Proteomic Analysis of Human Tooth Pulp: Proteomics of Human Tooth

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Abstract

Introduction: The unique pulp-dentin complex demonstrates strong regenerative potential, which enables it to respond to disease and traumatic injury. Identifying the proteins of the pulp-dentin complex is crucial to understanding the mechanisms of regeneration, tissue calcification, defense processes, and the reparation of dentin by dental pulp. The lack of knowledge of these proteins limits the development of more efficient therapies. **Methods:** The proteomic profile of human tooth pulp was investigated and compared with the proteome of human dentin and blood. The samples of tooth pulp were obtained from 5 sound permanent human third molars of 5 adults (n = 5). The extracted proteins were separated by 2-dimensional gel electrophoresis, analyzed by nano-liquid chromatography tandem mass spectrometry, and identified by correlating mass spectra to the proteomic databases. **Results:** A total of 342 proteins were identified with high confidence, and 2 proteins were detected for the first time in an actual human sample. The identified tooth pulp proteins have a variety of functions: structural, catalytic, transporter, protease activity, immune response, and many others. In a comparison with dentin and blood plasma, 140 (pulp/dentin) shared proteins were identified, 37 of which were not observed in plasma. It can be suggested that they might participate in the unique pulp-dentin complex. **Conclusions:** This proteomic investigation of human tooth pulp, together with the previously published study of human dentin, is one of the most comprehensive proteome lists of human teeth to date. *(J Endod 2014;40:1961–1966)*

Key Words

Dentin, human pulp, pulp-dentin complex, tandem mass spectrometry, tooth proteome, 2-dimensional gel electrophoresis

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The unique pulp-dentin complex, which makes the tooth alive, demonstrates strong regenerative potential, which enables it to respond to disease and traumatic injury (for example, dental pulp itself forms calcified tissue when transplanted subcutaneously) (1, 2). The identification of the bioactive proteins present in the pulp and/or dentin (review of bioactive dentin components [3]) has enabled their potential involvement in regenerative and other tissue responses to be better understood. These proteins could potentially offer paths to novel clinical therapies (3).

In the last decade, dental pulp stem cells have become a promising tool for the regeneration and repair of the pulp-dentin complex (4). There are 2 main teeth stem cell candidates suitable for dentin regeneration, dental pulp stem cells and stem cells from exfoliated deciduous teeth (1). It was demonstrated that rat dental pulp stem cells from a fractured incisal portion of tooth crowns differentiate to odontogenic cells and thus have regenerative capability (5). Zheng et al (6) demonstrated that porcine deciduous pulp stem/progenitor cells together with an appropriate scaffold provide preclinical evidence for stem/progenitor cell–based dentin regeneration. The development and characterization of the tooth slice/scaffold model of dental pulp tissue engineering have been reviewed by Sakai et al (7).

Only a few studies have described the proteome of human dental pulp to date (8, 9). The first complex proteomic study investigating human dental pulp used 2-dimensional (2D) gel electrophoresis followed by tandem mass spectrometry (8). The second proteomic study used difference gel electrophoresis to create a proteome reference map during the odontoblast-like differentiation of dental pulp cells *in vitro* (9). The proteomics not only of the dental pulp but of the whole tooth was also recently reviewed (10).

The main aim of our study was to create a detailed list of the proteins present in human dental pulp tissue and to study the proteins in the pulp-dentin complex. The benefit of this article is its connection of these samples and results with our previous study, where the proteome of the dentin has been described (11). This enabled us to compare the tooth pulp proteins with the dentin proteome, thus contributing toward improving current understanding of the composition and functions of human teeth.

Methods

Sample Preparation

Five healthy and completely erupted permanent human third molars with closed apex (n = 5) were extracted for clinical reasons from 5 adults aged 22–23 years (2 women and 3 men). These were exactly the same teeth from the same people as in our previous study of human dentin (11). The teeth were extracted in a dental clinic after acquiring the patient's informed consent for tooth donation for research and in accordance with the Code of Ethics of the World Medical Association for experiments involving humans.

