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The Spatiotemporal Expression Pattern of Syndecans in Murine Embryonic Teeth

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Abstract

The hierarchical interactions between the dental epithelium and dental mesenchyme represent a common paradigm for organogenesis. During tooth development, various morphogens interact with extracellular components in the extracellular matrix and on the cell surfaces to transmit regulatory signaling into cells. We recently found pivotal roles of FAM20B-catalyzed proteoglycans in the control of murine tooth number at embryonic stages. However, the expression pattern of proteoglycans in embryonic teeth has not been well understood. We extracted total RNA from E14.5 murine tooth germs for semi-quantitative RT-PCR analysis of 29 proteoglycans, and identified 23 of them in the embryonic teeth. As a major subfamily of FAM20B-catalyzed proteoglycans, Syndecans are important candidates being potentially involved in the tooth development of mice. We examined the expression pattern of Syndecans in embryonic teeth using *in situ* hybridization (ISH) and immunohistochemistry (IHC) approaches. *Syndecan-1* is mainly present in the dental mesenchyme at early embryonic stages. Subsequently, its expression expands to both dental epithelium and dental mesenchyme. Syndecan-2 is strongly expressed in the dental mesenchyme at early embryonic stages, then shifts to the stratum intermedium and inner dental epithelium at cap stages. Syndecan-3 shows a gradually increased expression that initially in the dental epithelium of both incisors and molars and then in the inner dental epithelium and stratum intermedium in molars alone. *Syndecan-4* is localized in the dental epithelium in incisors and the dental follicle mesenchyme in molars at early cap stage. The spatiotemporal expression pattern of Syndecans in murine embryonic teeth suggest potential roles of these proteoglycans in murine tooth morphogenesis.

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CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

Keywords

proteoglycans; syndecan; tooth development; gene expression pattern; *in situ* hybridization; immunohistochemistry; RT-PCR

1. INTRODUCTION

Tooth development has long been used as a model system for studying the molecular mechanisms regulating organogenesis. The hierarchical and reciprocal interactions between the dental epithelium and underlying mesenchyme represent a common paradigm for the development of multiple organs, such as hair follicles, mammary glands, lungs, and kidneys. Various signaling mediated by growth factors and transcriptional factors are iteratively used during organogenesis (Thesleff, 2003; Basson, 2012). The secreted morphogens must interact with extracellular components such as proteoglycans to transmit the extracellular signaling into cells.

Accumulating evidence shows that proteoglycans are pivotal signaling regulators for organogenesis. The glycosaminoglycan (GAG) chains covalently attached to the core protein of proteoglycans provide docking sites for various growth factors (Linhardt & Toida, 2004; Sarrazin et al., 2011; Mikami & Kitagawa, 2013). We recent found that disrupting GAGs in the dental epithelium by inactivating Fam20B (family with sequence similarity 20 member B), a newly identified xylose kinase essential for GAG assembly, led to supernumerary tooth formation in mice (Tian et al., 2016), indicating that proteoglycans play an important role in maintaining the signaling balance that governs tooth number in mice. Although sporadic reports have documented the expression of several proteoglycans in murine teeth (Thesleff et al., 1988; Vainio et al., 1989; Bernfield & Sanderson, 1990; Salmivirta et al., 1991; Vainio & Thesleff, 1992; Bai et al., 1994; Hikake et al., 2003; Yonemochi & Saku, 2006; Jiang et al., 2010; Ida-Yonemochi et al., 2011; Hayano et al., 2012; Wiweger et al., 2012), a systematic investigation is essential to determine the spatiotemporal expression pattern of FAM20B-catalyzed proteoglycans that are potentially associated with the tooth phenotype in *Fam20B*-mutant mice.

Syndecans compose a major family of FAM20B-catalyzed proteoglycans, which consists of four members: Syndecans 1-4. They all share a common feature of type I integral transmembrane protein with both heparan sulfates (HS) and chondroitin sulfates (CS) attached to the extracellular N-terminus of core protein (Saunders et al., 1989). Syndecan-1 and Syndecan-3 form a subfamily, while Syndecan-2 and Syndecan-4 constitute another (Makkonen et al., 2013). These proteoglycans interact with various extracellular signaling ligands via the GAG chains attached to the core protein. Their capacity of binding to a wide range of ligands is higher than growth factor receptors (Bernfield et al., 1992; Elfenbein and Simons, 2013; Szatmári et al., 2015). The signature transmembrane domain and the short C-terminal cytoplasmic domain of their core proteins contain highly conserved sequences, which is indicative of preserved functions (Bernfield et al., 1992). Accumulating evidence indicates that the conserved cytoplasmic domains of Syndecans can interact with signaling molecules and cytoskeletal components. This unique structure facilitates dimer and oligomer

formation, indicating that Syndecans are more than just co-receptors and have a potential to fulfill diverse functions (Dews & Mackenzie, 2007).

