# Dentin Sialophosphoprotein Signal in Dentinogenesis and Dentin Regeneration

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Dentinogenesis starts on odontoblasts which produce and secrete collagen and non-collagenous proteins (NCPs). When dentin is injured, dental pulp progenitors/mesenchymal stem cells (MSCs) can migrate into the injured area and differentiate into odontoblasts and facilitate formation of reactionary dentin. Dental pulp progenitor/MSC cell differentiation is controlled at given niches. Among dental NCPs, dentin sialophosphoprotein (DSPP) belongs to one of the SIBLINGs family members, sharing common biochemical features such as an arginine-glycine-aspartic acid (RGD) motif. DSPP expression is cell- and tissue-specific and highly seen in odontoblasts and dentin. DSPP mutations cause hereditary dentin diseases. DSPP is processed into dentin sialoprotein (DSP)/glycoprotein (DGP) and dentin phosphoprotein (DPP) by proteolytic cleavages. DSP is further catalyzed into the active molecules. DPP contains an RGD motif and abundant Ser-Asp/Asp-Ser repeat regions. The DPP-RGD motif binds to integrins αVβ3 and activates intracellular signaling via MAPK and FAK-Erk pathways. Unlike other SIBLING proteins, DPP lacks the RGD motif in some species. However, Ser-Asp/Asp-Ser repeat regions of DPP bind to calcium-phosphate deposition and promote hydroxyapatite crystal growth and mineralization via CaMKII cascades. Nevertheless, DSP lacks the RGD site, but contains signal peptides. The tripeptides of the signal domains interact with the cargo receptor within the endoplasmic reticulum (ER). The cargo receptor facilitates transportation of DSPP protein from ER to extracellular matrix (ECM). Furthermore, the middle- and COOH-terminal regions of DSP bind to cellular membrane receptors, integrin β6 and occludin, inducing cell differentiation. The review may shed light on roles
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Dentin sialophosphoprotein signal in dentinogenesis and dentin regeneration

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Abstract

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lacks the RGD motif in some species. However, Ser-Asp/Asp-Ser repeat regions of DPP bind to calcium-phosphate deposition and promote hydroxyapatite crystal growth and mineralization via CaMKII cascades. Nevertheless, DSP lacks the RGD site, but contains signal peptides. The tripeptides of the signal domains interact with the cargo receptor within the endoplasmic reticulum (ER). The cargo receptor facilitates transportation of DSPP protein from ER to extracellular matrix (ECM). Furthermore, the middle- and COOH-terminal regions of DSP bind to cellular membrane receptors, integrin β6 and occludin, inducing cell differentiation. The review may shed light on roles of DSPP during odontogenesis.

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Introduction

The tooth is a highly mineralized organ resulting from sequential and reciprocal interactions between dental oral epithelium and the underlying mesenchymal cells. The tooth is composed of enamel, dentin, and cementum as well as the soft connective tissues and periodontium (Fig. 1A) (Nanci, 2012; Mitsiadis et al., 2015). Dentin is a thick layer of highly mineralized tissue consisting of dentinal tubules and inter-tubular dentins beneath the enamel and serves as a second barrier of defense against infectious agents threatening the inner soft pulp tissue (Lopez-Cazaux et al., 2006). The dentinogenesis starts at the onset of odontoblast differentiation. Odontoblasts originate from the neural crest-derived mesenchymal cells, which then proceed in a series of cytodifferentiation to form odontoblasts in specific temporal-spatial patterns originating at the principal cusp tip and advancing toward the base of the teeth (Thesleff, 2003; Chen et al., 2008). Odontoblasts are post-mitotic cells organized as a layer of palisade cells along the interface between the dentin and dental pulp cavity. Odontoblasts synthesize and secrete the organic extracellular matrix (ECM) proteins (Linde and Goldberg, 1993; MacDougall et al., 1997). Dentin consists of the mostly hydroxyapatite (70% by weight) with the remaining approximately 12% of water as well as collagens and non-collagenous proteins (NCPs) (Linde and Goldberg, 1993; MacDougall et al., 1997). Odontoblasts are responsible for the formation of the physiological primary and secondary dentins. Also, odontoblasts maintain the dentin metabolism throughout the life of the tooth and serve as the first line of defense against dentin pathogen invasion by reactionary (reparative, RD, tertiary) dentin formation at the dentin-pulp interface beneath the carious infected dentin region (Couve et al., 2014).

Dental pulp is a loose connective and highly vascular tissue with a dense capillary plexus and an innervated tissue under the odontoblast layer. The blood vessels facilitate the exchange of nutrients and waste products in dental pulp (Tziafas et al., 2000; Lopez-Cazaux et al., 2006). Dental pulp cells (DPCs) are heterogeneous populations possessing a source for mesenchymal stem cells (MSCs) (Tirino et al., 2012). Maintenance of a healthy, vascularized, and innervated
dental pulp is necessary for a healthy tooth and dental regeneration (Huang et al., 2018). In the
dental pulp cavity, MSCs are thought to reside within perivascular microenvironments, termed
niches (Shi and Gronthos, 2003; Kaukua et al., 2014, Sui et al., 2019) and other localizations
(Gronthos et al., 2000; Miura et al., 2003; Seo et al., 2004; Morsczeck et al., 2005). However,
little is known on the exact locations and molecular regulations of the niches (Bluteau et al., 2008).
The role of specific local niches necessary for controlling cell fate specification, cell migration, and
differentiation in developmental and reactional events of dentin has been well recognized (Ruch,
1985). Dental progenitors/MSCs possess the ability to differentiate into new odontoblast-like cells,
which are capable of forming a dentin-like structure such as RD, for dentin repair after dentin
injury such as dental caries.

