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Dentin Sialophosphoprotein and Dentin Matrix Protein-1: Two Highly Phosphorylated Proteins in Mineralized Tissues

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Abstract

Dentin sialophosphoprotein (DSPP) and dentin matrix protein-1 (DMP-1) are highly phosphorylated proteins that belong to the family of small integrin-binding ligand *N*-linked glycoproteins (SIBLINGs), and are essential for proper development of hard tissues such as teeth and bones. In order to understand how they contribute to tissue organization, DSPP and DMP-1 have been analyzed for over a decade using both *in vivo* and *in vitro* techniques. Among the five SIBLINGs, the *DSPP* and *DMP-1* genes are located next to each other and their gene and protein structures are most similar. In this review we examine the phenotypes of the genetically engineered mouse models of DSPP and DMP-1 and also introduce complementary *in vitro* studies into the molecular mechanisms underlying these phenotypes. DSPP affects the mineralization of dentin more profoundly than DMP-1. In contrast, DMP-1 significantly affects bone mineralization and importantly controls serum phosphate levels by regulating serum FGF-23 levels, whereas DSPP does not show any systemic effects. DMP-1 activates integrin signaling and is endocytosed into the cytoplasm whereupon it is translocated to the nucleus. In contrast, DSPP only activates integrin-dependent signaling. Thus it is now clear that both DSPP and DMP-1 contribute to hard tissue mineralization and the tissues affected by each are different presumably as a result of their different expression levels. In fact, in comparison with DMP-1, the functional analysis of cell signaling by DSPP remains relatively unexplored.

Keywords

DSPP; DMP-1; genetically engineered mouse models; cell signaling molecules; astacin family proteinases

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1. Introduction

Dentin sialophosphoprotein (DSPP) and dentin matrix protein-1 (DMP-1) share many similarities in both their gene and protein structures, and it is now believed that DSPP arose from DMP-1 by a gene duplication event (1). Evolutionary analysis of DSPP genomic sequence indicated that the creation of ancestor DSPP by DMP-1 duplication event may be indispensable to toothed animals (2). DSPP and DMP-1 are both cleaved into two protein chains; the N-terminal regions are proteoglycans that contain chondroitin sulfate chains, and the C-terminal regions are highly phosphorylated (Figure 1) (3-9).

It is now obviously accepted that DSPP and DMP-1 play important roles in hard tissue development. DSPP and DMP1 are positive regulators of hard tissue mineralization with DSPP acting on dentin and DMP1 acting on both bone and dentin. Their essential roles for hard tissue development and mineralization *in vivo* have been analyzed utilizing many knockout and transgenic mouse models (summarized in Table 1 and 2).

Despite the similarities between DSPP and DMP-1, a recent *in vitro* study suggested that phosphorylated forms of both DSPP and DMP-1 would be able to act as nucleators of apatite crystal formation in the presence of collagen, with DSPP inducing highly organized intrafibrillar collagen mineralization and DMP-1 inducing the deposition of mineral particles along the collagen fibril axis (10). These discrete roles indicate the biochemical differences between DSPP and DMP-1.

2. The features of the SIBLING family and the properties of DSPP and DMP-1

The SIBLING family consists of five extracellular matrix proteins: DSPP, DMP-1, bone sialoprotein (BSP), matrix extracellular glycoposphoprotein (MEPE), and osteopontin (OPN). Human chromosome 4 (mouse chromosome 5) contains a SIBLING family gene cluster (4q21) located immediately proximal to a cluster of enamel matrix protein genes (4q13) (Figure 1). The SIBLING family was defined on the basis of their common structural, biochemical and genetic features (11). These include multiple phosphorylation sites, a highly acidic nature, the presence of an arginine-glycine-aspartic acid (RGD) integrin binding site, and a proteolytic-resistant acidic serine-aspartate-rich MEPE-associated (ASARM) motif (12, 13). Three SIBLINGs, DMP1, BSP, and OPN, can specifically bind to pro-matrix metalloproteinase-9 (proMMP-9), proMMP-2, and proMMP-3, respectively. This specific binding facilitates either a conformational changes or the release of tissue inhibitor of metalloproteinase (TIMP) from the MMP-TIMP complex thereby making the MMP proteolytic active site accessible (14). The activation of MMPs by binding of the SIBLINGs results in induction of specific cell invasion and migration (15, 16). Thus, SIBLINGs are extracellular matrix proteins that play important roles in controlling numerous cellular properties. The expression of SIBLINGs is increased in cancer cells and therefore they are considered biomarkers for prostate cancer (17-20). The mechanisms involved in the increased expression of SIBLINGs in cancer cells are not fully understood, however, a recent *in vitro* study suggested that DSPP has the potential to activate oral carcinogenesis (21).

