e-ISSN 3050-9432
Journal of Dental Health and Oral Research

ATHENAEUM SCIENTIFIC PUBLISHERS

Research Article

Does Enhanced Dentin Sialophosphoprotein (DSPP) Expression Affect Runx2 Expression in Tooth Development?

Wu KC[1], Gong T[2], Lee AM[1], Ma PX[2], Ritchie HH[1*]

[1]Department of Cariology, Restorative Sciences and Endodontics, School of Dentistry, University of Michigan, Ann Arbor, MI 48109, USA

[2]Department of Biomaterial Sciences, School of Dentistry, University of Michigan, Ann Arbor, MI 48109, USA

***Correspondence author:** Helena H Ritchie, Department of Cariology, Restorative Sciences and Endodontics, School of Dentistry, University of Michigan, Ann Arbor, MI 48109-1078, USA; E-mail: helenar@umich.edu

Citation: Wu KC, et al. Does Enhanced Dentin Sialophosphoprotein (DSPP) Expression Affect Runx2 Expression in Tooth Development? J Dental Health Oral Res. 2025;6(2):1-5.

https://doi.org/10.46889/JDHOR.2025.6206

Received Date: 07-05-2025

Accepted Date: 26-05-2025

Published Date: 03-06-2025

Abstract

Epithelial-mesenchymal interactions lead to Runx2 expression in mesenchyme in bud, cap and early bell stages. At late bell stage, DSPP expression appears in odontoblasts and dental pulp but no more Runx2 expression. Runx2 is required for proper tooth development from bud, cap to early and bell stages. Runx2 Knockout (KO) mice showed defective enamel organ lacked overt odontoblasts and ameloblast differentiation. It is interesting that while Runx2 expresses in bud, cap and early bell stages, no DSPP expression is detected in these stages. When DSPP expresses actively in mesenchyme at late bell stage, no Runx2 expression is detected in mesenchyme. This reciprocal expression of Runx2 and DSPP (i.e., Runx2 down and DSPP up in late Bell stage) posted the possibility that DSPP expression in late bell stage of Wild Type (WT) mice might down-regulate Runx2 expression. DSPP protein is cleaved into Dentin Sialoprotin (DSP) and Phosphophoryn (PP). Recently, we reported that DSP/PP can reduce cell proliferation and promote cell differentiation and DSP/PP can function as signal molecules during tooth development.

Objective: To better understand the mechanism for the exclusive expression patterns of Runx2 and DSPP expression during tooth development, we investigated (1) whether recombinant DSP/PP (recDSP/PP) can affect Runx2 expression in human dental pulp stem cells (hDPSCs) and (2) whether the absence of DSPP in DSPP KO changed Runx2 expression in molars and incisors.

Methods: recDSP/PP was used to test its effect on Runx2 expression in hDPSCs. Using immunohistochemical analyses and RealTime PCR analyses, we examined Runx2 expression in WT and DSPP KO mouse teeth.

Results: We found that the presence of recDSP/PP does not affect Runx2 expression *in-vitro*. Absence of DSPP expression in newborn DSPP-KO molar up regulates Runx2 expression *in-vivo.*

Conclusion: Appropriate temporal and spatial expression of Runx2 and DSPP are required for normal tooth development.

Keywords: Bud; Cap; Early Bell; Late Bell; Runx2 Expression; DSPP Expression

Introduction

Runx2 (Cbfa1) expresses in dental mesenchyme from the bud to early bell stages during epithelial-mesenchymal morphogenesis. In Wild Type (WT) mice, Runx2 expression is high in Cap stage, moderate in early Bell stage and not detectable in late Bell stage. Dental epithelium was shown to be required for Runx2 expression in mesenchyme via epithelium-mesenchyme recombination experiments. Runx2 Knockout (KO) showed tooth development stopped at cap stages. Runx2 KO mice showed misshapen and severely hypoplastic tooth organs, which lacked overt odontoblasts and ameloblast differentiation [1,2].

