DENTINE SIALOPHOSPHOPROTEIN SIGNAL IN DENTINEOGENESIS AND DENTINE REGENERATION

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Abstract

Dentineogenesis starts on odontoblasts, which synthesise and secrete non-collagenous proteins (NCPs) and collagen. When dentine is injured, dental pulp progenitors/mesenchymal stem cells (MSCs) can migrate to the injured area, differentiate into odontoblasts and facilitate formation of reactionary dentine. Dental pulp progenitor cell/MSC differentiation is controlled at given niches. Among dental NCPs, dentine sialophosphoprotein (DSPP) is a member of the small integrin-binding ligand N-linked glycoprotein (SIBLING) family, whose members share common biochemical characteristics such as an Arg-Gly-Asp (RGD) motif. DSPP expression is cell- and tissue-specific and highly seen in odontoblasts and dentine. DSPP mutations cause hereditary dentine diseases. DSPP is catalysed into dentine glycoprotein (DGP)/sialoprotein (DSP) and phosphoprotein (DPP) by proteolysis. DSP is further processed towards active molecules.

DPP contains an RGD motif and abundant Ser-Asp/Asp-Ser repeat regions. DPP-RGD motif binds to integrin αVβ3 and activates intracellular signalling via mitogen-activated protein kinase (MAPK) and focal adhesion kinase (FAK)-ERK pathways. Unlike other SIBLING proteins, DPP lacks the RGD motif in some species. However, DPP Ser-Asp/Asp-Ser repeat regions bind to calcium-phosphate deposits and promote hydroxyapatite crystal growth and mineralisation via calmodulin-dependent protein kinase II (CaMKII) cascades.

DSP lacks the RGD site but contains signal peptides. The tripeptides of the signal domains interact with cargo receptors within the endoplasmic reticulum that facilitate transport of DSPP from the endoplasmic reticulum to the extracellular matrix. Furthermore, the middle- and COOH-terminal...
regions of DSP bind to cellular membrane receptors, integrin β6 and occludin, inducing cell differentiation. The present review may shed light on DSPP roles during odontogenesis.

Keywords
Dentine; dental mesenchymal stem cells; dental caries; dentine regeneration; small integrin-binding ligand N linked glycoproteins; dentine sialoprotein; dentine glycoprotein; dentine phosphoprotein; dentine; dentine sialophosphoprotein

Introduction

The tooth is a highly mineralised organ resulting from the interactions between the dental oral epithelial and mesenchymal cells. It is composed of enamel, dentine, cementum, soft connective tissues and periodontium (Fig. 1a) (Mitsiadis et al., 2015; Nanci, 2012). The dentine is a thick highly mineralised tissue layer (present underneath the enamel) consisting of dentinal tubules and inter-tubular dentine and acts as a secondary barrier against infections of the dental pulp cavity (Lopez-Cazaux et al., 2006). Dentineogenesis starts at the onset of odontoblast differentiation. Odontoblasts originate from neural-crest-derived mesenchymal cells, which differentiate to form odontoblasts in specific temporal-spatial patterns, originating at the principal cusp tip and advancing toward the base of the teeth (Chen et al., 2008; Thesleff, 2003). Odontoblasts are mitotic cells organised as a layer of barrier cells along the edge between the dentine and dental pulp cavity. Odontoblasts synthesise and secrete the organic ECM proteins (Linde and Goldberg, 1993; MacDougall et al., 1997). Dentine is composed mostly of HA (70 % by weight), ~12 % water as well as collagens and NCPs (Linde and Goldberg, 1993; MacDougall et al., 1997). Odontoblasts in odontogenesis and dental caries participate in the physiological primary and secondary dentine formation. Also, odontoblasts maintain the dentine metabolism throughout the life of the tooth and serve as the first line of defence against dentine pathogen invasion by RD (reparative, tertiary) formation at the dentine-pulp interface beneath the carious infected dentine region (Couve et al., 2014).

The dental pulp is a loose connective tissue and contains blood vessels with abundant capillaries and an innervated tissue under the odontoblast layer. Blood vessels facilitate the exchange of nutrients and waste products in the dental pulp (Lopez-Cazaux et al., 2006; Tziafas et al., 2000). DPCs are a heterogeneous population retaining a source of MSCs (Tirino et al., 2012). Maintenance of a healthy, vascularised and innervated dental pulp is necessary for a healthy tooth and dental regeneration (Huang et al., 2018). In the dental pulp cavity, MSCs are known to dwell within peri-vascular microenvironments, termed niches (Kaukua et al., 2014; Shi and Gronthos, 2003; Sui et al., 2019), and other locations (Gronthos et al., 2000; Miura et al., 2003; Morsczeck et al., 2005; Seo et al., 2004). However, little is understood about exact localisations and signalling regulations of the niches (Bluteau et al., 2008). The role of specific local niches essential to regulate cell migration, differentiation and cell fate specification during developmental and reactional events of dentine is not well recognised (Ruch, 1985). Dental progenitors/MSCs are capable
of differentiating into new odontoblast-like cells, which can form a dentine-like structure such as RD, for dentine repair after a dentine injury such as dental caries.

**Dental caries and its management**

Dental caries, the most prevalent chronic infectious disease globally, is a biological irretrievable impairment of vulnerable dental hard tissues due to acids produced by bacterial glycolysis of dietary carbohydrates (Baker et al., 2021). The WHO has defined the early childhood caries as a worldwide problem, with a prevalence between 60% and 90%. In addition, more than 90% of all adults have experienced this disease (Bernabe et al., 2020; Griffin et al., 2008; Kazeminia et al., 2020). Tooth decay leads to dental pulpal infection, necrosis, loss of tooth function and vitality as well as eventual loss of the tooth. Various restorative materials have successfully been used to fill and replace injured or diseased dental tissues (Wang et al., 2020b). However, after restorative treatment, about 50% of cases demand revision in 5-10 years (Burke and Lucarotti, 2009; Chen et al., 2020). In addition, any traditional artificial restorative material might fail due to inappropriate physical, biocompatible and mechanical properties (Goldberg and Smith, 2004; Tziafas et al., 2000; Yang et al., 2020a). If the material pulls away from the cavity wall, a microleakage would form between the dentine layer and dental materials, causing secondary or recurrent caries (Askar et al., 2021; Goldberg and Smith, 2004; Tziafas et al., 2000). Therefore, despite several advances in dental restorative materials, it is required for new therapeutic restorative methods in dentistry to support a healthy dentition. Therapies using stem cells such as dental pulp MSCs, cell/tissue engineering and other biomaterial components have successfully been reported for replacing or regenerating destroyed and injured dental tissues (Han et al., 2021; Saoud et al., 2016). For instance, Vidovic et al. (2017) showed that when pulpectomy is performed in animal models, a group of dental pulp progenitor cells/MSCs can migrate to the injured areas, differentiating into odontoblast-like cells, forming an RD. Consequently, the growth factor BMP2 enhances dental pulp cell differentiation into odontoblast-like cells, which synthesise and secrete dental ECMs, forming an RD in the injured areas (Nakashima, 2005; Ni et al., 2018). Besides BMP/TGF-β signalling, recent studies have demonstrated that Wnt/β-catenin signalling induces progenitor cell/MSC growth and differentiation, promoting RD formation (Neves and Sharpe 2018; Zaugg et al., 2020). Furthermore, Han et al. (2021) reported that an artificial synthesised peptide, termed TVH-19, promotes human dental pulp cell differentiation and induces tertiary dentine formation in a rat model.

