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Comparative Analysis of the Proteomic Profile of the Dental Pulp in Different Conditions. A Pilot Study

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This study aimed to quantitatively compare the difference in protein expression in the progression of pulp pathogenesis, as well as to describe the biological functions of proteins identified in pulp tissue. Samples were obtained from six patients treated at the Araçatuba School of Dentistry and were divided into three groups: normal pulp - from teeth extracted for orthodontic indication; inflamed pulp and necrotic pulp - from patients diagnosed with irreversible pulpitis and chronic apical periodontitis, respectively. After previous proteomic preparation, dental pulp samples were processed for label-free quantitative proteomic analysis in a nanoACQUITY UPLC-Xevo QTof MS system. The difference in expression between the groups was calculated using the Protein Lynx Global Service software using the Monte Carlo algorithm. A total of 465 human proteins were identified in all groups. The most expressed proteins in the inflamed pulp group in relation to the normal pulp group were hemoglobin, peroxiredoxins and immunoglobulins, whereas the less expressed were the tubulins. Expression levels of albumins, immunoglobulins and alpha-2-macroglobulin were higher in the necrotic pulp group than in the inflamed pulp group. As for the qualitative analysis, the most prevalent protein functions in the normal pulp group were metabolic and energetic pathways; in the inflamed pulp group: cellular communication and signal transduction; and regulation and repair of DNA/RNA, while in the necrotic pulp group proteins were associated with the immune response. Thus, proteomic analysis showed quantitative and qualitative differences in protein expression in different types of pulp conditions.

Key Words: proteomic analysis, dental pulp, pulpitis, dental pulp necrosis, endodontics.

Introduction

Dental pulp is a complex specialized tissue with nourishing, restorative, sensorial and defensive functions (inflammatory or degenerative defenses, depending on the characteristics of the irritant). Although several etiological factors can cause pulp damage, the biological factor, represented by polymicrobial communities usually organized as biofilm, is the most relevant (1). The characteristic events of the inflammatory process, such as the recruitment of defense cells from the innate and adaptive immune response, and the presence of chemical mediators alter the physiology of the dental pulp in the attempt to remove the aggressive agent. Damage to the pulp tissue can occur in the persistence of the irritant agent, destructuring the tissue and leading to a process of necrosis. In this context, bacteria colonize the root canal system and start releasing potential antigens into the periapical tissues, thus triggering the development of apical diseases (2,3).

Proteomic analysis is the study of the whole set of proteins expressed by an organism in a particular environment during a specific stage of the cell cycle. It also covers their relative abundance, distribution, functions and interaction with other macromolecules (4). This approach has also been widely applied in medical microbiology, collecting information related to bacterial resistance and virulence, which has been used in the development of new diagnostic and therapeutic applications for the treatment of infectious diseases (5). Therefore, proteomic techniques are important tools for investigating the progression of both pulp alterations and study of the host / pathogen response to infections (6).

Characterization and expression of proteins using liquid chromatography (LC) / mass spectrometry (MS / MS) has gained prominence in proteomic analysis of pulp tissue, since this method provides the necessary technology for the study of small amounts of samples from complex biological systems (7). This method involves the proteolytic digestion of all proteins in the samples and their subsequent identification using a database of individual peptides, reducing sample handling time and eliminating the need for individual protein processing (8). The direct identification of proteins expressed in pulp and periapical diseases, involving descriptive analysis of pulp pathogenesis, focusing on:

primary and persistent infections (9); endodontic abscesses (10); and cases of failure of endodontic treatment (11) has allowed the identification of several human proteins, which were mainly related to cellular processes, metabolism and immune defense.

Although the above-mentioned studies provided significant contribution to the comprehension of endodontic infections, they employed qualitative identification approaches, which do not allow a direct quantitative comparison of the expression of proteins of human origin. Furthermore, to date no study has assessed the proteomic profile of the human pulp tissue with different degrees of microbial injury, especially determining quantitatively the down- and up-regulated proteins. Therefore, considering the scarcity of proteomic studies of pulp diseases, this pilot study aimed to compare the protein profile at different clinical stages during the progression of pulp diseases, as well as to describe the biological functions of each protein detected in the samples within the different pulp conditions (normal, inflamed or necrotic pulp tissue).

Material and Methods

Patient Selection

All patients signed an informed consent form prepared in accordance with the rules of the Research Ethics Committee of the Araçatuba School of Dentistry – UNESP (Nº 91331518.7.0000.5420). Samples were taken from patients with no history of systemic diseases, who attended the Endodontic Clinic of the Araçatuba School of Dentistry – UNESP for root canal treatment, and were divided into three groups: normal pulp group with pulp tissue samples obtained from teeth extracted for orthodontic indication (n=2); inflamed pulp group - samples obtained from patients diagnosed with irreversible pulpitis (n=2), and necrotic pulp group - samples obtained from patients diagnosed with chronic apical periodontitis (n=2). Clinical and radiographic characteristics and a detailed anamnesis of the patient's health conditions were recorded (Table 1).

Sample Collection

The collection was done aseptically. Firstly, the crown of the tooth to be sampled was cleaned with pumice paste and water, followed by removal of the restoration and/or carious tissue without exposing the root canals. Then, the tooth was individually isolated from the oral cavity with a rubber dam, except for the normal pulp sample. The tooth and the surrounding field were cleaned with 30% hydrogen peroxide and decontaminated with 2.5% sodium hypochlorite solution for 30 s each, followed by neutralization of the solution with 5% sodium thiosulfate (3). The access to the pulp cavity was performed with sterile carbide bur without water spray. Irrigation during the access phase was done with sterile saline solution.

Normal pulp - To obtain the normal pulp sample, teeth with healthy pulp and without periodontal disease, indicated for orthodontic extraction, were selected. Immediately after the extraction, the pulp tissue was carefully removed with the aid of sterile manual files (Hedstroem file size #20, Dentsply Sirona, Ballaigues, Switzerland), avoiding contamination and complete disruption of the pulp tissue.

Inflamed pulp - In cases of irreversible pulpitis, consistent pulp tissue was collected from the palatal or distal canal with Hedstroem file, complemented by three sterile paper points introduced into the apparent length of the canal determined on diagnostic radiographs, and held in place for 60 s each, without any irrigation.

Necrotic pulp - Samples of the teeth with pulp necrosis, with radiographic lesion (chronic apical periodontitis), were obtained immediately after exposure of the pulp chamber. A sterile K-type file was introduced with minimal instrumentation, without the use of any irrigant to disrupt biofilms of the canal wall; then three sterile paper points were introduced into the apparent length of the canal determined on diagnostic radiographs and held in place for 60 s each. If the canal was completely dry, a drop of sterile saline was placed before removing the paper point. In cases of teeth with more than one canal, the sample was

Table 1. Clinical and radiographic characteristics of patients included in each group

Groups	Gender	Tooth	Thermal stimuli	Radiographic examination	Clinical characteristics
Normal pulp	Female	15	Positive (normal)	Normal radiographic bone level with intact lamina dura and complete root formation	Absence of carious lesion or symptomatology and normal probing depth
	Male	34	Positive (normal)		
Inflamed pulp	Male	36	Positive (acute)	There was no periodontal ligament widening or periapical radiolucency and complete root formation	Carious lesion without pulp exposure, spontaneous pain and normal probing depth
	Female	16	Positive (acute)		
Necrotic pulp	Female	46	Negative	Periapical radiolucency on the mesial and distal roots	Extensive restoration with caries recurrence, absence of symptoms and normal probing depth
	Female	26	Negative	Extensive periapical radiolucency on the palatal root	

collected only from the widest canal, since it was associated with the apical lesion (otherwise the tooth would not be included in the study), to confine the analysis to a single environment.

After the collection, the paper points and tissue samples were placed in sterile, DNA-free and RNA-free cryotubes, which were frozen at -80° C until use for proteomic analysis.

Proteomic Analysis - Preparation of Pulp Samples

The paper points were cut and samples corresponding to the same groups were pooled. In the tubes containing the paper points an extraction solution containing 6 M urea, 2 M thiourea in 50 mM NH_4HCO_3 pH 7.8 was added until the papers were covered. The samples were then vortexed for 10 min at 4 °C, followed by sonication for 5 min and centrifugation at 20,817 × *g* for 10 min at 4 °C. The supernatant was collected, and this procedure was repeated once more. The papers were placed in filter tubes (Corning® Costar® Spin-X® Plastic Centrifuge Tube Filters Sigma-Aldrich, New York, USA) and centrifuged at 20,817 × *g* for 10 min at 4 °C. The supernatant was collected and added to the previously collected supernatant. Soon after, 1.5 mL of 50 mM NH_4HCO_3 was added to the samples. The samples were then placed in Falcon Amicon Ultra-4 10k tubes (Merck Millipore, Ireland) and centrifuged at 4,500 × *g* at 4°C to approximately 150 µL.

