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БЕЛКИ МИНЕРАЛИЗОВАННЫХ ТКАНЕЙ ЗУБОВ

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Резюме. Твердость зубных тканей является результатом биоминерализации внеклеточного матрикса: аморфное внеклеточное вещество становится микроструктурированным и постепенно насыщается соединениями кальция и фосфора до степени, позволяющей выдерживать высокие механические нагрузки. В этом процессе участвуют гидроксиапатит (HAP), образующий в матриксе громоздкие кристаллические структуры, и многочисленные белки, вырабатываемые специализированными клетками. Белки внеклеточного матрикса играют ключевую роль, как формирующую, так и регулирующую. Современная стоматология уверенно переходит на молекулярно-генетический уровень. В данном обзоре рассматриваются основные матричные белки минерализованных тканей зубов — белки эмали, дентина и цемента. Они являются уникальными агентами, задающими характер минерализации и специфическую функцию этих тканей в составе зуба. Большое внимание уделено роли генетических нарушений в стоматологической патологии. Обзор включает данные о нарушении развития зубов у лабораторных животных с искусственно нарушенной генетической регуляцией белков минерализованных тканей зуба. Среди протеаз матрикса дентина особое внимание уделено матриксным металлопротеазам, которые представляют собой группу кальцийзависимых цинксодержащих эндопептидаз, участвующих в основном в развитии и ремоделировании внеклеточного матрикса благодаря своей способности расщеплять органические молекулы. Обзор будет способствовать более глубокому пониманию врачами-стоматологами сути процессов, протекающих в матриксе зуба при его минерализации, и роли специфических белков в этих процессах.

Ключевые слова: белки; эмаль; дентин; цемент; кариес.

PROTEINS OF MINERALIZED DENTAL TISSUES

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Abstract. The hardness of dental tissues is a result of the extracellular matrix biomineralization; the amorphous extracellular substance becomes microstructured and gradually saturated with calcium and phosphorous compounds to the extent that allows withstanding high mechanical loads. This process involves hydroxyapatite (HAP), which forms bulky crystalline structures in the matrix, and numerous proteins produced by specialized cells. Proteins of the extracellular matrix play a pivotal role, both formative and regulatory. Modern stomatology is confidently passing on to the molecular genetic level. This review addresses the major matrix proteins of mineralized dental tissues — enamel, dentin and cementum proteins. They are unique agents that set the mineralization pattern and specific function of these tissues within a tooth. Much attention is paid to the role of genetic disorders in dental pathology. The review includes data on impaired dental development in laboratory animals with artificially disrupted genetic regulation of mineralized

dental tissue proteins. The review will promote deeper insight into the core of the processes proceeding in the dental matrix during its mineralization and the role of tooth-specific proteins in these processes in health and disease. Among dentin matrix proteases, special attention has been paid to matrix metalloproteases, that compose a group of calcium-dependent zinc-containing endopeptidases involved mainly in the development and remodeling of the extracellular matrix due to their ability to break down organic molecules.

Key words: proteins; enamel; dentin; cementum; caries.

INTRODUCTION

Teeth are regularly exposed to mechanochemical effects of dense substances during the initial, mechanical, stage of digestion — grinding food in the oral cavity. The hardness of dental tissues is a result of biomineralization of the extracellular matrix: the amorphous extracellular substance becomes microstructured and gradually saturated with calcium and phosphorous compounds to the extent that allows withstanding high mechanical loads. This process involves hydroxyapatite (HAP), which forms bulky crystalline structures in the matrix, and numerous proteins produced by specialized cells. Proteins of the extracellular matrix play a pivotal role, both formative and regulatory.

Human teeth are composed of three types of hard, i.e. mineralized tissues — enamel, dentin and cementum. The fourth type — pulp — a composite soft, i.e. non-mineralized, tissue is beyond the scope of this review. Enamel covers the crown of a tooth and is the hardest tissue in a human body. It is this tissue that forms a cutting edge which is the first to confront yet unground food. Cementum is an outer layer of the root of a tooth. Its structure (mineralization pattern) shares a considerable similarity with bone tissue. Together with the fibers of the periodontal ligament, cementum holds a tooth in the alveolar socket and prevents both an expansion of surrounding tissues and penetration of pathogenic microorganisms into a tooth. Dentin makes up the bulk of a tooth and separates enamel in the crown and cementum in the root from the pulp chamber and root canals. This dynamic calcified tissue also has a mineralization pattern which determines its density, elasticity, width, permeability for chemical substances, and regenerative ability.

This review addresses the major matrix proteins of mineralized dental tissues — unique agents that determine the mineralization pattern and specific function of these tissues. It is the authors' hope that their efforts will promote deeper insight into the processes that occur in the tissue matrix during its biomineralization and the role of proteins in these processes both in normal and pathological conditions. The most studied of these proteins are listed in Table 1.