**Dental pulp MSCs**

Stem cells are characterised by both self-renewal and differentiation potential. The self-renewal of stem cells can occur by symmetric cell divisions, generating two daughter cells with the same fate, or asymmetric cell divisions, where one daughter cell is identical to the mother cell, while the other develops into a different cell type (Götz and Huttner, 2005).

Stem cells are classified as ESCs, iPSCs and ASCs. ESCs originate from the inner cell mass of the blastocyst prior to implantation. ESCs possess unlimited self-renewal potential and can generate all the body cell types. iPSCs, generated by inducing the expression of defined transcription factors in somatic cells, are pluripotent and can differentiate towards all cell
types in given microenvironments. ASCs reside within different tissues such as the BM. Unlike ESCs and iPSCs, ASCs are limited in their potential to the cell types of the tissue they inhabit. Although stem cells normally remain in a quiescent, nondividing state, ASCs can proliferate and differentiate to replace damaged cells within their tissues and accelerate tissue healing following an injury (Cable et al., 2020; Pittenger et al., 1999; Yamanaka, 2020).

The BM contains numerous different cell types arising from HSCs, non-haematopoietic MSCs and other cell types, which are interconnected by a vascular and innervated network within the cavities of the BM. HSCs have the ability of self-renewal and differentiation into various cell types including erythrocytes, megakaryocytes, platelets, granulocytes, lymphocytes, osteoclasts, and dendritic cells (cells of the erythroid/myeloid lineages) and others. Subsequently, HSCs migrate to other haematopoietic or lymphoid organs giving rise to B lymphocytes, T lymphocytes, macrophages, and others. MSCs produce osteoblasts (bone-forming cells), adipocytes (fat cells) and other cell types, while osteoclasts (bone-resorbing cells) share a monocytic origin with macrophages. MSCs display a variable self-renewal and differentiation potential (Friedenstein et al., 1970; Pittenger et al., 1999; Wilkinson et al., 2020). They have been widely characterised in vitro as expressing various markers such as STRO-1, CD146 or CD44 (Pittenger et al., 1999). STRO-1 is a cell surface marker of osteogenic precursors, CD146 and CD44 are pericyte and mesenchymal stem cell markers, respectively. MSCs have self-renewal ability and potentially differentiate into mesodermal lineages, therefore originating cartilage, bone, fat, skeletal muscle and connective tissues (Pittenger et al., 1999; Wang et al., 2020a). Endothelial progenitor/stem cells play a principal role in BM angiogenesis as they have clonogenic capability and can be mobilised into the peripheral blood system, differentiating into mature endothelial cells in newly formed blood vessels after tissue injury. Thus, endothelial stem cells derived from the BM represent a source for the body vasculogenesis and angiogenesis.

Dental pulp contains progenitor cells/MSCs able to differentiate into adipocytes, chondrocytes, odontoblasts, osteoblasts and other cell type in given environments. During dentineogenesis and tertiary dentine formation, dental pulp progenitors/MSCs are able to differentiate into odontoblast-like and odontoblastic cells under appropriate signals (Gronthos et al., 2000; Miura et al., 2003; Sui et al., 2019). In a tooth, some cells can be either transit-amplifying cells or progenitors and commit to terminal differentiation. These transit-amplifying cells and progenitors have a limited lifespan thus, they can only produce a tissue for a given time (Walker et al., 2019). By contrast, dental MSCs are self-renewing and able to generate any of the tissues for their entire life span. In the dental pulp, dental MSCs include DPSCs, SHED and 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 MSCs (Gronthos et al., 2000). DPSCs lack unique markers, therefore generic MSC markers such as STRO-1, CD146, CD105 and CD44 are used for the identification and isolation of DPSCs (Pittenger et al., 1999; Wang et al., 2020a). DPSCs can 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 vitro and in vivo.

SHEDs were isolated from deciduous teeth, have fibroblastic features and express MSC specific markers including CD45, CD90, CD106, CD146, CD166 and STRO-1 but not haematopoietic and endothelial markers such as CD34 and CD31 (Miura et al., 2003). SHEDs have a high proliferation rate and can differentiate into adipogenic, chondrogenic, myogenic, neurogenic, odontogenic and osteogenic cells in vitro as well as induce formation of dentine and bone in vivo (Miura et al., 2003). SHEDs, neural-crest-derived stem cells, also express neural cell markers such as nestin, beta III tubulin and GFAP as well as several pluripotent markers including Oct4 and Nanog (Chai et al., 2000; Miura et al., 2003; Yang et al., 2019; Yang et al., 2020b). SHEDs express more osteocyte markers such as ALP, collagen type I and Runx2 than do BM MSCs in vitro. SHEDs were transplanted into the subcutaneous tissue in immunodeficient mice and promoted bone repair through inhibition of osteoclast activity in vivo (Yamaza et al., 2010). They are also capable of differentiating into vascular endothelial cells and form functional blood vessels by up-regulation of MEK1/ERK signalling (Bento et al., 2013). Due to their deciduous teeth origin, SHEDs exhibit several features similar to DPSCs. However, their proliferation and differentiation capacity are higher than that of DPSCs and BM MSCs (Bluteau et al., 2008).

SCAPs isolated from apical papilla cells at the root apex of teeth, display high proliferation rates and demonstrate an increase of migratory and regenerative capacities compared with other dental MSCs (Sonoyama et al., 2008). SCAPs are easily obtainable from human third molars. As SCAPs can be derived from the primary teeth, they express primitive embryonic markers including Sox2, Oct3/4, Nanog and others (Lee and Seo, 2016; Sonoyama et al., 2008). Among these markers, CD146 and STRO-1 co-expression is related to early-MSC phenotype. Certainly, CD146+/STRO-1+ SCAPs show superior colony-forming efficiency, with increased cumulative doubling compared with their counterpart (Nada and El Backly, 2018). CD24, another marker of the pluripotent population is considered to be a representative surface marker for SCAPs due to its absence in other dental MSCs (Kang et al., 2019). It is worth noting that the expression of the three markers CD146, STRO-1, CD24 declines with cell passaging, supporting their correlation with superior stemness. SCAPs are optimised for osteogenesis and odontogenesis; regarding that, SCAPs are considered to be odontoblast precursors in vivo (Du et al., 2020; Nada and El Backly, 2018). However, SCAPs are multipotent and give rise to mesenchymal cell lineages such as adipocytes and chondrocytes (Yang et al., 2020b). Taken together, SCAPs will hopefully gain a significant role in tissue repair and regeneration.