The cementum was removed, and each tooth was horizontally cut (below the level of the enamel). The roots were then crushed in a jaw vise into smaller fragments, and the dental pulp was carefully removed and washed with physiological saline. The pieces of pulp were lyophilized, frozen in liquid nitrogen, and stored for further experiments (up to 1 year at −80°C). The pulp sample taken from the roots of 1 tooth was about 2.5 mg dry weight.

The prepared lyophilized pulp samples (2 mg) were subjected to sonication (15 minutes, 20°C) in 360 μL lysis buffer (11), and the supernatant was taken for

subsequent 2-dimensional gel electrophoresis (2-DE) analysis, 125 μL (7-cm strip) or 300 μL (17-cm strip).

Separation by Gel Electrophoresis and Mass Spectrometry

Isoelectric focusing and separation by 2-DE were performed as described previously (11) on homogeneous 12.5% sodium dodecylsulfate–polyacrylamide gel, as well as in-gel digestion and extraction. The 2-DE gel analyses were performed on each sample twice.

Protein spots were excised from the coomassie-stained gels and then processed as described in Shevchenko et al (12). The resulting dried tryptic peptide extracts were stored at −80°C before analysis.

An analysis of the tryptic digests with nano-liquid chromatography tandem mass spectrometry (maXis, quadrupole-time of flight, as mass spectrometer) was performed as in the previous study (11).

Database Searches

Proteins were identified by correlating tandem mass spectra to the International Protein Index (v. 3.87) and SwissProt (v. 2/2012) databases by using the MASCOT online search engine for protein identification by using mass spectrometry data (http://www.matrixscience.com). The taxonomy was restricted to *Homo sapiens* to remove protein identification redundancy. Trypsin was chosen as the enzyme parameter. One missed cleavage was allowed, and an initial peptide mass tolerance of ±10.0 ppm was used for MS analysis and of ±0.05 Da for MS/MS analysis. Cysteines were assumed to be carbamidomethylated, proline and lysine to be hydroxylated, and serine, threonine, and tyrosine to be phosphorylated; methionine was allowed to be oxidated. All these possible modifications were set to be variable. The monoisotopic peptide charge was set to 1+, 2+, and 3+. Only significant hits (MASCOT score ≥ 60 for proteins and MASCOT score ≥ 20 for peptides) were accepted. The Peptide Decoy option was selected during the data-search process to remove false-positive results.

Results

The samples of human dental pulp were obtained from 5 third molar teeth (n = 5), and 342 proteins were detected in this tissue (Fig. 1, Supplemental Table S1). A comparison with the previously described dentin proteome (11) gave an overlap of 140 proteins (Fig. 2, Supplemental Table S1). Proteins shared with human plasma (natural or contaminant) were also recognized (168 pulp/plasma proteins) (Fig. 2, Supplemental Table S1) (13) (Supplemental Table S1 is available online at www.jendodon.com). Some of the proteins, 103, were detected in parallel in human pulp, dentin, and plasma; moreover, 37 shared pulp/dentin proteins were not observed in plasma (Table 1, Fig. 2) (13).

The human dental pulp proteins identified in this study have a variety of molecular functions and biological processes (Fig. 3, Supplemental Table S1) (Supplemental Table S1 is available online at

