The Role of DSPP in Dentine Formation and Hereditary Dentine Defects

Jie JIA[1,2], Zhuan BIAN[1], Yaling SONG[1]

The dentine sialophosphoprotein (DSPP) gene is the only identified causative gene for dentinogenesis imperfecta type 2 (DGI-II), dentinogenesis imperfecta type 3 (DGI-III) and dentine dysplasia type 2 (DD-II). These three disorders may have similar molecular mechanisms involved in bridging the DSPP mutations and the resulting abnormal dentine mineralisation. The DSPP encoding proteins DSP (dentine sialoprotein) and DPP (dentine phosphoprotein) are positive regulators of dentine formation and perform a function during dentinogenesis. The present review focused on the recent findings and viewpoints regarding the relationship between DSPP and dentinogenesis as well as mineralisation from multiple perspectives, involving studies relating to spatial structure and tissue localisation of DSPP, DSP and DPP, the biochemical characteristics and biological function of these molecules, and the causative role of the proteins in phenotypes of the knockout mouse model and in hereditary dentine defects.

Keywords: *dentine mineralisation, dentine sialophosphoprotein, hereditary dentine defects, mutation*

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Mineralised dentine consists of three components: collagen fibrils that determine the spatial structure and directly sustain the process of mineralisation[1,2]; multiple proteins that interact with each other during the process of mineralisation[3]; and carbon-apatite that forms hierarchically flaky ordered crystal structure .[4] A number of non-collagenous proteins (NCPs), accounting for 5% to 10% of the dentine extracellular matrix (DECM), are responsible for initiating and modulating the mineralisation of collagen fibres when predentine is converted to dentine.[5] Among these DECM proteins, dentine phosphoprotein (DPP) and dentine sialoprotein (DSP) appear to be the major dentine-specific proteins. They are encoded by the signal mRNA transcript of the dentine sialophosphoprotein (*DSPP*) gene.[6,7] The coding sequences for DSP are in the 5' end of the *DSPP* gene and those for DPP are in the 3' terminal (Fig 1). The intact DSPP peptide has never been identified[8-10], which indicates that DSPP would be catalysed after translation. Blocking the proteolytic processing of DSPP generated dentine hypomineralisation defects that are similar to those observed in *Dspp*-deficient mice models[11], indicating that the proteolytic processing of DSPP into different fragments would be vital in dentinogenesis.

DSPP plays an essential role in dentinogenesis and tissue development; however, there is contradicting evidence regarding the activity of DSPP and particularly its role in mineralisation. The present review presents the recent findings and viewpoints in detail regarding the relationship between DSPP and dentinogenesis as well as mineralisation from multiple perspectives involv-

1 State Key Laboratory of Oral & Maxillofacial Reconstruction and Regeneration, Key Laboratory of Oral Biomedicine Ministry of Education, Hubei Key Laboratory of Stomatology, School & Hospital of Stomatology, Wuhan University, Wuhan, P.R. China.
2 The First Affiliated Hospital of Henan University, Henan University School of Stomatology, Kaifeng, P.R. China.

Corresponding authors: Prof Yaling SONG (ORCID: https://orcid.org/0000-0003-0747-7381) and Prof Zhuan BIAN, State Key Laboratory of Oral & Maxillofacial Reconstruction and Regeneration, Key Laboratory of Oral Biomedicine Ministry of Education, Hubei Key Laboratory of Stomatology, School & Hospital of Stomatology, Wuhan University, Luoyu Road 237, Wuhan 430079, P.R. China. Tel: 86-27-87686142. Email: sningya@whu.edu.cn; bianzhuan@whu.edu.cn.