During dentinogenesis, DSPP is expressed in differentiated and mature odontoblasts and then secreted into the dentin matrix to direct dentin mineralization [3]. DSPP is translated from a DSPP mRNA to generate DSPP precursor protein, which is further cleaved into DSP and PP [4-6]. DSP/PP are well accepted as the major dentin matrix which are crucial for dentinogenesis. A

mutation in DSPP has been linked to DGI II [7,8]. Teeth from DSPP KO mice form thinner dentin, with low mineral density and enlarged pulp chambers [9]. Our lab reported poorly organized cuboidal odontoblasts and dental pulp cells in DSPP KO mice compared to those in WT mice. Furthermore, we identified chondrocyte-like cells in the dental pulp from KO mouse teeth [10]. These results suggest that DSPP affects dental pulp cell differentiation and the absence of DSPP might alter the dental pulp cell fate. The absence of DSPP likely affected odontoblast lineage differentiation. We found enamel and dentin junction was abnormal in postnatal 1-and 6-day first molar (M1) as well as the presence of a circular odontoblast layer. Safranin O is widely used to stain acidic proteoglycan in cartilage. Safranin O staining demonstrated the presence of acidic proteoglycan in 21-day DSPP KO dental pulp in contrast, no Safranin O staining is observed in the dental pulp of 21-day WT M2 teeth [10].

Recently, we reported DSP/PP can reduce cell proliferation and promote cell differentiation and DSP/PP can function as signal molecules during tooth development [11]. DSPP expression appeared in late Bell stage and continued to express strongly during subsequent developmental stages [1,2]. Runx2 expression is in bud stage, high in Cap stage, moderate in early Bell stage and not detectable in late Bell stage.

This reciprocal expression of Runx2 and DSPP (i.e., Runx2 down and DSPP up in late Bell stage) posted the possibility that DSPP expression in Wild Type (WT) mice might affect Runx2 expression. The Aim of the article is to test whether DSP/PP expression affected Runx2 expression and whether absence of DSPP in knockout (KO) mice changed Runx2 expression in teeth.

Methodology

Preparation of recombinant DSP/PP proteins (recDSP/PP)

pVL1392-DSP-PP_{240} cDNA construct was co-transfected with a linearized BaculoGold baculovirus DNA into insect Sf9 cells to obtain virus stock. To produce recombinant proteins, insect Sf9 cells infected with recombinant virus stock at a multiplicity of 10, were grown in Grace's insect cell medium (InVitrogen) supplemented with 10% fetal calf serum (FBS) to a density of $2x10^6$ cells/T25 flask. Supernatants were harvested on 6 days after phage infection [11].

Treatment of Human Dental Pulp Cells with recombinant DSP/PP proteins (recDSP/PP)

Human DPSCs (hDPSCs) were seeded in 6 well-plates with each well $30x10^4$ cells in growth medium (α-MEM, supplemented with L-glutamine and 1% penicillin-streptomycin; GIBCO) with 10% FBS. When the cells reached at 80% confluent the next day, cells were replaced with growth medium with 0.25% FBS for overnight, then added growth medium with 150µl/ml recDSP/PP, harvested the cells at 15 min and 30 min as well as 2 days [11].

Immunochemical Western Blot to Detect Whether the Addition of recDSP/PP Affects Runx2 Expression Dental Pulp Cells In-Vitro [12]

Proteins were extracted with the EpiQuik Whole Cell Extraction Kit (EpigenTek, Farmingdale, NY, USA). A total of 20µg protein was run through 12% NuPAGETM 4-12% Bis-Tris gel electrophoresis and transferred to a PVDF membrane. The blots were washed with 1x TBS and with 0.05% Tween-20, blocked in 5% BSA (Santa Cruz) and incubated overnight at 4^o with anti-Runx2 antibodies. Next, the blots were washed in TBST and incubated for 1 hour with goat anti-rabbit horseradish peroxidase-conjugated secondary antibody followed by treatment with chemiluminescence reagent. β-Actin is used as a control.

Immunohistochemical Analyses of Runx2 Expression In-Vivo [10].

Molars from 1-day Wild Type (WT) and DSPP KO mice were collected, fixed, embedded in parafilm. 5µm parafilm sections were performed immunohistochemical analyses with anti- Runx2 antibodies to determine Runx2 expression in 1-day WT and DSPP KO molars.

RealTime-PCR Analyses of Runx2 Expression in DSPP KO and WT Mouse Incisors

Incisors from one-month WT and DSPP KO mice were collected. Dental pulp cells were extracted with RNA extraction kit (Quiagen RNeasy Plus Universal Mini Kit). Bio-Rad Realtime PCR equipment and detection system was used [13].