ENAMEL PROTEINS

Enamel is a dense outermost sheath that covers the crown of a tooth. Structurally, like dentin, it is a mineralized framework of organic molecules, although much stronger and harder. About 96% of the MM of mature enamel is composed of HAP crystals, while the remaining 4% fall on water and organic compounds. Such a solid structure of enamel ensures its protective properties, as well as its major function in the digestive system — primary (mechanical) food processing in the oral cavity [120].

Enamel synthesis and formation. Enamel formation results from biominaralization of the amorphous organic matrix which is produced by epithelioid cells — ameloblasts. These cells secrete matrix components from the Tomes's processes — projections situated on their basal (secretory) end — toward the enamel-dentin junction. During this process, ameloblasts move away from the enamel and thus always remain at the periphery of the growing tissue (amelocytes and odontocytes prove to be separated by the enamel and dentin layers, respectively).

Enamel consists of prisms and a surrounding inter-prismatic substance which differ in the direction of HAP crystallization: intra-prismatic crystals are oriented along the prism's longitudinal axis, while inter-prismatic crystals are oriented otherwise (mainly perpendicular) [120].

Extracellular matrix proteins

ECM proteins include amelogenin, ameloblastin, and enamelin. On human chromosomes, the ameloblastin and enamelin genes are localized to h4q13, while the amelogenin genes (AMELX and AMELY) reside at Xp22 and Yp11, respectively. At the earlier stages of evolution, the genes of all three enamel matrix proteins were parts of the same gene which encoded a secretory calcium-binding phosphoprotein (SCPP) [110], i.e. the amelogenin gene got to sex chromosomes as a result of translocation, while the ameloblastin and enamelin genes retained their initial location thereon [53, 57, 108].

Our current knowledge of the function of the enamel matrix proteins is reduced to the following [2, 75, 76, 109]:

1) they are essential for the normal enamel formation and involved mainly in its maturation as being secreted by ameloblasts as part of the enamel amorphous substance and almost completely lacking in a fully mineralized matrix (i.e. in mature enamel);
2) amelogenin, ameloblastin and enamelin genes turned into pseudogenes in toothless (enamelless) mammals, indicating a specific role of these proteins in the enamel formation.

Amelogenin is the most common protein of the enamel ECM. In humans, 90% of the MM of amelogenin is transcribed from the X chromosome (PCR-based AMELY gene detection is used

Table 1

Dental proteins

Protein	Characteristics	Function	Pathology*
Extracellular matrix proteins			
Collagen I *Type I Collagen*	**TL** — enamel, dentin, cementum **G** — ? **MM** — ?	Fibrillar collagen — scaffold for HAP crystals and noncollagenous proteins	Systemic connective tissue diseases
DSP *Dentin sialoprotein*	**TL** – dentin **G** — 4q22.1 **MM** — 52.5	Omnipresent localization. In small amounts increases while in large decreases HAP crystallization	Dentin mineralization density increase during gene knockout in mice
DGP *Dentin glycoprotein*	**TL** — dentin **G** — 4q22.1 **MM** — 19	Involved in dentin biomineralization	Undetermined
DPP *Dentin phosphoprotein*	**TL** — intratubular dentin at the mineralization **G** — 4q22.1 **MM** — 140	HAP nucleation. Association with collagen	Dentin undermineralization in mouse gene knockouts
DMP-1 *Dentin matrix protein 1*	**TL** — dentin **G** — 4q22.1 **MM** — 53.5	High affinity for collagen. High ability to bind calcium. Regulation of gene DSPP transcription. Regulation of mineralization	Hypophosphatemic rickets. Dentin malformation due to predentin *dentinogenesis imperfecta* type III
BSP *Bone sialoprotein*	**TL** — dentin, cementum **G** — 4q22.1 **MM** — 60-80	HAP nucleation. Crystal face bonding. Cell signaling, differentiation and aggregation	Cementum thinning. Atypical mineralization sites in mouse gene knockouts
OPN *Osteopontin*	**TL** — dentin, cementum **G** — 4q22.1 **MM** — 34	HAP nucleation. Crystal face bonding. Cell signaling, differentiation and aggregation	Cementum thinning. Atypical mineralization sites in mouse gene knockouts
MEPE *Matrix extracellular phosphoglycoprotein*	**TL** — dentin, cementum **G** — 4q22.1 **MM** — 57	Inhibition of mineralization. Regulation of cell differentiation	X-associated hypophosphatemic rickets
Amelogenin	**TL** — enamel **G** — Xp22 и Yp11 **MM** — 24	HAP crystal growth regulation during enamel matrix maturation	Enamel malformation: *amelogenesis imperfecta*, soft enamel, poor enamel-dentin bonding → chipping
Ameloblastin	**TL** — enamel **G** — 4q13 **MM** — 48	Enamel prism formation at initial stages of amelogenesis	Enamel malformation in mouse gene knockouts
Enamelin	**TL** — enamel **G** — 4q13 **MM** — 65	Enamel matrix formation. Induction of biomineralization. KLK4 activation	*Amelogenesis imperfecta* (various phenotypes)
ON *Osteonectin*	**TL** — dentin, cementum **G** — 5q33.1 **MM** — 33	Inhibition of mineralization	
Osteocalcin	**TL** — dentin, cementum **G** — 1q25–q31 **MM** — 6	Inhibition of mineralization	
MGP *Matrix Gla protein*	**TL** — cementum **G** — 12p12.3 **MM** — 10	Inhibition of mineralization	