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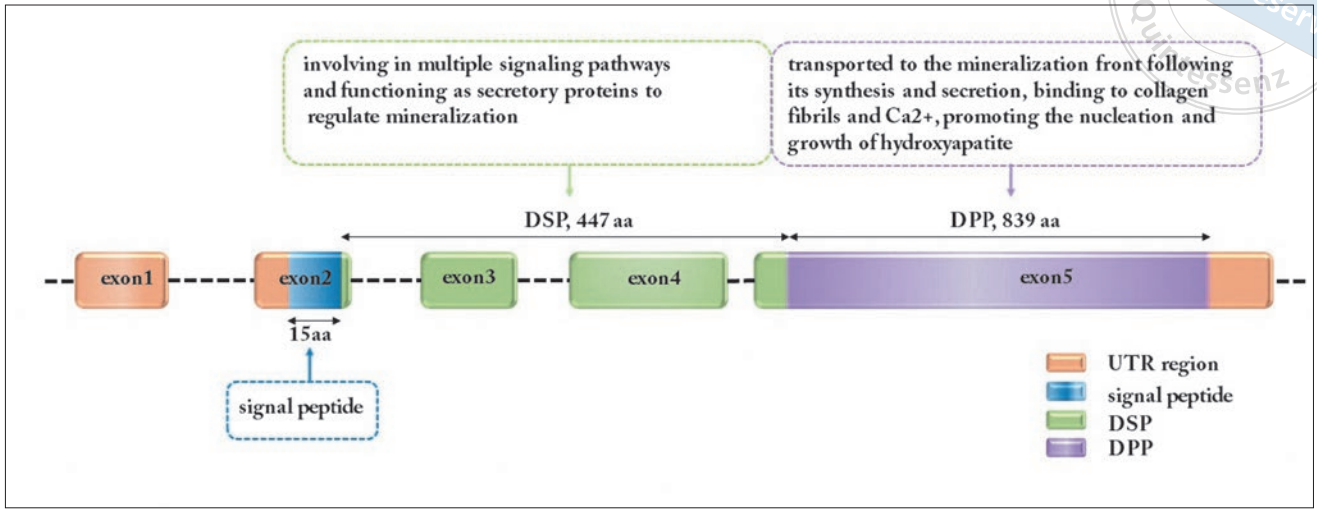

Fig 1 Diagrammatic representation of DSPP gene structure, encoding proteins and potential function.

ing studies at a molecular and cellular level, in animal models and in human dentine disorders.

General function of DSPP

DSPP belongs to sialic acid-rich glycoproteins, as a member of the small integrin-binding ligand N-linked glycoproteins (SIBLING) family. Immediately after the translation of full-length DSPP, the DSPP preproprotein in odontoblasts is proteolytically processed into separate daughter proteins with different properties.[12] The two major cleaved products are identified as DSP and DPP, which are secreted to the extracellular matrix by odontoblasts and predominantly but not exclusively expressed in odontoblasts and dentine.[13,14] Dentin glycoprotein (DGP) was found in the middle region of DSPP in procine[15], but it had not yet been reported in other species. DSP was found to be specifically expressed in odontoblasts, predentine, dentine and dental pulp[16-21], and was also detected in osteoblasts within alveolar bone, cellular cementum and periodontal ligament.[22,23] DPP was deposited directly at the advancing mineralisation front of dentine, whereas little phosphoprotein was detected in pulp.[24] DSP and DPP were also found to be expressed transiently in early ameloblasts adjacent to the dentine–enamel junction (DEJ).[25,26]

DSPP in dentine and pulp

The essential role of DSPP in tooth development and disease occurrence in vivo was analysed in knockout and transgenic mouse models. The *Dspp*$^{-/-}$ mice showed tooth defects similar to those seen in patients with DGI-III (dentinogenesis imperfecta type 3). The defects presented as widened predentine, sporadic unmineralised areas in irregular dentine and frequent pulp exposure, as well as mineralisation defects with globular dentine as a marker of abnormally mineralised dentine.[27] In addition, in the *Dspp*$^{-/-}$ mice the compromised DEJ was shown as a gap between enamel and dentine, which suggested a lack of DSPP would result in abnormal epithelial-mesenchymal interactions during early dental development.[28,29] The circular dentine formed within dental pulp and the altered dental pulp cells differentiating into chondrocyte-like cells were observed in the teeth of *Dspp*$^{-/-}$ mice.[29] The dosage of DSPP is critical to maintain tooth development and dentine formation. The transgenic mice that expressed *Dspp* mRNA at a level similar to that in wildtype mice can completely rescue the DSPP knockout defect; however, transgenic mouse incisors, with 10% *Dspp* mRNA expression, only partially rescued the DSPP knockout defect in mineral density.[30] *Dspp* heterozygous mice displayed dentine phenotypes similar to DD-II at the age of 12 and 18 months, which was characterised by compromised mineralisation of the dentine.[31] These studies suggested that DSPP would be required for normal pulp cell/odontoblast differentiation as well as dentine development and DEJ formation.