Then, 5 mM dithiothreitol (DTT) was added to the samples and they were incubated at 37°C for 40 min. After this time, 10 mM iodoacetamide (IAA) was added and the samples were incubated for 30 min in the dark. After the incubations, 100 µL of 50 mM NH_4HCO_3 were added and shortly thereafter the tryptic digestion was performed for 14 h at 37°C by the addition of 2% (w/w) trypsin (Promega, Madison, USA). After the digestion, 5% formic acid was added to stop the action of trypsin and the procedures were performed with the C18 spin column (Thermo Scientific, United States) for desalting and purifying the samples. Thus, an aliquot of each sample (1 µL) was removed and protein quantification was performed by the Bradford method (Bio-Rad Bradford Assays). The remnants were dried to approximately 1 µL in SpeedVac (Thermo Scientific, United States). After drying the samples were resuspended in 3% acetonitrile and 0.1% formic acid for the application to the nano Liquid Chromatography Electron Spray Ionization Tandem Mass Spectrometer (nLC-ESI-MS / MS) (12).

Shotgun Label-Free Quantitative Proteomic Analysis

Peptides identification was performed on a nanoACQUITY UPLC-Xevo QTof MS system (Waters, Manchester, New Hampshire, UK). The nanoACQUITY UPLC was equipped with nanoACQUITY HSS T3, analytical reverse phase column (75 µm X 150 mm, 1.8 µm particle size (Waters, Manchester, New Hampshire, UK). The column was equilibrated with mobile phase A (0.1% formic acid in water). Then, the peptides were separated with a linear gradient of 7-85% mobile phase B (0.1% formic acid in ACN) for 70 min at a flow rate of 0.35 µL/min. The column temperature was maintained at 55 °C. The Xevo G2 Q-TOF mass spectrometer was operated in positive nanoelectrospray ion mode and data were collected using the MSE method in elevated energy (19-45 V), which allows data acquisition of both precursor and fragment ions, in one injection. Source conditions used included capillary voltage, 2.5 kV; sample cone, 30 V; extraction cone, 5.0 V and source temperature, 80 °C. Data acquisition occurred over 70 min and the scan range was 50–2000 Da. The lock spray, used to ensure accuracy and reproducibility, was run with a [Glu1] fibrinopeptide solution (1 pmol/µL) at a flow rate of 1 µL/min, as a reference ion in positive mode at m/z 785.8427. ProteinLynx Global Server (PLGS) version 3.0 was used to process and search the LC-MSE continuum data. Proteins were identified with the embedded ion accounting algorithm in the software and a search of the Homo sapiens database (UniProtKB/Swiss-Prot) downloaded on April 2017 from UniProtKB (http://www.uniprot.org/).

For label-free quantitative proteome, three MS raw files from normal, inflamed and necrotic pulp groups were analyzed using the Protein Lynx Global Service (PLGS, v 2.2.5, Waters Co., Manchester, UK) software. All the proteins identified with a score with confidence greater than that 95% were included in the quantitative statistical analysis embedded in the PLGS software. Identical peptides from each triplicate by sample were grouped based on mass accuracy (<10 ppm) and on time of retention tolerance <0.25 min, using the clustering software embedded in the PLGS. Difference in expression among the normal and inflamed pulp groups and inflamed and necrotic groups was calculated using Monte-Carlo algorithm and expressed as $p< 0.05$ for proteins present in lower abundance and $1-p>0.95$ for proteins present in higher abundance.

In the quantitative analysis, two comparisons were made among the groups: The first comparison was between the normal and inflamed pulp groups, and the second was between the inflamed and necrotic pulp groups. Proteins expressed at a ratio >2.0 in relation to the group of comparison were regarded as up-regulated, while proteins expressed at a ratio <0.5 were regarded as down-regulated proteins. Proteins expressed with ratio between 0.5 and 2 were disregarded. The identified proteins were classified according to their biological functions using Homo sapiens database (UniProtKB/Swiss-Prot).

Results

Overall, 465 proteins were identified from the samples in all groups. Among them, 30 were common to all groups,

including six isoforms of Actin, Albumin, Alpha-1_4 glucan phosphorylase, three isoforms of Glycogen and six isoforms of Hemoglobin, Serum albumin, among other proteins such as Apolipoprotein A-II, Haptoglobin and three isoforms of Immunoglobulin (Fig. 1).

The proteins expression were divided into 12 categories: metabolism and energy pathways, immune response, transport, structure, DNA/RNA regulation and repair, cell communication and signal transduction, cell growth and/or maintenance, differentiation of neural cells, apoptosis, stress response, ions regulation and binding and proteins of unknown function (8) (Tables 2 and 3).

When comparing the inflamed pulp group with the normal pulp group, 39 proteins were found at higher levels in the first, among which 18 had more than a 2-fold increase, e.g. 4 subunits of Hemoglobin (100-fold higher), 2 isoforms of Peroxiredoxin, (20-fold higher), 3 isoforms of Immunoglobulin (10-fold higher), Glyceraldehyde-3-phosphate dehydrogenase (2-fold higher). On the other hand, 41 proteins were found at lower levels in inflamed pulp group in comparison with normal pulp group, among which 17 were isoforms of Tubulin, besides other cytoskeletal proteins, such as Desmin. Serum albumin and neurofilament proteins, such as Alpha-internexin, Neurofilament medium polypeptide were also reduced (Table 2).

When necrotic pulp group was compared with inflamed pulp group, 8 proteins were found at higher levels and 26 at lower levels in the necrotic pulp group. Among the high level proteins, 8 of them were more than 2-fold higher (2 isoforms of Serum albumin, Immunoglobulin heavy constant gamma 1 and Alpha-2-macroglobulin). As for the low level proteins, 13 were more than 2-fold lower (various isoforms of Hemoglobin, various isoforms of POTE ankyrin domain, various isoforms of Actin and Bromodomain-containing protein 3) (Table 2).

Table 3 shows the proteins exclusively identified in each one of the groups. Most of the proteins identified in the normal pulp group were involved in metabolic and energy pathways (20.4%) and in cell communication and signal transduction (20.4%). While in the inflamed pulp group, the cellular communication and signal transduction function (19.4%) also presented a higher percentage, followed by regulation and repair of DNA / RNA (17.9%). Finally, in the necrotic pulp group, most proteins were involved in the immune response (24.3%). (Fig. 2).

Alpha-2-macroglobulin, Transthyretin and Apolipoprotein A-I were not identified in the normal pulp group, while Beta-actin-like protein 2 was not found in necrotic pulp group. Isoforms of Neutrophil defensin were not found in the groups of normal and inflamed pulp, while Serotransferrin was identified in the groups of normal and necrotic pulp.

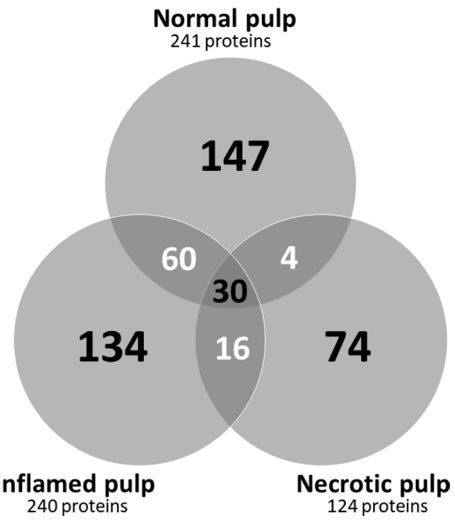

Figure 1. Venn diagram of proteins identified in all groups and the relation between them.

Discussion

Proteomic techniques have helped to improve the knowledge of the biology, function and pathology of the pulpal tissues. Tissue formation, diagnosis, identification of risk factors, tissue engineering and pathogenesis of endodontic infections represent some of the most important themes investigated in proteomic studies of the pulp. The understanding of proteins and their functions provide insights into the complex host-pathogen relationship and host antimicrobial strategies to combat infections (13). To our knowledge, this pilot study is the first to describe and quantify the proteome of the pulp tissue in relation to the progression of pulp diseases, comparing the protein profile of different pulp diagnoses and their relationship with the characteristic events of each diagnosis. The nLC-ESI-MS/MS method allowed the identification of 465 different proteins. The high sensitivity of the method makes it possible to identify and comparatively quantify proteins in tiny amounts of samples, such as samples for root canals, allowing the study of its pathological processes (14).