Ending of Table 1

Protein	Characteristics	Function	Pathology*
TIMPs *Tissue inhibitors of metalloproteinases*	**TL** — dentin **G** — TIMP1 — X11.3 **TIMP2** — 17q25.3, **TIMP3** — 22q12.3, **TIMP4** — 3p25.2 **MM** — ?	Inhibition of MMPs	
Extracellular matrix proteases			
AP *Alkaline phosphatase*	**TL** — cementum **G** — 2q37 **MM** — 140	Pyrophosphate hydrolysis. Proliferation of cementocytes	Thin acellular cementum. Sites of atypical structure
KLK4 *Kallikrein 4*	**TL** — cementum **G** — 19q13.41 **MM** — 27	Substrates: amelogenin, ameloblastin, enamelin. Protein degradation during enamel maturation	*Amelogenesis imperfecta* (various phenotypes)
Matrix metalloproteases			
MMP2 *Matrix matelloproteinase 2, Gelatinase A*	**TL** — dentin **G** — 16q12.2 **MM** — 72	Substrates: collagen, decorin	Underlie caries pathogenesis. Enzyme deficiency causes enamel malformation
MMP3 *Matrix matelloproteinase 3, Stromelysin-1*	**TL** — dentin **G** — 11q22.2 **MM** — 54	Substrates: proteoglycans and noncollagenous proteins	
MMP8 *Matrix matelloproteinase 8, Collagenase 8*	**TL** — dentin **G** — 11q22.2 **MM** — 53	Substrate: type I collagen helix	
MMP9 *Matrix matelloproteinase 9, Gelatinase B*	**TL** — dentin **G** — h20q13.12 **MM** — 92	Protease activity: C-terminal telopeptide, denatured collagen, decorin	
MMP20 *Matrix matelloproteinase 20, Enamelysin*	**TL** — enamel **G** — 11q22.2 **MM** — 54	Substrates: amelogenin, DSPP. Activation of KLK4	
Cathepsin B	**TL** — dentin **G** — 8p23.1 **MM** — 43-46	Substrates: N- and C-terminal collagen telopeptides and proteoglycans	MMP-like role in caries pathogenesis
Cathepsin D	**TL** — dentin **G** — 11p15.5 **MM** — 10	Substrates: proteoglycans	
Cathepsin K	**TL — dentin** **G — 1p23.1** **MM — 23.5**	Substrates: type I collagen, N- and C-terminal telopeptides, collagen helix. Fibrillar collagens	

Notes: Protein TL — localization in tissue; G — gene localization; MM — molecular mass, kDa. *Mouse gene knockout phenotypes referred to when human dental pathologies are not yet documented.

in criminalistics for sex identification) [103]. In mouse amelogenin gene knockouts, enamel retains its structure during development but has smaller HAP crystals [132], while in mouse enamelin [48] and ameloblastin [28] gene knockouts mature enamel does not develop whatsoever, indicating a role of amelogenin in maturation, not formation, of the ECM.

More than ten AMELX gene mutations that cause *amelogenesis imperfecta* (AI) have been identified, accounting for about 5% of all occurrences of AI. The mutant phenotype in women includes vertical (craze) lines on teeth and alternate normal and hypoplastic enamel regions. Men with such mutations exhibit a similar but more pronounced phenotype [11].

No occurrences of AI caused by a mutation in the AMELY gene are identified so far. Moreover, in two men with a detected mutation in this gene there were no AI manifestations [61]. In humans lacking the AMELX gene, like in mouse AMELX gene knockouts, enamel was found to be thin but well-formed [11].

Ameloblastin is the second most common protein of the enamel ECM [60]. Immunohistochemically, the ameloblastin breakdown products were localized to the sheath space which

surrounds each prism by a thin layer [12]. Full-length ameloblastin was detected in newly formed enamel where it accumulated on the surface of enamel prisms [48, 79]. From this, it can be inferred that the full-length molecule of the protein participates in the processes that occur in the depth of the mineralization front, while its breakdown products are involved in the formation of the sheath space [12].