Results

The Presence of recDSP/PP Does Not Affect Runx2 Expression

To investigate whether the up expression of DSPP affects Runx2 expression, the recDSP/PP was added to hDSPCs culture and followed Runx2 expression in hDSPCs. hDPSCs were exposed to recDSP/PP (150 µl/ml) for 15 min and 30 min and Runx2

expression was examined. We found that exposure of recDSP/PP to hDPSCs for 15 min and 30 min had no effect on Runx2 expression (Fig. 1). Also, no effect was detected after exposure of recDSP/PP to hDPSCs for 2 days (data not shown). Thus, recDSP/PP exposure did not affect Runx2 expression in hDPSCs. DSP/PP did not directly influence Runx2 expression. Likely DSP/PP indirectly affect Runx2 expression via other pathways.

Absence of DSPP Expression in DSPP-KO Mice Up Regulates Runx2 Expression In-Vivo

To test whether the absence of DSPP affects Runx2 expression, DSPP-KO mice was used to examine whether Runx2 expression *in-vivo*.

We examined Runx2 protein expression in 1-day WT and DSPP KO molars using immunohistochemical analysis with anti-Runx2 antibodies. We found that Runx2 was expressed in alveolar bones in WT mice, but not in odontoblasts and dental pulp of WT mice (Fig. 2). However, we found Runx2 expression in odontoblasts, dental pulp and alveolar bones in molars of DSPP KO mice (Fig. 2). Thus, the absence of DSP/PP in DSPP KO mice promoted Runx2 expression in molars.

Runx2 Expression is Upregulated in DSPP-KO Incisors Compared to that in WT Incisors in 1-Month Old Mice

In DSPP KO 1-month old incisors, while DSPP expression was absent in incisors, Runx2 expression was upregulated (Fig. 3). Real-time PCR data showed different gene expression profiles between WT and DSPP-KO incisors from 1-month-old mice (Fig. 3). We noticed that in DSPP KO mice, major odontoblast markers (Dmp1, Oc, BSP, Osx) were downregulated and there was no DSPP expression. This data supports that odontoblasts and dental pulp cells in DSPP-KO incisors lost their odontoblast cell fate.

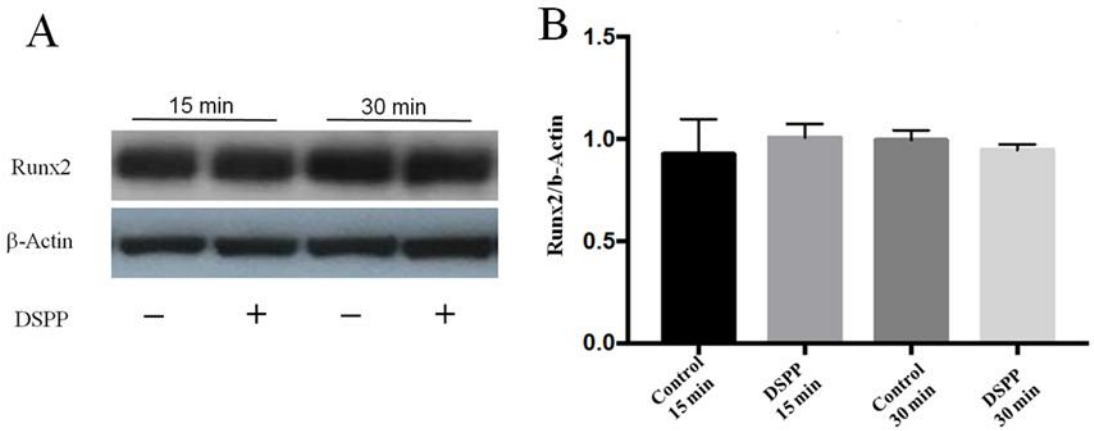

Figure 1: Exposure of recDSP/PP to hDPSCs for 15 min and 30 min had no effect on Runx2 expression. When the hDSPCs reached at 80% confluent the next day, cells were replaced with growth medium with 0.25% FBS for overnight, then added growth medium with 150μl/ml recDSP/PP, harvested the cells at 15min and 30minrec to examine whether recDSP/PP (150μl/ml) affected Runx2 expression.