In order comprehend the mechanisms related to the host in relation to the progression of the pulp disease, two comparisons were made between the groups. The first comparison (normal vs inflamed tissue) showed a significant up-regulation of 4 subunits of Hemoglobin. Approximately one third of the mass of a human red blood cell (RBCs) is hemoglobin (15). RBCs participate in the vascular system and its increase is related to the diagnosis of the pulp. The inflammation of the dental pulp causes an immediate increase in blood flow, along with vasodilation, increased blood supply and microcirculation (16).

Table 2. Human proteins that were more than 2-fold higher or lower in inflamed (I) compared with normal (NO) pulp group and necrotic (NE) compared with inflamed (I) pulp group

Accession	Description	Biologic process	Score	Ratio I:NO
P68871	Hemoglobin subunit beta [C]	Oxygen transport	25560	262.43
P02042	Hemoglobin subunit delta [C]	Oxygen transport	6892	164.02
G3V1N2	HCG1745306_ isoform CRA_a [C]	Oxygen transport	6068	139.77
P69905	Hemoglobin subunit alpha [C]	Oxygen transport	16796	139.77
Q14980	Nuclear mitotic apparatus protein [F]	Cell division	282	29.96
P32119	Peroxiredoxin-2 [F]	Cell redox homeostasis	558	24.05
Q06830	Peroxiredoxin-1 [F]	Cell redox homeostasis	558	22.20
P01876	Immunoglobulin heavy constant alpha 1 [B]	Adaptive immunity	338	14.44
P02652	Apolipoprotein A-II [C]	Transport	1124	12.94
P01877	Immunoglobulin heavy constant alpha 2 [B]	Adaptive immunity	338	12.81
P00738	Haptoglobin [B]	Acute phase. Immunity	842	11.94
P01857	Immunoglobulin heavy constant gamma 1 [B]	Adaptative immunity	822	11.70
Q9Y2L5	Trafficking protein particle complex subunit 8 [C]	Transport	426	9.39
Q7Z2K6	Endoplasmic reticulum metallopeptidase 1 [A]	Catalytic activity	573	6.05
P60174	Triosephosphate isomerase [A]	Glycolysis	216	3.19
Q9Y4G6	Talin-2 [D]	Structural constituent of cytoskeleton	333	2.48
P04406	Glyceraldehyde-3-phosphate dehydrogenase [J]	Oxidoreductase	312	2.23
Q562R1	Beta-actin-like protein 2 [D]	Structural constituent of cytoskeleton	2959	0.44
P17661	Desmin [D]	Cytoskeleton organization	4264	0.39
Q8TAI7	GTPase RhebL1 [F]	Transcription factor activity	277	0.39
Q16352	Alpha-internexin [H]	Differentiation. Neurogenesis	3973	0.38
P07197	Neurofilament medium polypeptide [D]	Structural constituent of cytoskeleton	3973	0.38
Q9BY44	Eukaryotic translation initiation factor 2A [D]	Translation regulation	444	0.36
Q9BUF5	Tubulin beta-6 chain [D]	Microtubule-based process	5168	0.35
A6NNZ2	Tubulin beta-8 chain-like protein LOC260334 [D]	Microtubule-based process	5281	0.34
P02768	Serum albumin [C]	Transport	14783	0.32
P68371	Tubulin beta-4B chain [D]	Microtubule-based process	23514	0.32
P04350	Tubulin beta-4A chain [D]	Microtubule-based process	21996	0.32
Q3ZCM7	Tubulin beta-8 chain [D]	Structural constituent of cytoskeleton	8805	0.32
Q9BVA1	Tubulin beta-2B chain [D]	Microtubule-based process	26225	0.28
P07437	Tubulin beta chain [D]	Microtubule-based process	26437	0.28
Q13885	Tubulin beta-2A chain [D]	Microtubule-based process	26225	0.28
Q13509	Tubulin beta-3 chain [D]	Microtubule-based process	19550	0.24
A0A0B4J269	Uncharacterized protein [D]	Microtubule-based process	8751	0.24
P08729	Keratin_ type II cytoskeletal 7 [D]	Structural constituent of cytoskeleton	478	0.23
Q9NY65	Tubulin alpha-8 chain [D]	Microtubule-based process	11965	0.23
Q71U36	Tubulin alpha-1A chain [D]	Microtubule-based process	38018	0.22

Accession	Description	Biologic process	Score	Ratio I:NO
G3V3R4	HCG1983504_ isoform CRA_c [D]	Microtubule-based process	16551	0.21
Q13748	Tubulin alpha-3C/D chain [D]	Microtubule-based process	12692	0.21
Q6PEY2	Tubulin alpha-3E chain [D]	Microtubule-based process	8817	0.21
P68363	Tubulin alpha-1B chain [D]	Microtubule-based process	37783	0.21
G3V2N6	HCG1983504_ isoform CRA_d [D]	Microtubule-based process	16551	0.21
P68366	Tubulin alpha-4A chain [D]	Microtubule-based process	12442	0.20
Q9BQE3	Tubulin alpha-1C chain [D]	Microtubule-based process	34846	0.20
F5H5D3	Tubulin alpha chain [D]	Microtubule-based process	34846	0.20
Q9UMX9	Membrane-associated transporter protein [F]	Sensory transduction	800	0.08
P02768	Serum albumin [C]	Transport	72906	25.53
C9JKR2	Albumin_ isoform CRA_k [C]	Transport	28569	18.17
P01857	Immunoglobulin heavy constant gamma 1 [B]	Adaptive immunity	12257	3.00
P01023	Alpha-2-macroglobulin [G]	Regulation of complement activation	1004	2.23
P68032	Actin_ alpha cardiac muscle 1 [D]	Structural constituent of cytoskeleton	1208	0.49
P68133	Actin_ alpha skeletal muscle [D]	Structural constituent of cytoskeleton	1190	0.49
P63267	Actin_ gamma-enteric smooth muscle [D]	Structural constituent of cytoskeleton	1208	0.49
P62736	Actin_ aortic smooth muscle [D]	Structural constituent of cytoskeleton	1208	0.48
Q6S8J3	POTE ankyrin domain family member [E L]	Unknown	1142	0.44
A5A3E0	POTE ankyrin domain family member [F L]	Unknown	1132	0.44
P0CG38	POTE ankyrin domain family member [I L]	Unknown	1085	0.41
P0CG39	POTE ankyrin domain family member [J L]	Unknown	568	0.35
P02042	Hemoglobin subunit delta [C]	Oxygen transport	3864	0.11
Q15059	Bromodomain-containing protein 3 [F]	Transcription regulation	2487	0.10
G3V1N2	HCG1745306_ isoform CRA_a [C]	Oxygen transport	3312	0.01
P69905	Hemoglobin subunit alpha [C]	Oxygen transport	8134	0.01
P68871	Hemoglobin subunit beta [C]	Oxygen transport	8969	0.00

Proteins were classified according to Uniprot database: A – Metabolism and energy pathways; B - Immune response; C – Transport; D – Structure; E - DNA/RNA regulation and repair; F - Cell communication and signal transduction; G - Cell growth and/or maintenance; H- Differentiation of neural cells; I- Apoptosis; J- Stress response; K- Ions regulation and binding; L- Unknown. *From accession number P02768 starts Ratio NE:I.

Table 3. Human proteins identified exclusively in normal, inflamed or necrotic pulp

Normal pulp			
Accession	Description	Biologic process	Score
E2QRG7	4-hydroxybenzoate polyprenyltransferase_ mitochondrial [A]	Ubiquinone biosynthesis	424.48
Q8IUX7	Adipocyte enhancer-binding protein 1 [F]	Transcription regulation	281.92
F2Z324	Aldehyde dehydrogenase 1 family member L1 isoform 2 [L]	Unknown	354.48
P02765	Alpha-2-HS-glycoprotein [B]	Acute-phase response	334
H0YMF8	Ammonium transporter Rh type C [D]	Component of membrane	751.41
P00966	Argininosuccinate synthase [A]	Arginine biosynthesis	178.45
H0YH81	ATP synthase subunit beta (Fragment) [A]	ATP synthesis	289.75