DSPP in reparative dentine formation

DSPP is mainly expressed in odontoblasts. It is a marker for the differentiation of dental pulp cells into odontoblast cells. *Dspp* expression was absent in the damaged odontoblasts 24 hours after tooth injury, and regenerating odontoblasts began to express *Dspp* at day 5 after

injury.[32] Immunohistochemical analyses in sclerotic dentine revealed a high expression of DSPP inside the tubules in reparative dentine, which was mainly found in the tubules in non-affected dentine of caries lesions and perhaps indicated a preventive defence against carious attack.[33] *Dspp* heterozygous mice (18 months) manifested excessive dentine attrition and excessive formation of reparative dentine, but much weaker DSPP signals within the reparative dentine indicated osteodentine rather than normally formed dentine.[31] Furthermore, DSPP was expressed together with bone sialoprotein (BSP) in the odontoblast-like cells of reparative dentine, which suggests that the newly formed reparative dentine possess both dentinogenic and osteogenic characteristics.[34]

In contrast with pulp capping agent calcium hydroxide, DPP/collagen composite demonstrated more rapid formation of reparative dentine with higher compactness, a greater ability to cover exposed pulp and less pulp inflammation.[35] Decellularised dentine matrix extracts contained DSPP, as pulp capping agents for miniature swine, regenerated complete dentine bridge and reactionary dentine, and this reparative dentine adjacent to pulp tissue showed dentinal tubules that were relatively similar to primary dentine.[36]

At 1 week after pulp capping with calcium hydroxide agent, strong DSPP signals often appeared in the early formed dentine bridge, demonstrating the rapid response of DSPP to the noxious external stimuli.[37] After pulp capping with different capping materials, human odontoblast-like cells and pulpal cells beneath the dentine bridge are capable of differentiating and producing new hard tissue that contains DSPP, ColI or Heme Oxygenase-1; thus, the newly formed hard tissue can be characterised as dentine rather than an unspecific hard organism.[38,39] Three weeks after calcium hydroxide paste was applied to wounded pulp tissue, only anti-DSP staining was visible in newly formed reparative predentine and dentine, indicating that DSPP rapidly performed its function in the formation of reparative dentine.[40]

In general, DSPP is able to respond rapidly to dental injury and could become an excellent biocompatible inducer for the formation of reparative dentine.

Interaction with mineralisation-related proteins

Tooth development requires the coordinated action of DSPP with other mineralisation-related proteins. TGF-β1, one of the negative regulation factors of DSPP, was reported early on to downregulate DSPP promoter activity. The expression of *Dspp* is significantly downregulated during tooth development in TGF-β1 transgenic mice with overexpressed active TGF-β1 predominantly in odontoblasts.[41] DPP and DSP binding to TGF-β1 were liberated in association with the degradation of DPP and DSP, and this combination might facilitate reparative dentine formation and affect cell behaviour in the dentine–pulp complex following tissue injury.[42]

DMP1, another member of the SIBLING family, is located on the same chromosome locus as the DSPP gene and shares similar biochemical and genomic DNA features.[43,44] It was proposed that DSPP arose from DMP1 as a result of a gene duplication event.[45] During early differentiation of rat odontoblasts, the COOH-terminal of DMP1 binds specifically with the DSPP promoter in the region between nt -450 and +80 and activates the transcription of DSPP.[46] Studies in mouse models showed that DSPP was reduced in *Dmp1*$^{-/-}$ mice at both mRNA and protein levels.[47,48] This finding was in agreement with the results in vitro studies, in which overexpression of *Dmp1* induced *Dspp* expression while blocking DMP1 expression inhibited the expression of *Dspp*.[49] Significantly, in *Dmp1*$^{-/-}$ mice, the transgenic *Dspp* expression elevated the expression of molecular markers (BSP, OPN and MEPE) and repaired the defects in dentine and alveolar bone.[48] Taken together, DSPP is not simply a downstream molecule; it also shows the capability of affecting the upstream factors and mineralisation.

Analyses of the mouse *Dspp* gene revealed three *Runx2*-binding sites in *Dspp* promoter.[21] In vitro studies suggested that *Runx2* upregulated DSPP gene expression in mouse preodontoblasts but decreased its activity in mature odontoblasts.[21,50] Overexpression of the *Runx2* gene in mouse dental papilla cells resulted in the downregulation of DSPP expression.[51] Thus, the effects of *Runx2* on DSPP expression depend on stages of cell differentiation, with *Runx2* activating DSPP expression in preodontoblast cells and repressing it at maturation stages.

Mouse *Dspp* promoter also has a BMP2-response element that physically interacts with BMP2. BMP2 upregulated DSPP expression in mouse preodontoblasts.[52] In conditional *Bmp2*$^{-/-}$ mice, the gene expression of Dspp in odontoblasts was reduced by 90%..[53] In addition, transcription factor *Dlx3* mutations causing tooth defects in humans indicated that DLX3 played a role in tooth development.[54,55] DSPP is directly regulated by DLX3 in odontoblasts and *Dlx3*$^{-/-}$ mice also showed major dentine defects and reduced DSPP expression.[56]

Overall, the cooperation between DSPP and other mineralization-related proteins is vital for the process of mineralisation. In summary, DSPP is a positive

regulator of hard tissue mineralisation, acting on both dentine and bone. Because of its unique structure, DSPP would obligatorily be proteolytically processed to segments. A general review of the two main separate daughter proteins of DSPP, DPP and DSP, will now be presented.

