

## DENTINE SIALOPHOSPHOPROTEIN SIGNAL IN DENTINOGENESIS 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  $\alpha V\beta 3$  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  $\beta 6$  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.

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List of Abbreviations		BSP	bone sialoprotein
ALP	alkaline phosphatase	CAMKII	calmodulin-dependent protein kinase II
Amg	amelogenin	CCD	cleidocranial dysplasia
ASC	adult stem cell	Col1 $\alpha$ 1	collagen type I alpha 1
BM	bone marrow	DD	dentine dysplasia
BMP	bone morphogenetic protein	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
DSPP	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
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/Jun-amino-terminal kinase
SBE	Smad binding element
SCAPs	stem cells from the apical part of the papilla
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SHED	human exfoliated deciduous teeth
SIBLING	small integrin-binding ligand N-linked glycoprotein
Surf4	surfeit locus protein 4
Tg	transgenic
TGF- $\beta$	transforming growth factor beta
TLR	Tolloid-like
UTR	untranslated region
WHO	World Health Organization

## 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; Morszeck *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; Kazemian *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- $\beta$  signalling, recent studies have demonstrated that Wnt/ $\beta$ -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,

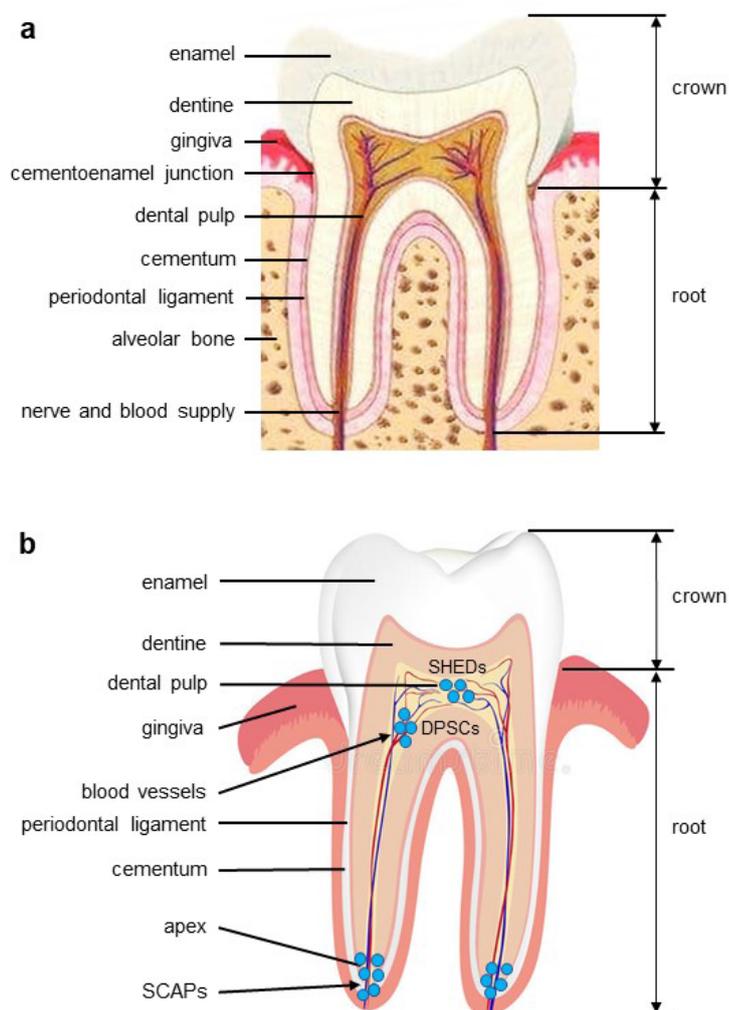
they can only produce a tissue for a given time (Walker *et al.*, 2019). By contrast, dental MSCs are self-renewing and able to generate any of the tissues for their entire life span. In the dental pulp, dental MSCs include DPSCs, SHED and SCAP (Fig. 1b) (Gronthos *et al.*, 2000; Miura *et al.*, 2003; 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,



**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 cemento-enamel 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.

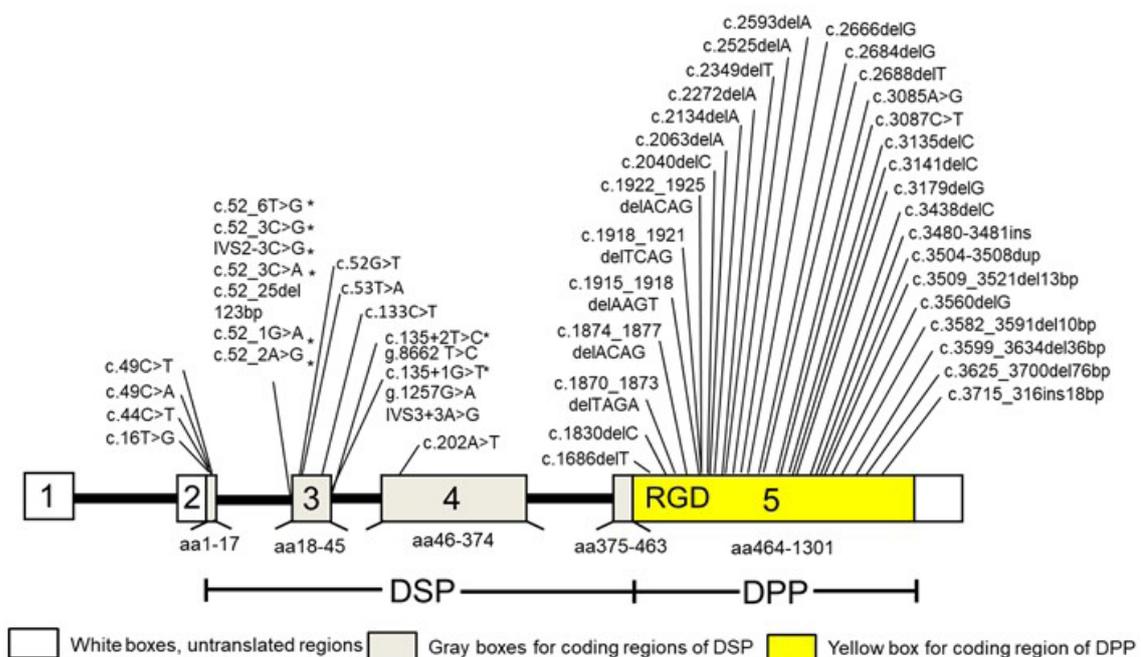
Nanog and others (Lee and Seo, 2016; Sonoyama *et al.*, 2008). Among these markers, CD146 and STRO-1 co-expression is related to early-MSc phenotype. Certainly, CD146<sup>+</sup>/STRO-1<sup>+</sup> SCAPs show superior colony-forming efficiency, with increased cumulative doubling compared with their counterpart (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 (Morsczech *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



**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.

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 (MacDougall *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 NH<sub>2</sub>-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 (Marschall 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 (Ser<sup>392</sup> to Gly<sup>472</sup>) located between DSP and DPP fragments. DGP contains 4 phosphorylated serine residues (Ser<sup>453</sup>, Ser<sup>455</sup>, Ser<sup>457</sup> and Ser<sup>462</sup>) and 1 glycosylated asparagine (Asn<sup>397</sup>). 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*<sup>-/-</sup>) 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  $\alpha v \beta 3$ ,  $\alpha v \beta 5$ ,  $\alpha v \beta 1$ ,  $\alpha 4 \beta 1$ ,  $\alpha 8 \beta 1$ ,  $\alpha 9 \beta 1$  and CD44 splice variants

**Table 1. Summary of DSP mutations associated with inherited dentine defects.** <sup>a</sup> 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.