Dental caries and its managements

Dental caries, the most prevalent chronic infectious disease globally, is a dynamic biological
process of irreversible destruction of susceptible dental hard tissues because of acids produced
by bacterial glycolysis of dietary carbohydrates (Baker et al., 2021). The World Health
Organization (WHO) has represented the early childhood caries as a worldwide problem with a
prevalence between 60% and 90%, and more than 90% of all adults have experienced this
disease (Griffin et al., 2008, Bernabe et al., 2020; Kazeminia et al., 2020). Tooth decay leads to
disease of the dental pulp with sequent pulpal infection, necrosis and loss of tooth vitality and
function as well as eventual loss of the tooth. Various restorative materials have successfully been
used to fill and replace diseased or injured dental tissues (Wang et al., 2020b). However,
approximately 50% of cases require revision within 5-10 years after restorative treatment (Burke
and Lucarotti, 2009; Chen et al., 2020). In addition, any traditional artificial restorative materials
might fail due to inappropriate physical, biocompatible, and mechanical properties (Tziafas et al.,
2000; Goldberg and Smith, 2004; Yang et al., 2020a). The material pulls away from the cavity
wall and microleakage would form between the dentin layer and dental materials, causing
secondary or recurrent caries (Tziafas et al., 2000; Goldberg and Smith, 2004; Askar et al., 2021). Therefore, despite several advances of dental restorative materials, it is required for novel therapeutic restorative approaches in dentistry to maintain a healthy dentition. Therapies using tissue engineering, stem cells such as dental pulp mesenchymal stem cells, and other biomaterial components have successfully been reported by regenerating or replacing diseased and injured dental tissues (Saoud et al., 2016; Han et al., 2021). For instance, when pulpectomy was performed in animal models, a group of dental pulp progenitor/MSC cells can migrate to the injured areas and differentiate into odontoblast-like cells, forming RD (Vidovic et al., 2017). Consequently, the growth factor BMP2 plus dental pulp cells enhanced the dental pulp cell differentiation into odontoblast-like cells, which synthesize and secrete dental ECMs, forming RD in the injured areas (Nakashima, 2005; Ni et al., 2018). Besides BMP/TGF-β signaling, recent study has found that Wnt/β-catenin signaling induced progenitor/MSC cell proliferation and differentiation as well as promoted the RD formation (Neves and Sharpe 2018; Zaugg et al., 2020). Furthermore, it was reported that an artificial synthesized peptide, termed TVH-19, promoted human dental pulp cell differentiation and induced tertiary dentin formation in the rat model (Han et al., 2021).

Dental pulp mesenchymal stem cells

Stem cells are defined by dual stylemark features of self-renewal and differentiation potential. The self-renewal of stem cells can appear by symmetric cell divisions, which generate two daughter cells with the same fate, or by asymmetric cell divisions, which likely characterize a stem cell mode of division where one daughter cell is identical to the mother cell while the other develops different cell type (Götz and Huttner, 2005).

Stem cells are classified as embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs) and adult stem cells (ASCs). ESCs are derived from the inner cell mass of the blastocyst prior to implantation. They retain indefinite self-renewal potential and have the ability to generate
all cell types within the body. iPSCs can be generated by inducing expression of defined transcription factors in somatic cells resulting in their dedifferentiation back to a pluripotent state, which can then be differentiated to all cell types in given microenvironments. ASCs are groups of cells residing within different tissues through the body such as the bone marrow (BM). Unlike ESCs and iPSCs, ASCs are limited in their potency to the cell types of the tissue in which they reside. Although they normally keep in a quiescent, nondividing state, these cells can proliferate and differentiate to replace damaged cells within their tissue and accelerate tissue healing in response to injury (Pittenger et al., 1999; Cable et al., 2020; Yamanaka, 2020).

BM contains numerous different cell types arising from hematopoietic stem cells (HSCs), non-hematopoietic mesenchymal stem cells (MSCs) and other cell types, which are interconnected by a vascular and innervated network within the cavities of the BM. HSCs are capable of self-renewal and differentiation into mature blood cells or following migration into other hematopoietic or lymphoid organs. MSCs give rise to bone-forming cells (osteoblasts) and fat cells (adipocytes) and others, whereas bone-resorbing cells (osteoclasts) share a monocytic origin with macrophages. MSCs display variable self-renewal and differentiation potential (Friedenstein et al., 1970; Pittenger et al., 1999; Wilkinson et al., 2020). Endothelial progenitor/stem cells play an important role in bone marrow angiogenesis due to their relevant clonogenic potential, and these cells can be mobilized into the peripheral blood, differentiating into mature endothelial cells in newly formed blood vessels after tissue injury. Thus, bone marrow-derived endothelial cells likely represent a reservoir for the entire body angiogenesis and vasculogenesis. MSCs have been extensively characterized in vitro by the expression of various markers such as STRO-1, CD146 or CD44 (Pittenger et al., 1999). STRO-1 is a cell surface antigen used to identify osteogenic precursors in BM, CD146 a pericyte marker, and CD44 a mesenchymal stem cell marker. MSCs have a high self-renewal and potential to differentiate into mesodermal lineages, thus forming bone, cartilage, skeletal muscle, adipose and connective tissues (Pittenger et al., 1999; Wang et al., 2020a).
It has been known that adult dental pulp contains progenitors/MSCs capable of differentiating odontoblasts, osteoblasts, chondrocytes, adipocytes, and others in given environments. During dentinogenesis and tertiary dentin formation, the dental pulp progenitors/MSCs can differentiate into odontoblasts or odontoblast-like cells under appropriate signals (Gronthos et al., 2000; Miura et al., 2003; Sui et al., 2019). In a tooth, some cells can be either progenitors or transit amplifying cells and commit to terminal differentiation. These progenitors and transit amplifying cells have a limited lifespan, therefore can only reconstitute a tissue for a short period of time (Walker et al., 2019). By contrast, dental MSCs are self-renewing, thus, can generate any tissues for a lifetime.

In dental pulp and dentin, dental MSCs include stem cells from dental pulp stem cells (DPSCs), human exfoliated deciduous teeth (SHED) and stem cells from the apical part of the papilla (SCAP) (Fig. 1B) (Gronthos et al., 2000; Miura et al., 2003; Sonoyama et al., 2008).

DPSCs were first isolated from human permanent third molar teeth and are the most common source of dental pulp MSCs (Gronthos et al., 2000). Due to the lack of specific DPSC markers, generic MSC markers such as STRO-1, CD146, CD105 and CD44 are generally used for the identification and isolation of DPSCs (Pittenger et al., 1999; Wang et al., 2020a). DPSCs are able to differentiate into odontoblasts (Gronthos et al., 2000), osteoblasts (d'Aquino et al., 2009), chondrocytes (Waddington et al., 2009), adipocytes (Gronthos et al., 2002; Waddington et al., 2009), myoblasts (Pisciotta et al., 2015), and neurogenic cells (Martens et al., 2014) in the in vitro and in vivo studies.