In this study, we performed a systematic investigation on the expression pattern of Syndecans 1-4 in the murine teeth at early embryonic stages, to evaluate the spatiotemporal distribution of Syndecan family members in the tooth germs of incisors and molars, by using immunohistochemistry and *in situ* hybridization approaches. The dynamic expression pattern of Syndecans suggests their potential involvement in the early-stage tooth development.

2. RESULTS

2.1. Twenty three proteoglycans are present in E14.5 murine tooth germs

With semi-quantitative RT-PCR method, we detected the transcripts of *Fam20B* and 23 proteoglycans in E14.5 murine teeth (Figure 1). Most of these proteoglycans are evenly expressed in molar, incisor and diastema regions, except for Serglycin, which is highly expressed in diastema region but barely seen in tooth germs.

2.2. The expression pattern of *Syndecan-1* in murine embryonic teeth

In embryonic lower incisors, *Syndecan-1* was localized at the distal side of dental mesenchyme at the early bud stage (E12.5) (Figure 2A). In molars, *Syndecan-1* showed a broader expression at both the buccal and lingual sides of the dental mesenchyme as well as in the dental epithelium (Figure 2B).

At the late bud stage (E13.5), *Syndecan-1* showed a strong and localized expression at the distal side of dental mesenchyme in the lower incisors (Figure 2C). The molars showed a broad expression in both the dental epithelium and mesenchyme, and the buccal side of dental mesenchyme had a stronger expression (Figure 2D).

At the cap stage (E14.5), both incisors and molars showed a broad expression in the enamel organ, dental papilla and dental follicle.

At the early bell stage, the expression of *Syndecan-1* became localized in the dental papilla and part of the enamel organ (Figures 2E–H).

2.3. The expression pattern of Syndecan-2 in murine embryonic teeth

At the early bud stage (E12.5), Syndecan-2 was broadly expressed in the dental epithelium and mesenchyme in both the incisors and molars. The lower incisors showed a higher expression at the distal side of the dental mesenchyme in comparison with the dental epithelium and the proximal side of the dental mesenchyme (Figures 3A and B).

At the late bud stage (E13.5), Syndecan-2 showed a strong and localized expression in the dental mesenchyme immediately surrounding the epithelial tooth bud in both incisors and molars (Figures 3C and D).

At the cap and early bell stages (E14.5-15.5), the incisors and molars showed differential expression pattern of Syndecan-2. The incisors showed a consistent pattern with E13.5 (Figures 3E and G), while in the molars Syndecan-2 switched back into the enamel organ (Figures 3F and H),

2.4. The expression pattern of Syndecan-3 in murine embryonic teeth

Syndecan-3 showed a mild expression in both incisors and molars throughout the embryonic stages. At E12.5, a weak expression was detected in both the dental epithelium and the dental mesenchyme (Figures 4A and B).

At E13.5, the incisors showed a more localized expression of Syndecan-3 in the dental epithelium. The molars had a mild expression in both the dental epithelium and dental mesenchyme (Figures 4C and D). At cap and early bell stages, Syndecan-3 expression was gradually reduced in the enamel organ of incisors (Figures 4E and G), while in the molars, the expression was increasingly localized in the inner dental epithelium (Figures 4F and H).

2.5. The expression pattern of *Syndecan-4* in murine embryonic teeth

At the early bud stage (E12.5), *Syndecan-4* was mainly detected in the oral and dental epithelium of both incisors and molars; the underlying dental mesenchyme showed very weak or sporadic expression (Figures 5A and B).

At the late bud stage (E13.5), *Syndecan-4* showed a broader expression in both the dental epithelium and the dental mesenchyme (Figures 5C and D).

At E14.5, *Syndecan-4* was specifically localized in the dental and oral epithelium in incisors (Figure 5E), while in molars, a strong expression was detected in both the dental epithelium and the dental follicle (Figure 5F).

At E15.5, *Syndecan-4* expression became broad again in both the dental epithelium and the dental mesenchyme (Figure 5G), while in molars, *Syndecan-4* turned to be localized in the dental epithelium and disappeared from the dental mesenchyme (Figure 5H).