Accession	Description	Biologic process	Score
P06576	ATP synthase subunit beta_ mitochondrial [C]	Transport	548.43
Q9NUQ8	ATP-binding cassette sub-family F member 3 [B]	Antiviral defense	217.99
P08237	ATP-dependent 6-phosphofructokinase_ muscle type [A]	Glycolysis	439.21
P49407	Beta-arrestin-1 [F]	Transcription regulation	243.29
P21810	Biglycan [D]	Extracellular matrix structural constituent	277.04
P22223	Cadherin-3 [F]	Sensory transduction	414.89
Q9H9S4	Calcium-binding protein 39-like [G]	Cell cycle arrest	785.13
P29762	Cellular retinoic acid-binding protein 1 [C]	Transport	1319.56
X6RIU2	Cilia- and flagella-associated protein 221 (Fragment) [D]	Component of cytoskeleton	158.14
Q96N23	Cilia- and flagella-associated protein 54 [D]	Component of cytoskeleton	864.57
P26441	Ciliary neurotrophic factor [H]	Differentiation. Neurogenesis. Growth factor	150.4
A0A0A0MT56	Cleavage stimulation factor subunit 2 (Fragment) [E]	mRNA-processing	428.34
Q9GZT6	Coiled-coil domain-containing protein 90B_ mitochondrial [D]	Mitochondrial membrane	271
J3QT66	COP9 signalosome complex subunit 7b [B]	Host-virus interaction	847.96
Q2NKJ3	CST complex subunit CTC1 [G]	Regulation of fibroblast proliferation	586.38
K7EJ26	CUGBP Elav-like family member 4 (Fragment) [E]	mRNA-processing	247.83
O75891	Cytosolic 10-formyltetrahydrofolate dehydrogenase [J]	Oxidoreductase	362.58
Q07507	Dermatopontin [D]	Extracellular matrix structural constituent	1412.86
O75907	Diacylglycerol O-acyltransferase 1 [A]	Acyltransferase. Transferase	290.99
Q16555	Dihydropyrimidinase-related protein 2 [H]	Differentiation. Neurogenesis	169.43
P53602	Diphosphomevalonate decarboxylase [G]	Regulation of cell proliferation	552.3
H7C0V2	DNA repair protein RAD50 (Fragment) [B]	Host-virus interaction	234.66
P38935	DNA-binding protein SMUBP-2 [F]	Transcription regulation	197.26
A0PK19	EPGN protein [G]	Regulation of cell proliferation	1566.9
Q6UW88	Epigen [G]	Regulation of cell proliferation	1566.9
P60842	Eukaryotic initiation factor 4A-I [B]	Host-virus interaction	175.34
Q04637	Eukaryotic translation initiation factor 4 gamma 1 [B]	Host-virus interaction	280.67
Q9NPD3	Exosome complex component RRP41 [E]	rRNA processing	340.1
Q9UK22	F-box only protein 2 [A]	Regulation of protein ubiquitination	280.14
Q969U6	F-box/WD repeat-containing protein 5 [A]	Regulation of protein ubiquitination	335.47
A0A0A0MS75	FGF receptor activating protein 1_ isoform CRA_e [D]	Integral component of membrane	229.6
Q06828	Fibromodulin [D]	Extracellular matrix structural constituent	484.9
G5E9X3	Fibronectin type III domain containing 3A_ isoform CRA_f [F]	Cell-cell adhesion	263.94
Q9Y2H6	Fibronectin type-III domain-containing protein 3A [F]	Cell-cell adhesion	283.11
H3BQN4	Fructose-bisphosphate aldolase [A]	Glycolytic process	577.74
P04075	Fructose-bisphosphate aldolase A [A]	Glycolytic process	591.64
P09382	Galectin-1 [I]	Apoptosis	4761.72
Q9UBS5	Gamma-aminobutyric acid type B receptor subunit 1 [A]	G-protein coupled receptor	433.53

Accession	Description	Biologic process	Score
Q99501	GAS2-like protein 1 [D]	Component of cytoskeleton	299.88
H7C4Q8	General transcription factor II-I repeat domain-containing protein 1 (Fragment) [F]	Transcription regulation	168.04
Q14687	Genetic suppressor element 1 [L]	Unknown	118.64
Q86VD9	GPI mannosyltransferase 4 [A]	GPI-anchor biosynthesis	218.44
G3V3J6	HCG1983504_ isoform CRA_b [D]	Microtubule-based process	10798.74
Q9H583	HEAT repeat-containing protein 1 [F]	Transcription regulation	391.17
Q86XA9	HEAT repeat-containing protein 5A [F]	Transcription regulation	238.55
P04792	Heat shock protein beta-1 [J]	Stress response	1700.43
Q9Y5N1	Histamine H3 receptor [A]	G-protein coupled receptor	188.39
Q96A08	Histone H2B type 1-A [B]	Inflammatory response	1914.55
Q14571	Inositol 1_4_5-trisphosphate receptor type 2 [C]	Transport	271.01
P19823	Inter-alpha-trypsin inhibitor heavy chain H2 [A]	Cellular protein metabolic process	371.22
V9GXZ7	Kin of IRRE-like protein 2 (Fragment) [L]	Unknown	462.22
Q96Q89	Kinesin-like protein KIF20B [G]	Cell division. mitosis	844.1
H0YEH0	Large neutral amino acids transporter small subunit 3 (Fragment) [D]	Integral component of membrane	736.6
O75845	Lathosterol oxidase [A]	Lipid metabolism	169.98
Q8N653	Leucine-zipper-like transcriptional regulator 1 [F]	Transcription factor	161.39
Q8NHJ6	Leukocyte immunoglobulin-like receptor subfamily B member 4 [B]	Adaptive immunity	315.03
P51884	Lumican [D]	Extracellular matrix structural constituent	5091.27
Q6UWQ5	Lysozyme-like protein 1 [B]	Defense response to bacteria	847.95
Q7Z4W2	Lysozyme-like protein 2 [B]	Defense response to bacteria	847.95
Q14168	MAGUK p55 subfamily member 2 [F]	Excitatory postsynaptic potential	700.69
Q9H3U5	Major facilitator superfamily domain-containing protein 1 [C]	Transport	167.24
C9JQX2	Mannosyltransferase [A]	Glycosyltransferase	210.23
E5RJR3	Methionine adenosyltransferase 2 subunit beta [A]	One-carbon metabolism	683.62
P25189	Myelin protein P0 [I]	Regulation of apoptotic process	366.82
P60660	Myosin light polypeptide 6 [K]	Calcium ion binding	430.43
Q9UK23	N-acetylglucosamine-1-phosphodiester alpha-N-acetylglucosaminidase [A]	Protein glycosylation	246.65
A0A087WYD0	NADH dehydrogenase (Ubiquinone) 1 beta subcomplex_ 5_ 16kDa_ isoform CRA_g [C]	Transport	379.52
O43674	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 5_ mitochondrial [C]	Transport	379.52
O00308	NEDD4-like E3 ubiquitin-protein ligase WWP2 [B]	Host-virus interaction	261.84
O00533	Neural cell adhesion molecule L1-like protein [H]	Differentiation. Neurogenesis	238.35
P12036	Neurofilament heavy polypeptide [D]	Structural constituent of cytoskeleton	471.82
P07196	Neurofilament light polypeptide [D]	Structural constituent of cytoskeleton	645.68
Q9NZ94	Neuroligin-3 [F]	Cell adhesion	470.51

Accession	Description	Biologic process	Score
Q7RTR2	NLR family CARD domain-containing protein 3 [B]	Regulation of inflammatory response	180.39
H3BLT9	NOD3 protein_ isoform CRA_d [D]	Microtubule organizing center	180.39
Q14980	Nuclear mitotic apparatus protein 1 [F]	Cell division	281.88
Q14686	Nuclear receptor coactivator 6 [F]	Transcription regulation	253.67
A0A126GWK9	Olfactory receptor [F]	Sensory transduction	305.24
O43869	Olfactory receptor 2T1 [F]	Sensory transduction	305.24
Q6IFN5	Olfactory receptor 7E24 [F]	Sensory transduction	220.6
H0Y2Y4	Palmitoyltransferase (Fragment) [A]	Kinase activity	212.83
O95497	Pantetheinase [J]	Response to oxidative stress	239.94
P26022	Pentraxin-related protein PTX3 [B]	Inflammatory response	159.12
Q9Y536	Peptidyl-prolyl cis-trans isomerase A-like 4A [A]	Catalytic activity	511.68
Q8NEB9	Phosphatidylinositol 3-kinase catalytic subunit type 3 [I]	Autophagy	518.24
Q9UHJ9	Post-GPI attachment to proteins factor 2 [A]	GPI-anchor biosynthesis	229.6
Q01860	POU domain_ class 5_ transcription factor 1 [F]	Transcription regulation	190.11
P02545	Prelamin-A/C [A]	Regulation of apoptotic signaling pathway	405.48
Q9UMS4	Pre-mRNA-processing factor 19 [E]	mRNA processing	353.75
Q96I59	Probable asparagine--tRNA ligase_ mitochondrial [A]	Protein biosynthesis	191.32
Q15034	Probable E3 ubiquitin-protein ligase HERC3 [A]	Ubl conjugation pathway	82.2
Q16651	Prostasin [K]	Regulation of sodium ion transport	275.33
H0YHR3	Protein phosphatase Slingshot homolog 1 (Fragment) [G]	Actin cytoskeleton organization	255.57
Q99497	Protein/nucleic acid deglycase DJ-1 [J]	Stress response	784.37
H3BRZ0	Putative sodium-coupled neutral amino acid transporter 7 (Fragment) [D]	Integral component of membrane	672.03
B4DNK4	Pyruvate kinase [A]	Kinase activity	458.75
P14618	Pyruvate kinase PKM [A]	Kinase activity	458.75
P26374	Rab proteins geranylgeranyltransferase component A 2 [C]	Intracellular protein transport	618.53
P21860	Receptor tyrosine-protein kinase erbB-3 [D]	Receptor of cell membrane	271.71
Q12913	Receptor-type tyrosine-protein phosphatase eta [D]	Receptor of cell membrane	436.47
E9PGT3	Ribosomal protein S6 kinase [F]	Intracellular signal transduction	415.96
Q15418	Ribosomal protein S6 kinase alpha-1 [F]	Intracellular signal transduction	430.79
Q6P3W7	SCY1-like protein 2 [C]	Endosome to lisossome transport	144.69
P59797	Selenoprotein V [L]	Unknown	3591.64
Q9H3S1	Semaphorin-4A [H]	Differentiation. Neurogenesis	898.66
O95754	Semaphorin-4F [H]	Differentiation. Neurogenesis	257.33
Q05519	Serine/arginine-rich splicing factor 11 [E]	mRNA processing	1467.45
Q8WU08	Serine/threonine-protein kinase 32A [F]	Intracellular signal transduction	292.95
Q9H2K8	Serine/threonine-protein kinase TAO3 [E]	DNA repair	607.16
O43147	Small G protein signaling modulator 2 [C]	Intracellular protein transport	738.5
Q8N4F4	Solute carrier family 22 member 24 [K]	Ion transport	317.43