3. The functional analysis of DSPP, DMP-1 by gene knockout and transgenic strategies

DSPP

The role of DSPP in dentin—The *Dspp* gene is primarily expressed in odontoblasts and, to a lesser extent, in osteoblasts. *Dspp* is also expressed in other tissues such as the salivary glands, sweat glands, lungs, kidneys, and nasal cartilage (22-29). Many point mutations in the human *DSPP* gene are linked to dentinogenesis imperfecta (DGI) and dentin dysplasia (DD), which are the two most common hereditary diseases affecting dentin, (30-35). *Dspp* null mice display severe tooth defects due to widened predentin and dentin hypomineralization (36), which are similar to the defects seen in patients suffering from DGI and DD. The teeth of these patients have irregular mineralization fronts and widened predentin concomitant with narrow dentin width. Their dentin also shows ectopic unmineralized spots. These observations suggest that the conversion of predentin to dentin is impaired by a lack of DSPP, resulting in defects in dentin maturation. *Dspp* has been thought just as a terminated odontoblast marker, however, recent study revealed that *Dspp* expression of odontoblasts was higher during primary dentinogenesis than during secondary dentinogenesis, suggesting more aggressive roles of DSPP for odontoblast differentiation and functions during primary dentinogenesis than ever thought (37). It is well known that the *DSPP* gene encodes two major dentin noncollagenous proteins, DSP (dentin sialoprotein) and DPP (dentin phosphoprotein, alternative termed dentin phosphophoryn or phosphophoryn) (38). In some species, DSP and DPP are the most abundant noncollagenous proteins in the dentin extracellular matrix (ECM) (39). DSP represents the amino-terminal part of DSPP and is deposited principally as a sialic acid-rich chondroitin sulfate-type proteoglycan in dentin, and less so in a non-proteoglycan form (5, 6, 40). DPP contains repeat sequences of serine-serine-aspartic acid (in humans there are over 200 tandem copies and in mice there are about 100 copies), and most of them are phosphorylated (33, 41). Porcine dentin glycoprotein (DGP) is found in the middle region of DSPP (42), however, it has not yet been detected in other species and thus its functions are still unknown.

The distinct functions of DSP and DPP in dentin—Since DSP and DPP are the cleaved products of DSPP, the phenotype of *Dspp* null mice does not shed much light on the individual roles of DSP or DPP in dentin mineralization. We have recently reported the generation of DPP conditional knockout (DPPcKO) mice, in which only DSP is expressed on a *Dspp* null background utilizing the DSP transgene under the control of the *Dspp* promoter (40). The teeth of DPPcKO mice show a partial rescue of the *Dspp* null phenotype with restored predentin width, an absence of irregular unmineralized areas in the dentin of molars, and less frequent pulp exposure. Micro-computed tomography (micro-CT) analysis of molars from DPPcKO mice further confirmed this partial rescue with a significant recovery in dentin volume, but not in dentin mineral density.

The *Dspp* promoter was able to express the DSP transgene in the molars in a proper spatiotemporal manner; however, the expression level appeared to be less in the incisors. When we compare the endogenous DSP and DPP staining intensities in dentin (or odontoblasts) between the molars and incisors from *Dspp*^{+/-} mice, these intensities were almost equivalent between the incisors and molars as shown in Fig. 2. These observations suggested that the endogenous DSP and DPP expression levels in incisors and molars were almost the same. Additionally, as reported earlier (36), dentin defect of DSPP knockout incisors was as severe as that of molars. Therefore, constant turn over of continuously growing incisor may not cause the expressional or phenotypic differences by the lack of endogenous DSPP. Instead, we speculated that the lower transgene expression level in incisor is caused by the feature of the DSPP promoter construct, i.e. *Dspp* gene expression is