Mutations in the ameloblastin (AMBN) gene that cause AI are not detected in humans. In mouse AMBN gene knockouts, dentin is covered by a thin layer of the aprismatic and crystalless substance, i.e. structurally differs from the normal enamel [28, 130]. Simultaneously, ameloblasts were observed to behave abnormally: during maturation, normal ameloblasts disrupted the underlying basement membrane and formed the Tomes's processes in its place. Instead, mutant cells, after the destruction of the basement membrane, came off, lost their polarity and, while clashing with each other, formed multilayered cell structures, indicative of a possible contribution of ameloblastin to cell adhesion [28].

Enamelin in humans is transcribed from the ENAM gene and in the form of a phosphorylated glycoprotein is secreted to the ECM, undergoing there several proteolytic treatments [26, 39, 44]. One of the enamelin fragments (32 kDa) was established to accumulate in the deep enamel layers [102]. It is this enamelin breakdown product that is most conserved in mammals [1], indicative of an important role of this domain in the enamel formation. Like the other enamel proteins, enamelin involved exclusively in the formation of the enamel matrix is not synthesized in toothless (enamelles) mammals, as indicated by the transformation of the gene into pseudogene [76]. This protein is not synthesized *in vitro* due to a great complexity caused by post-translational modification, as well as the presence of several glycosylation sites.

The function of enamelin was studied on mouse ENAM gene knockouts which exhibited multiple abnormalities of amelogenesis (up to its agenesis), indicative of a key role of enamelin in the crystallization and formation of the enamel matrix [48, 71, 104, 112]. In humans, damage to the ENAM gene (or its mutation) results in AI with different phenotypes:

1) after damage to a single allele, the phenotype either did not show up [56] or was characterized by enamel dysplasia with hollows and depressions [40], horizontal lines [70], thin enamel edge [98];
2) after damage to both alleles, enamel is extremely thin or absent at all [40].

In most of the registered occurrences, mutations in the ENAM gene caused autosomal dominant *amelogenesis imperfecta* [107].

Extracellular matrix proteases

As said above, most of the ECM proteins are present at the formative and developmental stages of amelogenesis, while in mature enamel their level is far lower: 30% during matrix secretion and 2% after complete maturation. Such an obvious difference is achieved due to activity of two major proteases of the enamel matrix — matrix metalloprotease 20 (MMP20), also called enamelysin, and a serine protease kallikrein 4 (KLK4), formerly called enamel matrix serine protease 1, (EMSP1) [10, 16, 22, 23, 47].

The maxima of MMP20 and KLK4 proteolytic activities are separated in time: MMP20 peaks at the earlier or secretory [10] and KLK4 at the later [21, 90, 110, 111, 118, 119] stages of amelogenesis.

MMP20 is a tooth-specific member of the metalloprotease family. Genetic and cytological studies revealed traces of MMP20 gene expression in none of the normal (benign) cells except ameloblasts [7] and odontoblasts, although its expression was detected in some malignant cells [59, 116, 117, 125]. This suggests a normal catalytic activity of MMP20 during amelo- and dentinogenesis [18]. The suggestion is further supported by the detection of the MMP20 pseudogene in mammals with reduced teeth or enamel [76].

MMP20 exhibits enzymatic activity toward amelogenin and ameloblastin at the early stages of the enamel matrix formation [5, 27, 63, 65, 78, 82, 101]. It was established *in vivo* that the 32 kDa enamelin is implicated in activation of KLK4 by splitting off its propeptide amino acid sequence. Experiments on MMP20 knockout mice demonstrated a characteristic aplastic phenotype: disrupted prismatic crystallization pattern, thin, exfoliated and chipped enamel [17]. The enamel thickness was reduced by 7−16%, degree of mineralization by 50%, enamel strength by 37% [6].

Seven MMP20 gene mutations are identified that cause several types of AI, including hypomaturation AI [1, 26, 102, 104] and mixed hypoplastic and hypomaturation AI [92, 129]. Being both homozygous and heterozygous, these mutations can be inherited in an autosomal recessive manner. These mutations provide a phenotype with normal-sized teeth but an obscure dentin-enamel borderline (X-ray image) and a tendency toward detaching enamel from dentin [30, 58, 62, 91, 92, 129].

Both the phenotype and structural alterations in enamel of mouse MMP20 gene knockouts and humans with a mutated MMP20 gene, as well as the presence of a pseudogene in mammals with reduced teeth or enamel, demonstrate the necessity of MMP20 for normal amelogenesis.