Accession	Description	Biologic process	Score
P00441	Superoxide dismutase [Cu-Zn] [J]	Antioxidant. Oxidoreductase	1233.87
Q9UMS6	Synaptopodin-2 [F]	Regulation of cell migration	289.93
Q9Y6H5	Synphilin-1 [A]	Cellular protein metabolic process	224.06
Q9NYW2	Taste receptor type 2 member 8 [A]	G-protein coupled receptor	173.03
Q8IWY7	Tau-tubulin kinase [A]	Kinase activity	231.03
P24821	Tenascin [F]	Cell adhesion	788.89
Q5R3I4	Tetratricopeptide repeat protein 38 [L]	Unknown	164.5
Q96FV9	THO complex subunit 1 [F]	Transcription regulation	165.16
Q14135	Transcription cofactor vestigial-like protein 4 [F]	Transcription regulation	320.85
Q15582	Transforming growth factor-beta-induced protein ig-h3 [F]	Cell adhesion	699.53
Q7Z6W1	Transmembrane and coiled-coil domain-containing protein 2 [D]	Integral component of membrane	383.03
Q9H4B7	Tubulin beta-1 chain [D]	Structural constituent of cytoskeleton	1944.92
Q9UJT1	Tubulin delta chain [D]	Structural constituent of cytoskeleton	493.65
A8MXF1	Tyrosine-protein phosphatase non-receptor type 5 [A]	Protein phosphatase	355.82
A0A0B4J269	Uncharacterized protein [D]	Microtubule-based process	8750.79
Q8NBR9	Uncharacterized protein C11orf72 [L]	Unknown	310.42
V9GY35	Uncharacterized protein C1orf109 (Fragment) [L]	Unknown	240.6
Q6ZUG5	Uncharacterized protein FLJ43738 [L]	Unknown	992.96
D6RBZ9	Uncharacterized protein FLJ43738 (Fragment) [L]	Unknown	990.13
Q92628	Uncharacterized protein KIAA0232 [L]	Unknown	583.39
D6REK0	Uncharacterized protein KIAA0232 [L]	Unknown	568.86
P10746	Uroporphyrinogen-III synthase [A]	Heme biosynthesis	212.17
P04004	Vitronectin [F]	Cell adhesion	728.59
Q06432	Voltage-dependent calcium channel gamma-1 subunit [K]	Calcium transport	219.37
Q5MNZ6	WD repeat domain phosphoinositide-interacting protein 3 [I]	Autophagy of nucleus	228.89
Q13105	Zinc finger and BTB domain-containing protein 17 [F]	Transcription regulation	302.68
Q6PJT7	Zinc finger CCCH domain-containing protein 14 [E]	Regulation of mRNA stability	331.85
Q09FC8	Zinc finger protein 415 [F]	Transcription regulation	192.42
Q96MU6	Zinc finger protein 778 [F]	Transcription regulation	562.65

Inflamed pulp

Accession	Description	Biologic process	Score
P62280	40S ribosomal protein S11 [F]	Translational initiation	351.39
Q13085	Acetyl-CoA carboxylase 1 [A]	Lipid metabolism	394.31
P78348	Acid-sensing ion channel 1 [K]	Ion transport	1387.23
A0A1X7SBU6	Adhesion G protein-coupled receptor V1 (Fragment) [D]	Integral component of membrane	208.16
Q9NVD7	Alpha-parvin [G]	Angiogenesis	174.54
Q86UQ4	ATP-binding cassette sub-family A member 13 (Fragment) [C]	Transport	281.18
O94911	ATP-binding cassette sub-family A member 8 (Fragment) [C]	Transmembrane transport	190.11
Q8WXE1	ATR-interacting protein [E]	Response to DNA damage	636.8

Accession	Description	Biologic process	Score
P17213	Bactericidal permeability-increasing protein [B]	Antibacterial humoral response	284.17
P02730	Band 3 anion transport protein [k]	Ion transport	243.95
Q96T60	Bifunctional polynucleotide phosphatase/kinase [E]	Response to DNA damage	229.66
P00915	Carbonic anhydrase 1 [K]	Bicarbonate transport	364.93
P00918	Carbonic anhydrase 2 [K]	Bicarbonate transport	641.72
Q9NS85	Carbonic anhydrase-related protein 10 [K]	Zinc ion binding	179.75
P51948	CDK-activating kinase assembly factor MAT1 [F]	Transcription regulation	228.15
P00451	Coagulation factor VIII [B]	Acute-phase response	548.5
H0YK65	Coiled-coil domain-containing 9B (Fragment) [L]	Unknown	372.31
Q9Y2V7	Conserved oligomeric Golgi complex subunit 6 [C]	Protein transport	382.37
Q8TEY5	Cyclic AMP-responsive element-binding protein 3-like protein 4 [F]	Transcription regulation	314.59
P55273	Cyclin-dependent kinase 4 inhibitor D [I]	Autophagic cell death	360.12
Q68DD2	Cytosolic phospholipase A2 zeta [A]	Lipid metabolism	279.68
P30038	Delta-1-pyrroline-5-carboxylate dehydrogenase_ mitochondrial [J]	Oxidoreductase. proline metabolism	191.36
P78352	Disks large homolog 4 [F]	Cell adhesion	233.8
P36507	Dual specificity mitogen-activated protein kinase 2 [A]	Activation of protein kinase activity	240.42
H0YD30	Dynein assembly factor 3_ axonemal (Fragment) [F]	Axonemal dynein complex assembly	309.68
Q14118	Dystroglycan [G]	Angiogenesis	383.01
Q8N2H9	E3 ubiquitin-protein ligase pellino homolog 3 [A]	Ubl conjugation pathway	1102.45
K7EII6	Echinoderm microtubule-associated protein-like 2 (Fragment) [F]	Regulation of microtubule nucleation	230.16
Q05BV3	Echinoderm microtubule-associated protein-like 5 [D]	Microtubule binding	308.9
P43897	Elongation factor Ts_ mitochondrial [G]	Protein biosynthesis	215.14
Q5T6L9	Endoplasmic reticulum membrane-associated RNA degradation protein [G]	Developmental protein	440.87
O15360	Fanconi anemia group A protein [E]	Response to DNA damage	156.29
Q14CZ7	FAST kinase domain-containing protein 3_ mitochondrial [A]	Protein kinase activity	216.49
O94887	FERM_ ARHGEF and pleckstrin domain-containing protein 2 [G]	Osteoclast differentiation	394.64
P02679	Fibrinogen gamma chain [F]	Hemostasis	117.71
Q4L180	Filamin A-interacting protein 1-like [I]	Regulation of apoptosis process	259.28
Q9NSN8	Gamma-1-syntrophin [F]	Cell communication	273.12
P36383	Gap junction gamma-1 protein [G]	Cell development. Vasculogenesis	179.45
Q5T442	Gap junction gamma-2 protein [G]	Cell development. Vasculogenesis	307.56
Q13630	GDP-L-fucose synthase [J]	Oxidoreductase	120.68
Q6UWF4	GLGQ5807 [D]	Membrane component	325.59
Q92805	Golgin subfamily A member 1 [D]	Structural component	197.06
A0A1W2PNZ5	GPI transamidase component PIG-T [I]	Neuron apoptotic process	369.7
P62826	GTP-binding nuclear protein Ran [G]	Cell division	306.27
A0A0A6YYF2	HCG1811249_ isoform CRA_e [F]	Regulation of cell adhesion	277.46
A0A0A0MTS5	HCG1811249_ isoform CRA_f [F]	Regulation of cell adhesion	274.02

