Matrix Biology 28 (2009) 221–229

Contents lists available at ScienceDirect

Matrix Biology

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

Dentin sialoprotein and dentin phosphoprotein have distinct roles in dentin mineralization

Shigeki Suzuki [a], Taduru Sreenath [a,1], Naoto Haruyama [a,2], Cherlita Honeycutt [a], Anita Terse [a], Andrew Cho [b], Thomas Kohler [c], Ralph Müller [c], Michel Goldberg [d], Ashok B. Kulkarni [a,*]

[a] Functional Genomics Section, Laboratory of Cell and Developmental Biology, National Institute of Dental and Craniofacial Research, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, USA
[b] Gene Targeting Facility, National Institute of Dental and Craniofacial Research, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, USA
[c] Institute for Biomechanics, ETH Zürich, Zürich, Switzerland
[d] Laboratoire Réparation et Remodelage des Tissues Orofaciaux EA 2496, Faculté de Chirurgie Dentaire, Université Paris Descartes, Montrouge, France

ARTICLE INFO

Article history:
Received 13 January 2009
Received in revised form 17 March 2009
Accepted 25 March 2009

Keywords:
Dentin sialophosphoprotein
Biomineralization
Dentin volume
Dentin mineral density
Chondroitin sulfate proteoglycan

ABSTRACT

Dentin sialophosphoprotein (DSPP), a major non-collagenous matrix protein of odontoblasts, is proteolytically cleaved into dentin sialoprotein (DSP) and dentin phosphoprotein (DPP). Our previous studies revealed that DSPP null mice display a phenotype similar to human autosomal dominant dentinogenesis imperfecta, in which teeth have widened predentin and irregular dentin mineralization resulting in sporadic unmineralized areas in dentin and frequent pulp exposure. Earlier in vitro studies suggested that DPP, but not DSP, plays a significant role in initiation and maturation of dentin mineralization. However, the precise in vivo roles of DSP and DPP are far from clear. Here we report the generation of DPPcKO mice, in which only DSP is expressed in a DSPP null background, resulting in a conditional DPP knockout. DPPcKO teeth show a partial rescue of the DSPP null phenotype with the restored predentin width, an absence of irregular unmineralized areas in dentin, and less frequent pulp exposure. Micro-computed tomography (micro-CT) analysis of DPPcKO molars further confirmed this partial rescue with a significant recovery in the dentin volume, but not in the dentin mineral density. These results indicate distinct roles of DSP and DPP in dentin mineralization, with DSP regulating initiation of dentin mineralization, and DPP being involved in the maturation of mineralized dentin.

Published by Elsevier B.V.

1. Introduction

Mineralization of dentin is a complex process regulated by the collagenous matrix which is largely comprised of type I collagen, many non-collagenous proteins (NCPs), and minerals. Dentin sialophosphoprotein (DSPP) is one of the key NCPs involved in tooth development and mineralization (Butler and Ritchie, 1995). It is highly expressed in odontoblasts and transiently expressed in ameloblasts (D'Souza et al., 1997; Bègue-Kirn et al., 1998; MacDougall et al., 1998). However, a low level of DSPP expression has also been reported in bones, kidneys, lungs, salivary glands, and sweat glands (Xiao et al., 2001; Qin et al., 2002, 2003a; Ogbureke and Fisher, 2004, 2005, 2007; Alvares et al., 2006; Verdelis et al., 2008). Many heterogeneous mutations in the human DSPP gene have been linked to the two most common hereditary diseases affecting dentin, dentinogenesis imperfecta (DGI), and dentin dysplasia (DD) (Hart and Hart, 2007; Kim and Simmer, 2007; McKnight et al., 2008). Patients with DGI and DD typically have amber-brown, opalescent teeth, and their tooth enamel is often broken off, exposing the underlying dentin, which can lead to pulp exposure and accelerated tooth attrition. We previously generated the *Dspp*$^{-/-}$ mice to delineate the precise functions of DSPP in dentinogenesis (Sreenath et al., 2003a). These *Dspp*$^{-/-}$ mice show tooth defects similar to those seen in patients suffering from DGI and DD, with widened predentin, irregular mineralization front, and hypomineralization resulting in frequent pulp exposure. The *Dspp*$^{-/-}$ phenotype confirmed the important roles of DSPP in dentin mineralization. Interestingly, the *Dspp*$^{+/-}$ mice did not show any obvious abnormalities, suggesting that haploinsufficiency does not cause any obvious phenotype in these mice.