SHEDs are derived from deciduous teeth and have a typical fibroblast morphology and express MSC specific markers such as CD45, CD90, CD106, CD146, CD166, and STRO-1 but not the hematopoietic and endothelial markers, CD34 and CD31 (Miura et al., 2003). SHEDs have a high proliferation rate capable of differentiating into odontogenic, myogenic, adipogenic, chondrogenic, osteogenic and neurogenic cells in vitro, and induce bone and dentin formation in vivo (Miura et al., 2003). As a member of neural crest-derived stem cells, SHEDs also express neural cell markers including Nestin, beta III tubulin and GFAP as well as some of the pluripotent
markers such as Oct4 and Nanog (Chai et al., 2000; Miura et al., 2003; Yang et al., 2019; Yang et al., 2020b). SHEDs exhibit increased gene expression patterns of osteocyte markers, including ALP, collagen type I, and runt-related transcription factor 2 (Runx2) as compared to BM MSCs in vitro. SHEDs were transplanted into the subcutaneous tissue in nude mice and promoted bone repair via inhibition of osteoclast activity in vivo (Yamaza et al., 2010). SHEDs can also differentiate into vascular endothelial cells forming functional blood vessels by up-regulation of the endogenous MEK1/ERK signaling (Bento et al., 2013). Due to their original deciduous teeth, SHEDs exhibit several characteristics similar to DPSCs. However, their proliferation and differentiation have high capacity compared to DPSCs and BM MSCs. (Bluteau et al., 2008).

SCAPs were isolated from the apical papilla at the root apex of the developing teeth and displayed highly proliferation rates and increased migratory and regenerative capacities compared with other dental MSCs (Sonoyama et al., 2008). Noticeably, SCAPs are easily accessible since they can be isolated from human third molars. As SCAPs are derived from the immature structure, they express primitive embryonic markers such as Sox2, Oct3/4 and Nanog accompanied with typical MSC markers (Sonoyama et al., 2008; Lee and Seo, 2016).

Among those markers, co-expression of CD146 and STRO-1 is found to be related to early MSCs. Indeed, CD146+/STRO-1+ SCAPs exhibit superior colony-forming efficiency with increased cumulative doubling compared with counterpart (Nada and El Backly, 2018). CD24, another marker for the pluripotent population, is regarded as a representative surface marker for SCAPs because of its absence in other dental MSCs (Kang et al., 2019). It is worth noting that these three markers tend to be declined with cell passaging, supporting their correlation with superior stemness. SCAPs are optimized for osteogenesis and odontogenesis considering that SCAPs are regarded as the precursors for primary odontoblast in vivo (Nada and El Backly, 2018; Du et al., 2020). However, SCAPs are multipotent and can also give rise to mesenchymal cell
lineages including adipocytes and chondrocytes (Yang et al., 2020b). Taken together, SCAPs are expected to exert significant roles in tissue regeneration and repair.

In addition to the dental MSCs (Gronthos et al., 2000; Miura et al., 2003), other MSC populations have been isolated from human dental tissues such as the periodontal ligament (Seo et al., 2004) and the dental follicle (Morsczeck et al., 2005). Stem/progenitor cells derived from oral cavity express several mesenchymal markers including CD29, CD73, CD90, and CD105 as well as embryonic markers such as Sox2, Nanog and Oct4 and can differentiate into multiple cell lineages (Miran et al., 2016). Importantly, some dental stem cells exhibit more embryonic-like features than bone marrow and umbilical cord stem cells (Miran et al., 2016; Sui et al., 2019). MSCs derived from the oral cavity are believed to be very important and valuable resources for eventual development for clinical/therapeutic applications not only in dentistry but also in medicine. However, little is known about how the progenitor/MSC cells differentiate into the given matured cells such as osteoblasts and odontoblasts as well as which niches promote these cell differentiations into the given cells.

SIBLINGs and dentin sialophosphoprotein (DSPP)

Niches can influence cell behavior and fate (Perry and Li L, 2007; Morrison and Spradling, 2008; Méndez-Ferrer et al., 2020). For instance, bone marrow ECM influences osteoblast differentiation into osteocytes while dental pulp ECM governs dental progenitor/MSC cell differentiation into odontoblastic cells (Chen et al., 2005; Chen et al., 2007; Guo et al., 2009; Vijaykumar et al., 2020). Bone and dentin are highly mineralized tissues and originate from osteoblasts and odontoblasts, which are derived from the mesenchymal cells. Both bone and dentin possess common characteristics and share similarities in their mineralization process. During this process, osteoblasts and odontoblasts synthesize and secrete ECM proteins into...
matrix forming osteoid and predentin, respectively. The organic matrix of osteoid and predentin is composed of collagens and NCP proteins necessary for the mineralization of collagen fibers during the conversion of osteoid and predentin to bone and dentin, respectively. The major common NCP proteins of bone and dentin include bone sialoprotein (BSP), osteopontin (OPN), matrix extracellular phosphoglycoprotein (MEPE), dentin sialophosphoprotein (DSPP) and dentin matrix protein 1 (DMP1) belonging to the SIBLINGs family (small integrin binding ligand N-linked glycoproteins) (MacDougall et al., 1997; Fisher and Fedarko, 2003; Bellahcène et al., 2008).

SIBLING genes are located on chromosome 4q21 in humans and chromosome 5q in mice, sharing a similar exon structure. The presence of the aspartate-glycine-aspartic acid (RGD) integrin binding motifs enables them to trigger intracellular signals by initiating integrin-mediated signaling. Although bones and teeth have common characteristics, the physical function between odontoblasts and osteoblasts reveals some differences (Chen et al., 2005; Chen et al., 2009; Vijaykumar et al., 2020). The specific functions of SIBLING family members in bone and dentin have been revealed through various genetic mouse models and a linkage to human diseases.