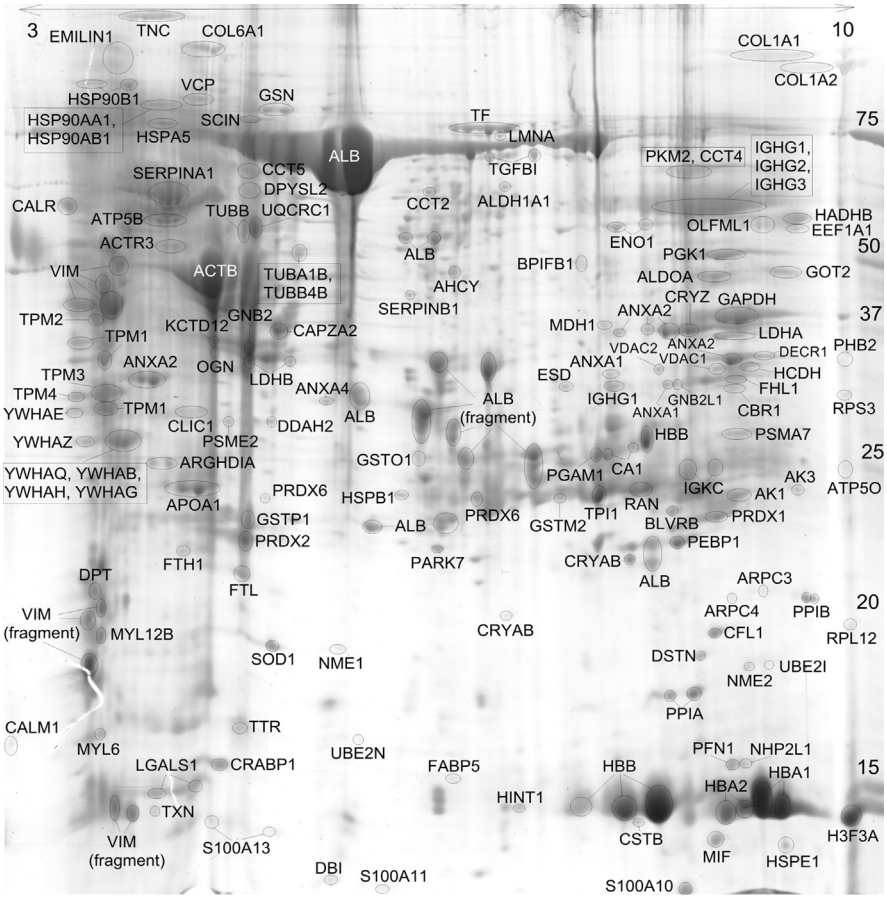

Figure 1. Representative 2-DE of human dental pulp from healthy human teeth: IPG strip pH 3-10 non-linear. All samples were separated in 12.5% sodium dodecylsulfate–polyacrylamide gels. Gels were stained with coomassie brilliant blue (CBB) dye. The symbols for the identified spots are characterized in Supplemental Table S1 (Supplemental Table S1 is available online at www.jendodon.com).

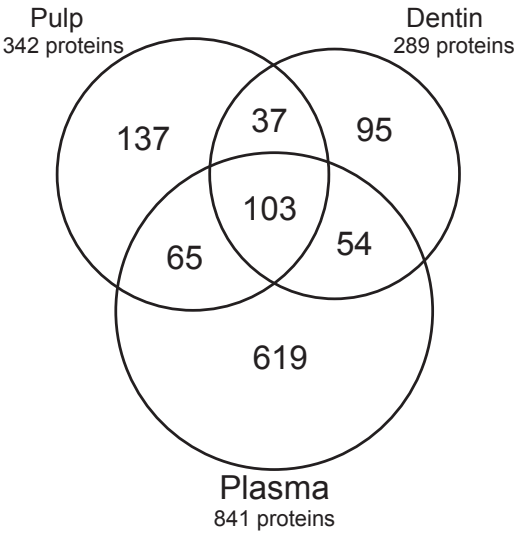

Figure 2. Venn diagram of proteins detected in human dental pulp, human dentin (11), and human plasma (13) by mass spectrometry.

www.jendodon.com). They were categorized according to the classification system used in the public database available at http://www.hprd.org.

The majority of the proteins identified in this study were involved in metabolism and energy pathways (23.7%) and cell growth and/or maintenance (20.5%). The next most significant functions were protein metabolism (14.0%), cell communication and signal transduction (12.3%), and immune response (7.9%). Some proteins had unknown functions (5.3%) (Fig. 3).