Accession	Description	Biologic process	Score
A0A0U1RR32	Histone H2A [E]	DNA-binding	176.75
P0C0S8	Histone H2A type 1 [E]	DNA-binding	176.75
Q96QV6	Histone H2A type 1-A [E]	DNA-binding	176.75
P04908	Histone H2A type 1-B/E [E]	DNA-binding	176.75
Q93077	Histone H2A type 1-C [E]	DNA-binding	176.75
P20671	Histone H2A type 1-D [E]	DNA-binding	176.75
Q96KK5	Histone H2A type 1-H [E]	DNA-binding	176.75
Q99878	Histone H2A type 1-J [E]	DNA-binding	176.75
Q6FI13	Histone H2A type 2-A [E]	DNA-binding	176.75
Q8IUE6	Histone H2A type 2-B [E]	DNA-binding	176.75
Q16777	Histone H2A type 2-C [E]	DNA-binding	176.75
Q7L7L0	Histone H2A type 3 [E]	DNA-binding	176.75
Q9BTM1	Histone H2A.J [E]	DNA-binding	176.75
Q71UI9	Histone H2A.V [E]	DNA-binding	176.75
P0C0S5	Histone H2A.Z [E]	DNA-binding	176.75
P16104	Histone H2AX [E]	DNA-binding	176.75
P62805	Histone H4 [E]	DNA-binding	1795.98
I3L3R1	Homeobox B8_ isoform CRA_a [F]	Transcription regulation	236.2
P17481	Homeobox protein Hox-B8 [F]	Transcription regulation	236.2
P31273	Homeobox protein Hox-C8 [F]	Transcription regulation	236.2
J3QL30	Hydrocephalus-inducing protein homolog (Fragment) [L]	Unknown	536.84
Q7Z5J1	Hydroxysteroid 11-beta-dehydrogenase 1-like protein [J]	Oxidoreductase	388.82
A0A0B4J2B6	Immunoglobulin heavy variable 2/OR16-5 (non-functional) (Fragment) [B]	Innate immune response	410.2
H0YBQ1	Integrator complex subunit 8 (Fragment) [E]	snRNA processing	244.63
Q8IU57	Interferon lambda receptor 1 [B]	Antiviral defense	262.8
A0A1B0GTI5	Interleukin-10 receptor subunit beta (Fragment) B	Inflammatory response	326.35
Q15811	Intersectin-1 [I]	Neuron apoptotic process	241.91
A0A1W2PQS2	IQ motif and SEC7 domain-containing protein 2 (Fragment) [D]	Structural component of cytoskeleton	385.21
Q16787	Laminin subunit alpha-3 [F]	Cell adhesion	282.82
Q16363	Laminin subunit alpha-4 [F]	Cell adhesion	215.34
Q2I0M4	Leucine-rich repeat-containing protein 26 [C]	Ion transport	231.68
Q9Y2P4	Long-chain fatty acid transport protein 6 [A]	Lipid metabolism	216.78
Q9Y561	Low-density lipoprotein receptor-related protein 12 [A]	Endocytosis	243.95
O95711	Lymphocyte antigen 86 [B]	Innate immune response	473.09
Q9BVV7	Mitochondrial import inner membrane translocase subunit Tim21 [C]	Protein transport	251.37
F8VYZ2	Monocarboxylate transporter 2 [C]	Transport	306.67
O75970	Multiple PDZ domain protein [F]	Cell adhesion	276.49
Q8IY17	Neuropathy target esterase [A]	Lipid metabolism	222.84

Accession	Description	Biologic process	Score
Q8NH81	Olfactory receptor 10G6 [F]	Sensory transduction	252.13
P09131	P3 protein [C]	Transport	201.68
O95428	Papilin [G]	Protease inhibitor	187.48
A6NIW5	Peroxiredoxin 2_ isoform CRA_a [J]	Cell redox homeostasis	776.51
P30041	Peroxiredoxin-6 [J]	Antioxidant. Lipid metabolism	1218.39
Q6IQ23	Pleckstrin homology domain-containing family A member 7 [D]	Cellular component	270.31
Q3KNV8	Polycomb group RING finger protein 3 [F]	Transcription regulation	192.63
Q5H9U9	Probable ATP-dependent RNA helicase DDX60-like [E]	RNA-binding	270.08
Q8IZL8	Proline-_ glutamic acid- and leucine-rich protein 1 [F]	Transcription	511.17
Q9H8V3	Protein ECT2 [H]	Differentiation. Neurogenesis	324.14
U3KQD2	Protein GPR107 [C]	Protein transport	186.68
Q9Y6F6	Protein MRVI1 [D]	Membrane component	270.56
Q8WXB1	Protein N-lysine methyltransferase METTL21A [F]	Transferase	236.44
A0A0A6YY99	Protein TNFSF12-TNFSF13 [B]	Immune response	490.49
O94855	Protein transport protein Sec24D [C]	Protein transport	271.08
Q69YN4	Protein virilizer homolog [E]	mRNA processing	321.84
A8MUN3	Putative uncharacterized protein ENSP00000381830 [L]	Unknown	672.41
Q96D71	RalBP1-associated Eps domain-containing protein 1 [K]	Calcium binding	277.02
Q92619	Rho GTPase-activating protein 45 [F]	Intracellular signal transduction	290.3
E5RI70	Rho GTPase-activating protein 7 (Fragment) [I]	Apoptosis process	1145.15
Q9NRP7	Serine/threonine-protein kinase 36 [A]	Kinase activity	197.99
Q96BR1	Serine/threonine-protein kinase Sgk3 [A]	Kinase activity	163.05
P30154	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A beta isoform [I]	Apoptotic process	273.47
Q8TE82	SH3 domain and tetratricopeptide repeat-containing protein 1 [L]	Unknown	248.71
Q8TCT6	Signal peptide peptidase-like 3 [A]	T cell receptor signaling pathway	203.96
O94813	Slit homolog 2 protein [H]	Differentiation. Neurogenesis	187.71
P05023	Sodium/potassium-transporting ATPase subunit alpha-1 [K]	Ion transport	328.76
Q8WUM9	Sodium-dependent phosphate transporter 1 [K]	Phosphate ion transport	571.51
P02549	Spectrin alpha chain_ erythrocytic 1 [D]	Structural component of cytoskeleton	335.76
A0A087WXB8	ST3 beta-galactoside alpha-2_3-sialyltransferase 6_ isoform CRA_b [A]	Protein glycosylation	277.21
Q8IVG5	Sterile alpha motif domain-containing protein 9-like [G]	Regulation of growth factor	456.51
P57105	Synaptojanin-2-binding protein [G]	Regulation of growth factor	169.06
Q86Y82	Syntaxin-12 [C]	Protein transport	371.79
Q6PGP7	Tetratricopeptide repeat protein 37 [A]	Catabolic process	262.47
A0A0U1RQW3	Three prime repair exonuclease 1_ isoform CRA_a [E]	DNA damage checkpoint	636.8
I3L3T4	TOM1-like protein 1 [C]	Intracellular protein transport	194.25
O75204	Transmembrane protein 127 [D]	Endosome organization	791.15
Q14CX5	Transmembrane protein 180 [D]	Membrane component	341.01