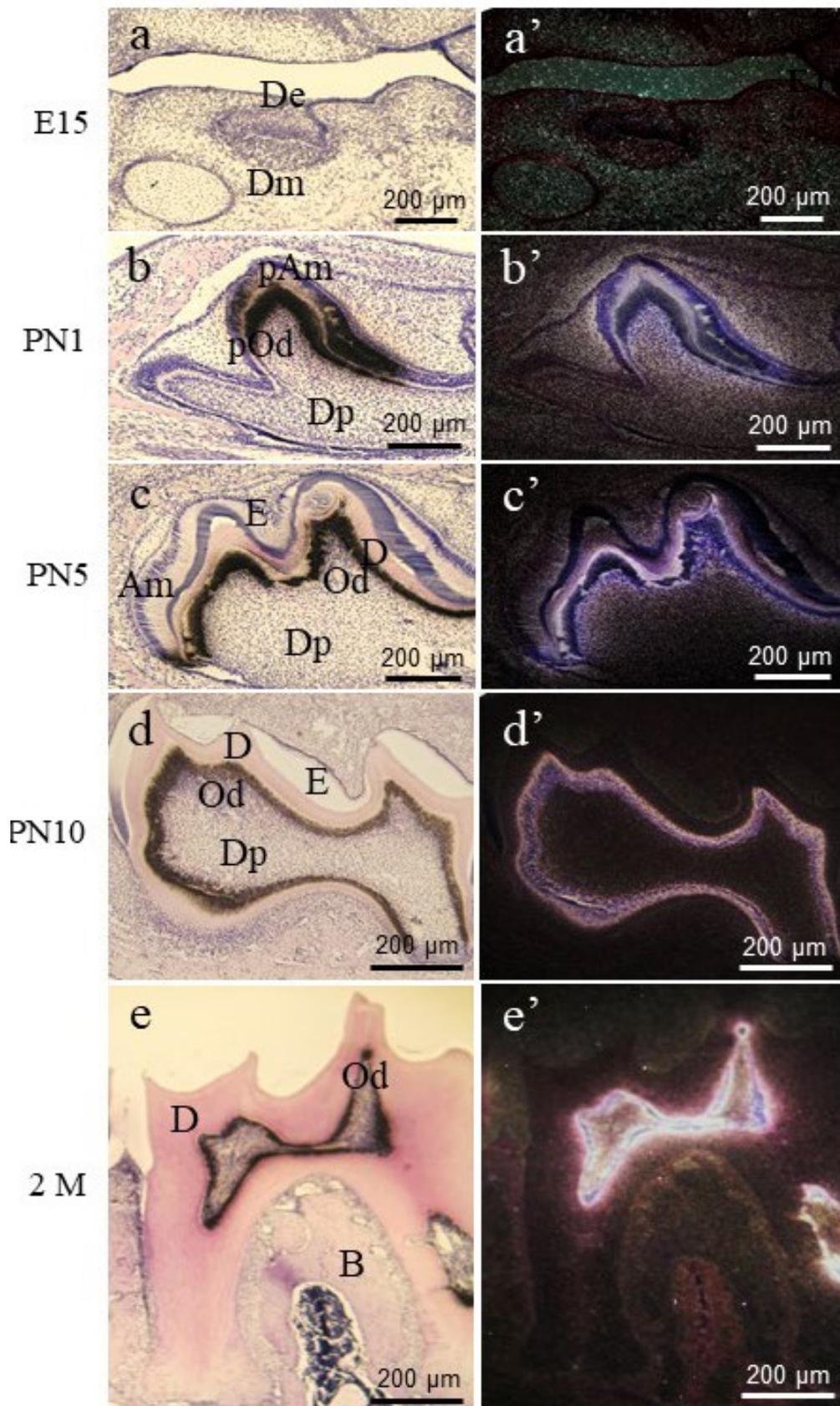
Location cDNA <sup>a</sup>	Protein	Ethnicity	Diagnosis	Mutation class	References
<b>Exon 2</b>					
c.16T > G g.16T > G	p.Y6D	Caucasian	DD-II	Missense	Rajpar <i>et al.</i> , 2002
c.44C > T g.44C > T	p.A15V	Caucasian	DGI-II	Missense	Malmgren <i>et al.</i> , 2004
c.49C > A g.49C > A	p.P17T	Chinese	DGI-II	Missense	Xiao <i>et al.</i> , 2001
c.49C > T g.49C > T	p.P17S	Chinese	DGI-II	Missense	Zhang <i>et al.</i> , 2007
		Caucasian	DGI-II	Missense	Hart <i>et al.</i> , 2007
		Caucasian	DGI-II	Missense	McKnight <i>et al.</i> , 2008
c.50C > T g.50 > T	p.P17L	Chinese	DGI-II	Missense	Li <i>et al.</i> , 2012
		Korean	DGI-II	Missense	Lee <i>et al.</i> , 2013
<b>Intron 2</b>					
c.52-6T > G g.1185T > G IVS2-6T > G	p.V18_Q45del	Korean	DGI-II	Splice site	Lee <i>et al.</i> , 2008
c.52-3C > G	p.V18_Q45del	Korean	DGI-II	Splice site	Kim <i>et al.</i> , 2004
g.1188C > G IVS2-3C > G		Korean	DGI-II	Splice site	Kim <i>et al.</i> , 2004
		Caucasian	DGI-II	Splice site	Kim <i>et al.</i> , 2004
c.52-3C > A g.1194C > A IVS2-3		Finnish	DGI-II	Splice site	Holappa <i>et al.</i> , 2006
c.52-25del23bp IVS2-3C-A		Chinese	DGI-II	Splice site	Wang <i>et al.</i> , 2009
c.52-1G > A		Chinese	DGI-II	Splice site	Liu <i>et al.</i> , 2016
c.52-2A > G	p.V18_Q45del	Chinese	DGI-III	Splice site	Li <i>et al.</i> , 2017
		Thai	DGI-II	Missense	Porntaveetus <i>et al.</i> , 2019
<b>Exon 3</b>					
c.52G > T g.1191G > T	p.V18F	Chinese	DGI-II	Missense	Xiao <i>et al.</i> , 2001
		Korean	DGI-III	Missense	Kim <i>et al.</i> , 2005
		Caucasian	DGI-III	Missense	Kim <i>et al.</i> , 2005
		Chinese	DGI-II	Missense	Song <i>et al.</i> , 2006
		Finnish	DGI-II	Missense	Holappa <i>et al.</i> , 2006
		Chinese	DGI-II	Missense	Li <i>et al.</i> , 2017
c.53T > A g.1192T > A	p.V18D	Japanese	DGI-II	Missense	Kida <i>et al.</i> , 2009
		Korean	DGI-II	Missense	Kim <i>et al.</i> , 2009
		Korean	DGI-II	Missense	Kim <i>et al.</i> , 2011
c.133C > T g.1272C > T	p.Q45X	Chinese	DGI-II	Missense	Zhang <i>et al.</i> , 2001
		Chinese	DGI-II	Missense	Song <i>et al.</i> , 2006
<b>Intron 3</b>					
c.135 + 2T > C g.8662 T > C		Chinese	DGI-II	Splice site	Zhang <i>et al.</i> , 2011
c.135 + 1G g.1275G > A IVS3 + 1G > A	p.V18_Q45del	Chinese	DGI-II	Splice site	Xiao <i>et al.</i> , 2001
c.135 + 1G > T	p.V18_Q45del	Caucasian	DGI-II	Splice site	McKnight <i>et al.</i> , 2008
c.135 + 3A > G IVS3 + 3A > G		Mongolian	DGI-II	Splice site	Bai <i>et al.</i> , 2010
<b>Exon 4</b>					
c.202A > T g.1474A > T	p.R68W	Caucasian	DGI-II	Missense	Malmgren <i>et al.</i> , 2004
		Finnish	DGI-II	Missense	Holappa <i>et al.</i> , 2006

**Table 2. Summary of DPP mutations associated with inherited dentin defects.** <sup>a</sup> Numbering assumes the A of the ATG start codon as nucleotide 1. Reference sequence eNM\_014208.3. <sup>b</sup> NSHL, familial nonsyndromic hearing loss. c. = cDNA; p. = protein; fs = frameshift; del = deletion; ins = insert.

Location cDNA <sup>a</sup>	Protein	Ethnicity	Diagnosis	Mutation class	Reference
<b>Exon 5</b>					
c.1686delT	p.D562EfsX752	Finnish	DD-II	Frameshift	Nieminen <i>et al.</i> , 2011
c.1830delC	p.S610RfsX704	French	DD-II	Frameshift	Nieminen <i>et al.</i> , 2011
			DD-II	Frameshift	McKnight <i>et al.</i> , 2008
c.1870_1873delTCAG	p.S624TfsX687	Caucasian	DD-II	Frameshift	McKnight <i>et al.</i> , 2008 <sup>b</sup>
c.1874_1877delACAG	p.D625AfsX687	Chinese	DDI-II	Frameshift	Li <i>et al.</i> , 2017
c.1915_1918delAAGT	p.K639QfsX67	Thai	DGI	Frameshift	Porntaveetus <i>et al.</i> , 2017
c.1918_1921delTCAG	p.S640TfsX671	Caucasian	DD-II	Frameshift	McKnight <i>et al.</i> , 2008 <sup>b</sup>
c.1918_1921delTCAG	p.S640TfsX673	Greek	DD-II	Frameshift	Nieminen <i>et al.</i> , 2011
c.1922_1925delACAG	p.D641AfsX672	Finnish	DD-II	Frameshift	Nieminen <i>et al.</i> , 2011
c.2040delC	p.S680fsX1313	Chinese	DD-II	Frameshift	Song <i>et al.</i> , 2008
c.2063delA	p.D688VfsX626	Finnish	DD-II	Frameshift	Nieminen <i>et al.</i> , 2011
c.2134delA	p.S712AfsX602	Turkish	DD-II	Frameshift	Lee <i>et al.</i> , 2019
c.2272delA	p.S758AfsX554	Caucasian	DGI-II/III	Frameshift	McKnight <i>et al.</i> , 2008 <sup>b</sup>
c.2349delT	p.S783RfsX531	Spanish	DDI-II	Frameshift	Nieminen <i>et al.</i> , 2011
c.2525delG	p.S842TfsX471	Caucasian	DGI-II/III	Frameshift	McKnight <i>et al.</i> , 2008 <sup>b</sup>
c.2593delA	p.S865fsx1313	Chinese	DDI-II	Frameshift	Song <i>et al.</i> , 2008
c.2666delG	p.S889TfsX425	Greek	DDI-II	Frameshift	Nieminen <i>et al.</i> , 2011
c.2684delG	p.S895fsx1313	Chinese	DDI-II	Frameshift	Song <i>et al.</i> , 2008
		Chinese	DDI-II	Frameshift	Li <i>et al.</i> , 2017
c.2688delT	p.N896EfsX418	Korean	DDI-II	Frameshift	Lee <i>et al.</i> , 2010
c.3085A>G	p.N1029D	Chinese	NSHLb	Missense	Li <i>et al.</i> , 2018
c.3087C>T	p.N1029D	Chinese	NSHLb	Missense	Li <i>et al.</i> , 2018
c.3135delC	p.S1045RfsX269	Caucasian	DD-II	Frameshift	McKnight <i>et al.</i> , 2008 <sup>a</sup>
c.3141delC		Caucasian	DD-II	Frameshift	McKnight <i>et al.</i> , 2008 <sup>b</sup>
c.3179delG	p.S1060TfsX254	Korean	DD-II	Frameshift	Lee <i>et al.</i> , 2019
c.3438delC	p.D1146fsX1313	Chinese	DDI-II	Frameshift	Song <i>et al.</i> , 2008
c.3480_3481insCTGCT	p.D1161LfsX155	Korean	DD-II	Frameshift	Lee <i>et al.</i> , 2019
c.3504_3508dup	p.D1170AfsX146	Chinese	DDI-II	Frameshift	Yang <i>et al.</i> , 2015
c.3509_3521del13bp	p.D1170AfsX139	Chinese	DDI-II	Frameshift	Li <i>et al.</i> , 2017
c.3546_3550delTAGCAinsG	p.D1182EfsX1312	Chinese	DDI-II	Frameshift	Song <i>et al.</i> , 2008
c.3560delG	p.S1187MfsX127	Korean	DDI-II	Frameshift	Lee <i>et al.</i> , 2011
c.3582_3591del10bp	p.D1194EfsX117 delCAGCAGCGAT	Finnish	DDI-II	Frameshift	Nieminen <i>et al.</i> , 2011
c.3599_3634del36bp c.3715_3716ins18bp del1160_1171 ins1198_1199		American	DGI-III	Frameshift	Dong <i>et al.</i> , 2005
c.3625_3700del76bp	p.D1209AfsX80	Vietnamese	DDI-II	Frameshift	Nieminen <i>et al.</i> , 2011