So far, a full-length DSPP protein has never been isolated or identified in dentin. DSPP is cleaved into dentin sialoprotein (DSP) and dentin phosphoprotein (DPP), also known as phosphophoryn (MacDougall et al., 1997). Interestingly, a third polypeptide, dentin glycoprotein (DGP), is cleaved out from the C-terminal end of porcine

* Corresponding author. Functional Genomics Section, LCDB, NIDCR, NIH, 30 Convent Dr., MSC 4395, Bethesda, MD, 20892, USA. Tel.: +1 301 435 2887; fax: +1 301 435 2888.
E-mail address: ak40m@nih.gov (A.B. Kulkarni).
[1] Present address: 1401 Rockville Pike Suite 200N, HFM-41 Rockville, MD 20852, USA.
[2] Division of Oral Dysfunction Science, Tohoku University, Graduate School of Dentistry4-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan.

0945-053X/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.matbio.2009.03.006

DSP by matrix metalloproteinases such as MMP-2 and MMP-20 (Yamakoshi et al., 2005a). DSP and DPP are the most abundant NCPs in dentin ECM (Yamakoshi et al., 2008), and some of the earlier studies suggest that DPP is more abundant than DSP in the mineralized dentin (Butler et al., 1981; Butler, 1998). DSP, the amino-terminal part of DSPP, is a sialic acid-rich glycosylated protein. It is a member of the SIBLING (Small Integrin-Binding Ligand N-linked Glycoproteins) family, which also includes bone sialoprotein (BSP), dentin matrix protein-1 (DMP-1), osteopontin (OPN), and matrix extracellular phosphoglycoprotein (MEPE). It has been reported that both porcine