Discussion

This study is unique in identifying the proteome of a significant part of sound permanent human teeth (pulp and dentin combined, 491 proteins). A total of 342 proteins were identified in human pulp samples (Fig. 1). A comparison of these proteins with the previously mentioned article (8) revealed an additional 274 proteins in dental pulp (Supplemental Table S1) (Supplemental Table S1 is available online at www.jendodon.com). Many of these proteins had been detected in other human or animal tissues. These results provide the most comprehensive proteome list for human teeth to date. Wei et al (9) created the proteome reference map during the odontoblast-like differentiation of human dental pulp cells *in vitro*, and 23 proteins were identified by mass spectrometry. Four proteins were confirmed by Western blot (9), and 2 of them were identified in dental pulp in this study, annexin 6 and collagen type VI. This study also determined the 140 shared pulp/dentin proteins (Supplemental Table S1, Fig. 2) and compared them with proteins in human blood plasma (pulp/plasma, 168 proteins; dentin/plasma, 157 proteins) (Supplemental Table S1 is available online at www.jendodon.com). The blood proteins could be possible contaminants of the pulp sample, but on the other hand, these proteins could also be natural compounds for this tissue. One of the frequent problems encountered by investigators in proteomics is cross-contamination (skin keratins or laboratory dust) (14). In this study, 11 types of keratins were detected. They could be an integral part of the protein matrix in dental pulp but could also be attributed to skin contaminants.

The proteome of human dental pulp and dentin was compared with an ultra-high confidence list of 841 human plasma proteins published by Schenk et al (13) (Fig. 2, Supplemental Table S1) (Supplemental Table S1 is available online at www.jendodon.com). A comparison of the proteomes of tooth pulp and dentin with human blood provides for the first time an idea of the number of proteins that cooperate in the processes between these tissues (Figs. 2 and 3, Table 1). In total, 37 shared pulp/dentin proteins had not been observed in plasma. It could be concluded that these proteins might be candidates to participate in the unique pulp-dentin complex and thus have potential in future regenerative approaches (Table 1, Fig. 2) (3, 11, 13). A large number of proteins in this group (11 proteins) are involved in cell growth and/or maintenance processes. Interestingly, 4 proteins participate in immune response processes (IGL@ protein, zinc-alpha-2-glycoprotein, secernin-1, and galectin-1), another 4 proteins have calcium-binding functions (annexin A1, A2, A5, and calmodulin), and 4 proteins are involved in protein/peptide degradation (cytosol aminopeptidase, secernin-1, alpha-1-antichymotrypsin, and ubiquitin carboxyl-terminal hydrolase isozyme L1) (Table 1). The molecular function and biological process of the polymeric glycoprotein olfactomedin-like protein 1 are unknown. Some of these proteins have already been found in several studies focused on analyzing teeth tissues (especially in connection to dental stem cells), and they might play an important role in the pulp-dentin complex. The more that is known about the protein composition of dental-pulp complex, the clearer the mechanism of dentin reparation and defense processes will be. Clarification of this mechanism is crucial and could open up new possibilities for novel clinical therapies.

The proteins in the tooth pulp of sound and fully erupted molars, obtained from young adults (aged 22–23 years) and previously exposed to the oral environment, were investigated. These healthy molars did not have caries and any injury or inflammation. This fact could influence the observed tooth pulp proteome and should be taken into consideration when interpreting the proteomic results of this study. Any changes in tooth pulp condition (eg, the pulp of injured/inflamed teeth or immature teeth) will most likely lead to changes in the pulp proteome. Proteins with published significance on the function and physiology of the pulp and/or pulp-dentin complex are further discussed.

In this study, 16 proteins were identified in dental pulp that were previously described in human cementum (15), and 5 of them contained shared pulp/dentin proteins that were not observed in plasma: annexin A2, adenosine triphospate synthase subunit beta, biglycan, myosin light polypeptide 6, and prelamin-A/C. The proteins carbonic anhydrase I and II were successfully visualized in the *in situ* pellicle layer (16). The proteins biglycan, lumican, and histone H2B were shown to directly interact with calcium phosphate minerals in a bovine bone biomineralization study (17). One recent study declared that asporin promotes osteoblast collagen mineralization (18). It can be speculated that these proteins might play an important role in tooth biomineralization. The actin-binding gelsolin-like protein adseverin was observed to be dramatically up-regulated during chondrocyte maturation from chicken embryos (19). It demonstrates that interdependence of cytoskeletal organization and chondrogenic gene expression is regulated, at least in part, by actin-binding proteins such as adseverin. It can be concluded that protein adseverin might play a similar role in tooth tissues. These findings could contribute to better understanding the mechanisms of the processes, which take place inside the oral cavity.