(Bellahcène *et al.*, 2008; Marschall and Fisher, 2008). OPN by thrombin cleavage separates the CD44<sup>-</sup> and integrin-binding domains, which in some cases promote adhesion over cell migration. Another example is the thrombin-cleaved NH<sub>2</sub>-terminal OPN segment that interacts with  $\alpha$ v $\beta$ 3 and  $\alpha$ v $\beta$ 5 integrins *via* the RGD motif (Bellahcène *et al.*, 2008; Furger *et al.*, 2003) or with  $\alpha$ 4 $\beta$ 1 and  $\alpha$ 9 $\beta$ 1 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  $\beta$ 1-containing integrins (Agnihotri *et al.*, 2001). OPN is also a substrate for plasma transglutaminase factor IIIa and liver transglutaminase (Prince *et al.*, 1991) and enhances cell adhesion, spreading and migration (Higashikawa *et al.*, 2007). The RGD domain of DMP1 only binds to  $\alpha$ v $\beta$ 3, while BSP-RGD motif not only interacts with  $\alpha$ v $\beta$ 3, but also with  $\alpha$ v $\beta$ 5 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; Steiglit *et al.*, 2004).



**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.

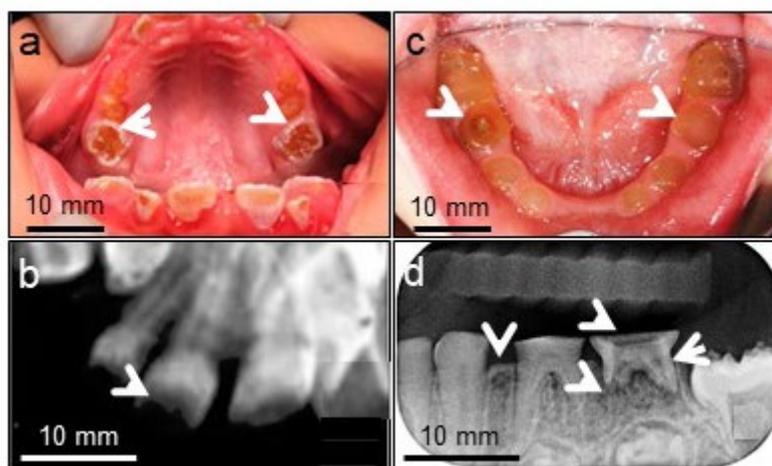
### DPP

DPP contains an RGD domain at the NH<sub>2</sub>-terminal site, acting as a ligand and binding to integrin  $\alpha$ V $\beta$ 3. DPP-RGD/integrin- $\alpha$ V $\beta$ 3 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 rudimentary (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<sup>2+</sup> release. This calcium flux promotes the activation of Ca<sup>2+</sup> 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

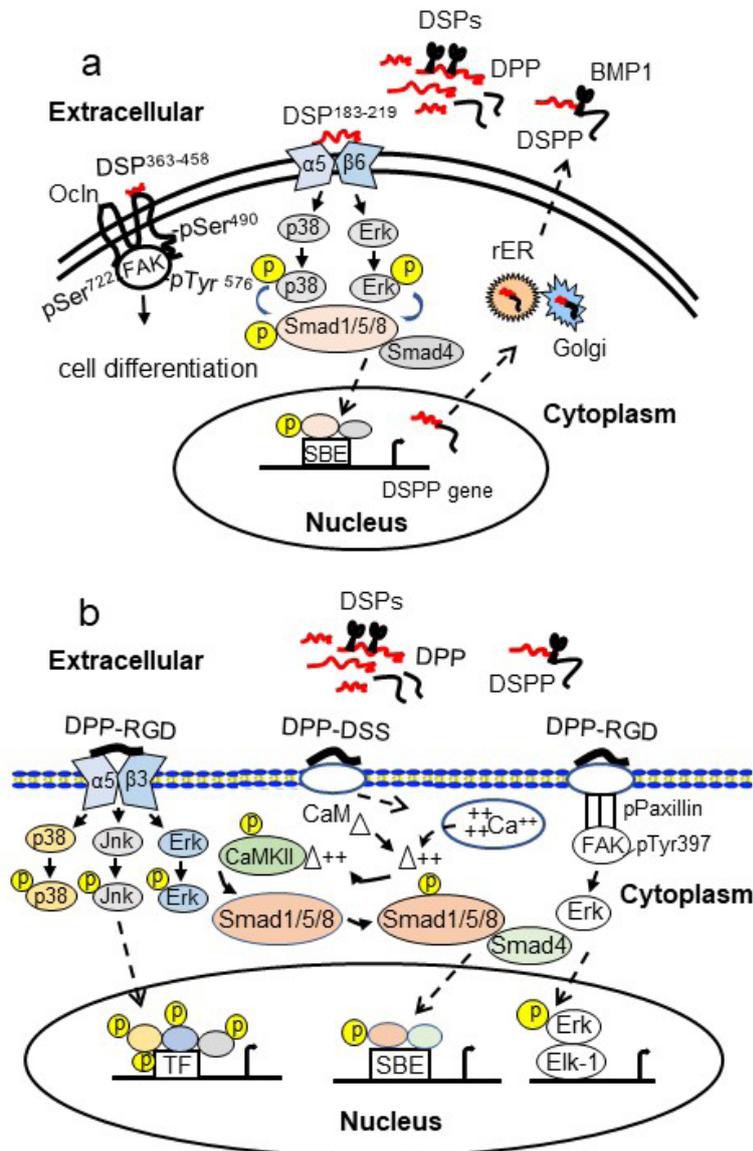


**Fig. 4. Clinical photographs and radiographs from DGI-II patients.** Clinical photograph of a 7 year-old boy showing (a) severe attritions (arrowheads). (b) Radiograph indicates severe enamel loss with decreased pulp space and reduced dental mineral density. (c) Intraoral photographs of a 5 year-old 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).

osteosarcoma (Saos-2) cells as well as promote cell differentiation (Gulseren *et al.*, 2019). The biological function of Dpp was narrowed-down to 3 Asp-Ser-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 *Col1a1* promoter (*Dpp-Col1a1* Tg) were crossed-bred with

*Dspp* KO (*Dspp*<sup>-/-</sup>) mice to generate *Dspp* KO/*Dpp-Col1a1* Tg mice (Zhang *et al.*, 2018). *Dspp* KO/*Dpp-Col1a1* 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-Col1a1* Tg mice. Scanning electron microscopy analysis demonstrated that the structure of dentinal tubules in *Dspp* KO/*Dpp-Col1a1* Tg mice was better organised than that of *Dspp* KO mice.



