Journal of Oral Biosciences ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Journal of Oral Biosciences

journal homepage: www.elsevier.com/locate/job

Review

Dentin sialophosphoprotein is a potentially latent bioactive protein in dentin

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ARTICLE INFO

Article history:
Received 1 July 2016
Accepted 1 August 2016

Keywords:
DSPP
DMP-1
BSP
Integrin binding site
Equine

ABSTRACT

Background: Dentin sialophosphoprotein (DSPP) belongs to the family of small integrin-binding ligand *N*-linked glycoproteins (SIBLINGs), which share common biochemical features such as an arginine-glycine-aspartic acid (RGD) integrin-binding site. However, amino acid sequence analyses suggest that DSPP has lost some common features, but acquired other unique features, such as repeat sequences of serine-serine-aspartic acid (SDrr) that are not observed in other SIBLINGs proteins.

Highlight: We review the biochemical features of DSPP using genetically modified mice and proteomic analyses. DSPP of some species lack the RGD sites unlike other SIBLING proteins such as dentin matrix protein-1 (DMP-1) and bone sialoprotein (BSP). We previously identified that mouse and human RGD domains in DSPP required the cleavage of an Ala-Ser peptide bond, next to the RGD domains, to become active. Other species such as bovine, sheep, and bears, possess a Thr-Ser bond next to the RGD domain, which is intrinsically unable to sequester the ability of the RGD domain. To predict the functional importance of certain proteins/domains based on evolutionary conservation rates, the RGD domain of DSPP did not appear to have pivotal roles compared to other SIBLINGs. However, upon investigating the peptide bond next to the RGD domains of DSPP in 37 species, we found most catarrhini, in which humans are classified, possess the Ala-Ser bond.

Conclusion: The functions of DSPP for integrin-mediated signaling possibly arize from the proteolytic cleavage of the peptide bonds close to the RGD domain and induce reactionary dentinogenesis *in vivo*.

Contents

Abbreviations: DSPP, dentin sialophosphoprotein; SIBLINGs, small integrin-binding ligand N-linked glycoproteins; RGD, arginine-glycine-aspartic acid; DMP-1, dentin matrix protein-1; BSP, bone sialoprotein; MEPE, matrix extracellular glycophosphoprotein; OPN, osteopontin; ASARM, a proteolytic-resistant acidic serine-aspartate-rich MEPE-associated motif; SDrr, Ser-Asp repeat region; FAM20C, family with sequence similarity 20, member C; FAK, focal adhesion kinase; MAPK, mitogen-activated protein kinase; ERK1/2, extracellular signal-regulated kinase 1/2; SAPK/JNK, stress-activated protein kinase/Jun-amino-terminal kinase; CAF, cancer-associated fibroblasts; BMP-1, bone morphogenetic protein 1; TLD, tolloid; DSP, dentin sialoprotein; PP, phosphophoryn; ADAM, a dizintegrin and metalloprotease; TGF-β1, transforming growth factor-1; HA, hydroxyapatite; DGI, dentinogenesis imperfecta; DEJ, dentin enamel junction; ATF4, activating transcriptional factor-4

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http://dx.doi.org/10.1016/j.job.2016.08.002
1349-0079/

Please cite this article as: Suzuki S, et al. Dentin sialophosphoprotein is a potentially latent bioactive protein in dentin. J Oral Biosci (2016), http://dx.doi.org/10.1016/j.job.2016.08.002

1. Introduction

Human chromosome 4 (mouse chromosome 5) contains a small integrin-binding ligand *N*-linked glycoprotein (SIBLING) family gene cluster (4q21) located immediately proximal to a cluster of enamel matrix protein encoding genes (4q13). The SIBLING family consists of five extracellular matrix proteins including dentin sialophosphoprotein (DSPP), dentin matrix protein-1 (DMP-1), bone sialoprotein (BSP), matrix extracellular glycophosphoprotein (MEPE), and osteopontin (OPN).

The DSPP gene is located at the end of the centromere side of the SIBLINGs gene cluster and was generated in toothed animals by a gene duplication event of the ancient DMP-1 gene [1]. The SIBLING family was defined based on common structural, biochemical, and genetic features [2]. These characteristics 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 [3,4]. In addition to these common domains, only DSPP has a Ser-Asp repeat region (SDrr), which is the most phosphorylated region of SIBLINGs. Comparative analyses of amino acid sequences, deduced by known nucleotide sequences from various species, indicated that DSPP of some species does not have the RGD domain [5]. A family with sequence similarity 20, member C (FAM20C) has been identified as the kinase for DMP-1, BSP, OPN, and MEPE. FAM20C mutations are frequently detected in patients with Raine syndrome, and inactivating mutations of FAM20C result in decreased transcriptional activation of the DMP-1 gene [6]. These results indicate that FAM20 modulates the activities of SIBLING proteins not only through post-translation modifications, but also by regulating promoter activity. Despite the key role of FAM20C for the expression of most SIBLINGs, its effects on DSPP have not been elucidated [7,8]. Therefore, after the gene duplication event of DMP-1 to generate DSPP, this protein might have lost some common features that are present in other SIBLINGs. Here, we review the key functions of DSPP in dentinogenesis, amelogenesis, cellular differentiation, proliferation, migration, and cancer progression, identified recently using genetically modified mouse models and proteomic studies.