Besides dental MSCs (Gronthos et al., 2000; Miura et al., 2003), other MSC populations have been isolated from human dental tissues including the periodontal ligament (Seo et al., 2004) and the dental follicle (Morsczeck et al., 2005). Progenitors/stem cells isolated from the oral cavity express a group of mesenchymal markers, such as CD29, CD73, CD90 and CD105, and embryonic markers, including Sox2, Nanog and Oct4, and can differentiate into multiple cell lineages (Miran et al., 2016). Noticeably, some dental stem cells demonstrate more embryonic-like characteristics than those of BM and umbilical cord stem cells (Miran et al., 2016; Sui et al., 2019). Oral cavity MSCs are an important and valuable resource
for dental and medical clinical/therapeutic applications. However, little is known about how progenitor cells/MSCs differentiate into specific mature cells, such as osteoblasts and odontoblasts, as well as which niches promote such differentiation.

SIBLINGS and DSPP

Niches can influence cell behaviour and fate (Méndez-Ferrer et al., 2020; Morrison and Spradling, 2008; Perry and Li, 2007). For instance, BM ECM influences osteoblast differentiation into osteocytes while dental pulp ECM governs dental progenitor cell/MSC differentiation into odontoblasts (Chen et al., 2005; Chen et al., 2007; Guo et al., 2009; Vijaykumar et al., 2020). Bone and dentine are highly mineralised tissues formed by osteoblasts and odontoblasts, which derive from mesenchymal cells. Both bone and dentine possess common characteristics and show similar features during mineralisation. During this process, odontoblasts and osteoblasts synthesise and secrete ECM proteins to form matrix-forming predentine and osteoid, respectively, which in turn are converted to bone and dentine. At the same time, the organic matrix of osteoid and predentine is composed of collagens and NCP proteins necessary for mineralisation of collagen fibres. The most common NCP proteins of bone and dentine include BSP, OPN, MEPE, DSPP and DMP1, belonging to the SIBLINGS family (Bellahcène et al., 2008; Fisher and Fedarko, 2003; MacDougall et al., 1997). SIBLING genes are located on chromosome 4q21 in humans and chromosome 5q in mice, sharing a similar exon structure. The presence of the RGD integrin-binding motifs enables them to trigger intracellular signals by initiating integrin-mediated signalling. Although bone and tooth show several common characteristics, the physical and biological functions of osteoblasts and odontoblasts exhibit several differences (Chen et al., 2005; Chen et al., 2009; Vijaykumar et al., 2020). The functions of the members of the SIBLINGS family in dentine and bone have been found through a linkage to human diseases and different genetic animal models. DSPP, ~ 143 kDa, is the largest of the SIBLING proteins, with 1,301 amino acids in humans and plays essential roles in dentineogenesis (de La Dure-Molla et al., 2015). DSPP contains 4 introns and 5 exons (Fig. 2) (MacDougall et al., 1997). Unlike other SIBLING protein family members, DSPP spatial-temporal expression is mainly seen in pre-ameloblasts and odontoblasts during tooth development and formation (Chen et al., 2009; D’Souza et al., 1997) and weakly detected in osteoblasts and non-mineralised tissues (Fig. 3) (Chaplet et al., 2006; Qin et al., 2002). For example, DSPP protein expression in odontoblasts and dentine is about 400-fold higher than that in osteoblasts and bone (Qin et al., 2002). DSPP is transcribed from a single gene (MacDouall et al., 1997) but full-length DSPP has hardly been found in cells or tissues, whereas its cleavage products, DSP and DPP in mice, rats and humans as well as DSP, DGP and DPP in pigs are the most abundant NCPs in dentine and odontoblasts (Qin et al., 2001; Yamakoshi et al., 2005; Yuan et al., 2012).

DSP is composed of partial exon 2, exon 3, exon 4 and partial NH2-terminal region of exon 5 of DSPP, while DPP consists of most DSPP exon 5 (Fig. 2). DSPP is first processed into DSP/DGP and DPP (also termed dentine PP) by BMP1, TLR metalloproteinases and astacin proteases (Marshall and Fisher, 2010; Steiglitz et al., 2004; Tsuchiya et al., 2011). Then, DSP is further catalysed into small active molecules by MMP-2, −9 and −20 to expose cryptic binding sites into active molecules (Yamakoshi et al., 2006; Yuan et al., 2017).
Mutations of the cleavage site between DSP and DPP regions result in DGI phenotypes in mice, indicating that DSPP needs to be cleaved into its active fragments, DSP and DPP (Zhu et al., 2012).

The porcine DGP has an 81 amino acid segment of DSPP (Ser392 to Gly472) located between DSP and DPP fragments. DGP contains 4 phosphorylated serine residues (Ser453, Ser455, Ser457 and Ser462) and 1 glycosylated asparagine (Asn397). DGP molecular weight is a 19 kDa in SDS-APGE gel by Coomassie Brilliant Blue staining, that is decreased to 16 kDa by glycopeptidase A digestion. The porcine DGP has the same number (12 each) of positively charged (Arg and Lys) and negatively charged (Asp and Glu) residues. This pig DGP contains abundant Ser (12) and Gly (13). Lacking post-translational modifications, DGP has a calculated isoelectric point of 6.7. Due to containing 4 phosphorylated Ser and sialic acids, the modified DGP has an increased affinity for HA, which most likely facilitates the binding to dentine crystals. The identity of the porcine DGP amino acid sequence (NP_99842.1) is conserved, with 58 (81 %) conserved amino acids in humans (F42472.1), 40 (49 %) in rats (L79813.1) and 38 (47 %) in mice (C12787.1) (Yamakoshi et al., 2005). How DSPP is catalysed into the porcine DGP by proteinases and DGP functions during dentineogenesis are yet to be determined.

DSP and DPP play unique biological roles during tooth development (Paine et al., 2005; Suzuki et al., 2009). DSP or DPP mutations in humans are associated with DGI-II (OMIM 125490) and DGI-III (OMIM 125500) as well as DD-II (OMIM 125420) and DD-I (MIM 125400) (Fig. 2, Table 1,2). Those hereditary dentine disorders are the most common dentine genetic diseases. Estimated incidences of DGI in humans is 1/6,000-8,000, while DD is 1/100,000 (Witkop, 1975). DGI-II is characterised by pulpal calcification, opalescent discoloured dentition and bulbous crown shape as well as impaired odontoblast cell differentiation and delayed conversion of predentine to dentine (Fig. 4). DGI-III was originally regarded as a Brandywine isolate (Witkop, 1975) and a severe form of DGI-II with multiple dental pulp exposures and shell-like teeth. DD-II is similar to DGI-II in the deciduous dentition, but tooth discolouration is minimal and dental pulp cavities are thistle-tube shaped with pulp stones in the permanent dentition. In DD-I, teeth are normal in shape and form, as well as consistent in the deciduous and permanent dentitions. In some cases, colour of the teeth may exhibit a slightly amber discolouration. However, the roots are short, and the pulp obliteration causes a crescent-shaped pulpal remnant in the permanent dentition and a total pulpal obliteration in the deciduous dentition. Using mouse models, it was confirmed that Dspp is required for dentineogenesis, as homogenous null mice (Dspp−/−) show tooth deficiency similar to those seen in patients suffering from DGI and DD, with enlarged pulp cavities, a wide predentine zone, reduced dentine volume, hypomineralisation and dental pulp exposure (Fig. 4) (de La Dure-Molla et al., 2015; Sreenath et al., 2003).