The immune defense role of dental pulp could play an important role in dental caries (20). Elevated levels of broad-spectrum antimicrobial proteins S100A8, S100A9, and S100A13 were detected in the pulp of carious teeth (21). McLachlan et al (20) characterized pulpal tissue with carious lesions, and the up-regulation of alpha-1-acid glycoprotein 1 was confirmed. Higher levels of zinc-alpha-2-glycoprotein and immunoglobulin (Ig) gamma-2 chain C region were increased in the whole unstimulated saliva of periodontitis patients (22). A total of 27 proteins participating in the immune response in dental pulp were found. Immunoglobulins (IGL@ protein, Ig alpha-1 chain C region, Ig gamma-2

TABLE 1. List of Human Dental Pulp/Dentin Shared Proteins Not Observed in Plasma (11)

Accession UniprotKB	Name	Molecular function	MASCOT score	No. of peptides	Sequence coverage (%)
P07355	Annexin A2	Calcium ion binding	1755	31	76
P68363	Tubulin alpha-1B chain	Structural constituent of cytoskeleton	1276	24	60
Q71U36	Tubulin alpha-1A chain	Structural constituent of cytoskeleton	1236	3	60
P27348	14-3-3 protein theta	Receptor signaling complex scaffold activity	1188	14	67
P08758	Annexin A5	Calcium ion binding	1173	23	75
P04083	Annexin A1	Calcium ion binding	1047	19	60
P02461	Collagen alpha-1(III) chain	Extracellular matrix structural constituent	1017	22	26
P06576	Adenosine triphosphate synthase subunit beta, mitochondrial	Transporter activity	1003	26	54
P21266	Glutathione S-transferase Mu 3	Glutathione transferase activity	778	19	75
P29762	Cellular retinoic acid-binding protein 1	Transporter activity	777	13	88
Q08257	Quinone oxidoreductase	Oxidoreductase activity	775	15	68
P01011	Alpha-1-antichymotrypsin	Protease inhibitor activity	607	13	37
P02545	Prelamin-A/C	Structural molecule activity	605	15	29
P07900	Heat shock protein HSP 90-alpha	Chaperone activity	592	13	26
Q16555	Dihydropyrimidinase-related protein 2	Cytoskeletal protein binding	515	14	35
P09382	Galectin-1	Receptor binding	484	9	72
Q6UWY5	Olfactomedin-like protein 1	Unknown	450	11	39
P08107	Heat shock 70 kDa protein 1 A/1B	Chaperone activity	444	11	22
Q6GMX3	IGL@ protein	Antigen binding	444	8	42
P48681	Nestin	Structural constituent of cytoskeleton	405	9	12
P62879	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2	GTPase activity	347	8	29
P13489	Ribonuclease inhibitor	Translation regulator activity	314	6	22
Q9Y6U3	Adseverin	Cytoskeletal protein binding	307	7	22
P28838	Cytosol aminopeptidase	Aminopeptidase activity	293	8	25
P49189	4-trimethylaminobutyraldehyde dehydrogenase	Catalytic activity	276	8	17
Q9BXN1	Asporin	Extracellular matrix structural constituent	249	4	20
P25311	Zinc-alpha-2-glycoprotein	Cell adhesion molecule activity	218	5	30
Q07507	Dermatopontin	Extracellular matrix structural constituent	217	6	28
P02458	Collagen alpha-1(II) chain	Extracellular matrix structural constituent	205	2	4
P21810	Biglycan	Extracellular matrix structural constituent	188	7	23
O95865	N(G),N(G)-dimethylarginine dimethylaminohydrolase 2	Hydrolase activity	180	4	21
Q12765	Secernin-1	Peptidase activity	167	2	15
Q9NRN5	Olfactomedin-like protein 3	Extracellular matrix structural constituent	151	5	12
P60660	Myosin light polypeptide 6	Structural constituent of cytoskeleton	147	4	48
P07108	Acyl-CoA-binding protein	Receptor binding	111	3	32
P62158	Calmodulin	Calcium ion binding	100	2	28
P09936	Ubiquitin carboxyl-terminal hydrolase isozyme L1	Ubiquitin-specific protease activity	79	2	15

chain C region, Ig gamma-3 chain C region) and zinc-alpha-2-glycoprotein, secernin-1, and galectin-1 could also play a role in protecting dental tissues against plaque bacteria and/or against the development of dental caries (Supplemental Table S1) (Supplemental Table S1 is available online at www.jendodon.com). The identification of these proteins participating in immune response in the pulp samples (in total, 7.9% of all identified pulp proteins) shows that these proteins probably act as an immunologic reservoir in sound tooth pulp tissue and can be important for the response to various pathological agents. Such knowledge could be very important for future investigations of defense mechanisms against tooth diseases.