DSPP protein is the largest one of the SIBLING proteins with 1,301 amino acids in human and approximately 143 kDa extracellular matrix protein with essential roles in dentinogenesis (de La Dure-Molla et al., 2015). The DSPP gene contains 4 introns and 5 exons (Fig. 2) (MacDougall et al., 1997). Unlike other SIBLING protein family members, spatial and temporal expression of DSPP is largely restricted in pre-ameloblasts and odontoblasts during tooth development (D’Souza et al., 1997; Chen et al., 2009) and weakly detected in osteoblasts and non-mineralized tissues (Fig. 3) (Qin et al., 2002; Chaplet et al., 2006). For example, DSPP expression in odontoblasts and dentin is approximately 400-fold higher than that of osteoblasts and bone (Qin et al., 2002). Although DSPP is transcribed from a single gene (MacDougall et al., 1997), full length of DSPP protein has scarcely been isolated from cells or tissues, whereas its cleavage products, dentin sialoprotein (DSP) and dentin phosphoprotein (DPP) in mice, rats and humans as well as
DSP, dentin glycoprotein (DGP) and DPP in porcine, are most abundant NCPs in odontoblasts and dentin (Qin et al., 2001; Yamakoshi et al., 2005; Yuan et al., 2012).

The DSP protein is composed of the partial exon 2, exons 3, 4 and partial NH₂-terminal region of exon 5 of the DSPP, while the DPP consists of the most region of exon 5 of the DSPP (Fig. 2). DSPP protein is first processed into the DSP/DGP and DPP [also termed dentin phosphoryn (PP)] by bone morphogenetic protein 1 (BMP1), Tolloid-like (TLR) metalloproteinases and Astacin proteases (Steiglitz et al., 2004; Marschall and Fisher, 2010; Tsuchiya et al., 2011). DSP is further processed into small molecular fragments by MMP-2, MMP-9, and MMP-20 to expose cryptic binding sites into active molecules (Yamakoshi et al., 2006; Yuan et al., 2016). Mutations of the cleaved site between the DSP and DPP regions resulted in dentinogenesis imperfecta (DGI) phenotypes of teeth in mice, indicating that DSPP is required to cleave into active fragments, DSP and DPP (Zhu et al., 2012).

The porcine DGP has an 81-amino acid segment of the DSPP (Ser³⁹² to Gly⁴⁷²) between the DSP and DPP domains. The DGP has four phosphorylated serine residues (Ser⁴⁵³, Ser⁴⁵⁵, Ser⁴⁵⁷, and Ser⁴⁶²) and one glycosylated asparagine (Asn³⁹⁷). The DGP is a stain-all positive protein with an apparent molecular mass on SDS-PAGE of 19 kDa, which is reduced by glycopeptidase A digestion to 16 kDa. The porcine DGP is an equal number (12 each) of negatively charged (Asp plus Glu) and positively charged (Arg plus Lys) residues. This pig DGP contains most abundant amino acids with glycine (13) and serine (12). In the absence of post-translational modifications, the DGP has a calculated isoelectric point of 6.7. Due to containing 4 phosphorylated serines and sialic acids, the modified DGP increases the affinity of the protein for hydroxyapatite, which most likely facilitates the binding to dentin crystals. The identity of the porcine DGP amino acid sequence (NP_99842.1) is conserved with 58 (81%) for humans (AAF42472.1), 40 (49%) for rats (AAL79813.1) and 38 (47%) for mice (AAC12787.1) (Yamakoshi et al., 2005). How the DSPP...
protein is processed into the porcine DGP by proteinases and the DGP functions during
dentinogenesis is yet to be determined.

DSP and DPP play unique biological functions during tooth development (Paine et al., 2005; Suzuki et al., 2009). Mutations of either DSP or DPP domain in humans are associate with
dentinogenesis imperfecta (DGI) type II (DGI-II, OMIM #125490) and type III (DGI-III, OMIM 125500) as well as dentin dysplasia (DD) type II (DD-II, OMIM 125420) and type I (DD-I, MIM 125400) (Fig. 2, Tables 1 and 2). Those hereditary dentin disorders are the most common dentin genetic diseases. DGI estimated incidences occurred in 1/6,000-8,000 in people, while DD was 1/100,000 people (Witkop, 1975). DGI-II is characterized by opalescent discolored dentition, pulpal calcification, and bulbous crown shape as well as impaired odontoblast cell differentiation and delayed conversion of predentin to dentin (Fig. 4). DGI-III was originally thought to be specific to the Brandywine isolate and a severe form of DGI-II with multiple pulp exposures and shell-like teeth. DD-II is similar to DGI-II in the deciduous dentition, but tooth discoloration is minimal and dental pulp chambers are thistle-tube shaped with pulp stones in the permanent dentition. In DD-I, teeth are normal in shape, form, and consistency in the deciduous and permanent dentitions. In some cases, color of the teeth may display a slight amber discoloration. However, the roots are short and the pulp obliteration results in a crescent-shaped pulpal remnant in the permanent dentition and a total pulpal obliteration in the deciduous dentition. In mouse models, it was confirmed that the DSPP gene is required for dentinogenesis as homogenous null mice of the DSPP (DSPP -/-) gene showed tooth defects similar to those seen in patients suffering from DGI and DD with enlarged pulp chambers, a wider predentin zone, decreased dentin width, hypomineralization and the prevalence of dental pulp exposure (Fig. 4) (Sreenath et al., 2003; de La Dure-Molla et al., 2015).

SIBLING-RGD motifs are capable of binding to cell surface integrins in normal tissues and function as signal transducers to promote cell adhesion, spreading, motility, proliferation,
differentiation and survival through activating kinase cascades and transcription factors. Also, the biological roles of SIBLINGs are modulated by proteolytic processing to reveal cryptic binding sites and can expose functional domains, therefore modulating cell adhesion and activity. For example, OPN binds to a variety of integrins, including αvβ3, αvβ5, αvβ1, α4β1, α8β1, α9β1 and CD44 splice variants (Bellahcene et al., 2008; Marschall and Fisher, 2008). Thrombin cleavage of OPN separates the integrin- and CD44-binding domains, which in some cases promote adhesion over migration. Another example is the thrombin-cleaved NH₂-terminal OPN fragment when it interacts with αvβ3 and αvβ5 integrins through the RGD motif (Furger et al., 2003; Bellahcene et al., 2008) or to α9β1 and α4β1 integrins through the cryptic SVVYGLR sequence (Rangaswami et al., 2006) and promotes cell adhesion and migration. The COOH-terminal region of OPN binds to CD44 variant 6 (CD44v6) and/or to CD44v3 by a heparin bridge (Teramoto et al., 2005). In addition, OPN is also catalyzed by MMP-3 and MMP-7, and the cleaved OPN fragments facilitate cell adhesion and migration in vitro through activation of β1-containing integrins (Agnihotri et al., 2001). OPN was also shown to be a substrate for liver transglutaminase and plasma transglutaminase factor IIIa (Prince et al.,1991) and enhanced cell adhesion, spreading and migration (Higashikawa et al., 2007). The RGD domain of DMP1 only bound to αvβ3, while BSP-RGD motif not only interacted with αvβ3, but also with αvβ5 and enhanced cell adhesion and migration (Marschall and Fisher, 2008). The DMP1 is a substrate of BMP1 and BMP1 generated DMP1 fragment had similar binding efficiency with the intact DMP1 protein in cell attachment and migration (Steiglitz et al., 2004; Marschall and Fisher, 2008).