KLK4 is a glycosylated serine protease synthesized in ameloblasts at the stage of enamel matrix maturation [45–47]. KLK4 expression occurs in teeth only at the developmental stage [82, 102]. KLK4 is secreted as a proenzyme and activated after splitting off the propeptide sequence by metalloprotease 20 [45]. However this is not the only mechanism of KLK4 activation, as evidenced by KLK4 activity detected in MMP20 gene knockout mice [136]. Cathepsin C (CTSC) is considered to be the best candidate for the role of KLK4 activator *in vivo*. At least, its ability to activate KLK4 *in vitro* has been proved quite recently [123].

KLK4 was reported to exhibit proteolytic activity toward amelogenin [54], ameloblastin [18] and enamelin [102]. The pattern of this activity (KLK4 splits the enamel matrix proteins into small fragments) indicates a role of KLK4 in the elimination of the protein matrix products as enamel hardens at the later stages of amelogenesis [18].

Studies on mice with both KLK4 gene alleles knocked out revealed that amelogenesis proceeded normally until the onset of the maturation stage. However, after that there were detected neither a breakdown of proteins nor their backward transport to ameloblasts. As a result, the enamel matrix retained proteins which caused its elevated softness and a tendency toward being destructed during food mastication. One of the plausible reasons may be concerned with a protein layer that separates prisms and the inter-prismatic matrix at the formative stage. The removal of this layer at the maturation stage precedes a tight contact between these structures. In KLK4 null mice, the prisms were separated from the inter-prismatic matrix, which could be due to the lack of KLK4 proteolytic activity [105, 106].

In humans, there were identified two types of KLK4 gene mutations causing AI:

1) the homozygous nonsense mutation near the sequence encoding the enzyme's catalytic domain that was detected in a twin couple and entailed the same phenotype in both twins: yellow or brown enamel, high dental sensitivity to temperature drops, chipped enamel on the chewing surfaces of teeth [39];
2) the single-nucleotide deletion in both alleles of the 9-year-old girl, sharing the same phenotype that was caused by the above mutation: yellow to brown enamel with multiple chips [129].

Similar phenotypes both in mice and humans imply a key role of KLK4 in the enamel maturation.

DENTIN PROTEINS

Dentin is a dense dental tissue covered with enamel in the crown and cementum in the root. It encloses the dental pulp and makes up a major part of a tooth. Structurally, dentin represents a highly mineralized matrix.

Synthesis of dentin. Dentin derives from the amorphous extracellular substance (predentin) which is secreted by odontoblasts toward the dentin-enamel and dentin-cementum junctions. Due to an interaction between proteins and matrix proteases, the organic framework results, beginning to get covered with hydroxyapatite [HAP; $Ca_5(PO_4)_3(OH)$] crystals. By the end of odontogenesis, mature dense dentin occupies most of a tooth, while its amorphous precursor, predentin, is reduced to a thin layer. The proportion of crystalline HAP in the dentin molecular mass is ~70%, organic components amount ~20% and water ~10% [120].

Extracellular matrix (ECM) of dentin consists of two components: the dentinal canaliculi (tubules), which transverse the dentin layer, and spaces in between them [120]. The dentinal tubules are formed by odontocyte processes; the number and size of them decrease as the tubules move away from the pulp toward dentin-enamel or dentin-cementum junctions. The dentin ECM is subdivided into intratubular (ITD) and peritubular (PTD) dentin. ITD components are randomly distributed along the fibers of the tubule's inner surface and have a crystallization axis which is parallel to the tubule and perpendicular to the peridentin crystallization axis. Demineralized ITD represents a network of collagen fibers (90% of the total MM) covered by noncollagenous proteins. Peridentin that completely ensheathes the tubules is also a mineralized framework, however it is thinner and has noncollagenous polypeptides and phospholipids instead of collagen fibers [35, 131].

Dentin matrix proteins

Type I collagen is the major dentin protein that makes up 90% of the MM of matrix organic substances. The type I collagen molecule assembles from two α_1 and one α_2 chains that are released individually to the ECM in vesicles and then wind together to form a triple helical tropocollagen. This assembly occurs with the involvement of dentin proteases. Collagen fibrils serve as a scaffold for intratubular dentin, and their ability to self-organize underlies the formation of calcospherites — mineralized globules of collagen fibrils. Inside of this structure, on the surface of and even within fibrils, there are crystalline HAP flakes. Collagen structures retain up to 56% of the HAP MM [120]. In predentin, fibers that are parallel to the mineralization front make up about 88% (proximal third), 74% (middle third) and 62% (distal third) of all collagen fibers, indicating an expulsion of collagen fibers toward the biomineralization region [34].