This study confirmed the presence of approximately 100 proteins that are/could be involved in dental pulp stem cell differentiation and/or other processes. These findings could contribute to future stem cell tissue engineering studies for the regeneration of tooth tissues. A recent study (23) compared the proteomes of mesenchymal stem cell–like populations derived from bovine:

1. Bone marrow
2. Periodontal ligament
3. Dental pulp

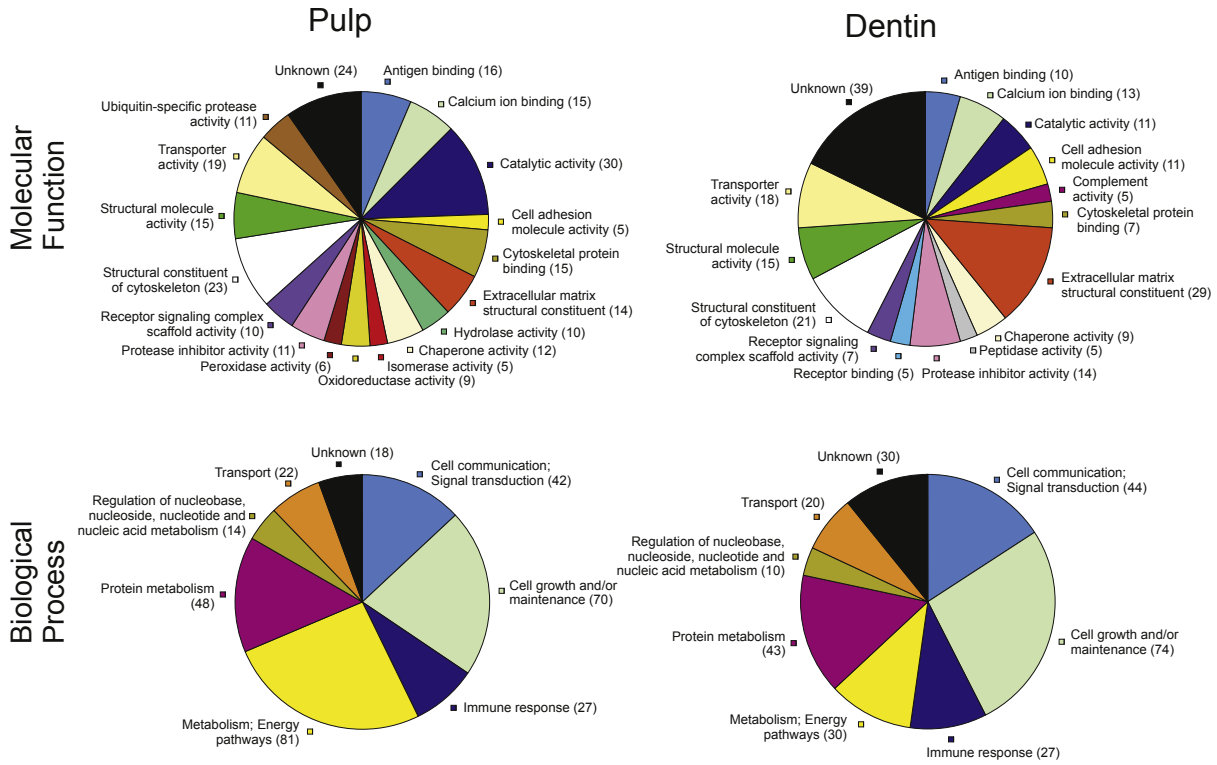

Figure 3. Distribution of molecular functions and biological processes of proteins found in human dental pulp and dentin. Molecular functions/biological processes of proteins to which at least 5 proteins were assigned are listed (the number of proteins is given in brackets). The functions of proteins in biological processes were categorized according to the classification system used in the public database available at http://www.hprd.org.