**Fig. 5. Hypothetical model of DSPP signalling during dentineogenesis and dentine regeneration.** (a) DSP<sup>183-219</sup> 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, DSP<sup>363-458</sup> as a ligand interacts with the extracellular loop2 of Ocln<sup>194-241</sup>, stimulating Ocln phosphorylation at Ser<sup>490</sup>. Furthermore, the DSP-Ocln complex stimulates FAK phosphorylation at Ser<sup>722</sup> and Tyr<sup>576</sup> 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 Tyr<sup>397</sup>. 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.

Dentine mineral deposition rate in *Dspp* KO/*Dpp-Col1 $\alpha$ 1* 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-Col1 $\alpha$ 1* Tg mice was lower compared to that of wild type mice. Moreover, both short and long bones were shorter in *Dpp-Col1 $\alpha$ 1* Tg mice compared to that of wild type mice. *Dpp-Col1 $\alpha$ 1* 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-Col1 $\alpha$ 1* 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 *Amg* 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*<sup>-/-</sup> mice present impairment of tooth formation, with progression only to the cap/early bell stages of tooth development. The teeth in *Runx2*<sup>-/-</sup> 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-canalculi-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*<sup>P19L/P19L</sup> 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*<sup>P19L/P19L</sup> 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 Dsp 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  $\beta 6$ , CD105 (endoglin) and collagen type IV (Li *et al.*, 2017; Wan *et al.*, 2016). Dsp<sup>183-219</sup> 36 amino acids are sufficient for interacting with the cellular membrane receptor integrin  $\beta 6$ . Dsp-integrin  $\beta 6$  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<sup>183-219</sup> peptide promotes dental cell spreading, migration, proliferation and differentiation. On the other hand, the COOH-terminal domain of Dsp<sup>363-458</sup> binds to the second loop<sup>194-241</sup> of Ocln, which is an integral membrane protein (Cong and Kong, 2020). Dsp domain phosphorylates Ocln on Ser<sup>490</sup> and FAK on Ser<sup>722</sup> and Tyr<sup>576</sup> through binding of Ocln to FAK. Dsp<sup>363-458</sup> facilitates mouse dental papilla mesenchymal and human dental pulp stem cell differentiation and mineralisation. Furthermore, in an *in vivo* study, Dsp<sup>363-458</sup> 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<sup>363-458</sup> is capable of inducing dental mesenchymal cell proliferation, cell differentiation and vasculogenesis (Fig. 5) (Li *et al.*, 2017).

For the *in vivo* study of the biological role of DSP, overexpression of *Dsp* Tg mice driven by the mouse *Dspp* promoter (*Dsp-Dspp* Tg) was generated (Suzuki *et al.*, 2009). *Dsp-Dspp* Tg mice were crossed-bred with the *Dspp*<sup>-/-</sup> mice. *Dspp*<sup>-/-</sup>/*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 *Dspp* KO mice. However, no rescue of dentine mineral density

was observed in these *Dspp*<sup>-/-</sup>/*Dsp-Dspp* Tg mice. This study implies that Dsp is related to the initiation of dentine mineralisation. In addition, overexpression of the *Dsp* Tg mice driven by the mouse *Amg* promoter (*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, *Dsp* driven by *Col1a1* promoter (*Dsp-Col1a1* Tg) Tg mice were crossed-bred with *Dspp* KO mice to generate *Dspp* KO/*Dsp-Col1a1* Tg mice (Gibson *et al.*, 2013). Unexpectedly, dentine of *Dspp* KO/*Dsp-Col1a1* Tg mice was much thinner, more poorly mineralised and remarkably disorganised than that of *Dspp*<sup>-/-</sup> mice. *Dspp* KO/*Dsp-Col1a1* Tg mice displayed more severe dentine defects than *Dspp*<sup>-/-</sup> mice. Furthermore, *Dspp* KO/*Dsp-Col1a1* Tg mice resulted in severely worse periodontal defects than that of *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 *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 (Marschall 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 (Suzuki *et al.*, 2016). DSP is a ligand and facilitates intracellular signalling *via* its cellular membrane receptors, integrin  $\beta 6$  and Ocln as well as induces dental pulp/ MSC cell differentiation and mineralisation. Dsp<sup>183-219</sup>- $\beta 6$  signal up-regulates *Dspp* expression, dental cell proliferation and differentiation *via* p-p38-pErk-Smad1/5/8 signal pathways, while Dsp<sup>363-458</sup>-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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### References

- Agnihotri R, Crawford HC, Haro H, Matrisian LM, Havrda MC, Liaw L (2001) Osteopontin, a novel substrate for matrix metalloproteinase-3 (stromelysin-1) and matrix metalloproteinase-7 (matrilysin). *J Biol Chem* **276**: 28261-28267.
- Alvares K, Stern PH, Arthur Veis A (2013) Dentin phosphoprotein binds annexin 2 and is involved in calcium transport in rat kidney ureteric bud cells. *J Biol Chem* **288**: 13036-13045.
- Askar H, Krois J, Göstemeyer G, Schwendicke F (2021) Secondary caries risk of different adhesive strategies and restorative materials in permanent teeth: systematic review and network meta-analysis. *J Dent* **104**: 103541. DOI: 10.1016/j.jdent.2020.103541.
- Bai H, Agula H, Wu Q, Zhou W, Sun Y, Qi Y, Latu S, Chen Y, Mutu J, Qiu C (2010) A novel DSPP mutation causes dentinogenesis imperfecta type II in a large mongolian family. *BMC Med Genet* **11**: 23. DOI: 10.1186/1471-2350-11-23.
- Baker JL, Morton JT, Dinis M, Alvarez R, Tran NC, Knight R, Edlund A (2021) Deep metagenomics examines the oral microbiome during dental caries, revealing novel taxa and co-occurrences with host molecules. *Genome Res* **31**: 64-74.
- Bellahcène A, Castronovo V, Ogbureke KUE, Fisher LW, Fedarko NS (2008) Small integrin-binding ligand N-linked glycoproteins (SIBLINGs): multifunctional proteins in cancer. *Nat Rev Cancer* **8**: 212-226.
- Bento L, Zhang Z, Imai A, Nör F, Dong Z, Shi S, Araujo F, Nör J (2013) Endothelial differentiation of SHED requires MEK1/ERK signaling. *J Dent Res* **92**: 51-57.
- Bernabe E, Marcenes W, Hernandez CR, Bailey J, Abreu LG, Alipour V, Amini S, Arabloo J, Arefi Z, Arora A, Ayanore MA, Bärnighausen TW, Bijani A, Cho DY, Chu DT, Crowe CS, Demoz GT, Demsie DG, Dibaji Forooshani ZS, Du M, El Tantawi M, Fischer F, Folayan MO, Futran ND, Geramo YCD, Haj-Mirzaian A, Hariyani N, Hasanzadeh A, Hassanipour S, Hay SI, Hole MK, Hostiuc S, Ilic MD, James SL, Kalhor R, Kemmer L, Keramati M, Khader YS, Kisa S, Kisa A, Koyanagi A, Laloo R, Le Nguyen Q, London SD, Manohar ND, Massenburg BB, Mathur MR, Meles HG, Mestrovic T, Mohammadian-Hafshejani A, Mohammadpourhodki R, Mokdad AH, Morrison SD, Nazari J, Nguyen TH, Nguyen CT, Nixon MR, Olagunju TO, Pakshir K, Pathak M, Rabiee N, Rafiei A, Ramezanzadeh K, Rios-Blancas MJ, Roro EM, Sabour S, Samy AM, Sawhney M, Schwendicke F, Shhmadi F, Shaikh MA, Stein C, Tovani-Palone MR, Tran BX, Unnikrishnan B, Vu GT, Vukovic A, Warouw TSS, Zaidi Z, Zhang ZJ, Kassebaum NJ (2020) Global, regional, and national levels and trends

in burden of oral conditions from 1990 to 2017: a systematic analysis for the global burden of disease 2017 study. *J Dent Res* **99**: 362-373.

Bluteau G, Luder HU, De Bari C, Mitsiadis TA (2008) Stem cells for tooth engineering. *Eur Cell Mater* **16**: 1-9.

Burke FJ, Lucarotti PS (2009) How long do direct restorations placed within the general dental services in England and Wales survive? *Br Dent J* **206**: 1-5.

Cable J, Fuchs E, Weissman I, Jasper H, Glass D, Rando TA, Blau H, Debnath S, Oliva A, Park S, Passegué E, Kim C, Krasnow MA (2020) Adult stem cells and regenerative medicine—a symposium report. *Ann N Y Acad Sci* **1462**: 27-36.

Chai Y, Jiang X, Ito Y, Bringas P, Han J, Rowitch DH, Soriano P, McMahon AP, Sucov HM (2000) Fate of the mammalian cranial neural crest during tooth and mandibular morphogenesis. *Development* **127**: 1671-1679.

Chaplet M, Waltregny D, Detry C, Fisher LW, Castronovo V, Bellahcène A (2006) Expression of dentin sialophosphoprotein in human prostate cancer and its correlation with tumor aggressiveness. *Int J Cancer* **118**: 850-856.

Chen H, Gu L, Liao B, Zhou X, Cheng L, Ren B (2020) Advances of anti-caries nanomaterials. *Molecules* **25**: 5047. DOI: 10.3390/molecules25215047.