differently regulated in molars and incisors. In incisors, *Dspp* regulatory region may exist upstream of our construct. This difference found in the molars and incisors was observed not only in two DSP transgenic mouse lines but also in the DSPP- α -galactosidase transgenic mouse line (43). Moreover, a similar phenomenon was observed when the amelogenin promoter was used for ameloblast-specific transgene expression (44). Thus, a comparison of the incisors and molars could be used to establish a correlation between the phenotypic severity and the level of *Dspp* promoter-driven transgene expression. The expression level of transgenic DSP in the molars is similar to endogenous DSP expression, but is much lower in the incisors (Figure 2). Biglycan accumulation in the unmineralized area occurs in the dentin and wider predentin areas of the DPPcKO incisors as well as in the DSPP null mice (Figure 3), which indicates that there is no obvious rescue of the *Dspp* null phenotype in the DPPcKO incisors. In contrast, the DPPcKO molars display much narrower predentin without any ectopic unmineralized dentin, indicating that the initiation of predentin-dentin conversion is dependent on the expression of DSP. These observations reveal the distinct roles of DSP and DPP in dentin mineralization. DSP regulates the initiation of dentin mineralization and DPP plays a role in the maturation of mineralized dentin.

The role of DSPP in bone—The bone phenotype of the *Dspp* null mice is subtle, most likely because of its lower expression in bone as compared to dentin but these mice have revealed the broad functions of DSPP (28). The *Dspp* null mice at five weeks have denser bones than age-matched wild type mice, and there is increased crystallinity. In contrast, at nine months old the *Dspp* null mice had bones that were much less dense than wild type mice. Therefore, unlike the dentin defects in *Dspp* null mice, which remained relatively constant for the entire lifespan, the bone phenotype is apparently age-dependent. However, the reasons why DSPP has opposite effects at five weeks and nine months are still unclear. It is possible that DSPP stimulates bone remodeling, which is more active at a younger age and could in turn lead to differences in the bone phenotype at different ages.

The role of DSPP in enamel—During the early stages of amelogenesis, *Dspp* is transiently expressed in the presecretory stage of ameloblasts at the time of the establishment of dentin enamel junction (DEJ) (45). The transient expression of DSPP is also seen in the presecretory ameloblasts of human teeth (46). Transgenic mice that express DSP or DPP under the amelogenin promoter were generated to analyze the functional significance of *Dspp* expression for enamel formation in ameloblasts. In particular, the effect on the construction of specialized first-formed harder enamel adjacent to the DEJ was examined (47, 48). Overexpression of DSP led to accelerated enamel mineralization, which significantly increased the enamel hardness (20%); however, the enamel morphology remained unaltered. The mechanism of DSP-mediated accelerated enamel hardness has not been analyzed yet, but it has been proposed that DSP acts as a nucleator of enamel crystallites. Similarly, the DSP-mediated initiation of mineralization was observed in the dentin of the mice with DSP expressed on a *Dspp* null background (40). In contrast, the overexpression of DPP resulted in “pitted” and “chalky” enamel without uniform thickness, and the inclusion of DPP weakened the enamel bulk. These findings indicate the positive effect of DSP, and the negative effect of DPP on enamel formation. The reasons for the negative effect of DPP remains unclear but *in vitro* experiments indicate that an excess concentration of DPP inhibits mineral crystallization, while a moderate concentration induces it (10). Thus, the expression level of DPP may be crucial in the formation of the appropriate enamel structures.

Potential roles of DSPP in cell signaling—The functional analysis in genetically altered mouse models indicates that the main function of DSPP is an inducer of mineralization in the extracellular matrix. Even though endogenous DSPP expression is