Dentin phosphoprotein (DPP)

DPP contains an RGD domain at the NH₂-terminal site of this protein, acting as a ligand, and binding to integrin αvβ3. The DPP-RGD/integrin-αvβ3 complex activated intracellular signaling pathways through up-regulating mitogen-activated protein kinase (MAPK), including p38, extracellular signal-regulated kinase½ (Erk½), and stress-activated protein kinase/Jun-amo-
terminal kinase (SAPK/JNK). Consequently, this complex up-regulated bone/dentin-related gene expression such as Runx2, Osterix (Osx), alkaline phosphatase (ALP), osteocalcin (Ocn) and Bsp as well as promoted cell differentiation and mineralization in human bone marrow stem cells (hBMSC), mouse osteoblastic cells (MC3T3-E1) and mouse fibroblastic (NIH3T3) cells (Jadlowiec et al., 2004; Jadlowiec et al., 2006). In addition, the DPP-RGD induced phosphorylation of paxillin and focal adhesion kinase (FAK), an activating transcription factor Elk-1 phosphorylation and up-regulated downstream gene transcription in mouse embryonic mesenchymal (C3H10T1/2) and primary dental pulp cells (Eapen et al., 2012) (Fig. 5). The flanking regions of the RGD motif influenced binding of the RGD to specific integrins and enhanced cell adhesion and migration (Marschall and Fisher, 2008; Suzuki et al., 2014). However, unlike other SIBLING family members, 46% (17/37) of DSPP genes from 37 species tested so far do not contain an RGD motif of the DPP protein, indicating that the RGD domain within the DPP may be rudimental (Suzuki et al., 2016).

In addition to these common domains, only the DPP domain of DSPP contains abundant serine-aspartic acid (Ser-Asp) or aspartic acid-serine (Asp-Ser) repeat regions, which are the most phosphorylated regions of the SIBLINGs and one of the most acidic proteins in species (Jonsson et al., 1978; Suzuki et al., 2016). The DPP binds to calcium ion and collagen type I, acting as an inductor of mineralization in ECMs and forming hydroxyapatite (HA) deposition and growth of vertebrate bones and teeth (He et al., 2005). The DPP is capable of interacting with cellular membrane, annexin 2 and 6, and facilitates calcium influx into cells (Alvares et al., 2013) while functioning as a cell-penetrating peptide promoting cellular uptake of components accompanying with the DPP and releasing different cargos intracellularly (Ravindran et al., 2013; Figueiredo et al., 2019). In addition, DPP-DSS (Asp-Ser-Ser) repeat regions can stimulate the release of intracellular Ca$^{2+}$. This calcium flux facilitated the activation of Ca$^{2+}$ calmodulin-dependent protein kinase II (CaMKII). Activated CaMKII enhanced the phosphorylation of
transcription factors, Smad^1/5/8^, and phosphorylated Smad^1/5/8^ proteins were translocated to the nucleus and up-regulated Smad^1/5/8^ downstream gene expression as well as promoted cell differentiation in murine pluripotent stem (C3H10T12) and hBMSC cells (Eapen et al., 2013) (Fig. 5). The study showed that the length of the Ser-Asp and/or Asp-Ser repeat regions varies among species but is not correlate with dentin hardness (Suzuki et al., 2016).

To analyze the relationship between length variations in Ser-Asp/Asp-Ser repeat regions and the role of the DPP in matrix mineralization, different lengths of the Ser-Asp/Asp-Ser repeat regions have been generated (Kobuke et al., 2015). The recombinant mouse DPP deleted 63.5 Ser-Asp repeat regions accounting for 36.5% of the length of the Ser-Asp repeat regions of the mouse full-length DPP were generated and the peptides were able to induce the precipitation of calcium-phosphate similar to that of the full-length DPP at the same molar concentration, whereas the flipped DPP deleted 63.5 Ser-Asp repeat regions had no effect on induction of the precipitation of calcium phosphate (Kobuke et al., 2015). The 8-repeat copy of Asp-Ser-Ser residues facilitated the calcium-phosphate precipitation and HA crystal growth, promoting the remineralization of demineralized human enamel and dentin tubule occlusion (Hsu et al., 2011).

The DPP-mimetic peptide molecules up-regulated expression of bone/dentin-related genes including Runx2, ALP, DMP1, OCN and collagen type I in human osteosarcoma (Saos-2) cells as well as promoted cell differentiation (Gulseren et al., 2019). The biological function of the DPP was narrowed to three Asp-Ser-Ser repeat peptides and the peptides were able to facilitate calcium-phosphate deposition on the human enamel surface and crystallographic structure of calcium-phosphate crystals in vitro (Chung et al., 2012).