SIBLING-RGD motifs are capable of binding to cell surface integrins in normal tissues and enhance cell adhesion, spreading, motility, proliferation, differentiation and survival via up-regulating kinase cascades and transcription factors. Also, the biological functions of SIBLINGS are regulated by proteolytic processing to uncover cryptic binding sites and expose functional domains, thus modulating cell adhesion and activity. For instance, OPN protein interacts with various integrins, such as αvβ3, αvβ5, αvβ1, α4β1, α8β1,
α9β1 and CD44 splice variants (Bellahcène et al., 2008; Marschall and Fisher, 2008). OPN by thrombin cleavage separates the CD44− and integrin-binding domains, which in some cases promote adhesion over cell migration. Another example is the thrombin-cleaved NH2-terminal OPN segment that interacts with αvβ3 and αvβ5 integrins via the RGD motif (Bellahcène et al., 2008; Furger et al., 2003) or with α4β1 and α9β1 integrins via the cryptic SVVYGLR sequence (Rangaswami et al., 2006) and promotes cell adhesion and migration. The COOH-terminal region of OPN interacts with CD44 variant 6 (CD44v6) and/or variant 3 (CD44v3) by a heparin bridge (Teramoto et al., 2005). In addition, OPN is also catalysed by MMP-3 and MMP-7 and the cleaved OPN domains promote cell adhesion and migration in vitro by activating β1-containing integrins (Agnihotri et al., 2001) and enhances cell adhesion, spreading and migration (Higashikawa et al., 2007). The RGD domain of DMP1 only binds to αvβ3, while BSP-RGD motif not only interacts with αvβ3, but also with αvβ5 and enhances cell adhesion and migration (Marschall and Fisher, 2008). DMP1 is a substrate of BMP1 and BMP1-generated DMP1 fragments have similar binding efficiency to the intact DMP1 protein in cell attachment and migration (Marschall and Fisher, 2008; Steiglitz et al., 2004).

**DPP**

DPP contains an RGD domain at the NH2-terminal site, acting as a ligand and binding to integrin αvβ3. DPP-RGD/integrin-αvβ3 complex activates intracellular signalling pathways through up-regulating MAPK, including SAPK/JNK, ERK1/2 and p38 in human and mouse cells. Consequently, this complex up-regulates bone/dentine-related gene expression such as RUNX2, OSX, ALP, OCN and BSP in human and mouse cells as well as promotes cell differentiation and mineralisation in hBMSC, mouse osteoblastic cells (MC3T3-E1) and mouse fibroblastic (NIH3T3) cells (Jadlowiec et al., 2004; 2006). In addition, DPP-RGD induces phosphorylation of paxillin, FAK and of the transcription factor Elk-1 and up-regulates 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 influence binding of RGD to specific integrins and enhance cell adhesion and migration (Marschall and Fisher, 2008; Suzuki et al., 2014). However, unlike other SIBLING family members, 17 out of 37 DSPP genes from 37 species tested do not contain the RGD motif of DPP, 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 Ser-Asp or Asp-Ser repeat regions, which are the most phosphorylated regions of SIBLING protein and one of the most acidic proteins in numerous species such as human, rat and mouse (Jonsson et al., 1978; Suzuki et al., 2016). DPP binds to calcium ion and collagen type I, acting as an inducer of mineralisation in ECMs and inducing HA deposition and growth of vertebrate bones and teeth (He et al., 2005). DPP can interact with the 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 attached to it and releasing different cargos intracellularly (Figueiredo et al., 2019; Ravindran et al., 2013). Additionally, DPP-DSS (Asp-Ser-Ser) repeat regions can
facilitate intracellular Ca\(^{2+}\) release. This calcium flux promotes the activation of Ca\(^{2+}\) CaMKII. Activated CaMKII enhances the phosphorylation of the transcription factors Smad1/5/8 and phosphorylated Smad1/5/8 proteins are translocated to the nucleus and up-regulate Smad1/5/8 downstream gene expression as well as promote cell differentiation in murine pluripotent stem cells (C3H10T12) and hBMSCs (Eapen et al., 2013) (Fig. 5). Eapen et al. (2013) showed that the length of the Ser-Asp and/or Asp-Ser repeat regions varies among species but is not correlated with dentine hardness (Suzuki et al., 2016).