much lower in bone than in dentin unlikely to DMP-1, the exogenous addition of DPP is clearly effective for undifferentiated mesenchymal cell and osteoblastic cells to differentiate into mature osteoblasts and/or odontoblasts as described below. DPP is reported to induce mineral nodule formation even in NIH3T3 fibroblast cells that do not have the potential to differentiate into osteoblast or odontoblast cells (49). NIH3T3 cells over-expressing DPP start to deposit calcified nodules after 4 days culture in mineralization-inducing medium containing ascorbic acid and -glycerophosphate. Moreover, phosphorylated DPP was identified in these cells and X-ray diffraction analysis indicated that the extracellular matrix of DPP-overexpressing NIH3T3 cells included hydroxyapatite crystals as same as pre-osteoblastic MC3T3-E1 cells. Thus, it was concluded that the presence of DPP alone was sufficient for the fibroblastic cells to differentiate into osteoblast-like cells. Several studies stated that recombinant DPP was able to induce osteogenic differentiation in osteoblastic cells and mesenchymal stem cells. This induction was induced by activation of the MAPK pathway as a result of recombinant DPP binding to cell membrane integrin thorough its RGD binding site (50, 51). Moreover, recombinant DPP was able to stimulate phosphorylation of Smad-1 and induce gene expression of bone/dentin differentiation markers. The phosphorylation of Smad-1 was not blocked by Noggin, which is the extracellular inhibitor of BMP ligands. This suggests that DPP activates Smad-1 via integrin signaling independently of BMP signaling (52). Also, an RGD peptide derived from porcine DPP promotes cellular migration of human dental pulp cells (53). Taken together, these observations indicate that DSPP functions as a cell-signaling molecule. Interestingly, RGD in the DSPP sequence is not conserved among different species (for example, rat, dog, and rabbit DSPP lack the RGD sequence), unlike the RGD in DMP-1 and other SIBLINGs (2). Therefore, further analysis is warranted to determine if DSPP plays a decisive role in cell differentiation or if DSPP has any other functional domains. For instance, it should be interesting to analyze whether DPP with a mutated RGD sequence can induce osteo/odontogenic differentiation as efficiently as normal DPP protein.

Opposite roles of biglycan and decorin in dentin mineralization—Biglycan (Bgn) and Decorin (Dcn) are major small leucine rich repeat proteoglycans (SLRPs) found in dentin and accumulate in the widened predentin of *Dspp* null mice (36). To examine the effect of SLRP accumulation in *Dspp* null predentin, we generated *Dspp*^{-/-}*Bgn*^{-/-} and *Dspp*^{-/-}*Dcn*^{-/-} null mice (54). Interestingly, we observed a reduction in the enlarged predentin in *Dspp*^{-/-}*Dcn*^{-/-} null mice and, conversely, wider predentin with an increased number of calcospherites in *Dspp*^{-/-}*Bgn*^{-/-} null mice (Figure 4). These observations suggest that Dcn and Bgn play opposite roles in the *Dspp* null background with Dcn inhibiting and Bgn stimulating the conversion of predentin to dentin. The effects may be due to other ECM molecules that bind to/interact with decorin and biglycan rather than the direct roles of these molecules. Although several mutations in the *DSPP* gene have been reported in DGI and DD patients, the severity of the dentin defects in these patients is not always correlated with the point mutations. This observation indicates that there are other factors that contribute to defect severity. Bgn and Dcn could influence the defects, since they significantly impact upon the dentin phenotypes on the *Dspp* null background. Most of the *DSPP* mutations observed in DGI and DD patients, such as mutations in the signal peptide and frame shift mutations in the DPP repeat sequence, are predicted to result in an accumulation of mutated DSPP proteins in the rough endoplasmic reticulum (rER) of odontoblasts (55). It is possible that these mutated DSPP precursor proteins are not properly degraded and therefore disturb the secretion of dentin ECM proteins. Indeed, the expression of *Bgn* and *Dcn* in the odontoblasts of *Dspp* null mice is increased as compared to the wild type control, probably resulting in the significant changes of SLRP expression (36). Thus, the secretion levels or patterns of BGN and DCN might be affected in each individual patient differently, leading to the varying spectrum of severity seen in this disease.