For the in vivo study regarding the role of the DPP in biological activity, overexpression of the DPP transgenic gene under the control of the type I collagen promoter (DPP-Col 1α1 Tg) was crossed-bred with the DSPP knockout (KO) (DSPP -/-) mice to generate DSPP KO/DPP-Col 1α1 Tg mice (Zhang et al., 2018). The DPP-Col 1α1 Tg mice had an increase of the dentin thickness
and restored the dentin mineral density in the DSPP KO mice. The histochemistry study showed that abnormal widening of the predentin in the DSPP KO mice was narrower in the DSPP KO/DPP-Col 1α1 Tg mice. Scanning electron microscopy analysis showed that the dentinal tubule structure in the DSPP KO/DPP-Col 1α1Tg mice was better organized than that of the DSPP KO mice. The dentin mineral deposition rate in the DSPP KO/DPP-Col 1α1 Tg mice was significantly improved compared to that of the DSPP null mice as analyzed by the double fluorochrome labeling. The overexpression of DPP partially rescued the dentin defects in the DSPP KO mice, suggesting that DPP may promote dentin formation during dentinogenesis. In contrast, the body weight in the DPP-Col 1α1 Tg mice was lighter than that of the wild type mice. In the DPP-Col 1α1 Tg mice, skeletons had small and long bones were short compared to that of the wild type mice. The DPP-Col 1α1 Tg mice exhibited reduced trabecular bone formation and narrow proliferative and chondroblast layers of the long bones. Histochemistry analysis demonstrated that the proliferative zone of the long bones in the DPP-Col 1α1 Tg mice reduced cell proliferation and increased gene expression of chondroblast differentiation markers, including type II collagen (Col II, a marker for the proliferating chondrocytes), type X collagen (Col X, a marker for the hypertrophic chondrocytes) and proteoglycan, but lacked obvious defects in the chondrocyte differentiation (Zhang et al., 2016). Spontaneously, an overexpression of the DPP gene driven by the mouse amelogenin (Amg) promoter (DPP-Amg Tg) was generated. The DPP-Amg Tg mice created pitted and chalky enamel of nonuniform thickness, which was more prone to wear. The teeth with the DPP-Amg Tg mice resulted in disruptions of the prismatic enamel structure and weakened enamel with uneven thickness (Paine et al., 2005; White et al., 2007). The reasons for dual effects of the DPP on different tissue development and formation remain unclear. It may explain that the biological mechanisms of the DPP are cell- and tissue-specific. Spatial-temporal DSPP expression was detected in pre-ameloblasts and pre-odontoblasts at early stages of tooth development. In mouse postnatal stages of tooth formation, this gene expression was barely detected in ameloblasts, but continuously seen in odontoblasts, predentin and dentin, maintaining
odontoblast and dentin metabolism and homeostasis (Fig. 3) (D’Souza et al., 1997; Chen et al., 2009). However, the DSPP gene is weakly expressed in osteoblasts, chondrocytes, and bones (Qin et al., 2002; Chen et al., 2009). This suggests that a dose-dependent tune of the DSPP gene expression plays an important role in the cell- and tissue-biological activity and behaviors. For instance, Runx2 is necessary for osteoblast differentiation and bone formation (Ducy et al., 1997). Mutations of Runx2 in humans are associated with cleidocranial dysplasia (CCD), displaying short stature, late closure of fontanels and sutures, aplasia of clavicles, hypertelorism and low nasal bridge as well as dental disorders with supernumerary teeth, abnormal tooth eruption, and tooth hypoplasia (Lee et al., 1997). Runx2 gene is expressed at dental mesenchymal cells at the early stages and down regulated in odontoblasts at the later stages during odontogenesis (Chen et al., 2009). Runx2 up-regulated the DSPP gene expression in mouse pre-odontoblastic cell lines, but down-regulated the DSPP expression in mouse odontoblast cells (Chen et al., 2005). Runx2 null mice impaired tooth formation, progressing only to the cap/early bell stages of tooth development. The teeth in Runx2 null mice were misshapen, severely hypoplastic and lacked odontoblast and ameloblast differentiation, while exhibiting the loss of normal dentin and enamel matrices (D’Souza et al., 1999). By contrast, transgenic mice of Runx2 (Runx2 Tg) gene showed that odontoblasts lost their columnar shape, and dentin was deposited around the odontoblasts, which were cuboid and/or flat in shape. The dentin in the Runx2 Tg mice was thin and possessed lacunae that contained osteoblast and bone canaliculi-like structures. Predentin and dentinal tubules were absent. In the Runx2 Tg mice, collagen type I expression was down regulated and DSPP expression was lost (Miyazaki et al., 2008). Thus, the function of Runx2 is likely cell and tissue type-specific or dependent on the stages of cytodifferentiation during tissue development.

**Dentin sialoprotein (DSP)**

DSP lacks an RGD domain and Ser-Asp/ Asp-Ser repeat regions (MacDougall et al 1997; Suzuki et al., 2016). Many DSPP gene mutations occurred in the DSP region (Fig. 2, Tables 1
The DSP and peptides derived from the DSP can regulate gene expression and protein phosphorylation as well as induce dental primary/stem cell differentiation (Lee et al., 2012; Ozer et al., 2013).

The starting site of the DSP contains the signal peptides, which are required for intracellularly trafficking the DSPP protein from the rough endoplasmic reticulum (rER) to ECMs. The point mutations of the signal peptides such as p.6Tyr>Asp, p.15Ala>Val, p.17Pro>Leu, and p.18Val>Asp together with frameshift mutations resulting in longer mutant hydrophobic domains of the DSPP protein are associated with DGI-II, DGI-III and DD-II (Fig. 2, Tables 1 and 2). In a mouse model, an amino acid on Pro19 of the signal peptides of the mouse DSPP protein was substituted by an amino acid on Leu19 (Liang et al., 2019). The mutant mice of the DSPP (DSPPP<sup>P19L/P19L</sup>) displayed the similar symptoms of DGI-II and DGI-III in humans, showing enlarged dental pulp chambers in the mutant young mice, while smaller dental pulp chambers in the older mutant mice. These mutant mice exhibited an increase of enamel attrition and an excessive deposition of peritubular dentin. DSPP<sup>P19L/P19L</sup> mice reduced the DSPP expression in the odontoblasts as compared to that of the wild type mice. The secretion of the mutated DSPP protein was impaired and the mutant DSPP protein accumulated within rER. The mechanisms of the mutations of the signal peptides associated with DGI and DD are not completely known.