Noncollagenous dentin matrix proteins (NMPs) are produced by odontoblasts and accumulate between collagen fibrils and at the periphery of dentinal tubules (in peritubular dentin). NMPs regulate intra-matrix processes, serving as crystallization nucleators, biomineralization stabilizers and inhibitors.

DSPP (dentin sialophosphoprotein) is a phosphorylated glycoprotein which is cleaved immediately upon secretion in odontocytes by matrix metalloproteases MMP2 and MMP20 into three daughter molecules [73, 134]:

1) DSP (dentin sialoprotein) — N-terminal DSPP fragment;
2) DGP (dentin glycoprotein) — central DSPP fragment;
3) DPP (dentin phosphoprotein or phosphophoryn) — C-terminal DSPP fragment.

Experiments on DSPP gene knockout mice demonstrated a close similarity between the associated pathology and *dentinogenesis imperfecta* type III, thus indicating a role of DSPP and its daughter molecules in the formation and development of dentin [114].

DSP (dentin sialoprotein) is an N-terminal fragment of the DSPP molecule which is low-phosphorylated, rich in aspartic and glutamic amino acids, as well as serine and glycine. It contains 350 amino acid residues and makes up ~5–8% of the NMP MM [135].

It was proved *in vitro* that DSP increases the number of HAP crystals at low (>25 μg/ml) and inhibit their accumulation at high (50–100 μg/ml) concentrations [13].

Using *in vivo* immunohistochemical methods, it was established that DSP is present both in dentin tubules and peritubular dentin. Experiments on mice with the retained ability to secrete DSP and suppressed ability to produce DPP showed an increase

in the total dentin volume, in contrast to DSP null mice (i.e. DSP gene knockouts) that exhibited a higher mineralization density of the dentin substance per its unit volume. Apparently, DSP is involved in the regulation and formation of an ECM, while DPP participates in the ECM initiation and maturation during mineralization [119].

DGP (dentin glycoprotein) is a central fragment of the DSPP molecule and may also play a role in dentinogenesis, although being found so far not in all mammalian species [134].

DPP (dentin phosphoprotein) and PP (phosphophorin) are products of the C-terminal DSPP fragment. DPP is a protein with a high content of phosphoserine and aspartic acid. It makes up about 50% of the dentinal NMP MM. DPP has a completely dephosphorylated C-terminal fragment of 244 amino acid residues, called DMP-2 (dentinmatrix protein 2). The calcium-binding ability of this molecule is far lower than in PP. While PP folds into a compact globular structure, DMP2 remains an intrinsically disordered protein. PP shows the *in vitro* ability to nucleate crystalline HAP flakes and interact with collagen fibrils [9]. Supposedly, the PP fragment of DPP is a mediator of mineralization [43], while DMP2 is irrelevant to this process [77]. *In vivo* immunohistochemical localization of DPP in intratubular dentin at the mineralization front also indicates its role as a dentinogenesis nucleator and mediator [96].

DMP1 (dentin matrix protein 1) is a highly phosphorylated acidic NMP due to a high level of serine and threonine. As a result of protease activity, it breaks down in dentin into the N- and C-terminal fragments. By interacting with other molecules, DMP1 regulates DSPP gene transcription [85]. Its molecules are localized to odontoblasts, microtubules and ameloblasts, being revealed immunohistochemically mainly at the mineralization front. DMP1 participates in the regulation of mineralization, as supported by its high calcium-binding ability and affinity for collagen fibrils. In *in vitro* experiments, DMP1 bound to collagen fibrils promotes precipitation of HAP crystal [30]. *In vivo* studies on DMP1 gene knockout mice also demonstrate a role of this molecule in biomineralization of the ECM [64]. DMP1 gene mutation is an important component of autosomal recessive hypophosphatemic rickets [95]. DMP1 ablation results in abnormal transformation of predentin into dentin, as well as hypomineralization, thus causing proliferation of the dental pulp and roots during postnatal development. The DMP1 mutant phenotype in mice strongly resembles dentinogenesis imperfect type III [137].

BSP (bone sialoprotein) is a glycoprotein in which carbohydrates make up about 50% of its MM, while the MM of the core protein is 33−34 kDa. The proportion of BSP in the MM of noncollagenous dentin proteins is ~1% [29]. BSP was shown to initiate *in vitro* crystallization, as achieved due to sufficient phosphorylation and the presence of sites bonding crystalline surfaces (faces) to each other [4].

OPN (osteopontin) is a partially phosphorylated ~34 kDa glycoprotein with a polyaspartate chain and phosphorylated Ser/Thr sites which mediate bonding of HAP crystals. OPN was demonstrated to be involved in inflammatory processes [113]. In OPN gene knockout mice, no changes in the dentin structure have been revealed. Also, the role of OPN in dentinogenesis still remains understudied. In *in vitro* experiments, it was shown that OPN with different degree of phosphorylation can be both an inhibitor and promoter of biomineralization [31].