It showed the up-regulation of 5 proteins in dental pulp compared with 2 other kinds of stem cells. Of these 5 proteins, the following 2 were detected in dental pulp: ubiquitin carboxyl-terminal hydrolase isozyme L1 and Rho GDP-dissociation inhibitor 1. Ubiquitin carboxyl-terminal hydrolase isozyme L1 is a neuronal de-ubiquitinating enzyme that participates in ubiquitin/proteasome-mediated protein degradation. The up-regulation of this protein in dental pulp stem cells possibly reflects the neural crest origin of these cells and is consistent with its demonstrated ability to differentiate into functionally active neurons under the appropriate inductive conditions (24).

The disodium EDTA extract from calcified tooth parts significantly enhanced dental pulp stem cell odontoblast differentiation and mineralization *in vitro*, but it only had a partial effect on bone marrow stem cells or adipose tissue stem cells. In total, 147 proteins were identified in this EDTA extract from calcified tooth parts (25). Presence of 76 of these proteins was confirmed in this study, and 10 of them were shared pulp/dentin proteins that were not previously observed in plasma: annexin A1, A2, and A5, biglycan, IGL@ protein, cellular retinoic acid binding protein 1, asporin, prelamin A/C, olfactomedin-like protein 1, and nestin. These findings are strong evidence of the importance of specific interactions inside the dental-pulp complex (25). Annexins A1, A2, and A5 belong to a family of calcium-dependent phospholipid membrane-binding proteins. Annexins seem to play a role in various cellular activities such as vesicle trafficking, calcium signaling, cell division, cell growth regulation, and apoptosis (26). Bonnamain et al (27) observed the expression of tubulin beta-3 chain and nestin in the non-adherent cell population of human dental pulp stem cells (nestin is a marker of neural stem/progenitor cells). These spheroid non-adherent cells seem to be more involved in the odontoblastic lineage than the adherent cell populations. Vimentin was proposed to act as a quality standard for pulp regeneration and pulp cell function in a study of pulp stem/progenitor cells (28). Vimentin is an intermediate filament protein that organizes a number of critical proteins implicated in adhesion, migration, and cell signaling. A recent study has shown that vimentin regulates epithelial-to-mesenchymal transition-associated induced migration (29). The similar role of vimentin might be expected in dental pulp tissue. Asporin plays an important role in predentin mineralization (calcium deposition), because its expression is high in the early phase, and then it decreases during the late phase of the odontogenic differentiation of human adult pulp stem cells (30). The results of the study by Lee et al (31) suggest that a preameloblast-conditioned medium (from mouse apical bud cells) induces the odontogenic differentiation of human dental pulp stem cells and promotes dentin formation *in vivo* and also *in vitro*. In total, 23 proteins were identified in this preameloblast-conditioned medium (31), and the following 10 proteins were detected in human pulp: collagen alpha-1(III) chain, prelamin-A/C, 14-3-3 protein beta/alpha, 14-3-3 protein epsilon, actin cytoplasmic 1, fructose-bisphosphate aldolase A, collagen alpha-1(I) chain, pyruvate kinase isozymes M1/M2, alpha-actinin-1, heat shock protein HSP 90-beta, and elongation factor 2 (Supplemental Table S1) (Supplemental Table S1 is available online at www.jendodon.com).

In addition, 2 proteins were detected in the human body for the first time in the present study, a putative tropomyosin alpha-3 chain-like protein (TPM3L_HUMAN) and the putative uncharacterized protein DKFZp686I15196 (Q6N096_HUMAN) (Supplemental Table S1) (Supplemental Table S1 is available online at www.jendodon.com). Identification of these proteins and their localization in the human dental pulp could contribute in future tooth investigation about their function in this tissue.

In conclusion, this study improves current understanding and provides the broadest and most comprehensive proteome map of

human tooth pulp to date. A total of 342 proteins were identified; many of them had not been previously detected in human tooth pulp.

Acknowledgments

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The authors deny any conflicts of interest related to this study.

Supplementary Material

Supplementary material associated with this article can be found in the online version at www.jendodon.com (http://dx.doi.org/10.1016/j.joen.2014.07.001).

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