3	cDNA FLJ90670 fis, clone PLACE1005539	1766533	41641333.33	23.57234508
J3KNB4	Cathelicidin antimicrobial peptide	7159300	23662000	3.305071725
B0LPF3	Growth factor receptor-bound protein 2	0	463583.3333	#DIV/0!
D3DRR6	Inter-alpha-trypsin inhibitor heavy chain H2	20304.67	1162200	57.23807335
A5PL27	Ceruloplasmin	1453307	14883666.67	10.24124296
A0N071	Hemoglobin subunit delta	0	2260466.667	#DIV/0!
A0N5G3	Rheumatoid factor G9 light chain (Fragment)	707150	1658600	2.345471258
C0JYY2	Apolipoprotein B-100	38411.33	588156.6667	15.31206068
P32320	Cytidine deaminase	3287540	21085000	6.413610177
P02647	Apolipoprotein A-I	38379333	166900000	4.34869461
B2RDN9	X-ray repair cross-complementing protein 6	0	122306.6667	#DIV/0!
P02679	Fibrinogen gamma chain	12508500	106470333.3	8.511838616
D3DRP5	Golgi-associated plant pathogenesis-related protein 1	0	371813.3333	#DIV/0!
P01024	Complement C3	6955233	48595000	6.986825268
B7ZKJ8	Inter-alpha-trypsin inhibitor heavy chain H4	94181	1998166.667	21.21623965

O95498	Vascular non-inflammatory molecule 2	0	921016.6667	#DIV/0!
Q6N094	Putative uncharacterized protein DKFZp686O01196	138710	1112800	8.022492971
P02652	Apolipoprotein A-II	12016167	33074000	2.752458493
Q562Z4	ctin-like protein (Fragment)	132960	21197333.33	159.4263939
A8K9E4	Neutrophil collagenase	1075367	6952366.667	6.465112675
O14818	Proteasome subunit alpha type-7	267363.3	637753.3333	2.385343291
D9YZU5	Hemoglobin subunit beta	17177000	463056666.7	26.95794764
B4E1Z4	Complement factor B	456356.7	2216600	4.857165813
E9PF41	Actin-related protein 2	3865200	13812000	3.573424402
Q03591	Complement factor H-related protein 1	47350	698620	14.75438226
Q9BRF8	Calcineurin-like phosphoesterase domain-containing protein 1	406256.7	1769200	4.354882381
P05164	Myeloperoxidase	19076333	52458333.33	2.749917
P19827	Inter-alpha-trypsin inhibitor heavy chain H1	55176.67	1265366.667	22.93300308
E7EWW9	Glutathione S-transferase Mu 1	0	842656.6667	#DIV/0!
P30520	Adenylosuccinate synthetase isozyme 2	47301.67	357546.6667	7.558859801
P02763	Alpha-1-acid glycoprotein 1	42126000	130010000	3.086217538
B4E1F0	Plasma protease C1 inhibitor	797206.7	4350633.333	5.457346903
P52790	Hexokinase-3	511966.7	1306100	2.551142653
B1ALS2	Receptor-type tyrosine-protein phosphatase C	0	70701.66667	#DIV/0!
P02649	Apolipoprotein E	51762	453176.6667	8.755006891
Q6J1Z7	Putative uncharacterized protein	0	3886700	#DIV/0!
A8K5T0	Complement factor H	214541	2927933.333	13.64743025
Q6UX06	Olfactomedin-4	780783.3	3004533.333	3.848101266
P09960	Leukotriene A-4 hydrolase	4033467	11955666.67	2.964116889
Q9BQ22	Alpha-2-macroglobulin (Fragment)	0	10348633.33	#DIV/0!
P25774	Cathepsin S	447133.3	1302466.667	2.912926793
P36871	Phosphoglucomutase-1	37410.33	1082053.333	28.92391585
B4DE31	Transketolase	25076667	54846666.67	2.187159378
C9JF17	Apolipoprotein D	330870	2197866.667	6.642689475
Q6P4A8	Phospholipase B-like 1	342750	916826.6667	2.674913688
P35754	Glutaredoxin-1	1396933	8304233.333	5.94461678
Q68D08	Protein FAM49B	66553.33	1062536.667	15.96519082
Q9BS91	Probable UDP-sugar transporter protein SLC35A5	0	1041173.333	#DIV/0!
P00915	Carbonic anhydrase 1	0	1555300	#DIV/0!
P11684	Uteroglobin	1499033	53602000	35.75771052