MEPE (matrix extracellular phosphoglycoprotein). Experiments on MEPE gene knockout mice demonstrated that suppression of MEPE molecule secretion leads to matrix hypermineralization. Apparently, MEPE is an inhibitor of biomineralization. In *in vivo* experiments, the central fragment of the MEPE molecule (dentoin) was shown to participate in differentiation of dental pulp cells into odontocytes [14]. MEPE gene mutation causes X-associated hypophosphatemic rickets. In MEPE mutant mice, dentin shows intraglobular cavities that accumulate ECM molecules, while under normal conditions these molecules are diffusely distributed throughout dentin [15].

Dentin matrix proteases

MMPs (matrix metalloproteases) compose a group of calcium-dependent zinc-containing endopeptidases involved mainly in the development and remodeling of the ECM due to their ability to break down organic molecules. All known 23 forms of MMPs share the following structure [83]: they represent a signaling peptide containing a propeptide domain with a cysteine residue and a catalytic domain with a zinc-binding motif, as well as a C-terminal fragment resembling the hemopexin molecule. Dentin contains the following MMPs:

1) MMP2 (gelatinase A) — substrates: C-terminal telopeptide, denatured collagen, decorin;
2) MMP3 (stromelysin 1) — substrates: proteoglycans and other noncollagenous proteins;
3) MMP9 (gelatinase B) — substrates: C-terminal telopeptide, denatured collagen, decorin;
4) MMP8 (neutrophil collagenase) — substrate: helical portion of type I collagen;
5) MMP20 (enamelysin) — substrates: DSPP, amelogenin.

MMPs are synthesized in odontoblasts and secreted as proenzymes to the ECM where they are activated by other proteases, low pH [87], or some other agents, including reactive oxygen species. During odontogenesis, activity of MMPs is regulated by tissue inhibitors of metalloproteases (TIMPs), resulting in the attaining in the ECM an optimal balance between matrix biomineralization and matrix destruction [84]. Activity of MMPs is minimum during matrix assembly and increases when waste organic fragments of proteins and proteoglycans are to be eliminated from the ECM.

MMPs are involved in the pathogenesis of caries. After ultimate mineralization, the dentin ECM still harbors some residual inactive proforms of MMPs. Carious infection is accompanied by a decreasing pH which activates MMPs, including those disrupting type I collagen. This process leads to a slow destruction of dentin and deeper invasion of pathogenic microorganisms [72].

TIMPs (tissue inhibitors of metalloproteases) are specific protein agents that are expressed at the formative and remodeling stages of the tissue genesis. In vertebrates, four types of TIMPs

are described (TIMP1−4). All of them contain the N- and C-terminal domains. The N-terminal fragment provides a substrate-specific interaction of the molecule with the MMP's active center, thus terminating the proteolytic activity [24, 80, 128].

Cysteine cathepsins make up another group of endopeptidases also responsible for disrupting and remodeling the ECM. The following types of cysteine cathepsins dominate in dentin:

1) cathepisin B — substrates: type I collagen, terminal N- and C-telopeptides, some fragments of collagen chains; carious teeth was shown to have elevated CTSB levels [86];
2) cathepsin D — substrate: proteoglycans;
3) cathepsin K — substrates: type I collagen, terminal N- and C-telopeptides and some fragments of collagen chains [3, 122].

CEMENTUM PROTEINS

Cementum is a mineralized dental tissue structurally resembling bone tissue and covering dentin in the dental root. Together with the periodontium, cementum holds teeth in the alveolar bone and forms a protective layer separating root dentin from surrounding tissues.

Forty-five or fifty percent of the total MM of cementum are made up of HAP crystals, while the organic component (50%) is represented by type I collagen (90%) and noncollagenous matrix proteins. Other types of collagen are represented in smaller amounts, e.g., type III collagen is revealed at the odontogenetic stage or during reparative processes [33, 120].

Cementum protein fibers are subdivided into (a) the extrinsic or Sharpey's fibers deriving from the periodontium and arranged perpendicular to the root surface, and (b) the cementum's own fibers. Cementum is subdivided into cellular, acellular and intermediate. Acellular cementum covers the dental root as a thin layer. Cellular cementum contains cementocytes and covers exclusively root apices. Intermediate cementum is located in area of the cementum-enamel junction [37, 38].

Osteopontin (OPN) and bone sialoprotein (BSP) are noncollagenous proteins that perform similar functions in cementum. These highly phosphorylated proteins are nucleators and regulators of HAP crystal growth. Presumably, OPN and BSP are required for the initiation of crystallogenesis on mature type I collagen chains [100].