DMP-1

The role of DMP-1 in dentin—DMP-1 was initially identified in odontoblasts during embryonic and postnatal development. Unlike DSPP, which is predominantly expressed in odontoblasts, *Dmp-1* is also highly expressed by osteocytes, chondrocytes, and preosteoblasts (56). *Dmp-1* null mice develop obvious tooth phenotypic changes during postnatal period including partial failure to mature from predentin to dentin, enlargement of pulp chambers concomitant with increased predentin width, and dentin hypomineralization (57). These phenotypic features of *Dmp-1* null mice are quite similar to those seen in *Dspp* null mice. However, *Dmp-1* null features in teeth are less severe than those of *Dspp* null mice. Interestingly, in DMP-1 null mice, the tooth development of the lower third molar is delayed (58). *Dmp-1* is initially expressed in differentiating odontoblasts and osteoblasts and facilitates the expression of the osteoblast and odontoblast differentiation marker genes (59). Subsequently, DMP-1 is deposited into the extracellular space as an ECM molecule. To determine the developmental stage at which DMP-1 expression is critical for the phenotypes of *Dmp-1* null mice, *Dmp-1* was expressed during early to late stages of odontogenesis using the *Col1a* promoter, or during the late stage of odontogenesis using the *Dspp* promoter. When *Dmp-1* was expressed using the *Col1a* promoter on the *Dmp-1* null background, the defects in mineralization, dentinal tubules, and third molar development seen in *Dmp-1* null mice were completely rescued. These defects were not completely rescued in molars when *Dmp-1* was reintroduced using the *Dspp* promoter on the *Dmp-1* null background. These results suggest that DMP-1 expression is required in both the early and late stages of odontogenesis (58).

The role of DMP-1 in bone—Unlike *Dspp* null mice, the *Dmp-1* null mice display severe defects in bones and cartilage. The lack of DMP-1 in bone results in decreased mineral properties (mineral-to-matrix ratio) and increased crystal size, suggesting that DMP-1 directly induces bone mineralization and acts as a nucleator of mineralization. Moreover, a lower Ca × P concentration was observed in the bones of *Dmp-1* null mice, which indicates that DMP-1 is also involved in calcium and phosphate metabolism (60). *Dmp-1* null mice also develop severe defects in cartilage formation during postnatal chondrogenesis (61). Secondary ossification centers were deformed and the growth plate was wider and had an irregular mineralization front, which results in shortened and widened vertebrae and long bones. These phenotypes are similar to the dwarfism associated with chondrodysplasia. This phenotype results from increased cell proliferation in the proliferating zone and reduced apoptosis in the hypertrophic zone. These findings show that DMP-1 is essential for normal postnatal chondrogenesis and subsequent osteogenesis. Several *Dmp-1* transgenic mouse models have confirmed the important roles of DMP-1 in bone development. To test the *in vivo* roles of the C-terminal fragment, transgenic mice overexpressing either full-length DMP-1 or a 57-kDa (C-terminal) fragment using the *Col1a* promoter were generated. Both of the transgenic mice rescued the bone phenotypes of *Dmp-1* null mice, suggesting that the 57-kDa C-terminal fragment is able to recapitulate the function of intact DMP-1 *in vivo* (62).

The role of DMP-1 in cell signaling—Interestingly, DMP-1 is known to be endocytosed into preosteoblasts and preodontoblasts by binding to the 78-kDa glucose-regulated protein (GRP78) that has been newly identified as the DMP-1 plasma membrane receptor (63–65). GRP78 belongs to the heat shock protein 70 (HSP70) family and is a stress response protein. GRP78 is localized in the endoplasmic reticulum (ER) and act as a molecular chaperone to monitor the proper folding and assembly of secretory proteins (66). In certain cell types, GRP78 is also localized to the cell surface (66–68). Chondrocytes and osteocytes express GRP78 and some GRP78 protein is secreted into extracellular space where it is deposited in the bone matrix *in vivo* (65). The expression of GRP78 is up-regulated during osteoblast

differentiation *in vitro* and recent *in vitro* assays suggest that GRP78 is able to bind to type I collagen and DMP1 directly to facilitate calcium phosphorus deposition (65). Unexpectedly, the binding of DMP-1 to GRP78 is dependent on the N-terminal half of DMP-1 protein, which had been thought to be a less functional domain compared to C-terminal region, which has the nuclear localization signal, RGD sequence, and phosphorylation sites. (63). Endocytosed DMP-1 results in the release of calcium from ER stores leading to an increased intracellular calcium level, which induces the activation of p38 pathway and Runx2 nuclear translocation. Runx2 activity up-regulates the transcription of tooth and bone-related genes such as *osteocalcin*, *Runx-related transcription factor 2 (Runx2)*, and *Osterix*. The pathway from the uptake of extracellular DMP-1 to the up-regulation of differentiation markers is blocked by an anti-GRP78 antibody, inositol 1,4,5-triphosphate (IP3) receptor inhibitor, dominant negative p38 and calcium ion chelator. This suggests that the function of DMP-1 as a cell signaling molecule is highly dependent on calcium-mediated signaling (63-66). Previous *in vitro* studies suggested that DMP-1 itself could localize in the nucleus, bind to the *Dspp* promoter region and regulate *Dspp* gene expression (57, 69). Therefore, it is likely that the tooth phenotype of *Dmp-1* null mice is at least partially caused by diminished expression of *Dspp*.