Recently, Yin et al. found that surfeit locus protein 4 [Surf4, also termed as ER-derived vesicles protein 29 (Erv29p)] is the cargo receptor, which has a high affinity of binding to the triple amino acids, IPV, within the signal peptides of the DSPP, but weakly binding to the mutant amino acids of the signal peptides. The wild type of the DSPP is exited from rER lumen of cytoplasm to ECMs. Specific changes in a single amino acid of the tripeptide of the DSPP resulted in aggregate formation within rER and failure to efficiently DSPP-traffic cargo out of the rER. The mutant signal peptide (s) of the DSPP accumulated in rER lumen, forming the damaging aggregates and degradation (Yin et al., 2018).
The DSP is an ECM protein and activates intracellular signaling when dental cells are treated with this protein (Lee et al., 2012; Ozer et al., 2013). How the DSP domain and its cleaved products facilitate intracellular signaling is unknown. Using DSP as bait for screening a dental cell protein library, we found that the DSP as a ligand, interacted with 4 cellular membrane proteins including occludin (Ocln), integrin β6 (β6), CD105 (endoglin) and collagen type IV (Wan et al., 2016, Li et al., 2017). The 36 amino acid residues of DSPaa183-219 were sufficient to bind to the cellular membrane receptor, β6, and the DSP-β6 complex stimulated phosphorylation of p38 and Erk1/2 protein kinases and phosphorylated transcription factors, Smad1/5/8 (pSmad1/5/8). The pSmad1/5/8 interacted with Smad4 and were translocated to the nucleus and bound to the DSPP regulatory region and up regulated the DSPP transcription as well as positively feedbacked the DSPP expression and odontoblast cell homeostasis. Also, DSPaa183-219 peptides promoted dental cell spreading, migration, proliferation, and differentiation. On the other hand, the COOH-terminal domain of the DSPaa363-458 bound to the second loopaa194-241 of the Ocln, which is an integral membrane protein (Cong and Kong, 2020). The DSP domain phosphorylated Ocln on serineaa490 and FAK on serineaa722 and tyrosineaa576 through binding of Ocln to FAK. The DSPaa363-458 facilitated human dental pulp stem cell and mouse dental papilla mesenchymal cell differentiation and mineralization. Furthermore, the in vivo study showed that the DSPaa363-458 was mixed with agarose beads (DSP-beads) and the DSP-beads compound was implanted into mouse dental chambers. The histological analysis showed that in the DSP-beads treated mice, dental pulp mesenchymal cell proliferation and cell differentiation were significantly intense around the DSP-beads compared to that of the control mice. The dental pulp mesenchymal cells in the DSP-beads treated groups secreted dental ECMs and formed a layer between the resin and dental pulp chamber. Interestingly, in the DSP-beads treated group, there were many newly formed blood vessels and less inflammatory cells around the DSP-beads, along with the dental pulp mesenchymal cells and blood vessels which migrated into the DSP-beads. This study indicated
that the DSP induces dental mesenchymal cell differentiation and vasculogenesis (Fig. 5) (Li et al., 2017).

For the in vivo study of the role of the DSP in biological activity, overexpression of the DSP transgenic mice under the control of the mouse DSPP promoter (DSP-Dspp Tg) was generated (Suzuki et al., 2009). The DSP-Dspp Tg mice were crossed-bred with the DSPP KO (-/-) mice. The DSPP KO/DSP-Dspp Tg mice resulted in partial rescue of restored predentin width, decrease of frequent dental pulp chamber exposure, and partial recovery in the dentin volume compared to that of the DSPP KO mice. However, teeth in these DSPP KO/DSP-Dspp Tg mice did not rescue the dentin mineral density. This study implies that the DSP is involved in the initiation of dentin mineralization. In addition, overexpression of the DSP transgenic mice driven by the mouse amelogenin gene promoter (DSP-Amg Tg) caused significantly and uniformly increased enamel hardness and an increased rate of enamel mineralization, but the enamel morphology was not significantly altered. These studies demonstrated that the DSP made a unique contribution to the physical properties of the dentin-enamel junction (DEJ) and facilitated enamel formation (Paine et al., 2005; White et al., 2007). In contrast, overexpression of the DSP transgenic mice under the control of the type I collagen promoter (DSP-Col 1α1 Tg) was crossed-bred with the DSPP KO mice to generate DSPP KO/DSP-Col 1α1 Tg mice (Gibson et al., 2013). Unexpectedly, dentin of the DSPP KO/DSP-Col 1α1 Tg mice was much thinner, more poorly mineralized, and remarkably disorganized as compared with that of the DSPP KO mice. The DSPP KO/DSP-Col 1α1 Tg mice displayed more severe dentin defects than the DSPP KO mice. Furthermore, the DSPP KO/DSP-Col 1α1 Tg mice resulted in severely worse periodontal defects than that of the DSPP KO mice and a greater reduction of the alveolar bone, more remarkably altered canalicular systems around the osteocytes, less cementum, more radical migration of the epithelial attachment towards the apical direction, and more severe inflammation in molar furcation region than that of the DSPP KO mice (Gibson et al., 2014). It suggests that the DSP
mediates an inhibitory role in the periodontium formation. The different DSP effects on hard tissue development and formation may rely on the control of given tissue gene promoters.

Conclusions and future perspectives

In this review, we provided a brief overview of the DSPP expression, proteolysis, pathophysiology, and biological functions of the cleaved products, the DSP/DGP and DPP based on the recent literatures. Dentin is a highly mineralized tissue and derived from odontoblasts. When dentin is injured such as pulpotomy and dental caries, dental pulp progenitors/MSCs can migrate to the injured areas and differentiate into odontoblast-like cells (Vidovic et al., 2017). The differentiation of the dental pulp progenitors/MSCs is controlled at the given niches (Morrison and Spradling, 2008, Méndez-Ferrer et al., 2020). During dentinogenesis, the odontoblasts synthesize and secrete dental ECMs, which bind to calcium-phosphate, finally forming predentin and dentin. Dental ECMs are composed of collagens and NCPs (MacDougall et al., 1997). Among NCPs, the DSPP is highly expressed in the odontoblasts and dentin (Fig. 3) (D'Souza et al., 1997; Chen et al. 2009). DSPP protein is processed into the DSP/DGP, DPP by BMP1 and Tolloid-like proteinases (Yamakoshi et al., 2006; Marschall and Fisher, 2010). Mutations of the DSP and DPP domains are associated with DGI-II, DGI-III, DD-I and DD-II and the most common genetic dentin diseases (Fig. 2, Tables 1 and 2). The DSP and DPP play distinct roles during odontogenesis.