3. DISCUSSION

Tooth development involves hierarchical interactions between the dental epithelium and the dental mesenchyme. Numerous growth factors, such as BMPs, FGFs, HHs, and WNTs, compose a regulatory network governing tooth morphogenesis. The cell-cell interactions derived by these secreted signaling molecules need extracellular matrix (ECM) to shape diffusion gradients and facilitate ligand-receptor interactions. Proteoglycans are ubiquitously present on the cell surface and in the ECM. These heavily glycosylated proteins play critical roles in mediating the cell-matrix signaling through glycosaminoglycan (GAG) chains attached to their core proteins. Previous studies have identified dynamic expression pattern of several proteoglycans and glycosaminoglycans in tooth germs, implying potential implication of these heavily glycosylated molecules with tooth development (Table 1). We recently found that FAM20B-catalyzed proteoglycans have important roles in maintaining the signaling balance that governs murine tooth number (Tian et al., 2016), which made us

to further investigate the molecular mechanism underlying this phenotype. As a beginning, we systematically examined the expression pattern of FAM20B-catalyzed proteoglycans in murine embryonic teeth.

Through semi-quantitative RT-PCR analysis, we identified 23 proteoglycans in murine embryonic teeth. Some of them, such as Glypican 1 (Glypican), NG2, Syndecan 4 and Testican, were not detected in the developing teeth in previous studies (Hikake et al., 2003), which may be related to the different methods and probes used. Glypicans and Syndecans have been recognized for their regulatory roles in the signaling transduction of various tissues (Linhardt & Toida, 2004). As a major sub-family of FAM20B-catalyzed proteoglycans, Syndecans are important candidates that are potentially associated with the signaling cascades governing tooth number in mice. Among the Syndecan members, Syndecan-1 is the most investigated to date. Previous studies have spotted it in the epithelium of several murine tissues (Hayashida et al., 2006). However, our current study together with the results of Bai et al (1994) and Filatova et al. (2015), indicate that Syndecan-1 has a different expression pattern in embryonic teeth: it is mainly present in the dental mesenchyme at bud stages and then spreads to both the enamel organ and the dental mesenchyme at cap stages. This dynamic expression pattern suggests a potential implication with the reciprocal interactions between the dental epithelium and dental mesenchyme during tooth morphogenesis.

Syndecan-2 showed a different expression pattern from Syndecan-1 as revealed by IHC staining. It was initially expressed in both the dental epithelium and dental mesenchyme. Then the expression was localized in the dental mesenchyme, and eventually switched into the dental epithelium in molars and displayed differential pattern between different types of teeth. This suggests that Syndecan-2 may also be involved in the dynamic signaling switch between the dental epithelium and dental mesenchyme and may have a role in the differential development between incisors and molars. The localized expression of Syndecan-2 in the dental mesenchyme immediately underneath the inner dental epithelium suggests a potential role of Syndecan-2 in the development of odontoblasts. This is supported by a recent finding that Syndecan-2 was present in alveolar bone and differentiating odontoblasts and involved in the formation of dentin and dental pulp that originate from the dental mesenchyme (Bai et al., 1994).

Syndecan-3 was found to be important for chondrocyte proliferation and function during limb skeletogenesis (Pacifici et al., 2005). There is no investigation about its role in tooth development to date. We detected a strong and specific expression of Syndecan-3 in the inner dental epithelium and stratum intermedium of molars at the late-cap stage, suggesting a potential role of this proteoglycan in the ameloblast differentiation in molars.

Syndecan-4 is a transmembrane heparan sulfate proteoglycan. It has been found to regulate the morphogenesis in a number of tissues and organs (Elfenbein & Simons, 2013). We found that *Syndecan-4* was primarily expressed in the dental epithelium in both incisor and molar tooth germs, suggesting that Syndecan-4 may have a role in the signaling cascade in the dental epithelium. This is consistent with a recent finding that Syndecan-4 is expressed in the stratum intermedium cells of enamel organ and in the cervical loop at later stage of E18

(Yan et al., 2014). We also identified a strong temporal expression of *Syndecan-4* in the dental follicle of molars at E14.5, reminiscent of a potential role in the development of periodontium in molars. The differential expression pattern of *Syndecan-4* between molars and incisors also suggests its diverse roles in the morphogenesis of molars and incisors.