To identify the functional significance of DMP-1 cleavage into two proteins, transgenic mice that express a mutated cleavage-resistant DMP-1 were generated (70-72). These transgenic mice were unable to rescue the bone abnormalities of DMP-1 null mice as well as the abnormalities of dentin and jaw formation, which indicates that DMP-1 cleavage is essential for its biological functions. The reasons for the lack of function of uncleaved DMP-1 remain unclear. It is possible that the binding of DMP-1 to GRP78, and posttranslational modifications such as phosphorylation and attachment of the chondroitin sulfate chain are altered or the RGD sequences might be sequestered in full-length DMP-1. Structural analysis of the DMP-1 protein will be essential to answer these questions.

Recently, it was reported that a homozygous mutation in the *DMP-1* gene caused an autosomal recessive form of hypophosphatemia (ARHP). In some of the ARHP patients with DMP-1-inactivating mutations, the serum level of fibroblast growth factor-23 (FGF-23) was clearly increased. FGF-23 is an osteocyte-derived phosphaturic hormone that decreases the serum phosphate level with enhancement of renal phosphate excretion by decreasing its re-absorption within the proximal tubules. FGF-23 also suppresses biosynthesis and metabolism of vitamin D (73, 74). Moreover, hypophosphatemia phenotypes observed in *Dmp-1* null mice were rescued by the disruption of FGF-23 expression, suggesting that an abnormally high expression of FGF-23 was a contributing factor in the phenotypes of ARHP patients (75). The mechanism of DMP-1 control of FGF-23 expression remains undetermined. An *in vitro* assay of FGF-23 promoter-luciferase reporter gene activity indicated that DMP-1 overexpression in the ROS17/2.8 osteoblast cell line does not affect FGF-23 promoter activity, suggesting that DMP-1 indirectly affects FGF-23 expression. One possible explanation is that the hypomineralized bone caused by the lack of DMP-1 easily releases FGF-23, resulting in high circulating levels (76). The transgenic mice that expressed the same *DMP-1* deletion mutation concordant with the human ARHP patient on the *Dmp-1* null background displayed a mild rachitic tooth phenotype and did not mimic the severe dentin and enamel phenotypes observed in the human ARHP patient (77). An *in vitro* study indicated that this mutant DMP-1 protein maintains partial functional activity of DMP-1. Thus, the mechanisms that generate the tooth phenotypes in ARHP patients remain to be elucidated.

4. The Cleavage of DSPP and DMP-1 by astacin family proteinases

Bone morphogenetic protein 1 (BMP-1) and its alternatively spliced isoform, tolloid (TLD) cleave full-length DMP-1 and DSPP proteins into two proteins (78-82). There are four mammalian BMP1/TLD-like proteinases: BMP-1, which is the shorter product of the *Bmp1* gene; TLD, which is the longer product of the *Bmp1* gene; TLD-like 1 and 2 (TLL1 and TLL2), which are transcribed from distinct genes (83). The BMP1/TLD-like proteinases contain a conserved astacin family of metzincin metalloproteinases domain and therefore belong to the astacin proteinase family. Recent studies suggest that meprin A, (MEP1B), which is a member of the astacin family can cleave the DSPP precursor protein to generate DPP (82). MEP1B but not BMP-1 can degrade DPP protein *in vitro*. Therefore, MEP1B may be an essential factor that controls free calcium concentration during dentin mineralization at the mineralization front by releasing calcium from degraded DPP protein. Since these proteinases are critical for collagen fibrillogenesis, odontoblasts that secrete collagen matrix should express these proteinases. Unfortunately, because of antibody cross-reactivity, these proteinases have not as yet been localized *in vivo*.