To analyse the relationship between length variations in Ser-Asp/Asp-Ser repeat regions and the role of DPP in matrix mineralisation, different lengths of the Ser-Asp/Asp-Ser repeat regions have been generated (Kobuke et al., 2015). Recombinant mouse Dpp deleted 63.5 Ser-Asp repeat regions, accounting for 36.5 % of the length of the Ser-Asp repeat region, were generated and these peptides were able to induce calcium-phosphate precipitation similarly to the full length Dpp at the same concentration. In contrast, the inverted Dpp deleted 63.5 Ser-Asp repeat regions had no effect on the induction of calcium phosphate precipitation (Kobuke et al., 2015). The 8-repeat copy of Asp-Ser-Ser residues facilitates calcium-phosphate precipitation and HA crystal growth, promoting the remineralisation of demineralised human enamel and dentine tubule occlusion (Hsu et al., 2011). Dpp-mimetic peptide molecules upregulate the expression of bone/dentine-related genes including RUNX2, ALP, DMP1, OCN and collagen type I in human osteosarcoma (Saos-2) cells as well as promote cell differentiation (Gulseren et al., 2019). The biological function of Dpp was narrowed-down to 3 Ser-Asp-Ser repeat peptides that are 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 of the role of DPP, mice overexpressing Dpp transgenic gene driven by Coll1α1 promoter (Dpp-Coll1α1 Tg) were crossed-bred with Dspp KO (Dspp\(^{-/-}\)) mice to generate Dspp KO/Dpp Coll1α1 Tg mice (Zhang et al., 2018). Dspp KO/Dpp-Coll1α1 Tg mice had an increase in dentine thickness and restored dentine mineral density compared with Dspp KO mice. Histochemistry showed that abnormal widening of the predentine was narrower in Dspp KO/Dpp-Coll1α1 Tg mice. Scanning electron microscopy analysis demonstrated that the structure of dentinal tubules in Dspp KO/Dpp-Coll1α1 Tg mice was better organised than that of Dspp KO mice. Dentine mineral deposition rate in Dspp KO/Dpp-Coll1α1 Tg mice was significantly enriched compared to that of Dspp KO mice as analysed by double fluorochrome labelling. The overexpression of Dpp partially rescued the dentine deficiency in Dspp KO mice, indicating that Dpp may facilitate dentine development during dentineogenesis. In contrast, the body weight of Dpp-Coll1α1 Tg mice was lower compared to that of wild type mice. Moreover, both short and long bones were shorter in Dpp-Coll1α1 Tg mice compared to that of wild type mice. Dpp-Coll1α1 Tg mice presented reduced trabecular bone formation and exhibited narrow proliferative and chondroblast layers in long bones. Histochemistry analysis demonstrated that the proliferative zone of long bones in Dpp-Coll1α1 Tg mice was characterised by reduced cell proliferation and increased gene expression of chondroblast differentiation markers such as type II collagen (a marker of proliferating chondrocytes), type X collagen (a marker of hypertrophic chondrocytes) and proteoglycan, but there were no obvious defects in chondrocyte differentiation (Zhang et al., 2016). Transgenic mice of an overexpression of
Dpp driven by the mouse Ang promoter (Dpp-Amg Tg) were generated. Dpp-Amg Tg mice showed a pitted and chalky enamel with nonuniform thickness that tended to wear more easily. In mice, Dpp-Amg transgene results in disruptions of the prismatic enamel structure and weakened enamel with uneven thickness (Paine et al., 2005; White et al., 2007). The reasons for the different effects of Dpp on different tissue development and formation remain unclear. A reason might be that the biological mechanisms of Dpp are cell- and tissue-specific. Spatial-temporal expression of Dspp is detected in preodontoblasts and preameloblasts at early stages of tooth development. During mouse tooth formation at postnatal stages, Dspp expression is barely detected in ameloblasts, but continuously seen in odontoblasts, predentine and dentine, maintaining odontoblast and dentine metabolism and homeostasis (Fig. 3) (Chen et al., 2009; D’Souza et al., 1997). However, Dspp is weakly expressed in osteoblasts, chondrocytes and bones (Chen et al., 2009; Qin et al., 2002). This suggests that a dose-dependent tuning of Dspp expression plays important roles in cell- and tissue-biological activity and behaviour. For instance, Runx2 is a key factor necessary for osteoblast differentiation and bone formation (Ducy et al., 1997). RUNX2 mutations in humans are related to CCD, with affected subjects displaying short stature, late closure of fontanels and sutures, aplasia of clavicles, hypertelorism, low nasal bridge and dental defects including tooth hypoplasia supernumerary teeth and abnormal tooth eruption (Lee et al., 1997). Runx2 is expressed by dental mesenchymal cells at the early stages and downregulated in odontoblastic cells at the later stages during odontogenesis (Chen et al., 2009). Runx2 stimulates Dspp expression in mouse preodontoblastic cell lines but represses its expression in mouse odontoblastic cells (Chen et al., 2005). Runx2−/− mice present impairment of tooth formation, with progression only to the cap/early bell stages of tooth development. The teeth in Runx2−/− mice are misshapen, severely hypoplastic and lack odontoblast and ameloblast differentiation, while exhibiting loss of normal dentine and enamel matrices (D’Souza et al., 1999). In contrast, in Runx2 Tg mice, odontoblasts lose their normal columnar shape and dentine is surrounded by odontoblasts that are flat or and cuboid in shape. In Runx2 Tg mice, dentine is thin and retains lacunae, which display osteoblast and bone-canaliculi-like structures. Structure of dentinal tubules and pre-dentine is invisible. Moreover, collagen type I expression is decreased and Dspp expression is undetectable (Miyazaki et al., 2008). Therefore, Runx2 function is related to cell- and tissue-type-specific or dependent on the stages of cytodifferentiation during tissue development.

DSP

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

The starting site of DSP contains the signal peptides, which are required for intracellularly trafficking of DSPP from the rER to the ECM. Point mutations of the signal peptides such as Tyr 6 to Asp, Ala 15 to Val, Pro 17 to Leu and Val 18 to Asp together with frameshift mutations resulting in longer mutant hydrophobic domains of DSPP are associated with DD-II, DGI-II and DGI-III (Fig. 2, Table 1,2). In a mouse model, an amino acid on Pro 19 of
the signal peptides of Dspp was substituted by an amino acid on Leu 19 (Liang et al., 2019). The mutant mice Dspp_{P19L/P19L} displayed symptoms similar to human DGI-II and DGI-III, showing enlarged dental pulp chambers in mutant young mice and smaller dental pulp chambers in older mutant mice. These mutant mice exhibited an increase in enamel attrition and an undue deposition of peritubular dentin. Dspp_{P19L/P19L} mice presented a decrease in Dspp expression in odontoblasts as compared to the wild type mice. The secretion of the mutated Dspp was impaired and the mutant Dspp protein accumulated within the rER. The traffic mechanisms of Dspp protein from rER to ECM related to the mutations in the signal peptides associated with DGI and DD are not completely known. Recently, Yin et al. (2018) found that Surf4 (also named Erv29p) is the cargo receptor, which has a high affinity for binding the triple amino acids, IPV, within the signal peptides of DSPP but weakly binds the mutant amino acids of the signal peptides. The wild type DSPP is transported from the rER lumen to the ECM. Specific alterations in a single amino acid of the tripeptide of Dspp result in inadequate aggregate formation of Dspp within the rER and failure to efficiently transport Dspp out of the rER. The mutant signal peptide(s) of Dspp protein accumulate in the rER lumen, forming damaging aggregates and degradation by proteinases within rER (Yin et al., 2018).

DSP is an ECM protein that activates intracellular signalling pathways when dental cells are treated with it (Lee et al., 2012; Ozer et al., 2013). How DSP domain and its cleaved products facilitate intracellular signalling is unknown. Dsp protein was used as a bait for seeking its partner(s) through screening a dental cell protein library and it was found that Dsp acts as a ligand and interacts with 4 cellular membrane proteins including Ocln, integrin β6, CD105 (endoglin) and collagen type IV (Li et al., 2017; Wan et al., 2016). Dsp_{183-219} 36 amino acids are sufficient for interacting with the cellular membrane receptor integrin β6. Dsp-integrin β6 complex stimulates p38 and Erk1/2 phosphorylation and phosphorylated transcription factors Smad1/5/8 (pSmad1/5/8). pSmad1/5/8 interacts with Smad4 and both are translocated into the nucleus, bind to Dspp regulatory region, upregulate Dspp transcription and have a positive feedback on Dspp expression and odontoblast cell homeostasis. Also, Dsp_{183-219} peptide promotes dental cell spreading, migration, proliferation and differentiation. On the other hand, the COOH-terminal domain of Dsp_{363-458} binds to the second loop_{194-241} of Ocln, which is an integral membrane protein (Cong and Kong, 2020). Dsp domain phosphorylates Ocln on Ser$^{490}$ and FAK on Ser$^{722}$ and Tyr$^{576}$ through binding of Ocln to FAK. Dsp_{363-458} facilitates mouse dental papilla mesenchymal and human dental pulp stem cell differentiation and mineralisation. Furthermore, in an in vivo study, Dsp_{363-458} was mixed with agarose beads (Dsp-beads) and the Dsp-beads compound was implanted into mouse dental pulp chambers. The histological analysis showed that in Dsp-beads-treated mice, dental pulp mesenchymal cell proliferation and cell differentiation were significantly improved around the Dsp-beads compound 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 dental pulp chamber and resin. More interestingly, there were a lot of 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$^{363-458}$ is capable of inducing dental mesenchymal cell proliferation, cell differentiation and vasculogenesis (Fig. 5) (Li et al., 2017).