DPP and dentine mineralisation

Relationship between DPP conformation and mineralisation

As the polyanionic macromolecules, DPP is the major noncollagenous DECM protein in dentine[57,58], which can function in biological mineralisation by binding to the matrix of structural protein, nucleating hydroxyapatite (HA) crystallisation and modulating crystal growth.[2,59] In the majority of species, DPP possesses a unique composition with aspartyl and seryl residues comprising at least 75% of amino acid residues and with 85% to 90% of the phosphorylated seryl residues.[58] DPP isolated from dentine extract was capable of initiating the formation of HA in an in vitro gel diffusion system without HA[60]; however, recombinant full-length PP (rPP-full) required HA co-embedding to induce mineralisation.[61] One interpretation was that rPP might be less phosphorylated than DPP from dentine.[15] It was considered that the length of the serine/aspartic acid-rich repeats (SDrr) of DPP could be associated with phosphorylation and tooth mineralisation[62]; however, inter- and intraspecies length polymorphisms in SDrr have been reported in toothed animals.[15,63] Several recombinant mouse DPP proteins possessing 62.4% and 36.5% of the length of rPP-full induced the precipitation of calcium phosphate similar to rPP-full, whereas the induction ability of the vector without SDrr repeats was significantly lower than that of rPP-full.[61] Thus, interspecies length variation in SDrr may not result in different abilities of individual DPP for tooth mineralisation, but a different phosphorylated extent of DPP determines the capacity for mineralisation.

Comparison of DPP sequences among toothed and toothless animals showed that although there was a significant difference among the species, the BMP1-cleavage motif and RGD (Arg-Gly-Asp) integrin-binding domain were defined in two conservation domains in DPP.[62] The BMP1-cleavage motif is conserved among mammals[62], and mutations within the BMP1-cleavage site would block, impair or accelerate the efficiency of DSPP cleavage.[9,64] In in vivo studies, the normal DSPP transgene fully repaired the dentine defects of *Dspp*$^{-/-}$ mice, whereas the D452A-DSPP mutant transgene with a mutation in the BMP1-cleavage region failed to do so.[11] These studies imply that the proteolytic processing of DSPP through the BMP1-cleavage motif is indispensable for DSPP to exert its biological function during dentinogenesis.

The RGD motif is another characteristic domain of DSPP. RGD domain in DPP can bind to integrins on the cell surface of undifferentiated mesenchymal stem cells and pulp cells, promoting their terminal differentiation into odontoblast-like cells.[65] In addition, DPP containing RGD motif induced the differentiation and mineralisation of mouse dental papilla cell line 23 (MDPC-23) cells[66], enhanced the survival and proliferation of rat immortalised preodontoblast cells, and promoted the terminal differentiation of preodontoblasts to functional odontoblasts.[67] Moreover, an RGD peptide derived from porcine DPP promotes cellular migration of human dental pulp cells.[68] All these findings indicate that the RGD motif integrating the surface of dental pulp cells plays a vital role in cell migration or differentiation; however, native rat DPP protein displays no RGD-induced dental pulp cell migration and differentiation.[69]

Conformational analysis suggested that DPP be thought as a unique structure with a nonplanar, folded and modified *trans*-extended backbone chain, in which the repeat aspartic acid/serine/serine (DSS) domain in DPP constituted the extended backbone structures with long ridges of carboxylate and phosphate groups respectively on each side of the peptide backbone, forming two well-defined ionic ridges.[58] Such a specific spatial arrangement could produce highly negatively charged aggregation areas for binding calcium ions. The bound calcium ions on the two ionic ridges could be bridged between the parallel chains or interact with a hydroxyapatite surface array of calcium ions.[58] The spatial structure of DPP directly generated a dual behaviour that is entirely compatible. On one hand, phosphorylated DPP shows an affinity for the surface of hydroxyapatite and octacalciumphosphate and meanwhile maintains the ability to sequester Ca^{2+} ions.[70] On the other hand, DPP have an avid affinity for binding the collagen gap zones where collagen fibrils aggregate in the mineralisation front, providing the connecting interfaces for mineralised crystal and collagen fibrils.[71,72] At low DPP concentrations, the Ca^{2+} ions might be folded back on themselves, creating partially ordered and internally bridged structures; only at higher concentrations, DPP would adopt an open conformation that provides a structural freedom for interaction as previously mentioned, both with collagen and with

surfaces of cell membranes and mineral crystals.[73] The conformational state of DPP with dual interaction with collagen and crystal might be responsible for facilitating well-defined mineral deposition.