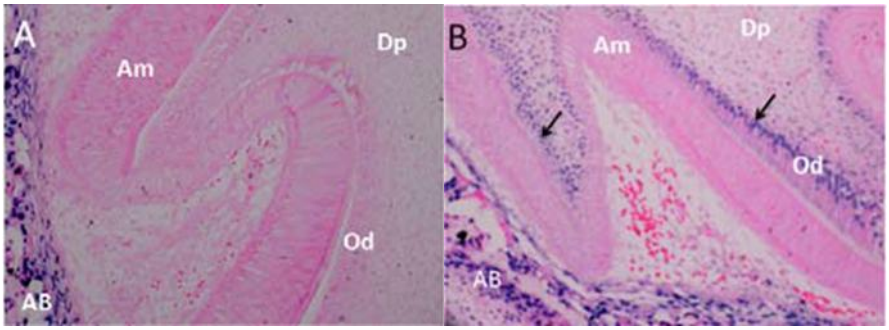

Figure 2: A: 1d Wild Type (WT) molar tooth section stained with anti-Runx2 antibodies and counterstained with Eosin, 1d WT molar 1 (M1) Runx2 protein expression in alveolar bone but no Runx2 expression in M1 odontoblasts; B: 1d DSPP KO molar tooth section stained with anti-Runx2 antibodies and counterstained with Eosin. Runx2 protein was robustly expressed in odontoblasts (as indicated by arrow) and dental pulp. Runx2 protein expression in alveolar bones in 1d molar 1 from DSPP KO

mice. AB: Alveolar Bone. Am: Ameloblast. Dp: Dental pulp; Od: Odontoblast.

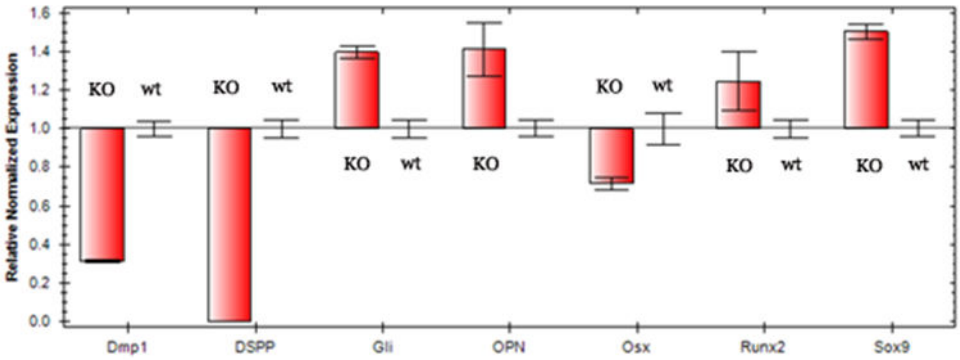

Figure 3: Real-time PCR data of 1-month-old WT and DSPP KO incisors. Using β-Actin mRNA as an internal reference gene, relative mRNA expression in DSPP KO incisors to those of WT controls is shown above. Odontoblast lineage marker genes Dmp1, Osx, BSP (not shown) and Osteocalcin (Oc) (not shown) and DSPP mRNAs were downregulated (represented by downward red bar, essentially absence) relative to the WT controls. The WT control gene expression for each gene was set at 1.0 with standard error bars.

Discussion

The Exposure of recDSP/PP to hDPSCs Did Not Affect Runx2 Expression. Likely DSP/PP Does Not Directly Affect Runx2 Expression

No effects were detected after exposure of recDSP/PP to hDPSCs for 15 min, 30 min and 2 days. recDSP/PP exposure might not affect Runx2 expression in hDPSCs *in-vitro*. DSP/PP might not directly influence Runx2 expression. Possibly DSP/PP indirectly affect Runx2 expression via other pathways. Future works are needed for identifyin the other factors that affect Runx2 expression.

The Lack of DSP/PP Expression in DSPP KO Mice Showed the Up Regulation of Runx2 Expression

In the molar one of wild type mouse at day 1, while DSPP is actively synthesis in bell stage, Runx2 expression is not present in odontoblasts and dental pulp cells. In other words, WT molar 1 shows high DSPP expression with no Runx2 expression in bell stage [1,14]. We would like to know whether the absence of DSPP can affect Runx2 expression in late bell stage. The immunohistochemical analyses showed that the absence of DSP/PP in DSPP KO mice promoted Runx2 expression *in-vivo* which fits the exclusiveness of DSPP and Runx2 expression pattern (Fig. 2). One month old incisors from DSPP KO mice showed upregulation of Runx2 compared to that from WT mice (Fig. 3).

The Presence of Runx2 in Late Stage of Tooth Development Inhibited DSPP Expression In-vivo

In-vivo DSPP promoter directed Runx2 expression in late stage of tooth development, it led to Runx2 expression in the late stage of tooth development.