For the \textit{in vivo} study of the biological role of DSP, overexpression of \textit{Dsp} Tg mice driven by the mouse \textit{Dspp} promoter (\textit{Dsp-Dspp} Tg) was generated (Suzuki et al., 2009). \textit{Dsp-Dspp} Tg mice were crossed-bred with the \textit{Dsp}r$^{-/-}$ mice. \textit{Dsp}r$^{+/-}$/\textit{Dsp-Dspp} Tg mice resulted in partial rescue of restored predentine width, decrease of frequent dental pulp chamber exposure and partial recovery in dentine volume compared to \textit{Dspp} KO mice. However, no rescue of dentine mineral density was observed in these \textit{Dsp}r$^{+/-}$/\textit{Dsp-Dspp} Tg mice. This study implies that Dsp is related to the initiation of dentine mineralisation. In addition, overexpression of the \textit{Dsp} Tg mice driven by the mouse \textit{Amg} promoter (\textit{Dsp-Amg} Tg) causes significantly and uniformly increased enamel hardness and an increased rate of enamel mineralisation but did not significantly alter enamel morphology. These studies demonstrated that Dsp significantly contributes to the physical properties of the dentine-enamel junction and facilitates enamel formation (Paine et al., 2005; White et al., 2007).

In contrast, \textit{Dsp} driven by \textit{Col}1\textit{a}1 promoter (\textit{Dsp-Col1\textit{a}1} Tg) Tg mice were crossed-bred with \textit{Dspp} KO mice to generate \textit{Dspp} KO/\textit{Dsp-Col1\textit{a}1} Tg mice (Gibson et al., 2013). Unexpectedly, dentine of \textit{Dspp} KO/\textit{Dsp-Col1\textit{a}1} Tg mice was much thinner, more poorly mineralised and remarkably disorganised than that of \textit{Dsp}r$^{-/-}$ mice. \textit{Dspp} KO/\textit{Dsp-Col1\textit{a}1} Tg mice displayed more severe dentine defects than \textit{Dsp}r$^{-/-}$ mice. Furthermore, \textit{Dspp} KO/\textit{Dsp-Col1\textit{a}1} Tg mice resulted in severely worse periodontal defects than that of \textit{Dspp} KO mice and a greater decrease of alveolar bone, more remarkably altered canalicular structures 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 \textit{Dspp} KO mice (Gibson et al., 2014). Overall, this suggests that the Dsp mediates an inhibitory role in 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**

The present review provides a brief overview of DSPP expression, proteolysis, pathophysiology and biological functions of the cleaved products, DSP/DGP and DPP, based on the recent literature. Dentine is a highly mineralised tissue and derives from odontoblasts. When dentine is injured, such as in cases of 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 (Méndez-Ferrer et al., 2020; Morrison and Spradling, 2008). During dentineogenesis, odontoblasts synthesise and secrete dental ECMs, which bind to calcium-phosphate, finally forming predentine and dentine. Dental ECMs are composed of collagens and NCPs (MacDougall et al., 1997). Among NCPs, DSPP expression is highly visible in odontoblasts and dentine (Fig. 3) (Chen et al. 2009; D’Souza et al., 1997). DSPP is catalysed into DSP/DGP, DPP by BMP1 and TLR proteinases (Marshall and Fisher, 2010; Yamakoshi et al., 2006). Mutations of DSP and DPP domains are associated with DD-I, DD-II, DGI-II, DGI-III and the most common genetic dentine diseases (Fig. 2, Table 1,2). DSP and DPP play unique roles during odontogenesis. DSP promotes the initial effect on early dentine development while DPP is related to HA crystal growth and mineralisation.
DSP is a ligand and facilitates intracellular signalling via its cellular membrane receptors, integrin β6 and Ocln as well as induces dental pulp/MSC cell differentiation and mineralisation. Dsp\textsuperscript{183-219}-β6 signal up-regulates Dspp expression, dental cell proliferation and differentiation via p-p38-pErk-Smad1/5/8 signal pathways, while Dsp\textsuperscript{363-458}-Ocln complex promotes dental mesenchymal cell/MSC differentiation and biomineralisation through FAK cascades (Fig.5a). Overexpression of Dsp partially rescues dentine defects in Dspp KO mice (Suzuki et al., 2009). In addition, DPP-RGD activates downstream gene expression and cell differentiation through integrin-MAPKs and paxillin-FAK signal pathways. Moreover, DPP contains Ser-Asp/Asp-Ser repeat regions, which mediate intracellular calcium store flux and trigger CaMKII-Smad1/5/8 activations, facilitating cell differentiation and mineralisation (Fig.5b). Dpp overexpression partially rescues dentine defects in Dspp KO mice (Zhang et al., 2018). Nevertheless, overexpression of Dsp or Dpp driven by the given gene promoter(s) results in impairment of certain tissues’ development (Gibson et al., 2014; White et al., 2007). How DSP and DPP play dual roles in different tissues is not completely understood and needs to be further studied. Although biological roles of DSPP have made the advanced achievements in odontoblast differentiation and mineralisation during tooth development, mechanisms of DSPP during tooth development and formation remain still unknown. For instance, where cleavages of 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 cell- and tissue-specific expression of DSPP is not completely understood although DSPP expression is controlled by several growth factors, transcriptional factors and materials (Chen et al., 2008; Suzuki et al., 2016). However, understanding the mechanisms of DSPP spatial-temporal expression in odontoblastic cells at different stages during tooth formation and progenitor cell/MSC differentiation to odontoblasts may be a potential novel avenue during dentine development and regeneration.

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List of Abbreviations

- ALP: alkaline phosphatase
- Amg: amelogenin
- ASC: adult stem cell
- BM: bone marrow
- BMP: bone morphogenetic protein
- BSP: bone sialoprotein

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| Abbreviation | Full Form                                      |
|-------------|-----------------------------------------------|
| CAMKII      | calmodulin-dependent protein kinase II        |
| CCD         | cleidocranial dysplasia                       |
| Col1α1      | collagen type I alpha 1                       |
| DD          | dentine dysplasia                             |
| DD-I        | DD type 1                                     |
| DD-II       | DD type 2                                     |
| DGI         | dentineogenesis imperfecta                    |
| DGI-II      | DGI type 2                                    |
| DGI-III     | DGI type 3                                    |
| DGP         | dentine glycoprotein                          |
| DMP1        | dentine matrix protein 1                      |
| DPC         | dental pulp cell                              |
| DPP         | dentine phosphoprotein                        |
| DPSC        | dental pulp stem cell                         |
| DSP         | dentine sialoprotein                          |
| DSPPP       | dentine sialophosphoprotein                   |
| ECM         | extracellular matrix                          |
| ER          | endoplasmic reticulum                         |
| Erk1/2      | extracellular signal-regulated kinase1/2      |
| Erv29p      | ER-derived vesicles protein 29                |
| ESC         | embryonic stem cell                           |
| FAK         | focal adhesion kinase                         |
| GFAP        | glial fibrillary acidic protein               |
| HA          | hydroxyapatite                                |
| hBMSC       | human bone marrow stem cell                   |
| HSC         | haematopoietic stem cell                      |
| iPSC        | induced pluripotent stem cell                 |
| KO          | knockout                                      |
| MAPK        | mitogen-activated protein kinase              |
| Abbreviation | Description |
|--------------|-------------|
| MEPE         | matrix extracellular phosphoglycoprotein |
| MMP          | matrix metalloproteinase |
| MSC          | mesenchymal stem cell |
| NCP          | non-collagenous protein |
| Ocln         | occludin |
| Ocn          | osteocalcin |
| OPN          | osteopontin |
| Osx          | osterix |
| PP           | phosphoryn |
| RD           | reactionary dentine |
| rER          | rough ER |
| RGD          | arginine-glycine-aspartic acid |
| Runx2        | runt-related transcription factor 2 |
| SAPK/JNK     | stress-activated protein kinase/Junamino-terminal kinase |
| SBE          | Smad binding element |
| SCAPs        | stem cells from the apical part of the papilla |
| SDS-PAGE     | sodium dodecyl sulphate-polyaaylamide gel electrophoresis |
| SHED         | human exfoliated deciduous teeth |
| SIBLING      | small integrin-binding ligand N-linked glycoprotein |
| Surf4        | surfeit locus protein 4 |
| Tg           | transgenic |
| TGF-β        | transforming growth factor beta |
| TLR          | Tollloid-like |
| UTR          | untranslated region |
| WHO          | World Health Organization |