Chen S, Rani S, Wu Y, Unterbrink A, Gu TT, Gluhak-Heinrich J, Chuang HH, Macdougall M (2005) Differential regulation of dentin sialophosphoprotein expression by Runx2 during odontoblast cytodifferentiation. *J Biol Chem* **280**: 29717-29727.

Chen S, Gluhak-Heinrich J, Martinez M, Li T, Wu Y, Chuang HH, Chen L, Dong J, Gay I, MacDougall M (2008) Bone morphogenetic protein 2 mediates dentin sialophosphoprotein expression and odontoblast differentiation *via* NF- $\kappa$ B signaling. *J Biol Chem* **283**: 19359-19370.

Chen S, Gluhak-Heinrich J, Wang YH, Wu YM, Chuang HH, Chen L, Yuan GH, Dong J, Gay I, MacDougall M (2009). Runx2, Osx and Dspp in tooth development. *J Dental Res* **88**: 904-909.

Chen XD, Dusevich V, Feng JQ, Manolagas SC, Jilka RL (2007) Extracellular matrix made by bone marrow cells facilitates expansion of marrow-derived mesenchymal progenitor cells and prevents their differentiation into osteoblasts. *J Bone Miner Res* **22**: 1943-1956.

Chung HY, Li CC, Chia-Chan Hsu CC (2012) Characterization of the effects of 3DSS peptide on remineralized enamel in artificial saliva. *J Mech Behav Biomed Mater* **6**: 74-79.

Cong X, Kong W (2020) Endothelial tight junctions and their regulatory signaling pathways in vascular homeostasis and disease. *Cell Signal* **66**: 109485. DOI: 10.1016/j.cellsig.2019.109485.

Couve E, Osorio R, Schmachtenberg O (2014) Reactionary dentinogenesis and neuroimmune response in dental caries. *J Dent Res* **93**: 788-793.

d'Aquino R, De Rosa A, Lanza V, Tirino V, Laino L, Graziano A, Desiderio V, Laino G, Papaccio G (2009) Human mandible bone defect repair by the grafting of dental pulp stem/progenitor cells and collagen sponge biocomplexes. *Eur Cell Mater* **18**: 75-83.

de La Dure-Molla M, Philippe Fournier B, Berald A (2015) Isolated dentinogenesis imperfecta and dentin dysplasia: revision of the classification. *Eur J Hum Genet* **23**: 445-51.

Dong J, Gu TT, Jeffords L, MacDougall M (2005) Dentin phosphoprotein compound mutation in dentin sialophosphoprotein causes dentinogenesis imperfecta type III. *Am J Med Genet A* **132A**: 305-309.

D'Souza RN, Cavender A, Sunavala G, Alvarez J, Ohshima T, Kulkarni AB, MacDougall M (1997) Gene expression patterns of murine dentin matrix protein 1 (Dmp1) and dentin sialophosphoprotein (DSPP) suggest distinct developmental functions *in vivo*. *J Bone Miner Res* **12**: 2040-2049.

D'Souza RN, Aberg T, Gaikwad J, Cavender A, Owen M, Karsenty G, Thesleff I (1999) Cbfa1 is required for epithelial-mesenchymal interactions regulating tooth development in mice. *Development* **126**: 2911-2920.

Du J, Lu Y, Song M, Yang L, Liu J, Chen X, Ma Y, Wang Y (2020) Effects of ERK/p38 MAPKs signaling pathways on MTA-mediated osteo/odontogenic differentiation of stem cells from apical papilla: a *vitro* study. *BMC Oral Health* **20**: 50. DOI: 10.1186/s12903-020-1016-x.

Ducy P, Zhang R, Geoffroy V, Ridall AL, Karsenty G (1997) Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation. *Cell* **89**: 747-754.

Eapen A, Ramachandran A, George A (2012) Dentin phosphoprotein (DPP) activates integrin-mediated anchorage-dependent signals in undifferentiated mesenchymal cells. *J Biol Chem* **287**: 5211-5224.

Eapen A, Kulkarni R, Ravindran S, Ramachandran A, Sundivakkam P, Tiruppathi C, George A (2013) Dentin phosphophoryn activates Smad protein signaling through Ca<sup>2+</sup>-calmodulin-dependent protein kinase II in undifferentiated mesenchymal cells. *J Biol Chem* **288**: 8585-8595.

Figueiredo P, Sipponen MH, Lintinen K, Correia A, Kiriazis A, Yli-Kauhaluoma J, Österberg M, George A, Hirvonen J, Kostianen MA, Santos HA (2019) Preparation and characterization of dentin phosphophoryn derived peptide-functionalized lignin nanoparticles for enhanced cellular uptake. *Small* **15**: e1901427. DOI: 10.1002/smll.201901427.

Fisher LW, Fedarko NS (2003) Six genes expressed in bones and teeth encode the current members of the SIBLING family of proteins. *Connect Tissue Res* **44** Suppl 1: 33-40.

Friedenstein AJ, Chailakhjan RK, Lalykina KS (1970) The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. *Cell Tissue Kinet* **3**: 393-403.

Furger KA, Allan AL, Wilson SM, Hota C, Vantghem SA, Postenka CO, Al-Katib W, Chambers

- AF, Alan B Tuck AB (2003)  $\beta 3$  integrin expression increases breast carcinoma cell responsiveness to the malignancy-enhancing effects of osteopontin. *Mol Cancer Res* **1**: 810-819.
- Gibson MP, Liu Q, Zhu Q, Lu Y, Jani P, Wang X, Liu Y, Paine ML, Snead ML, Feng JQ, Qin C (2013) Role of the NH<sub>2</sub>-terminal fragment of dentin sialophosphoprotein in dentinogenesis. *Eur J Oral Sci* **121**: 76-85.
- Gibson MP, Jani P, Wang X, Lu Y, Qin C (2014) Overexpressing the NH<sub>2</sub>-terminal fragment of dentin sialophosphoprotein (DSPP) aggravates the periodontal defects in DSPP knockout mice. *J Oral Biosci* **56**: 143-148.
- Goldberg M, Smith AJ (2004) Cells and extracellular matrices of dentin and pulp: a biological basis for repair and tissue engineering. *Crit Rev Oral Biol Med* **15**: 13-27.
- Götz M, Huttner WB (2005) The cell biology of neurogenesis. *Nat Rev Mol Cell Biol* **6**: 777-788.
- Griffin SO, Jones K, Gray SK, Malvitz DM, Gooch BF (2008) Exploring four-handed delivery and retention of resin-based sealants. *J Am Dent Assoc* **139**: 281-289.
- Gronthos S, Mankani M, Brahim J, Robey PG, Shi S (2000) Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*. *Proc Natl Acad Sci U S A* **97**: 13625-13630.
- Gronthos S, Brahim J, Li W, Fisher LW, Cherman N, Boyde A, DenBesten P, Robey PG, Shi S (2002) Stem cell properties of human dental pulp stem cells. *J Dent Res* **81**: 531-535.
- Gulseren G, Tansik G, Garifullin R, Tekinay AB, Guler MO (2019) Dentin phosphoprotein mimetic peptide nanofibers promote biomineralization. *Macromol Biosci* **19**: e1800080. DOI: 10.1002/mabi.201800080
- Guo W, He Y, Zhang X, Lu W, Wang C, Yu H, Liu Y, Li Y, Zhou Y, Zhou J, Zhang M, Deng Z, Jin Y (2009) The use of dentin matrix scaffold and dental follicle cells for dentin regeneration. *Biomaterials* **30**: 6708-6723.
- Han S, Peng X, Ding L, Lu J, Liu Z, Wang K, Zhang L (2021) TVH-19, a synthetic peptide, induces mineralization of dental pulp cells *in vitro* and formation of tertiary dentin *in vivo*. *Biochem Biophys Res Commun* **534**: 837-842.
- Hart PS, Hart TC (2007) Disorders of human dentin. *Cells Tissues Organs* **186**: 70-77.
- He G, Ramachandran A, Dahl T, George S, Schultz D, Cookson D, Veis A, George A (2005) Phosphorylation of phosphophoryn is crucial for its function as a mediator of biomineralization. *J Biol Chem* **280**: 33109-33114.
- Higashikawa F, Eboshida A, Yokosaki Y (2007) Enhanced biological activity of polymeric osteopontin. *FEBS Lett* **581**: 2697-2701.
- Holappa H, Nieminen P, Tolva L, Lukinm PL, Alaluusua S (2006) Splicing site mutations in dentin sialophosphoprotein causing dentinogenesis imperfecta type II. *Eur J Oral Sci* **114**: 381-384.
- Hsu, CC, Chung HY, Yang JM, Shi W, Wu B (2011) Influence of 8DSS peptide on nano-mechanical behavior of human enamel. *J Dent Res* **90**: 88-92.
- Huang CC, Narayanan R, Warshawsky N, Ravindran S (2018) Dual ECM biomimetic scaffolds for dental pulp regenerative applications. *Front Physiol* **9**: 495. DOI: 10.3389/fphys.2018.00495.
- Jadlowiec J, Koch H, Zhang X, Campbell PG, Seyedain M, Sfeir C (2004) Phosphophoryn regulates the gene expression and differentiation of NIH3T3, MC3T3-E1, and human mesenchymal stem cells *via* the integrin/MAPK signaling pathway. *J Biol Chem* **279**: 53323-53330.
- Jadlowiec JA, Zhang X, Li J, Campbell PG, Sfeir C (2006) Extracellular matrix-mediated signaling by dentin phosphophoryn involves activation of the Smad pathway independent of bone morphogenetic protein. *J Biol Chem* **281**: 5341-5347.
- Jonsson M, Fredriksson S, Jontell M, Linde A (1978) Isoelectric focusing of the phosphoprotein of rat incisor dentin in ampholine and acid pH gradients. Evidence for carrier ampholyte protein complexes. *J Chromatogr* **21**: 234-42.
- Kang J, FanW, Deng Q, He H, Huang F (2019) Stem cells from the apical papilla: a promising source for stem cell-based therapy. *BioMed Res Int* **2019**: 6104738. DOI: 10.1155/2019/6104738.
- Kaukua N, Shahidi MK, Konstantinidou C, Dyachuk V, Kaucka M, Furlan A, An Z, Wang L, Hultman I, Ahrlund-Richter L, Blom H, Brismar H, Lopes NA, Pachnis V, Suter U, Clevers H, Thesleff I, Sharpe P, Ernfors P, Fried K, Adameyko I (2014) Glial origin of mesenchymal stem cells in a tooth model system. *Nature* **513**: 551-554.
- Kazemina M, Abdi A, Shohaimi S, Jalali R, Vaisi-Raygani A, Salari N, Mohammadi M (2020) Dental caries in primary and permanent teeth in children's worldwide, 1995 to 2019: a systematic review and meta-analysis. *Head Face Med* **16**: 22. DOI: 10.1186/s13005-020-00237-z.
- Kida M, Tsutsumi T, Shindoh M, Ikeda H, Tadashi Ariga T (2009) *De novo* mutation in the DSPP gene associated with dentinogenesis imperfecta type II in a Japanese family. *Eur J Oral Sci* **117**: 691-694.
- Kim JW, Nam SH, Jang KT, Lee SH, Kim CC, Hahn SH, Hu JC, Simmer JP (2004) A novel splice acceptor mutation in the DSPP gene causing dentinogenesis imperfecta type II. *Hum Genet* **115**: 248-254.
- Kim JW, Hu JC, Lee JI, Moon SK, Kim YJ, Jang KT, Lee SH, Kim CC, Hahn SH, Simmer JP (2005) Mutational hot spot in the DSPP gene causing dentinogenesis imperfecta type II. *Hum Genet* **116**: 186-191.
- Kobuke S, Suzuki S, Hoshino H, Haruyama N, Nishimura F, Shiba H (2015) Relationship between length variations in Ser/Asp-rich repeats in phosphophoryn and *in vitro* precipitation of calcium phosphate. *Arch Oral Biol* **60**: 1263-1272.
- Lee B, Thirunavukkarasu K, Zhou L, Pastore L, Baldini A, Hecht J, Geoffroy V, Ducy P, Karsenty G (1997) Missense mutations abolishing DNA binding