The DSP promotes the initial effect on early dentin development while the DPP is related to HA crystal growth and mineralization (Suzuki et al., 2016). DSP acts as a ligand and facilitates intracellular signaling pathways via its cell membrane receptors, integrin β6 and Ocln as well as induces dental pulp/MSC cell differentiation and mineralization. DSP\textsuperscript{aa183-219}-β6 signal up-regulates the DSPP expression, dental mesenchymal cell proliferation and differentiation via p-p38-pErk-Smad\textsuperscript{1/5/8} signal pathways, while DSP\textsuperscript{aa363-458}-Ocln complex facilitates dental mesenchymal/MSC cell differentiation and biomineralization through FAK cascades (Fig.5A).

Overexpression of the DSP partially rescued dentin defects in the DSPP KO mice (Suzuki et al.,
2009). In addition, the DPP-RGD activated the downstream gene expressions and cell differentiation through integrin-MAP kinases and paxillin-FAK signal pathways. Moreover, the DPP contains Ser-Asp/Asp-Ser repeat regions, which mediated intracellular calcium store flux and triggered the CaMKII-Smad1/5/8 activations, facilitating cell differentiation and mineralization (Fig.5B). The DPP overexpression partially rescued dentin defects in the DSPP KO mice (Zhang et al., 2018). Nevertheless, overexpression of the DSP or DPP under the control of the given gene promoter (s) resulted in impairment of certain tissue development (White et al., 2007; Gibson et al., 2014). How the DSP and DPP play dual roles in different tissues is not completely understood and needs to be further studied. Although biological roles of the DSPP have made the advanced achievements during tooth development, mechanisms of the DSPP during tooth development and formation remain still unknown. For instance, where cleavages of the DSPP occur in cytoplasm and/or ECMs needs to be further investigated. Differences of three-dimensional structures between wild type and mutant DSPP and its cleaved products have not been described and need to be studied. Control of the spatial-temporal DSPP gene in cell- and tissue-specific expression is not completely understood although it has been reported that expression of the DSPP is regulated by several growth factors, transcriptional factors and other materials (Chen et al., 2008; Suzuki et al., 2016). However, understanding mechanisms of the DSPP in the progenitor/MSC cell differentiation to odontoblasts may be a potential novel avenue during dentin development and regeneration.

Acknowledgments

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References


Proliferation and Differentiation of Human Periodontal Ligament Stem Cells. Plos One 8 (12): e81655.


Figure Legends

Figure 1. Schematic of a molar and mesenchymal stem cells found in the teeth. A. The crown of the tooth is covered with enamel whereas the root is covered with cementum. The cementoenamel junction is located between the enamel and root. The root is attached to the alveolar bone by periodontal ligament. The dentin surrounds the dental pulp. Blood vessels and nerves enter the dental pulp from the apical foramen of the tooth and provide innervation and nutrition to the odontoblasts and dental pulp. B. DPSCs, dental pulp stem cells; SCAPs, stem cells from the apical papilla; SHEDs, stem cells from human exfoliated deciduous teeth.

Figure 2. Diagram of the DSPP mutations associated with inherited dentin defects. The structure of the human DSPP gene is shown. The exons are represented as boxes numbered 1–5 with the amino acid numbers encoded by each exon being shown below. The introns are represented by lines. The locations of the DSPP mutations are indicated above the gene structure. The white color indicates the 5′ untranslated region sequence (UTR). The gray color shows the dentin sialoprotein (DSP) protein sequences, while the yellow color indicates dentin phosphoprotein (DPP) protein sequences. The asterisks indicate mutations affecting splice sites according to splice-site recognition software.

Figure 3. Dspp expression pattern in developing mouse teeth from embryonic days 15 to postnatal 2 months. Dspp mRNA was not detected in the dental and osteogenic mesenchyme as well as the dental epithelium at embryonic days 15 (E15) (A'). At postnatal day 1 (PN1), Dspp was expressed in pre-ameloblasts, pre-odontoblasts and weakly in the dental pulp (B'). Dspp expression was mostly restricted in odontoblasts from PN5 to 2 months (M) after birth (C', D' E'). Dspp expression was barely seen in bones. A, B, C, D and E show the bright images. Am, Ameloblasts, B, Bone, D, dentin, De, dental epithelium, Dm, dental mesenchyme, Dp, dental pulp, E, enamel, Od, Odontoblasts, pAm, pre-ameloblasts, pOd, pre-odontoblasts.

Figure 4. Clinical photographs and radiographs from the DGI-II patients. The clinical photograph taken of age 7 years-old boy (A) shows severe attritions (arrowheads) and radiograph indicates severe enamel loss with reduced pulp space and decreased dental mineral density (B). The intraoral photographs taken of age 5 years-old girl showed severe attrition of the primary dentition to the gingiva level and teeth with yellow-brown color and a translucent appearance (arrowheads) (C). Radiograph shows that dentin was thin with severe occlusal attrition and periapical abscess (arrowheads) (D).

Figure 5. Hypothetical model of DSPP signal during dentinogenesis and dentin regeneration. A. DSPaa183–219 binds to integrin β6 and forms a complex, activating phosphorylation of p38, Erk1/2 and Smad1/5/8. Phosphorylated Smad1/5/8 interact with Smad4. The complex is translocated into the nucleus. Phosphorylated Smad1/5/8 in coordination with Smad4 bind to Smad binding elements (SBEs) in the DSPP gene promoter and stimulate DSPP gene transcription. On the other hand, DSPaa363–458 acts as a ligand and interacts with the extracellular loop2 of Oclnaa194–241, activating Ocln.
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**Intron 2**

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- c.52-3G>C, IVS2-3C>G
- c.52-3C>A, IVS2-3
- c.52-1G>A, IVS3+1G>A

**Exon 3**

- c.202A>T, IVS3+3A>G
- c.135+2T>C, IVS3+3A>G

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^All numbering assumes the A of the ATG start codon as nucleotide1. Reference sequence NM_014208.3.
Table 2. Summary of DPP Mutations Associated with Inherited Dentin Defects

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*a* All numbering assumes the A of the ATG start codon as nucleotide1. Reference sequenceNM_014208.3.

*NSHL, familial nonsyndromic hearing loss*