Relationship between DPP and collagen and its role in dentine formation

As the most abundant noncollagenous protein in dentine, DPP is deposited directly at the advancing mineralisation front of dentine while newly synthesised collagen is deposited at the advancing predentine border.[74,75] The key function of DPP is its collagen-binding capacity[75], and as the concentration of PP increases, more DPP will bind to the collagen fibrils.[76] The close interlinking of DPP and collagen is directly associated with mineral deposition during dentine formation.[77] Previous studies showed that DPP covalently crosslinked to type I collagen (Col1) significantly promoted the growth of hydroxyapatite crystals in vitro.[2,78] Phosphorylated DPP induced highly organised mineralisation of collagen fibrils in which the deposited mineral particles were fully crystalline and organised with their c-axes coaligned with the collagen fibril axis, whereas non-phosphorylated DPP stabilised amorphous calcium phosphate (ACP) at higher concentrations without organised crystallization.[4,79] Progressive enzymatic removal of immobilised phosphophoryn led to an increase in mineralisation induction time, and when 90% of the phosphate of dentine collagen was removed, mineralisation was no longer induced.[77] These findings definitively suggested that the phosphate esters of DPP correlated with collagen were indispensable for the initiation of mineralisation.

As a mineralisation scaffold, Col1 rarely appears in the mature hypermineralised peritubular dentine[80,81]; however, our previous studies found that amounts of collagen fibres were around dentine tubules in hypomineralised peritubular dentine in DGI specimens with *DSPP* mutation.[82] Higher Col1 expression and lower mineralisation were found in Dspp mutation cells than in normal Dspp cells.[83] These findings indicate that abnormal DSPP would be associated with the alteration not only of dentine mineralisation but also of the amount of collagen. Altogether, the self-aggregating properties of collagen fibrils contribute to the transition of matrix vesicles forming a larger, mineralised structure.[84,85] In the absence of DPP, collagen fibrils are not directly involved in mineralisation. The interplay between the self-assembled type I collagen matrix and the noncollagenous DPP serves as a template for dentine mineral nucleation and growth.[86,87]

DSP and mineralisation

DSP and its fragments

DSP is further processed into small molecular fragments that are segregated into specific compartments within odontoblasts and dentine[8], and later DSP was discovered to be a novel substrate of matrix metalloproteinase 9.[88,89] DSP NH2-terminal fragments are highly localised in predentine, whereas the COOH-terminal fragments are mainly distributed to the mineralised dentine.[90] The distinct distribution pattern of the two terminal fragments of DSP in different compartments of teeth suggests that they might play unique functions during dentinogenesis. The C-terminal of recombinant human DSP (rh-DSP$^{376\text{-}462}$) was reported to have a novel signalling function of rh-DSP for the promotion of growth, migration and differentiation in HDPCS via the BMP/Smad, JNK, ERK, MAPK and NF-κB signalling pathways.[91] The C-terminal of recombinant mouse DSP (rC-DSP183-457) facilitates attachment, migration, proliferation and differentiation of human periodontal ligament stem cells (PDLSCs) significantly, by regulating gene expression of tooth-/bone-related markers, transcription factors and growth factors.[88] The middle domain (DSPaa183-219) of the N-terminal fragments was bound to integrin β6, forming a complex to stimulate the phosphorylation of Smad1/5/8 proteins; then, the phosphorylated Smad1/5/8 proteins would be bound to DSPP gene promoter to activate the expression of DSPP and DMP1 and induce dental mesenchymal cell differentiation and biomineralisation.[92]

Few studies clarified the nature of the carbohydrate moieties of DSP. Qin et al[93] first proposed a new concept, high molecular weight DSP (HMW-DSP) from the extracellular matrix of rat dentine, which was an isoform of DSP. HMW-DSP absorbed much more water than DSP, which is consistent with the hypothesis that HMW-DSP contains more carbohydrate than DSP. They found that HMW-DSP possess extremely large amounts of carbohydrates and great heterogeneity in glycosylation, and proposed that HMW-DSP could be the functional form of DSP.[93] In fact, HMW-DSP is the proteoglycan form of DSP and was renamed DSP-PG, which appeared to be more abundant than DSP.[93,94] DSP-PG consists of two glycosaminoglycan (GAG) chains. Investigations have revealed the roles of these GAGs, including binding calcium ions[95] and inhibiting hydroxyapatite crystal growth.[96] The GAG side-chains for the DSP-PG made of chondroitin-sulphate inhibited the formation and growth of hydroxyapatite crystals in collagen gels.[97,98]