of the osteoblast-specific transcription factor OSF2/CBFA1 in cleidocranial dysplasia. *Nat Genet* **16**: 307-310.

Lee JH, Seo SJ (2016) Biomedical application of dental tissue-derived induced pluripotent stem cells. *Stem Cells Int* **2016**: 9762465. DOI: 10.1155/2016/9762465.

Lee JW, Hong J, Seymen F, Kim YJ, Kang J, Koruyucu M, Tuloglu N, Bayrak S, Song JS, Shin TJ, Hyun HK, Kim YJ, Lee JC, Park JC, Hu J, Simmer J, Kim JW (2019) Novel frameshift mutations in DSPP cause dentin dysplasia type II. *Oral Dis* **25**: 2044-2046.

Lee KE, Kang HY, Lee SK, Yoo SH, Lee JC, Hwang YH, Nam KH, Kim JS, Park JC, Kim JW (2011) Novel dentin phosphoprotein frameshift mutations in dentinogenesis imperfecta type II. *Clin Genet* **79**: 378-384.

Lee SK, Hu JCC, Lee KE, Simmer JP, Kim JW (2008) A dentin sialophosphoprotein mutation that partially disrupts a splice acceptor site causes type II dentin dysplasia. *J Endod* **34**: 1470-1473.

Lee SK, Lee KE, Hwang YH, Kida M, Tsutsumi T, Ariga T, Park JC, Kim JW (2011) Identification of the DSPP mutation in a new kindred and phenotype-genotype correlation. *Oral Dis* **17**: 314-319.

Lee SK, Lee KE, Song SJ, Hyun HK, Lee SH, Kim JW (2013) A DSPP mutation causing dentinogenesis imperfecta and characterization of the mutational effect. *Biomed Res Int* **2013**: 948181. DOI: 10.1155/2013/948181.

Lee SY, Kim SY, Park SH, Kim JJ, Jang JH, Kim EC (2012) Effects of recombinant dentin sialoprotein in dental pulp cells. *J Dent Res* **91**: 407-412.

Li D, Du X, Zhang R, Shen B, Huang Y, Valenzuela RK, Wang B, Zhao H, Liu Z, Li J, Xu Z, Gao L, Ma J (2012) Mutation identification of the DSPP in a Chinese family with DGI-II and an up-to-date bioinformatic analysis. *Genomics* **99**: 220-226.

Li F, Liu Y, Liu H, Yang J, Zhang F, Feng H (2017) Phenotype and genotype analyses in seven families with dentinogenesis imperfecta or dentin dysplasia. *Oral Dis* **23**: 360-366.

Li W, Chen L, Chen Z, Wu L, Feng J, Wang F, Shoff L, Li X, Donly KJ, MacDougall M, Chen S (2017) Dentin sialoprotein facilitates dental mesenchymal cell differentiation and dentin formation. *Sci Rep* **7**: 300. DOI: 10.1038/s41598-017-00339-w.

Li WX, Peng H, Yang L, Hao QQ, Sun W, Ji F, Guo WW, Yang SM (2018) Familial nonsyndromic hearing loss with incomplete partition type II caused by novel DSPP gene mutations. *Acta Otolaryngol* **138**: 685-690.

Liang T, Zhang H, Xu Q, Wang S, Qin C, Lu Y (2019) Mutant dentin sialophosphoprotein causes dentinogenesis imperfecta. *J Dent Res* **98**: 912-919.

Linde A, Goldberg M (1993) Dentinogenesis (review) *Crit Rev Oral Biol Med* **4**: 679-728.

Liu Y, Huang Y, Gao J, Li S, Zhao X, Zhang X (2016) Identification of a novel mutation of DSPP gene in a Chinese family affected with dentinogenesis imperfecta shields type II. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* **33**: 34-37.

Lopez-Cazaux S, Bluteau G, Magne D, Lieubeau B, Guicheux J, Alliot-Licht B (2006) Culture medium modulates the behavior of human dental pulp-derived cells: technical note. *Eur Cell Mater* **11**: 35-42.

MacDougall M, Simmons D, Luan X, Nydegger J, Feng J, Gu TT (1997) Dentin phosphoprotein and dentin sialoprotein are cleavage products expressed from a single transcript coded by a gene on human chromosome 4. Dentin phosphoprotein DNA sequence determination. *J Biol Chem* **272**: 835-842.

Malmgren B, Lindskog S, Elgadi A, Norgren S (2004) Clinical, histopathologic, and genetic investigation in two large families with dentinogenesis imperfecta type II. *Hum Genet* **114**: 491-498.

Marschall VZ, Fisher LW (2008) Dentin matrix protein-1 isoforms promote differential cell attachment and migration *J Biol Chem* **283**: 32730-32740.

Marschall VZ, Fisher LW (2010) Dentin sialophosphoprotein (DSPP) is cleaved into its two natural dentin matrix products by three isoforms of bone morphogenetic protein-1 (BMP1). *Matrix Biol* **29**: 295-303.

Martens W, Sanen K, Georgiou M, Struys T, Bronckaers A, Ameloot M, Phillips J, Lambrechts I (2014) Human dental pulp stem cells can differentiate into Schwann cells and promote and guide neurite outgrowth in an aligned tissue-engineered collagen construct *in vitro*. *FASEB J* **28**: 1634-1643.

McKnight DA, Simmer JP, Hart PS, Hart TC, Fisher LW (2008a) Overlapping DSPP mutations cause dentin dysplasia and dentinogenesis imperfecta. *J Dent Res* **87**: 1108-1111.

McKnight DA, Hart PS, Hart TC, Hartsfield JK, Wilson A, Wright JT, Fisher LW (2008b) A comprehensive analysis of normal variation and disease-causing mutations in the human DSPP gene. *Hum Mutat* **29**: 1392-404.

Méndez-Ferrer S, Bonnet D, Steensma DP, Hasserjian RP, Ghobrial IM, Gribben JG, Andreeff M, Krause DS (2020) Bone marrow niches in haematological malignancies. *Nat Rev Cancer* **20**: 285-298.