2. RGD domains in integrin-mediated signaling

The RGD domain is one of the most well studied integrin ligands, and is found in many extracellular matrix proteins such as vitronectin and fibronectin [9]. The RGD domain has been identified in all SIBLINGs from many species. RGD domains bind to cell surface integrin receptors to increase the internalization of integrin receptors [10]. Internalization of integrin receptors is mediated through clathrin-dependent and clathrin-independent mechanisms and is associated with cellular migration and tumor cell invasiveness [11,12]. The interaction between RGD domains and integrin receptors also activates integrin-mediated intracellular signaling to regulate cellular proliferation, differentiation, and migration. This occurs in various cells through the activation of downstream signaling pathways such as focal adhesion kinase (FAK) and mitogen-activated protein kinase (MAPK), which includes p38, extracellular signal-regulated kinase 1/2 (ERK1/2), and stress-activated protein kinase/jun-amino-terminal kinase (SAPK/JNK) [13,14]. In particular, secretion of SIBLINGs from cancer cells and cancer-associated fibroblasts (CAFs) contribute to cancer metastasis by inducing cellular migration and matrix destruction through both paracrine and autocrine mechanisms [15–17]. In general, the accessibility of integrin to the RGD domains is influenced by the flanking amino acid sequences of the RGD domain [9]. Each RGD domain of DMP-1 (DMP1-RGD), BSP (BSP-RGD), and OPN (OPN-RGD) has been shown to bind to specific integrin partners. DMP1-RGD, BSP-RGD, and OPN-RGD activate integrin αvβ3 and αvβ5, αvβ5, and αvβ3, respectively [18,19]. Marschall and Fisher showed that the replacement of four flanking amino acids of the DMP1-RGD domain with those of the BSP-RGD domain resulted in increased binding to the BSP receptor, integrin αvβ5 [18].

Bone morphogenetic protein 1 (BMP-1) and its alternatively spliced isoform tolloid (TLD) cleave full-length DSPP and DMP-1 proteins into two proteins. The N- and C-terminal cleaved products of DSPP precursor are called dentin sialoprotein (DSP) and phosphophoryn (PP) (alternatively referred to as dentin phosphoprotein or dentin phosphophoryn), respectively [18,20–23]. The RGD domain of DSPP is localized to the N-terminal side of the PP portion. In contrast to those of DMP1, BSP, and OPN, the intact RGD domain of DSPP (DSPP-RGD) has nominal ability to activate integrin-mediated signaling [19]. By utilizing a mammalian expression system, we previously generated recombinant PP (rPP), containing the RGD domain, and analyzed its cell-migratory and adhesive effects in human dental pulp cells, MG-63 human osteosarcoma cells, and MC3T3-E1 murine preosteoblastic cells by comparing with simultaneously purified recombinant DMP-1 (rDMP-1). We demonstrated that only rDMP-1, and not rPP, had these abilities. Subsequently, by performing cell adhesion analyses utilizing several PP peptides and truncated rPP, which ended with Ala next to the RGD domain, the Ala^{482}-Ser^{483} flanking sequence was identified as the key peptide bond that allowed DSPP-RGD to be sequestered.

DSPP-RGD is not well conserved among species, in contrast to other SIBLING members. As shown in Table 1, among 37 species from which DSPP nucleotides and deduced amino acid sequences are available from Ensembl and NCBI sites, only 20 species possess the RGD domain in their DSPP. In contrast, as shown in Table 2, among the same 37 species, 33 species possess the RGD domain in DMP-1; for three species, this information is unavailable, and only one species, the Chinese soft-shell turtle, lacks the RGD sequence in this protein. The armadillo genome possesses one parental DSPP/DMP-1 gene (Ensembl Protein Num. PTHR23400), indicating that the duplication event has not occurred. The RGD domains in BSP and DMP-1 are well conserved. Among the 37 species, 35 species possess the RGD domain in BSP, for one species, this information was lacking, and for the other species, the shrew, this domain is absent from this protein. Thus, the relatively low evolutionary conservation of DSPP-RGD might indicate its RGD-dependent role was abrogated when DSPP appeared during evolution.

However, if we compare the amino acid sequences next to the DSPP-RGD domain, among species with DSPP-RGD, the species can be classified into four groups. The first, second, third, and fourth groups have Ala, Thr, Asp, and other amino acids, respectively, next to the RGD domain. The first group (described as Group 1) includes humans, mice, bush babies, chimpanzees, gibbons, gorillas, horses, macaques, microbats, and sperm whales. Among haplorhini species, catarrhini, but not platyrrhini, examined here are included in the Group 1. The second group (Group 2) includes pigs, sheep, and polar bears. The third group (Group 3) contains the African savanna elephant. Previous studies showed that the exogenous addition of pig DSPP-RGD peptide, which has Thr next to the RGD domain, induced cellular migration in human dental pulp cells [24], unlike the mouse and human DSPP-RGD, which is followed by Ala, and was incapable of activating integrin-mediated signaling [19]. Therefore, we previously determined whether point mutations in Ala and/or Ser altered the activity of the DSPP-RGD domain. Using recombinant PP proteins without SDrr (rPP-ΔSDrr), we examined six types of mutations and found that all converted rPP-ΔSDrr into a cell-adhesive protein. We used rPP-ΔSDrr instead of rPP simply based on the experimental purpose [25]. We compared the cell-adhesive

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