Li, et al., generated transgenic mice that expressed Runx2 specifically in odontoblasts to test the function of Runx2 in the late stage of tooth development [15]. In one week and one month, the odontoblasts in transgenic mice lost their tall columnar shape and polarization as well as no tubules with similarities to DSPP KO mice including enlarged pulp chamber and thinner dentin. Briefly, the presence of Runx2 in late stages of tooth development inhibited DSPP expression. *In-vitro* studies, forced overexpression of Runx2 induced increases of endogenous DSPP protein levels in preodontoblast cells but reduced its expression in mature odontoblast cells [16]. Runx2 should be inhibited in odontoblasts to encourage normal cell maturation, differentiation and dentinogenesis [15].

Conclusion

The addition of recDSP/PP to hDPSCs did not change Runx2 expression *in-vitro*. One day old molar (at the late bell stage) showed no Runx2 expression in wild type mice and strong Runx2 expression in DSPP KO mice. Even in one month old DSPP KO incisor, upregulation of Runx2 mRNA was observed. Taken together, these findings again demonstrated the exclusiveness of proper Runx2 and DSPP expression patterns is important during tooth development.

Conflict of Interest

The authors declare that they have no conflicts of interest with the contents of the article.

Funding
None.

Reference

1. Åberg T, Wozney J, Thesleff I. Runx2 mediates FGF signalling from epithelium to mesenchyme during tooth morphogenesis. Dev Biol. 2004;270:76-93.
2. James M, Kadesch T, Ito Y, Atchley WR, de Crombrugghe B. Different roles of Runx2 during early neural crest-derived bone and tooth development. J Bone Miner Res. 2006;21:1034-44.
3. Ritchie H. The functional significance of dentin sialoprotein-phosphophoryn and dentin sialoprotein. Int J Oral Sci. 2018;10:31.
4. Ritchie HH, Wen LY, Wang LH, Li W, Butler WT. DSP-PP precursor protein cleavage by Tolloid-related-1 protein and by bone morphogenetic protein-1. PLoS One. 2012;7(7):e41110.
5. Sun Y, Lu Y, Chen JJ, Du W, Zhang J, Qian X, et al. Key proteolytic cleavage site and full-length form of DSPP. J Dent Res. 2010;89:498-503.
6. von Marschall Z, Fisher LW. Dentin sialophosphoprotein (DSPP) is cleaved into its two natural dentin matrix products by three isoforms of Bone Morphogenetic Protein-1 (BMP1). Matrix Biol. 2010;29:295-303.
7. Xiao S, Yu C, Chou X, Yuan W, Wang Y, Bu L, et al. Dentinogenesis imperfecta 1 with or without progressive hearing loss associated with distinct mutations in DSPP. Nat Genet. 2001;27:201-4.
8. Zhang X, Zhao J, Li C, Gao S, Qiang B, Guo W, et al. DSPP mutation in dentinogenesis imperfecta shields type II. Nat Genet. 2001;27:151-2.
9. Sreenath TL, Suzuki S, Lee Y, Nakamura Y, Yamada Y, Kulkarni AB. Dentin sialophosphoprotein knockout mouse teeth display widened predentin zone and develop defective dentin mineralization similar to human dentinogenesis imperfecta type III. J Biol Chem. 2003;278:24874-80.
10. Guo S, Lim D, Dong Z, Saunders TL, Ma PX, Li W, et al. Dentin sialophosphoprotein: a regulatory protein for dental pulp stem cell identity and fate. Stem Cells Dev. 2014;23(23):2883-94.
11. Chuang S, Hsu H, Lin Y, Lin F, Lin C. Phosphophoryn and dentin sialoprotein effects on dental pulp cell migration, proliferation and differentiation. Dent J. 2018;6:70.
12. Lee SY, Lim J, Wang C, Li W, Lin Y, Lu Y, et al. Effects of recombinant dentin sialoprotein in dental pulp cells. J Dent Res. 2012;91(4):407-12.
13. Wagner E. Monitoring gene expression: Quantitative real-time RT-PCR. Methods Mol Biol. 2013;1027:19-45.
14. D'Souza RN, Aberg T, Gaikwad J, Cavender A, Owen M, Karsenty G, et al. Cbfa1 is required for epithelial-mesenchymal interactions regulating tooth development in mice. Development. 1999;126:2911-20.
15. Li S, Kong H, Yao N, Yu Q, Han D, Liu Y, et al. The role of runt-related transcription factor 2 (Runx2) in the late stage of odontoblast differentiation and dentin formation. Biochem Biophys Res Commun. 2011;410:698-704.
16. Chen S, Gluhak-Heinrich J, Wang Y, Wu Y, Chuang H, Chen L, et al. Differential regulation of dentin sialophosphoprotein expression by Runx2 during odontoblast cytodifferentiation. J Biol Chem. 2005;280:29717-27.