Miran S, Mitsiadis TA, Pagella P (2016) Innovative dental stem cell-based research approaches: the future of dentistry. *Stem Cells Int* **2016**: 7231038. DOI: 10.1155/2016/7231038.

Mitsiadis TA, Orsini G, Jimenez-Rojo L (2015) Stem cell-based approaches in dentistry. *Eur Cell Mater* **30**: 248-257.

Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, Shi S (2003) SHED: stem cells from human exfoliated deciduous teeth. *Proc Natl Acad Sci U S A* **100**: 5807-5812.

Miyazaki T, Kanatani N, Rokutanda S, Yoshida C, Toyosawa S, Nakamura R, Takada S, Komori T (2008) Inhibition of the terminal differentiation of odontoblasts and their trans-differentiation into osteoblasts in Runx2 transgenic mice. *Arch Histol Cytol* **71**: 131-146.

- Morrison SJ, Spradling AC (2008) Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. *Cell* **132**: 598-611.
- Morszeck C, Gotz W, Schierholz J, Zeilhofer F, Kuhn U, Mohl C, Sippel C, Hoffmann KH (2005) Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. *Matrix Biol* **24**:155-165.
- Nada OA, El Backly RM (2018) Stem cells from the apical papilla (SCAP) as a tool for endogenous tissue regeneration. *Front Bioeng Biotechnol* **6**: 103. DOI: 10.3389/fbioe.2018.00103.
- Nakashima M (2005) Bone morphogenetic proteins in dentin regeneration for potential use in endodontic therapy. *Cytokine Growth Factor Rev* **16**: 369-376.
- Nanci A (2012) Ten Cate's oral histology: development, structure, and function (8th edition). Mosby, USA. pp: 1-13.
- Neves VCM, Sharpe PT (2018) Regulation of reactionary dentine formation. *J Dent Res* **97**: 416-422.
- Ni SL, Zhang J, Liu X, Li XW, Sun YJ, Zhang X, Wang L, Lu JJ, Cui Y, Zheng CY, Han B, Sun HC (2018) Effects of human bone morphogenetic protein 2 (hBMP2) on tertiary dentin formation. *Am J Transl Res* **10**: 2868-2876.
- Nieminen P, Papagiannoulis-Lascarides L, Waltimo-Siren J, Ollila P, Karjalainen S, Arte S, Veerkamp J, Walton VT, Küstner EC, Siltanen T, Holappa H, Lukinm PL, Alaluusua S (2011) Frameshift mutations in dentin phosphoprotein and dependence of dentin disease phenotype on mutation location. *J Bone Miner Res* **26**: 873-880.
- Ozer A, Yuan GH, Yang GB, Wang F, Li W, Yang Y, Guo F, Gao QP, Shoff L, Chen Z, Gay I, Donly KJ, MacDougall M, Chen S (2013) Domain of dentine sialoprotein mediates proliferation and differentiation of human periodontal ligament stem cells. *Plos One* **8**: e81655. DOI: 10.1371/journal.pone.0081655.
- Paine ML, Luo W, Wang HJ, Bringas P Jr, Ngan AY, Miklus VG, Zhu DH, MacDougall M, White SN, Snead ML (2005) Dentin phosphoprotein overexpression during amelogenesis. *J Biol Chem* **280**: 31991-31998.
- Perry JM, Li L (2007) Disrupting the stem cell niche: good seeds in bad soil. *Cell* **129**: 1045-1047.
- Pisciotta A, Riccio M, Carnevale G, Lu A, De Biasi S, Gibellini L, La Sala GB, Bruzzesi G, Ferrari A, Huard J, De Pol A (2015) Stem cells isolated from human dental pulp and amniotic fluid improve skeletal muscle histopathology in mdx/SCID mice. *Stem Cell Res Ther* **6**: 156. DOI: 10.1186/s13287-015-0141-y.
- Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR (1999) Multilineage potential of adult human mesenchymal stem cells. *Science* **284**: 143-147.
- Porntaveetus T, Nowwarote N, Osathanon T, Theerapanon T, Pavasant P, Boonprakong L, Sanon K, Srisawasdi S, Suphapeetiporn K, Shotelersuk V (2019) Compromised alveolar bone cells in a patient with dentinogenesis imperfecta caused by DSPP mutation. *Clin Oral Investig* **23**: 303-313.
- Prince CW, Dickie D, Krumdieck CL (1991) Osteopontin, a substrate for transglutaminase and factor XIII activity. *Biochem Biophys Res Commun* **177**: 1205-1210.
- Qin C, Cook RG, Orkiszewski RS, Butler WT (2001) Identification and characterization of the carboxyl-terminal region of rat dentin sialoprotein. *J Biol Chem* **276**: 904-909.
- Qin C, Brunn JC, Cadena E, Ridall A, Tsujigiwa H, Nagatsuka H, Nagai N, Butler WT (2002) The expression of dentin sialophosphoprotein gene in bone. *J Dent Res* **81**: 392-394.
- Rajpar MH, Koch MJ, Davies RM, Mellody KT, Kieley CM, Dixon MJ (2002) Mutation of the signal peptide region of the bicistronic gene DSPP affects translocation to the endoplasmic reticulum and results in defective dentine biomineralization. *Hum Mol Genet* **11**: 2559-2565.
- Rangaswami H, Bulbule A, Kundu GC (2006) Osteopontin: role in cell signaling and cancer progression. *Trends Cell Biol* **16**: 79-87.
- Ravindran S, Sneek PT, Ramachandran A, George A (2013) Acidic domain in dentin phosphoprotein facilitates cellular uptake. *J Biol Chem* **288**: 16098-109.
- Ruch JV (1985) Odontoblast differentiation and the formation of the odontoblast layer. *J Dent Res* **64**: 489-498.
- Saoud TMA, Ricucci D, Lin LM, Gaengler P (2016) Regeneration and repair in endodontics-a special issue of the regenerative endodontics-a new era in clinical endodontics. *Dent J (Basel)* **4**: 3. DOI: 10.3390/dj4010003.
- Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahimi J, Young M, Robey PG, Wang CY, Shi S (2004) Investigation of multipotent postnatal stem cells from human periodontal ligament. *Lancet* **364**: 149-155.
- Shi S, Gronthos S (2003) Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. *J Bone Miner Res* **18**: 696-704.
- Sonoyama W, Liu Y, Yamaza T, Tuan RS, Wang S, Shi S, Huang GT (2008) Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. *J Endod* **34**: 166-171.
- Song YL, Wang CN, Fan MW, Su B, Z Bian Z (2008) Dentin phosphoprotein frameshift mutations in hereditary dentin disorders and their variation patterns in normal human population. *J Med Genet* **45**: 457-464.
- Sreenath T, Thyagarajan T, Hall B, Longenecker G, D'Souza R, Hong S, Wright JT, MacDougall M, Sauk J, Ashok B, Kulkarni AB (2003) Dentin sialophosphoprotein knockout mouse teeth display widened predentin zone and develop defective dentin mineralization similar to human dentinogenesis imperfecta type III. *J Biol Chem* **278**: 24874-24880.