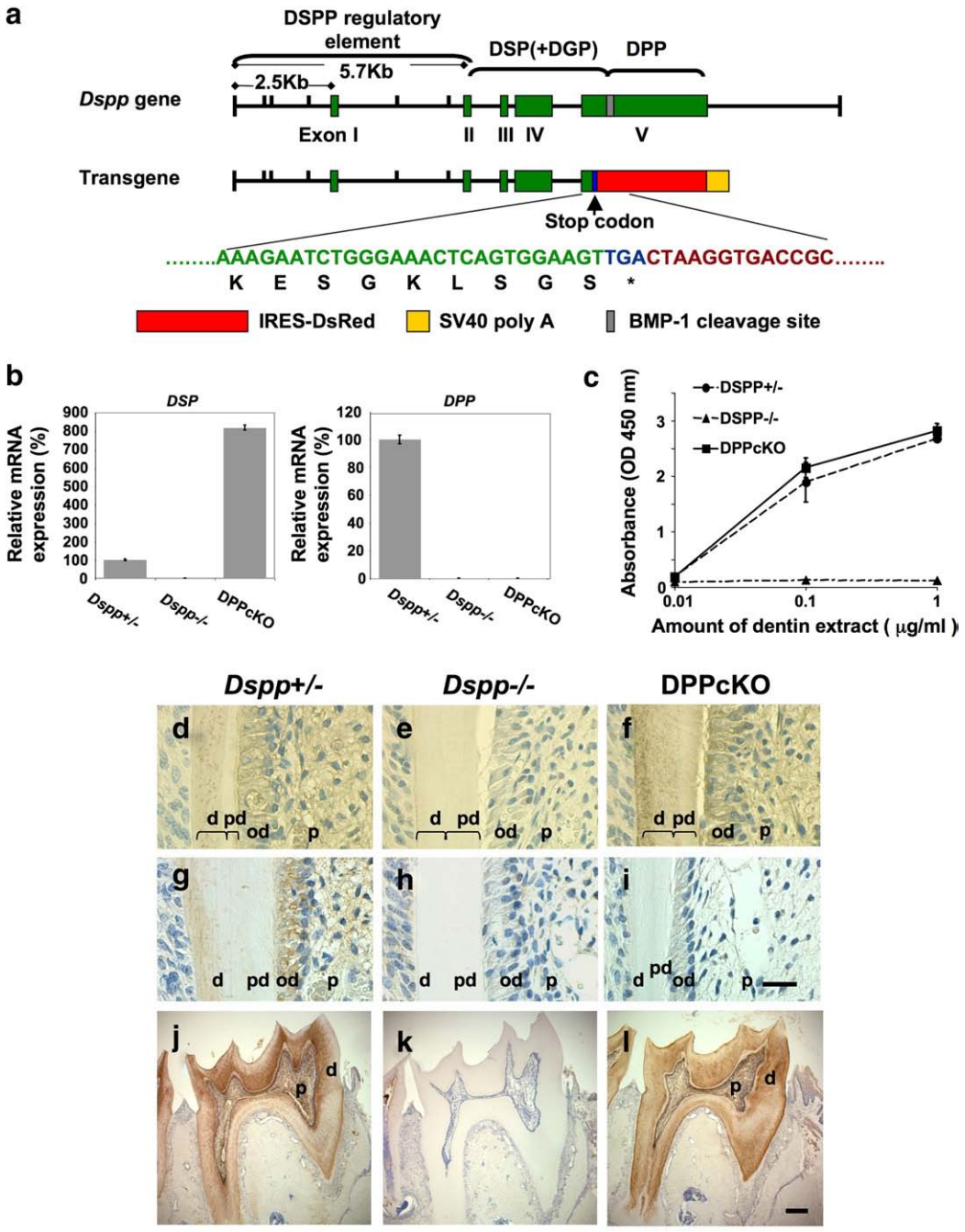

Fig. 1. Generation of DPPcKO mice. a, A diagrammatic representation of the transgenic DSP construct showing the genomic DSP sequence (5.7-kb regulatory element and DSP domain) followed by a stop codon, IRES-DsRed, and SV 40 ploy(A). Exons are colored green, stop codon is blue, BMP-1 cleavage site is gray, IRES-DsRed is red, and SV40 poly(A) is yellow. b, Relative mRNA expression of *Dsp* and *Dpp* analyzed by real-time PCR using specific primer pairs. mRNA expression of *Dsp* in DPPCKO-B molars is eight folds higher than in $Dspp^{+/-}$ molars. *Dsp* is not detectable in $Dspp^{-/-}$ molars. *Dpp* is only detectable in $Dspp^{+/-}$ molars. *Dsp* and *Dpp* expression level was normalized using *Gapdh* expression level. c, DSP protein levels in dentin of $Dspp^{+/-}$, $Dspp^{-/-}$, and DPPcKO-B molars. EDTA fraction of dentin proteins, isolated from molars of P20 mice ($n>40$), were coated on the individual wells of 96-well plates at different concentrations. DSP was determined using anti-DSP antibody. $Dspp^{+/-}$ and DPPcKO-B dentins contain almost same level of DSP where as $Dspp^{-/-}$ dentin does not contain any. Each column represents the mean ± SD. d–l, Expression pattern of DSP and DPP in $Dspp^{+/-}$, $Dspp^{-/-}$, and DPPcKO-B molars. DSP expression (d, e, f) in P16 mice shows endogenous DSP (d) and transgenic DSP (f) expression in odontoblasts, dentin, and pulp, but not in $Dspp^{-/-}$ (e) molars. DPP expression (g, h, i) in P16 mice shows only endogenous DPP expression (g) in $Dspp^{+/-}$ molars but none in $Dspp^{-/-}$ (h) or DPPcKO-B (i) molars. Expression of DSP (j–l) in lower first molars of 3-month-old mice shows endogenous DSP (j) and transgenic DSP (l) equally deposited in the primary dentin. No positive staining is observed in $Dspp^{-/-}$ molars (k). d, dentin; pd, predentin; od, odontoblast; p, pulp. Bars in d–i = 25 μm. j–l = 200 μm.