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Fig. 1. Schematic representation of a molar and MSCs found in the teeth.
(a) The crown of the tooth is covered with enamel, while the root is covered with cementum. The cementoenamel junction is located at the enamel and root. The root is surrounded by the alveolar bone through periodontal ligaments. The dentine surrounds the dental pulp. Nerves and blood vessels enter the dental pulp from the apical foramen of the tooth and provide nutrition and innervation to odontoblasts and dental pulp. (b) DPSCs; SCAPs; SHEDs
Fig. 2. Diagram of DSPP mutations associated with genetic dentine diseases.
The structure of the human DSPP is shown. Exons are shown as boxes numbered 1-5, with
the amino acids (aa) encoded by each exon indicated below. Introns are represented by lines.
Locations of DSPP mutations are indicated. White colour indicates the 5’ UTR, gray colour DSP
sequences, yellow colour the DPP sequence. Asterisks show mutations affecting splice
sites according to a splice-site recognition software (https://www.phenosystems.com/www/
index.php). c. = cDNA; g. = genomic; del = deletion; dup = duplication; ins = insertion; IVS
= intervening sequence.
Fig. 3. DSPP expression pattern in developing mouse teeth from embryonic day 15 to postnatal month 2.

(a') DSPP mRNA expression was not seen in the dental and osteogenic mesenchyme as well as the dental epithelium at embryonic day 15 (E15). (b') At postnatal day 1 (PN1), DSPP expression was detected in pre-ameloblasts, pre-odontoblasts and weakly in the dental pulp. (c'-e') DSPP expression was mostly restricted to odontoblasts from PN5 to 2 months (M) after birth while DSPP expression was barely seen in bones, (a-e) Brightfield images.

Am, ameloblasts; B, bone; D, dentine; De, dental epithelium; Dm; dental mesenchyme, Dp, dental pulp; E, enamel; Od, odontoblasts; pAm, pre-ameloblasts; pOd, pre-odontoblasts
Fig. 4. Clinical photographs and radiographs from DGI-II patients.
Clinical photograph of a 7 year-old boy showing (a) severe attritions (arrowheads). (b) Radiograph indicates severe enamel loss with decreased pulp space and reduced dental mineral density. (c) Intraoral photographs of a 5 year-old girl exhibiting severe attrition of the primary dentition to the gingiva level and teeth with yellow-brown colour and a translucent appearance (arrowheads). (d) Radiograph shows that dentine was thin, with severe occlusal attrition and periapical abscess (arrowheads).
Fig. 5. Hypothetical model of DSPP signalling during dentineogenesis and dentine regeneration. (a) DSP183-219 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 SBEs in the DSPP regulatory region and activate DSPP transcription. On the other hand, DSP363-458 as a ligand interacts with the extracellular loop2 of Ocln194-241, stimulating Ocln phosphorylation at Ser490. Furthermore, the DSP-Ocln complex stimulates FAK phosphorylation at Ser722 and Tyr576 and then induces dental mesenchymal cell differentiation and mineralisation. (b) DPP binds to integrin α5β3 through its RGD domain. DPP-α5β3 activates MAPK signal pathway and up-regulates gene expression and cell differentiation. The RGD domain of DPP phosphorylates paxillin and FAK at Tyr397. Phosphorylated FAK activates Erk and phosphorylated Erk is translocated into the nucleus and activates transcription factor Elk-1 and downstream gene expression. In addition, the...
DSS repeat region of DPP mediates intracellular calcium store flux and triggers CaMKII activation, resulting in Smad1/5/8 signalling cascade. DSS, Asp-Ser-Ser; RGD, Arg-Gly-Asp.
Table 1.

Summary of DSP mutations associated with inherited dentine defects.

| Location cDNA<sup>a</sup> | Protein | Ethnicity | Diagnosis | Mutation class | References |
|---------------------------|---------|-----------|-----------|----------------|------------|
| **Exon 2**                |         |           |           |                |            |
| C.16T > G g.16T > G      | p.Y6D   | Caucasian | DD-II     | Missense       | Rajpar et al., 2002 |
| c.44C > T g.44C > T      | p.A15V  | Caucasian | DGI-II    | Missense       | Malmgren et al., 2004 |
| c.49C > A g.49C > A      | p.P17T  | Chinese   | DGI-II    | Missense       | Xiao et al., 2001 |
| c.49C > T g.49C > T      | p.P17S  | Caucasian | DGI-II    | Missense       | Hart et al., 2007 |
| c.50C > T g.50 > T       | p.P17L  | Korean    | DGI-II    | Missense       | Li et al., 2012 |
|                          |         | Korean    | DGI-II    | Missense       | Lee et al., 2013 |
| **Intron 2**             |         |           |           |                |            |
| C.52-6T > G g.1185T > G  | p.V18_Q45del | Korean | DGI-II  | Splice site    | Lee et al., 2008 |
| C.52-3C > G              | p.V18_Q45del | Korean | DGI-II  | Splice site    | Kim et al., 2004 |
| g.1188C > G g.1194C > A  | Korean  | DGI-II    | Splice site |                | Kim et al., 2004 |
| IVS2-3C > G              | Caucasian| DGI-II    | Splice site |                | Kim et al., 2004 |
| C.52-3A > A g.1194C > A  | Finnish | DGI-II    | Splice site |                | Holappa et al., 2006 |
| IVS2-3C-A                |         |           |           |                |            |
| c.52-25del23bp IVS2-3C-A | Chinese | DGI-II    | Splice site |                | Wang et al., 2009 |
| C.52-1G > A              | Chinese | DGI-II    | Splice site |                | Liu et al., 2016 |
| C.52-2A > G              | p.V18_Q45del | Thai     | DGI-II    | Missense       | Li et al., 2017 |
|                          |         |           |           |                | Porntaveetus et al., 2019 |
| **Exon 3**               |         |           |           |                |            |
| c.52G > T g.1191G > T   | p.V18F  | Chinese   | DGI-II    | Missense       | Xiao et al., 2001 |
|                          |         | Korean    | DGI-III   | Missense       | Kim et al., 2005 |
| Location       | Protein     | Ethnicity | Diagnosis | Mutation class | References       |
|----------------|-------------|-----------|-----------|----------------|------------------|
| cDNA           |             |           |           |                |                  |
|                |             | Caucasian | DGI-III   | Missense       | Kim et al., 2005 |
|                |             | Chinese   | DGI-II    | Missense       | Song et al., 2006|
|                |             | Finnish   | DGI-II    | Missense       | Holappa et al., 2006|
|                |             | Chinese   | DGI-II    | Missense       | Li et al., 2017   |
| c.53T > A      | p.V18D      | Japanese  | DGI-II    | Missense       | Kida et al., 2009 |
| g.1192T > A    |             | Korean    | DGI-II    | Missense       | Kim et al., 2009  |
|                |             | Korean    | DGI-II    | Missense       | Kim et al., 2011  |
| C.133C > T     | p.Q45X      | Chinese   | DGI-II    | Missense       | Zhang et al., 2001|
| g.1272C > T    |             | Chinese   | DGI-II    | Missense       | Song et al., 2006 |
| Intron 3       |             |           |           |                |                  |
| c.135 + 2T > C |             | Chinese   | DGI-II    | Splice site    | Zhang et al., 2011|
| g.8662 T > C   |             |           |           |                |                  |
| c.135 + 1G     | p.V18_Q45del| Chinese   | DGI-II    | Splice site    | Xiao et al., 2001|
| g.1275G > A    |             |           |           |                |                  |
| IVS3 + 1G > A  |             |           |           |                |                  |
| c.135 + 3A > G | p.V18_Q45del| Caucasian | DGI-II    | Splice site    | McKnight et al., 2008|
| IVS3 + 3A > G  |             |           |           |                |                  |
| Exon 4         |             |           |           |                |                  |
| c.202A > T     | p.R68W      | Caucasian | DGI-II    | Missense       | Malmgren et al., 2004|
| g.1474A > T    |             | Finnish   | DGI-II    | Missense       | Holappa et al., 2006|