Supplementary Table 2. Down-regulated proteins in saliva obtained from CP patients

Protein IDs	Protein names	C/ iBAQaverage	CP/ iBAQaverage	Ratio (CP/C)
Q99935	Proline-rich protein 1	1725300	0	0
Q9Y6R7	IgGFc-binding protein	10981667	2535733.333	0.230906056
A2JA16	Anti-mucin1 light chain variable region (Fragment)	2253733	0	0
Q07654	Trefoil factor 3	1.11E+08	36903333.33	0.332842282
A8K2M4	Transmembrane protease serine 11D	8895033	306656.6667	0.034475044
Q9HC84	Mucin-5B	2.26E+08	52207000	0.231096454
B1N7G0	Alpha7 nicotinic receptor isoform 15 (Fragment)	5202500	0	0
B2R6A3	Na(+)/H(+) exchange regulatory cofactor NHE-RF1	277830	0	0
P20061	Transcobalamin-1	1.47E+08	64322333.33	0.437061448
P31947	14-3-3 protein sigma	73504000	22213000	0.302201241
P28325	Cystatin-D	9.11E+08	429323333.3	0.471523077
Q8TDL5	BPI fold-containing family B member 1	33251667	6335733.333	0.19053882
Q13835	Plakophilin-1	2375100	412636.6667	0.173734439
P04280	Basic salivary proline-rich protein 1	5277633	498260	0.094409742
Q96FQ6	Protein S100-A16	9793333	2319500	0.236844792
Q8N4F0	BPI fold-containing family B member 2	1.96E+08	39055666.67	0.199773231
Q08188	Protein-glutamine gamma-glutamyltransferase E	68477667	13845666.67	0.202192442
P27482	Calmodulin-like protein 3	41409000	10675066.67	0.257795809
P06311	Ig kappa chain V-III region IARC/BL41	4589600	1274580	0.277710476
H0UI06	Cytochrome c oxidase subunit 7A2, mitochondrial	5608500	754863.3333	0.134592731
Q59FR8	Galectin-3	9228367	1104250	0.119658228
Q86TT1	Full-length cDNA clone CS0DD006YL02 of Neuroblastoma of Homo sapiens	37131667	8977433.333	0.24177297
P63261	Actin, cytoplasmic 2	1395567	669413.3333	0.47967134
P12109	Collagen alpha-1(VI) chain	120326.7	9139.666667	0.075957117
B3EWG3	Protein FAM25A	18090333	2075833.333	0.114748208
Q96JD1; P06319; P06318	Ig lambda chain V-VI region EB4	2464467	0	0
P101638	Basic salivary proline-rich protein 4	7724233	2175400	0.281633129
Q9HCY8	Protein S100-A14	10324067	4075866.667	0.39479275
Q6UWP8	Suprabasin	12308000	2435266.667	0.19786047
P02812	Basic salivary proline-rich protein 2	49217000	11277766.67	0.229143724
P62269	40S ribosomal protein S18	950566.7	0	0
B4E1S8	cDNA FLJ59147	23767667	1099866.667	0.046275753
P04792	Heat shock protein beta-1	96485667	15803000	0.163785985

Q9UL96	Myosin-reactive immunoglobulin heavy chain variable region (Fragment)	6501467	556850	0.085649905
A0N5G7	Rheumatoid factor D5 heavy chain (Fragment)	6059867	0	0
B3KQF4	Metalloproteinase inhibitor 1	44844667	21439000	0.478072458
P29508	Serpin B3	9564633	2486666.667	0.259985572
P04083	Annexin	2.44E+08	113150000	0.463267506
A2N7P4	Immunoglobulin mu-chain D-J4-region (Fragment)	2820800	1217966.667	0.431780582
P40926	Malate dehydrogenase	8801500	1851800	0.210395955
P01763	Ig heavy chain V-III region WEA	10622467	3605266.667	0.339400139