5. Common questions regarding DSPP and DMP-1

The targeted expression of DSP on a *Dspp* null background resulted in the partial rescue of predentin-dentin conversion and DMP-1 endocytosis is reliant on the presence of its N-terminal domain, however, in general, the functions of DSPP and DMP-1 are mainly dependent on their phosphorylated C-terminal protein region. There are a number of important questions regarding DSPP and DMP-1 that need to be addressed. What is the functional role of the N-terminal portions proteoglycan forms? Do these N-terminal portions facilitate full-length and C-terminal protein folding and/or secretion? Why are these different nature proteins transcribed from the same genes? When does the attachment of proteoglycans and protein phosphorylation occur? In order to answer these questions, it would be useful to firstly monitor the intracellular transport process of DSPP and DMP-1. The precursor proteins are synthesized and transported to the extracellular matrix via the endoplasmic reticulum, golgi, and secretory vesicles. During this process, active BMP-1, which is processed by furin convertase, cleaves full-length DSPP and DMP-1. Recently, small amounts of full-length DSPP and DMP-1 were identified from bone and dentin but it is unclear whether they are simply remainders of uncleaved products or if they are retained and play a specific role (79, 84). As we described the above, the expression level of *DSPP* and *DMP-1* is different in bone and dentin despite the similarities such as its mineralization step and compositions, i.e., 1) Dentin or bone ECM is constructed as non mineralized status, called predentin or osteoid, and then mineral deposit into them; 2) Approximately 70% of dentin and bone tissue are mineral and the main organic matrix is type I collagen. Regulatory mechanisms for the gene expression of DSPP and DMP-1 have not been fully revealed. Previous reports disclosed that DMP-1 directly induced DSPP gene expression (57, 58, 69). However this phenomenon would not be able to explain the different expression level of DSPP and DMP-1 in dentin and bone. Interestingly, Runx2 transgenic mouse utilizing *Col1a* promoter which is active through entire odontoblast differentiation process, showed inhibition of terminal odontoblast differentiation and induction of transdifferentiation into osteoblast. Therefore, strict spatiotemporal expression pattern of Runx2 and its co-factors may control the fate of undifferentiated mesenchymal cells.

6. Conclusions

It is now clear that DSPP and DMP-1 play important roles in hard tissue development. Importantly, compared to DMP-1, the effects of DSPP during cell signaling have not been well analyzed. It is interesting to look at how phosphorylated recombinant DPP proteins

influence osteo/odontoblastic cell and mesenchymal cell differentiation. In summary, DSPP and DMP-1 have overlapping but distinct functions in mineralized tissues, not only as extracellular proteins but also as cell signaling molecules.

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Abbreviations

DSPP	dentin sialophosphoprotein
DSP	dentin sialoprotein
DPP	dentin phosphoprotein Alternative names
Dentin	phosphophoryn or phosphophoryn
DMP-1	dentin matrix protein-1
SIBLINGs	small integrin-binding ligand <i>N</i> -linked glycoproteins
FGF-23	fibroblast growth factor-23
BSP	bone sialoprotein
MEPE	matrix extracellular glycoprophosphoprotein
OPN	osteopontin
RGD	arginine-glycine-aspartic acid
ASARM	acidic serine-aspartate-rich MEPE-associated
MMP	matrix metalloproteinase
TIMP	tissue inhibitor of metalloproteinases
DGI	dentinogenesis imperfect
DD	dentin dysplasia
ECM	extracellular matrix
DGP	dentin glycoprotein
DPPcKO	DPP conditional knockout mouse
SLRP	small leucine rich repeat (SLRP)
rER	rough endoplasmic reticulum
MAPK	mitogen-activated protein kinase
BMP	bone morphogenetic protein
GRP78	glucose-regulated protein
HSP70	heat shock protein 70
IP3	inositol 1,4,5-triphosphate
ARHP	autosomal recessive form of hypophosphatemia
TLD	tolloid
MEP1B	meprin A

GAG glycosaminoglycans

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Figure 1.

The genomic location and exon-intron structure of mouse DSPP and DMP-1. SIBLING proteins are located next to each other on human chromosome 4 and mouse chromosome 5. Two glycosaminoglycans (GAG) sites have been reported in mouse DSPP (serine242 and serine254) and one GAG site in DMP1 (serine 89) (8, 9). The amino acids are numbered after signal peptide removal. SSD repeats: serine-serine-aspartic acid repeats sequence.

Figure 2.