In vitro mineralisation analyses showed that DSP without GAG chains did not have a significant effect on mineral formation and growth.[99] *Dspp*$^{-}$/*Dsp*$^{+}$ mice showed more severe dentine defects than *Dspp*$^{-/-}$ mice.[100] Based on the above investigations, it was suggested that DSPPG might serve as an antagonist of DPP, preventing the predentine from being mineralised too rapidly during dentinogenesis[100], and that this proteoglycan might be the functional form of the N-terminal fragment of DSPP during biomineralisation.

Thus, DSP is critical for tooth development and could be processed further into small fragments in odontoblast cells, and some proteolytic processing of DSP could be the activation step of biological function and/or degradation functions.

DSP and pulp cell differentiation

Generally, DSP has similar characteristics to other SIBLING members due to similar amino acid composition. Earlier studies proposed that DSP could nominally affect the formation and growth of hydroxyapatite crystals in vitro[99]; however, recent studies reported new findings on DSP function. Spatially, *DSP-only* transcripts appeared to be localised in cells at the areas subjacent to the odontoblast layer and in the dental pulp rather than expressed in the columnar mature odontoblasts in rats. Stro-1 protein, a stem cell marker, was also identified in cells at the areas subjacent to odontoblasts and in dental pulp.[101] The presence of *DSP-only* transcripts containing no PP sequence was also reported in porcine teeth.[16] DSP was found to enhance the mechanical properties of the DEJ in vivo.[102] The temporal expression of DSP mRNA coincides with odontoblast secretory activity and dentine matrix deposition during dentinogenesis in mouse molars.[103] DSP facilitates initiation of hydroxyapatite formation along or inside the collagen fibrils, leading to the conversion of predentine to dentine at the mineralisation front.[19] The molars in *Dspp*$^{-}$/*Dsp*$^{+}$ mice display narrower predentine without any ectopic unmineralised dentine, indicating that the initiation of predentine-dentine conversion is dependent on the expression of DSP.[104] In conclusion, the functions of DSP-only transcripts remain to be determined.

DSP could involve multiple signalling pathways, as well as functioning as secretory proteins to regulate mineralisation. Peptides derived from DSP had the ability to regulate gene expression and protein phosphorylation of BMP-dependent pathway-related molecules, and the signalling function of DSP via the BMP/Smad, JNK, ERK and NF-κB signalling pathways was revealed.[91] DSP was capable of binding to integrin β6 and phosphorylated transcription factors Smad1/5/8, which upregulated expression of DSPP and DMP1 genes and induced dental mesenchymal cell attachment, differentiation and mineralisation through P38 and ERK1/2 protein kinases.[92] Furthermore, as a ligand, DSP could also bind to the cell surface receptor Occludin (Ocln) that binds to focal adhesion kinase (FAK), and it induced differentiation and mineralisation of human dental pulp stem cells and mouse dental papilla mesenchymal cells through the Ocln-FAK signalling pathway.[105] Silencing *DSPP* expression altered the levels of signalling molecules Runx2, Numb, Notch1 and Gli1 in developing molars of *Dspp*$^{-/-}$ mice.[106] All these findings support the view that the regulatory role of DSP was to orchestrate the process of dentine formation.

DPP binding with collagen improves its structural stability[15], and similarly, as DSP is the most abundant proteoglycan in dentine, it is also likely to interact with collagen.[42] Recombinant human DSP protein (rhDSP) facilitates human dental pulp stem cell differentiation and induces Col I and endogenous DSPP upregulation.[107] Recombinant mouse DSP fusion protein promotes Col I expression in calvarial bone and osteoblasts.[108] Col I mRNA was downregulated in primary calvarial cells without *Dsp* expression of *Dspp*$^{-/-}$ mice in an in vitro culture system.[106] Thus, it is speculated that DSP, as the upstream factor of Col I, ensures the normal differentiation function of odontoblasts.

Although DSP exerts a positive influence in dentine mineralisation, recent studies have shown different results. *Dspp*$^{-}$/*Dsp*$^{+}$ mice presented more severe defects in dentine compared to *Dspp*$^{-/-}$ mice, indicating that DSP might inhibit dentine mineralisation or restrain the accelerating action of DPP and prevent predentine from being mineralised too rapidly during dentinogenesis.[100] Although studies have shown that DSP is critical for dentine mineralisation, findings concerning the biological roles played by DSP in dentinogenesis are renewed continuously and further studies are warranted to delineate the detailed functions and mechanisms.