Both proteins contain acidic poly(amino acid) domains (polyaspartate in OPN and two polyglutamine domains in BSP) that promote calcium binding to crystal surfaces. Acidic arginine-glycine-aspartate sequences (RGD motifs) of these proteins serve as cell aggregation mediators [89, 113]. In *in vitro* experiments, it was proved that BSP can "stick together" growing HAP crystals, acting as a nucleator [52]. Localization of BSP and OPN on the surface of cementocytes during cementogenesis indicates their role in chemotaxis, cell-cell adhesion and differentiation of cementocytes [67–69].

Experiments on BSP gene shutdown in mice demonstrated a prime importance of this protein in the formation of acellular cementum. The mutant phenotype was characterized by multiple defects of the cementum structure, low degree of mineralization, and considerable thinning of the acellular cementum layer up to its complete disappearance [25].

Gla-containing proteins (or gamma-carboxyglutamate-containing proteins) are represented in cementum mainly by a vitamin K-dependent matrix gamma-carboxyglutamic protein (MGP) and osteocalcin.

Both of these proteins are secreted by cells of dense tissues (bone, dentin, cementum) into the ECM where they regulate the process of crystallization, as supported in particular by morphological alterations in acellular cementum in mice with relevant gene knockouts [55].

Based on the observed hypercalcification of the aorta and cardiac valves, it can be inferred that osteocalcin and MGP function as crystallization inhibitors. Osteocalcin was shown to inhibit the transformation of a phosphate mineral brushite into HAP [41, 66, 81, 93].

Osteonectin (ON) is an acidic protein of mineralized tissues, including dentin and cementum. In the latter, ON is secreted by cementocytes [99]. Based on the decelerated growth of HAP crystals in the presence of ON, as observed *in vitro*, it was suggested that this protein plays an inhibitory role in biomineralization [74].

Alkaline phosphatase (AP) is a nonspecific tissue hydrolase enzyme transcribed from several genes, including h2q37 [97]. AP exhibits an enzymatic activity toward the phosphate groups at pH 8 and also inhibits pyrophosphatase and ATPase at neutral pH. As in other tissues, AP is involved in proliferation of cementocytes [51, 133], phosphate metabolism and cementogenesis [8, 9, 36]. A special role of AP in the latter process is indicated by the phenotype of AP gene knockout mice. The relevant morphological alterations include a reduced layer of acellular cementum with numerous spot-like inclusions of atypical cementum around the attachment sites of the extrinsic Sharpey's fibers. In humans, mutation in the AP gene is associated with hypophosphatasia, and its specific phenotype is distinguished from that of the knockout mice by aplasia of the entire cementum, not only its acellular layer [126, 127]. A considerable role of AP in cementogenesis may relate to pyrophosphate hydrolysis which inhibits HAP crystallization [42, 88, 121].

ABBREVIATIONS

AI — amelogenesis imperfecta
AP — alkaline phosphatase
BSP — bone sialoprotein
CTSB — cysteine cathepsin B
CTSC — cathepsin C
CTSD — cysteine cathepsin D
CTSK — cysteine cathepsin K
CTSs — cysteine cathepsins (B, D and K)
DGP — dentin glycoprotein (DSPP)
DMP1 — dentin matrix protein 1
DPP — dentin phosphoprotein, phosphophoryn (C-terminal DSPP fragment)

DSP — dentin sialoprotein (N-terminal DSPP fragment)
DSPP — dentin sialophosphoprotein (DSP, DGP and DPP precursor)
ECM — extracellular matrix
EMSP1 — enamel matrix serine protease 1 (current name: KLK4)
HAP — hydroxyapatite
ITD — intratubular dentin
KLK4 — kallikrein 4 (former name: EMSP1)
MEPE — matrix extracellular phosphoglycoprotein
MGP — matrix Gla protein
MM — molecular mass
MMP2 — matrix matelloprotease 2
MMP3 — matrix matelloprotease 3
MMP8 — matrix matelloprotease 8
MMP9 — matrix matelloprotease 9
MMP20 — matrix matelloprotease 20
MMPs — matrix matelloproteases (2, 3, 8, 9 and 20)
ON — osteonectin
OPN — osteopontin
PP — phosphophoryn (product of C-terminal DSPP fragment)
PTD — peritubular dentin
SCPP — secretory Ca-binding phosphoprotein
SIBLINGs — small integrin-binding ligand N-linked glycoproteins
TIMP1 — tissue inhibitor of metalloproteases 1
TIMP2 — tissue inhibitor of metalloproteases 2
TIMP3 — tissue inhibitor of metalloproteases 3
TIMP4 — tissue inhibitor of metalloproteases 4
TIMPs — tissue inhibitors of metalloproteases (1–4)

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