Immunohistochemical analysis of DSP expression (a-f) in odontoblasts of molars (a-c) and incisors (d-f) in P16 mice. Endogenous DSP expression is seen in molars (a) and incisors (d). Transgenic DSP expression is also seen in molars (c) but much less in incisors (f), and not in *Dspp*^{-/-} molars (b) or incisors (e). Immunohistochemical analysis of DPP expression (g-l) in P16 mice shows only endogenous DPP expression in molars (g) and incisors (j) of *Dspp*^{+/-} mice, but none in molars (h) or incisors (k) of *Dspp*^{-/-} mice or in molars (i) or incisors (l) of DPPcKO mice. D, dentin; P, pulp; arrowhead, odontoblasts layer Bar=50 m

Figure 3.

Immunohistochemical analysis of biglycan expression in molars (a-c) and in incisors (d-f) of *Dspp*^{+/-} (a and d), *Dspp*^{-/-} (b and e), and DPPcKO (c and f) 3-month-old mice show ectopic expression in dentin of *Dspp*^{-/-} molar (b) and *Dspp*^{-/-} and DPPcKO incisors (e and f arrowheads). D, dentin; P, pulp. Bar=100 μm

Figure 4.

Immunohistochemical analysis of 12-month-old incisors from wild-type (WT) (a and d), *Dspp*^{-/-}*Bgn*^{-/0} (b and e), and *Dspp*^{-/-}*Dcn*^{-/-} mice (c and f) with anti-Bgn (a-c) or anti-Dcn (d-f) antibodies.

In wild-type mice, Bgn and Dcn are predominantly distributed in predentin and odontoblast cell bodies (a and d). Neither Bgn nor Dcn could be detected in *Dspp*^{-/-}*Bgn*^{-/0} (b and e) and *Dspp*^{-/-}*Dcn*^{-/-} (c and f) double null mice. E, enamel; D, dentin; pd, predentin; od, odontoblasts. Bar=50 μm

Table 1

Summary of phenotypes and observations of DSPP and DMP-1 knockout mice.

Affected hard tissues in knockout mice		DSPP	DMP-1
Target gene	Affected tissues	Dentin Bone	Dentin Bone Cartilage
		The phenotypes	The potential mechanisms
DSPP	Dentin	Widened predentin, irregular mineralization front and dentin hypomineralization (36)	DSPP affects the initiation and maturation of hard tissue mineralization, DSPP may affect bone remodeling resulting in age-dependent phenotypes in bone.
	Bone	Younger age, bone is dense on the other hand, elder age, bone is reduced dense. (28)	
DMP-1	Dentin	Widened predentin and dentin hypomineralization (57)	Reduced expression of Osterix and Dspp in odontoblasts. (57,58)
	Bone	Decrease of Mineral-to-matrix ratio, increase crystal size and lower Ca × P concentration (60)	DMP-1 directly induces mineralization and involves calcium and phosphate metabolism.
	Cartilage	Shortened and widened vertebrae and long bones (61)	Increased cell proliferation in the proliferating zone and reduced apoptosis in the hypertrophic zone. (61)

Table 2

Summary of phenotypes and observations of DSPP and DMP-1 related transgenic mice and knockout mice rescue studies.

Transgene	Analyzed tissues	The constructs	The phenotypes
DSPP	Dentin	DSPP-DSP Tg/DSPP null background (Only DSP expression in DSPP knockout mouse)	Restoration of the dentin volume but no rescue of the dentin mineral density (40)
	Enamel	Amelogenin-DSP Tg Amelogenin-DPP Tg	Increase of enamel hardness in DSP Tg but decrease in DPP Tg (47, 48)
DMP-1	Dentin	<u>Comparative Study</u> Col1a-DMP1 Tg I DMP1 knockout mouse and DSPP-DMP1 Tg/DMP1 knockout mouse.	Complete rescue by the reintroduction of Col1a-DMP1 Tg I but only partial rescue byt the DSPP-DMP1 Tg (58)
		Col1a-DMP1 C terminal fragment Tg/DMP1 knockout mouse.	Complete rescue of the phenotypes of DMP-1 knockout mouse (62)
	Dentin and Bone	Col1a-mutated (ARHP mimicking) DMP1 Tg/DMP1 knockout mouse. Col1a-mutated (full length DMP-1 cleavage resisted) DMP1 Tg/DMP1 knockout mouse	No recapture of human ARHR phenotypes but showing mild rachitic tooth phenotype. (77) No rescue (70,71,72)