Accession	Description	Biologic process	Score
Q9NX78	Transmembrane protein 260 [D]	Membrane component	121.58
Q9BTW9	Tubulin-specific chaperone D [D]	Microtubule cytoskeleton organization	328.09
Q2QBA2	Tumor necrosis factor (Ligand) superfamily member 13 transcript variant delta [B]	Immune response	490.49
O75888	Tumor necrosis factor ligand superfamily member 13 B	Immune response	490.49
Q9Y274	Type 2 lactosamine alpha-2_3-sialyltransferase [A]	Metabolic process	277.21
A0A0B4J269	Uncharacterized protein [H]	Neuron differentiation	526.49
S4R451	WD repeat-containing protein 11 [A]	Signaling pathway to ciliogenesis	385.34
Q9UII5	Zinc finger protein 107 [F]	Transcription regulation	162.73
Q8NHY6	Zinc finger protein 28 homolog [F]	Transcription regulation	415.19
F8WAL3	Zinc finger protein 528 [F]	Transcription regulation	269.74
Q86YE8	Zinc finger protein 573 [F]	Transcription regulation	246.76
Q03923	Zinc finger protein 85 [F]	Transcription regulation	289.34
	Necrotic pulp		
P31946	14-3-3 protein beta/alpha [B]	Host-virus interaction	731.1
Q6N063	2-oxoglutarate and iron-dependent oxygenase domain-containing protein 2 [J]	Oxidoreductase activity	350.12
Q86U10	60 kDa lysophospholipase [A]	Lipid metabolism	653.91
E9PNY0	Adenine DNA glycosylase [E]	DNA repair	1337.74
P55196	Afadin [F]	Cell adhesion	546.31
P02763	Alpha-1-acid glycoprotein 1 [B]	Regulation of immune system	588.98
P04217	Alpha-1B-glycoprotein [B]	Neutrophil degranulation	418.04
P01019	Angiotensinogen [G]	Growth factor activity	772.78
Q6ZTN6	Ankyrin repeat domain-containing protein 13D [F]	Ubiquitin-binding protein	491.4
K7EL63	Ankyrin repeat domain-containing protein 29 (Fragment) [L]	Unknown	358.5
C9JP59	Ankyrin repeat_ SAM and basic leucine zipper domain-containing protein 1 (Fragment) [L]	Unknown	859.6
Q01484	Ankyrin-2 [C]	Protein transport	58.02
Q9Y2F9	BTB/POZ domain-containing protein 3 [H]	Neurogenesis	645.18
Q9BXU9	Calcium-binding protein 8 [K]	Calcium ion binding	451.01
B4E1Z4	cDNA FLJ55673_ highly similar to Complement factor B (EC 3.4.21.47) [B]	Complement activation	503.52
A0A087X2B6	Cell cycle and apoptosis regulator protein 2 [I]	Regulation of apoptotic process	704.06
A6PVI9	Centrosomal protein 250kDa [G]	Cell cycle	461.65
Q9BV73	Centrosome-associated protein CEP250 [G]	Cell cycle	490.84
P00450	Ceruloplasmin [K]	Ion transport	161
H3BN91	C-Jun-amino-terminal kinase-interacting protein 3 (Fragment) [H]	Axon regeneration	327.76
P01024	Complement C3 [A]	Complement pathway	788.64
P00751	Complement factor B [B]	Innate immunity	503.52
O15315	DNA repair protein RAD51 homolog 2 [E]	DNA repair	292.13
Q7L591	Docking protein 3 [F]	Signal transduction	282.57

Accession	Description	Biologic process	Score
Q9NRD9	Dual oxidase 1 [J]	Oxidoreductase	1227.04
Q0PNE2	Elongator complex protein 6 [F]	Transcription regulation	406.73
E7EU71	Ephrin type-A receptor 6 [D]	Membrane component	466.19
Q9NRG7	Epimerase family protein SDR39U1 [J]	Oxidoreductase	346.17
I6L9I8	EPN3 protein [L]	Unknown	628.04
Q9H201	Epsin-3 [F]	Lipid-binding	650.19
C9JLC0	F-box/SPRY domain-containing protein 1 [H]	Neurogenesis	326.5
P02675	Fibrinogen beta chain [B]	Innate immunity	518.5
P00739	Haptoglobin-related protein [I]	Regulation of cell death	1026.1
A0A0B4J1V2	Immunoglobulin heavy variable 2-26 [B]	Adaptive immunity	663.4
P01834	Immunoglobulin kappa constant [B]	Adaptive immunity	3257.26
P01619	Immunoglobulin kappa variable 3-20 [B]	Adaptive immunity	613.37
P0CG04	Immunoglobulin lambda constant 1 [B]	Adaptive immunity	4803.8
P0DOY2	Immunoglobulin lambda constant 2 [B]	Adaptive immunity	4803.8
P0DOY3	Immunoglobulin lambda constant 3 [B]	Adaptive immunity	2697.85
P0CF74	Immunoglobulin lambda constant 6 [B]	Adaptive immunity	1652.51
A0M8Q6	Immunoglobulin lambda constant 7 [B]	Adaptive immunity	402.02
B9A064	Immunoglobulin lambda-like polypeptide 5 [B]	Innate immune response	4803.8
Q53G59	Kelch-like protein 12 [A]	Ubl conjugation pathway	237.11
P02788	Lactotransferrin [K]	Ion transport	256.65
Q6ZSS7	Major facilitator superfamily domain-containing protein 6 [D]	Membrane component	148.16
B5MC10	MpV17 mitochondrial inner membrane protein isoform 2 [J]	Regulation of reactive oxygen	563.58
E9PK80	NAD-dependent protein deacetylase [A]	Catalytic activity	1042.07
Q9NTG7	NAD-dependent protein deacetylase sirtuin-3_ mitochondrial [A]	Catalytic activity	1060.08
Q9UBB6	Neurochondrin [F]	Signal transduction	490.42
P59665	Neutrophil defensin 1 [B]	Antiviral defense	7785.6
P59666	Neutrophil defensin 3 [B]	Antiviral defense	1433.54
P08246	Neutrophil elastase [A]	Catalytic activity	614.34
Q15155	Nodal modulator 1 [D]	Membrane component	523.61
Q5JPE7	Nodal modulator 2 [D]	Membrane component	45.57
P69849	Nodal modulator 3 [D]	Membrane component	533.01
A0A0J9YWI0	Peroxisomal 2_4-dienoyl-CoA reductase (Fragment) [J]	Oxidoreductase	424.99
P27986	Phosphatidylinositol 3-kinase regulatory subunit alpha [I]	Regulation of apoptotic process	462.89
O00443	Phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit alpha [C]	Endocytosis	586
A0A0U1RQS1	Probable global transcription activator SNF2L2 (Fragment) [H]	Neurogenesis	986.63
Q9BVM2	Protein DPCD [D]	Formation of ciliated cells	225.08
C9K0C0	Protein FAM71F2 [L]	Unknown	1745.9
P06702	Protein S100-A9 [I]	Apoptosis	4809.99
Q96I85	Putative uncharacterized protein C14orf144 [L]	Unknown	1071.1

Accession	Description	Biologic process	Score
Q86TS7	Putative UPF0730 protein encoded by LINC00643 [L]	Unknown	506.3
Q9UHV5	Rap guanine nucleotide exchange factor-like 1 [H]	Nervous system development	657.95
P52565	Rho GDP-dissociation inhibitor 1 [I]	Regulation of apoptotic process	241.48
J3KPQ4	Rho GTPase activating protein 9_ isoform CRA_a [F]	Signal transduction	421.35
Q9BRR9	Rho GTPase-activating protein 9 [F]	Signal transduction	421.35
O15393	Transmembrane protease serine 2 [A]	Catalytic activity	300.54
A0A1W2PQJ5	Uncharacterized protein [L]	Unknown	331.97
H0Y8H3	Uncharacterized protein C3orf67 (Fragment) [L]	Unknown	864.21
P02774	Vitamin D-binding protein [C]	Transport	470.82
E9PNL3	V-type proton ATPase 21 kDa proteolipid subunit [K]	Ion transport	222.07

Proteins were classified according to Uniprot database: A – Metabolism and energy pathways; B - Immune response; C – Transport; D – Structure; E - DNA/RNA regulation and repair; F - Cell communication and signal transduction; G - Cell growth and/or maintenance; H- Differentiation of neural cells; I- Apoptosis; J- Stress response; K- Ions regulation and binding; L- Unknown.

The immunoinflammatory response is intended to restore the structural and functional integrity of the injured tissue by eliminating irritants as quickly as possible (17). Indicating the activity of the immune response in inflamed pulp, three immunoglobulin isoforms were present in greater amounts in the inflamed tissue than in the healthy tissue samples. The increase of Nuclear mitotic apparatus protein indicates cell division, probably related to the proliferation of immunoinflammatory cells.