To conclude, DSP has the unique biological characteristic of inducing initial dentine mineralisation and participating in differentiation in some mineralisation cells. DSP is involved in multiple signalling transduction pathways in regulating the process of mineralisation.

DSPP mutations and human hereditary dentine disorders

Hereditary dentine disorders are mainly separated into two categories: dentinogenesis imperfecta (DGI) with

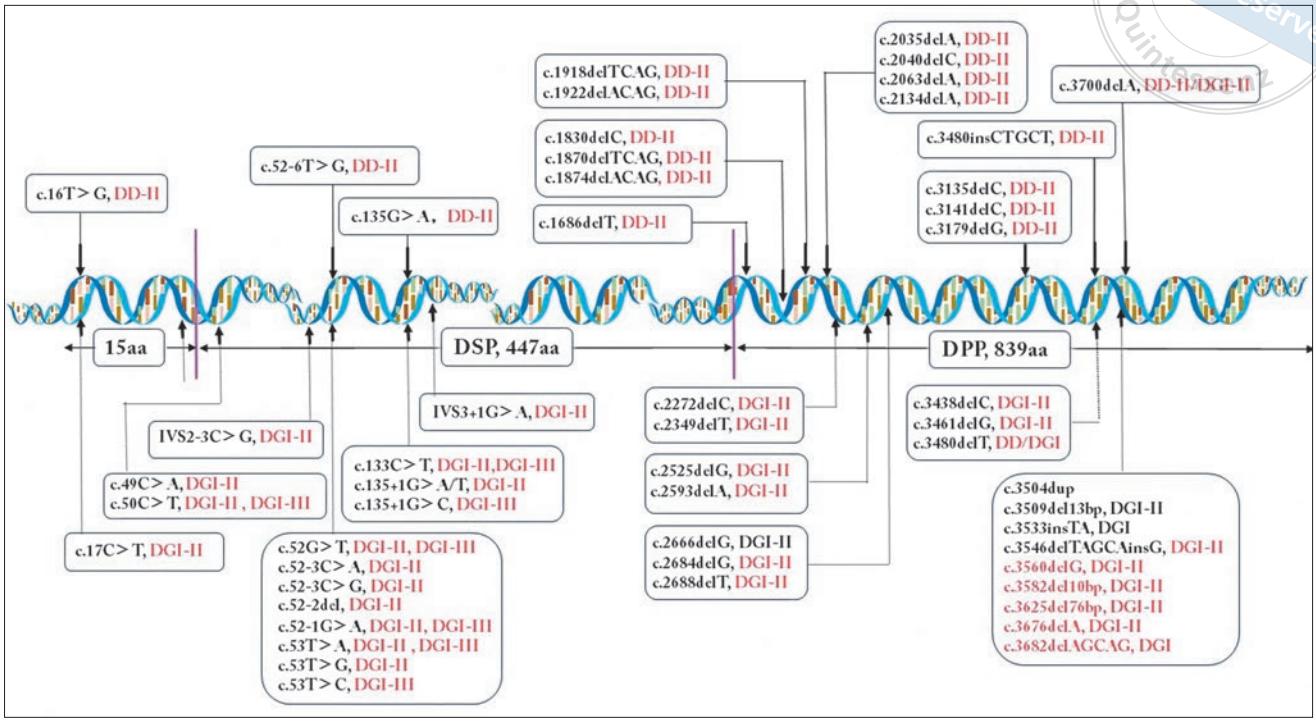

Fig 2 DSPP gene diagram showing mutations identified in families with dentinogenesis imperfecta.[111-113,115,118,119,121,126-132]

DGI-I, DGI-II and DGI-III subtypes, and dentine dysplasia (DD) with DD-I and DD-II subtypes. Among them, DGI-II, DGI-III and DD-II are isolated dentine disorders usually with an autosomal dominant inheritance, and *DSPP* is the only identified causative gene for them.