- Steiglitz BM, Ayala M, Narayanan K, George A, Greenspan DS (2004) Bone morphogenetic protein-1/Tolloid-like proteinases process dentin matrix protein-1. *J Biol Chem* **279**: 980-986.
- Sui B, Chen C, Kou X, Li B, Xuan K, Shi S, Jin Y (2019) Pulp stem cell-mediated functional pulp regeneration. *J Dent Res* **98**: 27-35.
- Suzuki S, Sreenath T, Haruyama N, Honeycutt C, Terse A, Cho A, Kohler T, Müller R, Goldberg M, Kulkarni AB (2009) Dentin sialoprotein and dentin phosphoprotein have distinct roles in dentin mineralization. *Matrix Biol* **28**: 221-229.
- Suzuki S, Kobuke S, Haruyama N, Hoshino H, Kulkarni AB, Nishimura F (2014) Adhesive and migratory effects of phosphophoryn are modulated by flanking peptides of the integrin binding motif. *PLoS One* **9**: e112490. DOI: 10.1371/journal.pone.0112490.
- Suzuki S, Nakanishi J, Yoshida K, Shiba H (2016) Dentin sialophosphoprotein is a potentially latent bioactive protein in dentin. *J Oral Biosci* **58**: 134-142.
- Teramoto H, Castellone MD, Malek RL, Letwin N, Frank B, Gutkind JS, Lee NH (2005) Autocrine activation of an osteopontin-CD44-Rac pathway enhances invasion and transformation by H-RasV12. *Oncogene* **24**: 489-501.
- Thesleff I (2003) Epithelial-mesenchymal signaling regulating tooth morphogenesis. *J Cell Sci* **116**: 1647-1648.
- Tirino V, Paino F, De Rosa A, Papaccio G (2012) Identification, isolation, characterization, and banking of human dental pulp stem cells. *Methods Mol Biol* **879**: 443-463.
- Tsuchiya S, Simmer JP, Hu JC, Richardson AS, Yamakoshi F, Yamakoshi Y (2011) Astacin proteases cleave dentin sialophosphoprotein (Dspp) to generate dentin phosphoprotein (Dpp). *J Bone Miner Res* **26**: 220-228.
- Tziafas D, Smith AJ, Lesot H (2000) Designing new treatment strategies in vital pulp therapy. *J Dent* **28**: 77-92.
- Vijaykumar A, Dyrkacz P, Vidovic-Zdrilic I, Maye P, Mina M (2020) Expression of BSP-GFP<sup>tpz</sup> transgene during osteogenesis and reparative dentinogenesis. *J Dent Res* **99**: 89-97.
- Vidovic I, Banerjee A, Fatahi R, Matthews BG, Dymont NA, Kalajzic I, Mina M (2017)  $\alpha$ SMA-expressing perivascular cells represent dental pulp progenitors *in vivo*. *J Dent Res* **96**: 323-330.
- Waddington RJ, Youde SJ, Lee CP, Sloan AJ (2009) Isolation of distinct progenitor stem cell populations from dental pulp. *Cells Tissues Organs* **189**: 268-274.
- Walker JV, Zhuang H, Singer D, Illsley CS, Kok WL, Sivaraj KK, Gao Y, Bolton C, Liu Y, Zhao M, Grayson PRC, Wang S, Karbanová J, Lee T, Ardu S, Lai Q, Liu J, Kassem M, Chen S, Yang K, Bai Y, Tredwin C, Zambon AC, Corbeil D, Adams R, Abdallah BM, Hu B (2019) Transit amplifying cells coordinate mouse incisor mesenchymal stem cell activation. *Nat Commun* **10**: 3596. DOI: 10.1038/s41467-019-11611-0.
- Wan C, Yuan G, Luo D, Zhang L, Lin H, Liu H, Chen L, Yang G, Chen S, Chen Z (2016) The dentin sialoprotein (DSP) domain regulates dental mesenchymal cell differentiation through a novel surface receptor. *Sci Rep* **6**: 29666. DOI: 10.1038/srep29666.
- Wang HY, Hou YN, Cui YX, Huang YF, Shi YC, Xia XY, Lu HY, Wang YH, Li XJ (2009) A novel splice site mutation in the dentin sialophosphoprotein gene in a Chinese family with dentinogenesis imperfecta type II. *Mutat Res* **662**: 22-27. DOI: 10.1016/j.mrfmmm.2008.11.019.
- Wang L, Dorn P, Zeinali S, Froment L, Berezowska S, Kocher GJ, Alves MP, Brügger M, Esteves BIO, Blank F, Wotzkow C, Steiner S, Amacker M, Peng RW, Marti TM, Guenat OT, Bode PK, Moehrlen U, Schmid RA, Hall SRR (2020a) CD90(+)/CD146(+) identifies a pulmonary mesenchymal cell subtype with both immune modulatory and perivascular-like function in postnatal human lung. *Am J Physiol Lung Cell Mol Physiol* **318**: L813-L830. DOI: 10.1152/ajplung.00146.2019.
- Wang Y, Zhu M, Zhu XX (2020b) Functional fillers for dental resin composites. *Acta Biomater* **122**: 50-65.
- White SN, Paine ML, Ngan AYW, Miklus VG, Luo W, Wang HJ, Malcolm L, Snead, ML (2007) Ectopic expression of dentin sialoprotein during amelogenesis hardens bulk enamel. *J Biol Chem* **282**: 5340-5345.
- Wilkinson AC, Igarashi KJ, Nakauchi H (2020) Haematopoietic stem cell self-renewal *in vivo* and *ex vivo*. *Nat Rev Genet* **21**: 541-554.
- Witkop Jr CJ (1975) Hereditary defects of dentin. *Dent Clin North Am* **19**: 25-45.
- Xiao S, Yu C, Chou X, Yuan W, Wang Y, Bu L, Fu G, Qian M, Yang J, Shi Y, Hu L, Han B, Wang Z, Huang W, Liu J, Chen Z, Zhao G, Kong X (2001) Dentinogenesis imperfecta 1 with or without progressive hearing loss is associated with distinct mutations in DSPP. *Nat Genet* **27**: 201-204.
- Yamakoshi Y, Hu JC, Fukae M, Zhang H, Simmer JP (2005) Dentin glycoprotein: the protein in the middle of the dentin sialophosphoprotein chimera. *J Biol Chem* **280**: 17472-17479.
- Yamakoshi Y, Hu JC, Iwata T, Kobayashi K, Fukae M, Simmer JP (2006) Dentin sialophosphoprotein is processed by MMP-2 and MMP-20 *in vitro* and *in vivo*. *J Biol Chem* **281**: 38235-38243.
- Yamanaka S (2020) Pluripotent stem cell-based cell therapy-promise and challenges. *Cell Stem Cell* **27**: 523-531.
- Yamaza T, Kentaro A, Chen C, Liu Y, Shi Y, Gronthos S, Wang S, Shi S (2010) Immunomodulatory properties of stem cells from human exfoliated deciduous teeth. *Stem Cell Res Ther* **1**: 5. DOI: 10.1186/scrt5.
- Yang J, Kawasaki K, Lee M, Reid BM, Nunez SM, Choi M, Seymen F, Koruyucu M, Kasimoglu Y, Estrella-Yuson N, Lin BP, Simmer JP, Hu JC (2015) The dentin phosphoprotein repeat region and inherited defects of dentin. *Mol Genet Genomic Med* **4**: 28-38.

- Yang JW, Shin YY, Seo Y, Kim HS (2020b) Therapeutic functions of stem cells from oral cavity: an update. *Int J Mol Sci* **21**: 4389. DOI: 10.3390/ijms21124389.
- Yang SY, Choi JW, Kim KM, Kwon JS (2020a) Prevention of secondary caries using resin-based pit and fissure sealants containing hydrated calcium silicate. *Polymers (Basel)* **12**: 1200. DOI: 10.3390/polym12051200.
- Yang X, Ma Y, Guo W, Yang B, Tian W (2019) Stem cells from human exfoliated deciduous teeth as an alternative cell source in bio-root regeneration. *Theranostics* **9**: 2694-2711.
- Yin Y, Garcia MR, Novak AJ, Saunders AM, Ank RS, Nam AS, Fisher LW (2018) Surf4 (Erv29p) binds amino-terminal tripeptide motifs of soluble cargo proteins with different affinities, enabling prioritization of their exit from the endoplasmic reticulum. *PLoS Biol* **16**: e2005140. DOI: 10.1371/journal.pbio.2005140.
- Yuan G, Yang G, Song G, Chen Z, Chen S (2012) Immunohistochemical localization of the NH(2)-terminal and COOH-terminal fragments of dentin sialoprotein in mouse teeth. *Cell Tissue Res* **349**: 605-614.
- Yuan G, Chen L, Feng JS, Yang G, Ni Q, Xu XP, Wan C, Lindsey M, Donly KJ, MacDougall M, Chen Z, Chen S (2017) Dentin sialoprotein is a novel substrate of matrix metalloproteinase 9 *in vitro* and *in vivo*. *Sci Rep* **7**: 42449. DOI: 10.1038/srep42449.
- Zaugg LK, Banu A, Walther AR, Chandrasekaran D, Babb RC, Salzlechner C, Hedegrd MAB, Gentleman E, Sharpe PT (2020) Translation approach for dentine regeneration using GSK-3 antagonists. *J Dent Res* **99**: 544-551.
- Zhang H, Liu P, Wang S, Liu C, Jani P, Lu Y, Qin C (2016) Transgenic expression of dentin phosphoprotein inhibits skeletal development. *Eur J Histochem* **60**: 2587. DOI: 10.4081/ejh.2016.2587.
- Zhang H, Xie X, Liu P, Liang T, Lu Y, Chunlin Qin C (2018) Transgenic expression of dentin phosphoprotein (DPP) partially rescued the dentin defects of DSPP-null mice. *PLoS One* **13**: e0195854. DOI: 10.1371/journal.pone.0195854.
- Zhang J, Wang J, Ma Y, Du W, Zhao S, Zhang Z, Zhang X, Liu Y, Xiao H, Wang H, Jin L, Liu J (2011) A novel splicing mutation alters DSPP transcription and leads to dentinogenesis imperfecta type II. *PLoS One* **6**: e27982. DOI: 10.1371/journal.pone.0027982.
- Zhang X, Zhao J, Li C, Gao S, Qiu C, Liu P, Wu G, Qiang B, Lo WH, Shen Y (2001) DSPP mutation in dentinogenesis imperfecta shields type II. *Nat Genet* **27**: 151-152.
- Zhang X, Chen L, Liu J, Zhao Z, Qu E, Wang X, Chang W, Xu C, Wang QK, Liu M (2007) A novel DSPP mutation is associated with type II dentinogenesis imperfecta in a Chinese family. *BMC Med Genet* **8**: 52. DOI: 10.1186/1471-2350-8-52.
- Zhu Q, Gibson MP, Liu Q, Liu Y, Lu Y, Wang X, Feng JQ, Qin C (2012) Proteolytic processing of dentin sialophosphoprotein (DSPP) is essential to dentinogenesis. *J Biol Chem* **287**: 30426-30435.

**Editor's note:** There were no questions from reviewers for this paper, therefore there is no Discussion with Reviewers section. The Scientific Editor responsible for this paper was Thimios Mitsiadis.