The release of reactive oxygen species (ROS) by

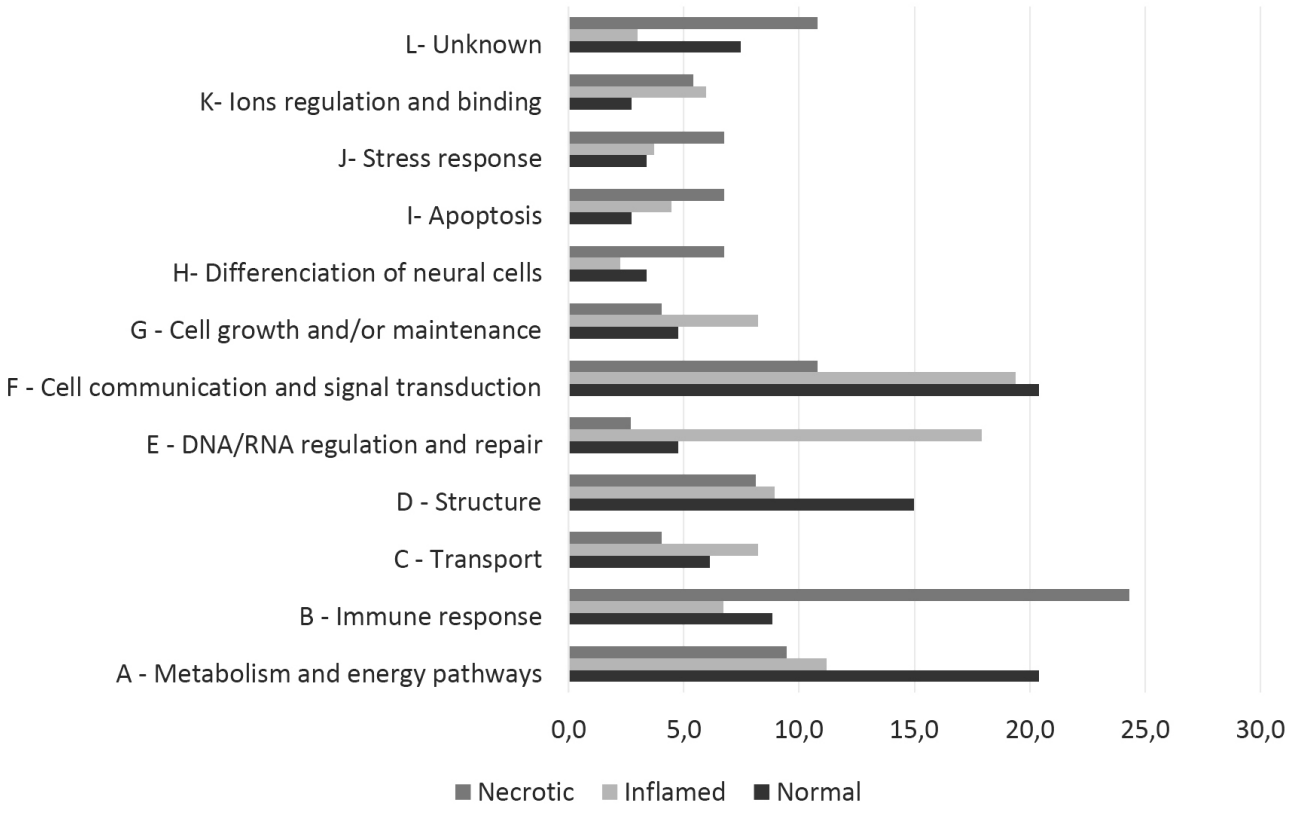

Figure 2. Biological function (Uniprot database) of proteins exclusively identified in normal, inflamed and necrotic pulp groups (%).

disintegrated neutrophils and macrophages within the pulp tissue might damage these tissues. ROS are mandatory byproducts of the metabolic activities of living aerobic organisms (18). Removal of ROS is done by peroxiredoxins - a family of antioxidant proteins that catalyze these substances. Two isoforms of Peroxiredoxins were up-regulated in the inflamed pulp group when compared to the normal tissue, which are responsible for the protection of cellular components against oxidative damage. They are involved in processes such as cell proliferation and differentiation, protection of free radical-sensitive proteins, hemoglobin metabolism and intracellular signaling (19).

Throughout the inflammatory process maintained by the aggressive agent, damage to the pulp tissue occurs, with consequent cell death and destruction of the extracellular matrix. The down-regulation of 17 isoforms of Tubulin in the inflamed pulp group shows the disorganization and destruction of the structural portion of the cell in front of this process (20). Neurofilament proteins, such as Alpha-internexin and Neurofilament medium polypeptide were also down-regulated, which could be predicted due to the intense inflammation present in irreversible pulpitis. Some other proteins were also found with a high level expression in inflamed pulpal tissue, most of them participating in biological processes related to transport, and metabolism and energy pathways.

As a result of the evolution of the inflammatory process of the pulp, the vital functions of the pulp are compromised, followed by hypoxia and tissue necrosis. In this context, there are also changes in blood microcirculation that led to reduced pulp blood flow, explaining the down-regulation of hemoglobin in the necrotic pulp group, when compared with the inflamed one (21). The down-regulation of 4 isoforms of actins show that the mortification process of the pulp tissue leads to destruction of the cytoskeleton and rupture of actin microfilaments. Actin is a protein involved in structuring the cytoskeleton. The actin microfilaments participate in the generation of forces and cell adhesion, stabilizing the cell and determining the shape of the plasma membrane (22).

Among the proteins up-regulated in the necrotic group, serum albumin, albumin, immunoglobulin, and alpha-2-macroglobulin were found. Proteins derived from albumin and serum albumin are constituents of fluids and exudates that infiltrate the apical and lateral foramen of the root canal. Albumin and immunoglobulins may be related to the immune response as they participate in reducing the diffusion of antigens when they adhere to the dentinal tubules (23).

Immunity-related proteins, such as immunoglobulins and protease inhibitors, involved in antigen presentation, defense cell activation and stress response can be identified in necrotic pulps, suggesting that host cells react to root canal system infections (24). One of the proteins found in high level in this group, Alpha-2-macroglobulin (α2M), protects the body against bacterial endotoxins, regulating apoptosis and inhibiting the generation of hydrogen peroxide. In addition, α2M can be used as a biomarker for the diagnosis and prognosis of various diseases (25).

The most recurrent biological processes found in normal pulp tissue provide tissue balance, including maintenance, renewal and energy supply for cellular interaction. In the inflamed pulp group, there was an increase in the percentage of proteins involved in the regulation and repair of DNA / RNA in order to allow cell viability. This may have occurred due to the damage suffered by the cells of the pulp tissue during the inflammatory process. Meanwhile, the increase of proteins associated with metabolism and energetic pathways is directly related to the greater cellular activity for the elimination of the aggressive agent. Moreover, samples representing the infected pulp had a higher percentage of proteins with biological function related to the immune response, which was also described by Provenzano et al. (24), revealing the presence of viable host cells at the site of infection. These results contribute to the understanding of the complex pathogen-host relationship, the host's antimicrobial strategies to fight the infections and shed light into the pathogenesis of the disease.

The present study was the first report to analyze both qualitative and quantitatively proteins differently expressed in normal, inflamed and necrotic pulp, thus providing important data that might not only contribute to the understanding of the complex pathogen-host relationship involved in the progression of pulp diseases, but also to direct future researches. Moreover, the present study reported higher levels of proteins that are considered constitutively produced and ideally should be constant such as beta actin, Glyceraldehyde-3 phosphate dehydrogenase, macroglobulin and tubulin. Therefore, future studies using quantitative methods such as Real time-PCR and Western Blotting analysis should avoid targeting such genes/proteins as reference when analyzing samples related to pathosis of the pulp.

In conclusion, this proteomic analysis showed quantitative differences in protein expression in different types of pulp conditions and revealed that pulp inflammation induced a high-level expression of proteins related to cellular communication and signal transduction. Nevertheless, with the progression to pulp necrosis, the proteins were associated with immune response.

Resumo

Este estudo teve como objetivo comparar quantitativamente a diferença da expressão de proteínas na progressão da patogênese pulpar, bem

como descrever as funções biológicas das proteínas identificadas no tecido pulpar. As amostras foram obtidas de seis pacientes atendidos na Faculdade de Odontologia de Araçatuba e divididas em três grupos: polpa normal - dentes extraídos por indicação ortodôntica; polpa inflamada e polpa necrótica – pacientes diagnosticados com pulpite irreversível e periodontite apical crônica, respectivamente. Após o preparo proteômico prévio, as amostras de polpa dentária foram processadas para análise proteômica quantitativa livre de marcadores em um sistema nanoACQUITY UPLC-Xevo QTof MS. A diferença de expressão entre os grupos foi calculada usando o software Protein Lynx Global Service através do algoritmo de Monte Carlo. Um total de 465 proteínas humanas foram identificadas em todos os grupos. As proteínas mais expressas no grupo polpa inflamada em relação ao grupo polpa normal foram hemoglobinas, peroxirredoxinas e imunoglobulinas, enquanto as menos expressas foram as tubulinas. Os níveis de expressão de albuminas, imunoglobulinas e alfa-2-macroglobulina foram maiores no grupo polpa necrótica do que no grupo de polpa inflamada. Quanto à análise qualitativa, as funções proteicas mais prevalentes no grupo polpa normal foram vias metabólicas e energéticas; no grupo polpa inflamada: comunicação celular e transdução de sinal; e regulação e reparo de DNA / RNA, enquanto no grupo polpa necrótica as proteínas foram associadas à resposta imune. Assim, a análise proteômica mostrou diferenças quantitativas e qualitativas na expressão de proteínas em diferentes tipos de condições pulpares.

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