*Numbering assumes the A of the ATG start codon as nucleotide 1. Reference sequence NM_014208.3. c. = cDNA; g. = genomic; p. = protein; del = deletion; IVS = intervening sequence.*
Table 2.

Summary of *DPP* mutations associated with inherited dentin defects.

| Location cDNA<sup>a</sup> | Protein     | Ethnicity | Diagnosis | Mutation class | Reference          |
|---------------------------|-------------|-----------|-----------|----------------|--------------------|
| Exon 5                    |             |           |           |                |                    |
| c.1686delT                | p.D562fsX752| Finnish   | DD-II     | Frameshift     | Nieminen et al., 2011 |
| c.1830delC                | p.S610fsX704| French    | DD-II     | Frameshift     | Nieminen et al., 2011 |
| c.1870_1873delTCAG        | p.S624fsX707| Caucasian | DD-II     | Frameshift     | McKnight et al., 2008 |
| c.1874_1877delACAG        | p.D625fsX687| Chinese   | DD-II     | Frameshift     | Li et al., 2017     |
| c.1915_1918delAAGT        | p.K639fsX67 | Thai      | DOI       | Frameshift     | Pompavert et al., 2017 |
| c.1918_1921delTCAG        | p.S640fsX67 | Caucasian | DD-II     | Frameshift     | McKnight et al., 2008 |
| c.1918_1921delTCAG        | p.S640fsX673| Greek     | DD-II     | Frameshift     | Nieminen et al., 2011 |
| c.1922_1925delACAG        | p.D641fsX672| Finnish   | DD-II     | Frameshift     | Nieminen et al., 2011 |
| c.2040delC                | p.S680fsX67 | Chinese   | DD-II     | Frameshift     | Song et al., 2008   |
| c.2063delA                | p.D688fsX626| Finnish   | DD-II     | Frameshift     | Nieminen et al., 2011 |
| c.2134delA                | p.S712fsX602| Turkish   | DD-II     | Frameshift     | Lee et al., 2019    |
| c.2272delA                | p.S758fsX554| Caucasian | DGI-II/III| Frameshift     | McKnight et al., 2008 |
| c.2349delTC                | p.S783fsX531| Spanish   | DGI-II/III| Frameshift     | Nieminen et al., 2011 |
| c.2525delG                | p.S842fsX471| Caucasian | DGI-II/III| Frameshift     | McKnight et al., 2008 |
| c.2593delA                | p.S865fsX133| Chinese   | DGI-II/III| Frameshift     | Song et al., 2008   |
| c.2666delG                | p.S889fsX425| Greek     | DGI-II/III| Frameshift     | Nieminen et al., 2011 |
| c.2688delG                | p.S895fsX133| Chinese   | DGI-II/III| Frameshift     | Song et al., 2008   |
| c.2688delG                | p.S895fsX133| Chinese   | DGI-II/III| Frameshift     | Li et al., 2017     |
| c.2688delG                | p.N896fsX418| Korean    | DGI-II/III| Frameshift     | Lee et al., 2010    |
| c.3085A>G                 | p.N1029D    | Chinese   | NSHFb     | Missense       | Li et al., 2018     |
| c.3087C>T                 | p.N1029D    | Chinese   | NSHFb     | Missense       | Li et al., 2018     |
| c.3135delI                | p.S1045fsX269| Caucasian| DD-II     | Frameshift     | McKnight et al., 2008 |
| c.3141delC                | p.S1045fsX269| Caucasian| DD-II     | Frameshift     | McKnight et al., 2008 |

<sup>a</sup>Mutation class: Frameshift

<sup>b</sup>Mutation class: Missense
| Location cDNA | Protein | Ethnicity | Diagnosis | Mutation class | Reference |
|--------------|---------|-----------|-----------|----------------|-----------|
| c.3179delG   | p.S1060TfsX254 | Korean | DD-II | Frameshift | Lee et al., 2019 |
| c.3438delC   | p.D1146fsX1313 | Chinese | DDI-II | Frameshift | Song et al., 2008 |
| c.3480_3481insCTGCT | p.D1161fsX155 | Korean | DD-II | Frameshift | Lee et al., 2019 |
| c.3503_3509delG | p.D1170fsX146 | Chinese | DDI-II | Frameshift | Yang et al., 2015 |
| c.3509_3513del13bp | p.D1170fsX139 | Chinese | DDI-II | Frameshift | Li et al., 2017 |
| c.3546_3550delG | p.D1182fsX132 | Chinese | DDI-II | Frameshift | Song et al., 2008 |
| c.3560delG   | p.S1187MfsX127 | Korean | DDI-II | Frameshift | Lee et al., 2011 |
| c.3582_3591del10bp | p.D1194fsX117 | Finnish | DDI-II | Frameshift | Nieminen et al., 2011 |
| c.3599_3614del13bp | p.D1209fsX80 | Vietnamese | DDI-II | Frameshift | Nieminen et al., 2011 |

*aNumbering assumes the A of the ATG start codon as nucleotide 1. Reference sequence eNM_014208.3.*

*bNSHL, familial nonsyndromic hearing loss. c. = cDNA; p. = protein; fs = frameshift; del = deletion; ins = insert.*