Xiao et al[109] and Zhang et al[110] first reported that mutations of the *DSPP* gene are associated with DGI-II. To date, many *DSPP* mutations have been identified in families with dentine disorders.[111,112] A large number of these were in the DSP region, with most being missense, nonsense or splicing mutations. Successful sequencing of the highly repetitive DPP region marked a breakthrough in identifying the mutations in the DPP region in hereditary dentine defects. A previous investigation conducted by our group first revealed frameshift mutations in DPP region resulting in hereditary dentine defects in five Chinese families with DGI-II or DD-II and found 14 in-frame indel polymorphisms and 11 single nucleotide polymorphisms (SNPs) in the DPP region in the normal population.[113] Subsequently, other groups reported similar findings.[114-116] In animal models, mice that deleted a "G" to cause a -1 frameshift following the first four amino acids of DPP exhibited severe dentine defects.[117] The results indicated that the reading frame-preserving length variations and single missense alteration in the DPP coding region had no apparent effects on its function, and functional aberrations would not take place unless a frameshift or nonsense mutation occurred in DPP.

DSPP can be secreted normally outside the cell, usually requiring the guidance of a signal peptide. Tyrosine to aspartic acid mutations in the signal peptide region of *DSPP* would affect the secretion of both DSP and DPP owing to the significantly reduced ability to translocate the primary translated product into the endoplasmic reticulum (ER).[118] The result is that either the accumulation of the mutant protein in the cytosol or the continued occupation on the ER may indirectly bring about insufficient processing of the normal DSPP with concomitant defective biomineralisation.

Given the proximity to the border of exon 3 and splicing acceptor site, the mutational "hotspot" was suspected to have some influence on normal pre-mRNA splicing, causing skipping of exon 3.[119,120] Closely adjacent to the membrane-associated signal peptide peptidases cleavage site, the first three amino acids of the mature protein DSPP are Ile Pro Val (IPV), which is highly conserved within almost all animal species. Excepting mutations in the signal peptide region, all mutations in this conserved DSP region would result in a change of the proposed IPV domain.[120] The mutations with deleted exon 3 of DSPP, lacking the conserved IPV motif at the N-terminus after cleavage of the signal peptide, would generate varying degrees of clinical

phenotypes. The expression level of the DSPP exon 3 deletion transcript correlated with the severity of the dentine defects, the weaker the quantity of mutant protein, the milder the clinical phenotype.[121] Mutations in or adjacent to the IPV domain would cause mutant protein to be retained within the ER[122], which can be captured by the autophagy-lysosome system for degradation (ER-phagy).[123] The quantity of mutant protein accumulated in the odontoblast ER was positively associated with the clinical phenotype.[124] Recent studies proposed that the encoding amino acids in the DPP repeat domain with -1bp frameshift mutations result in longer mutant hydrophobic domains that anchor the mutant protein within the rough endoplasmic reticulum (rER) membrane. Meanwhile, the dominant negative effects caused by the retained mutant proteins form entropy-driven, multivalent cation (Ca^{2+}) bridges between each other and then entrap WT DSPP through its own calcium-binding domain.[125] In summary, mutations in either the DSP or DPP domain lead to DGI-II or DGI-III and DD-II. A diagram of *DSPP* gene mutations identified in families with dentinogenesis imperfecta is shown in Fig 2.[111-113,115,118,119,121,126-132] The dentine defect phenotype caused by DSPP mutations would contribute to revealing the functional role of DSPP and lead to a better understanding of dentine mineralisation and homeostasis.

Conclusion and prospects

Since the discovery of DSPP and the degradation products DSP and DPP, considerable progress has been made regarding their biological characteristics. The findings have greatly enhanced our understanding of their function in the process of biomineralisation. The major discoveries to date include the reiwpoints that DSPP was cleaved into DSP, DGP and DPP, and the distinct features of the proteolytic products suggest that these proteins play different roles in biomineralisation; that DPP is transported to the mineralisation front following its synthesis and secretion; that DPP binds to collagen fibrils and Ca^{2+}, promoting the nucleation and growth of hydroxyapatite; that the proteoglycan form of DSP is the functional segment of the NH_2-terminal fragment of DSPP; and that many heterogeneous mutations in the human DSPP gene have been linked to hereditary dentine defects. These discoveries also present challenges, such as whether proteolytic processing of DSPP is an activation step, which components participate in the process of DSPP proteolysis and what biological role they play; what the specific characterisation of the proteoglycan form of DSP is; what the potential roles of DSP/DPP are in regulating signal pathways and what effect they exert on downstream molecules; and which proteins are associated with biomineralisation in dentine other than DSPP, whether DSP/DPP interacts physically with these proteins and what the precise mechanism is. As such advancements in research enrich our knowledge regarding DSP, DPP and DSPP, they also point to new directions for further exploration.

Conflicts of interest

The authors declare no conflicts of interest related to this study.

Author contribution

Drs Jie JIA and Yaling SONG designed the study, drafted and revised the manuscript; Dr Zhuan BIAN supervised the study.

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