It is worth to note that proteoglycans are ubiquitously expressed in almost all cells and tissues, thus it's understandable to see some backgrounds in the ISH and IHC staining, and expression beyond tooth germs (such as in the diastema regions). In this regards, a relatively specific/localized expression usually hints a spatiotemporal function. The dynamic expression pattern of Syndecans revealed in this study suggests that these proteoglycans may have active roles in regulating the development of murine embryonic teeth.

4. MATERIALS AND METHODS

4.1. Animals and tissue processing

All of the animal experiments were carried out according to the protocol approved by the Institutional Animal Care and Use Committee of Texas A&M University College of Dentistry (Dallas, TX, USA), and performed in accordance with the NIH *Guide for the Care and Use of Laboratory Animals*.

Eight-week old C57BL/6 mice were crossbred to obtain the embryos. The embryonic age was determined by the vaginal plug (E 0.5 day) and further confirmed by morphological criteria. Embryos were harvested at E12.5-E15.5.

For immunohistochemistry and *in situ* hybridization analyses, the embryo heads were collected and fixed in 4% paraformaldehyde (PFA) prepared in 0.1% diethyl pyrocarbonate (DEPC)-treated PBS at 4°C, and dehydrated in a serial gradient of ethanol solutions overnight. Paraffin embedding and sectioning were carried out as previously described (Wang et al., 2010).

4.2. Semi-quantitative RT-PCR

For RT-PCR analysis, the tooth germs of lower molars and incisors were isolated from the mandibles of E14.5 C57BL6/J embryos under stereomicroscope (Olympus America Inc., Waltham, MA, USA) using Dumont tweezers (Ted Pella Inc., Redding, CA, USA). Oral epithelium and mandibular mesenchyme surrounding the tooth germs were carefully trimmed off to avoid tissue contamination. The boundary between the dental epithelium and oral epithelium was determined by their different light refraction under the stereomicroscope. However, the boundary between the dental mesenchyme and surrounding mandibular mesenchyme was difficult to discern. To solve this problem, we over-trimmed the mesenchyme surrounding the tooth germ to minimize tissue contamination. Additionally, tissue grafts isolated from the diastema regions were used as controls for RT-PCR analysis. Total RNAs were extracted from the tooth germs and diastema grafts using an Rneasy Mini Kit (Qiagen, Valencia, CA, USA). The RNAs were reversely transcribed into first strand cDNAs using a Reverse Transcription Kit (Qiagen). Semi-quantitative RT-PCR was performed to amplify 10 ng cDNA for each proteoglycan and Fam20B using corresponding primers (Table 2). The PCR condition was 94°C for 2 min., then 28 cycles of 94°C for 30

sec., 56°C for 30 sec. and 72°C for 40 sec., and finally 72°C for 4 min. The intensity of each PCR product was normalized to GAPDH using ImageJ.

4.3. Immunohistochemistry (IHC)

Immunohistochemistry was performed on 4 gm-thick coronal sections using a DAB substrate kit (Vector Laboratories, Burlingame, CA, USA) as previously described (Wang et al., 2010). The anti-Syndecan-2 and anti-Syndecan-3 antibodies (Santa Cruz Biotechnology, Dallas, TX, USA) were used at a dilution of 1:200. Methyl green was used for counterstaining. Primary antibody was omitted or replaced by non-immune rabbit serum in the control experiments.

4.4. *In situ* hybridization (ISH)

Plasmids containing the cDNAs of mouse Syndecan-1 and -4 were kindly provided by Dr. Shunichi Shibata at Tokyo Medical and Dental University (Hayashida et al., 2006). ISH is performed according to the protocol previously described (Wu et al., 2019). Briefly, ISH probes were prepared using an RNA Labeling Kit (Roche; Indianapolis, IN). Syndecan transcripts were detected in embryonic teeth with the digoxigenin (DIG)-labeled antisense RNA probes by an enzyme-linked immunoassay with a specific anti-DIG-AP antibody conjugate (Roche), and stained with BM Purple (Roche) for positive signals. In the negative control experiments, the DIG-labeled sense probes were used in place of the antisense probes.

4.5. Statistics

The data was expressed as mean ± SD of at least 6 determinations in all experiments unless otherwise indicated. ANOVA was employed to detect any differences among the groups. When a difference was identified, 2-sample t test was used to evaluate pairs of samples. A P value of <0.05 was considered to indicate statistically significant differences.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

- Twenty three proteoglycans are identified in murine embryonic teeth.
- Syndecans show dynamic spatiotemporal expression during the development of murine embryonic teeth.
- Syndecans show differential expression pattern between embryonic molars and incisors.
- The expression pattern of Syndecans suggests potential roles in tooth morphogenesis.

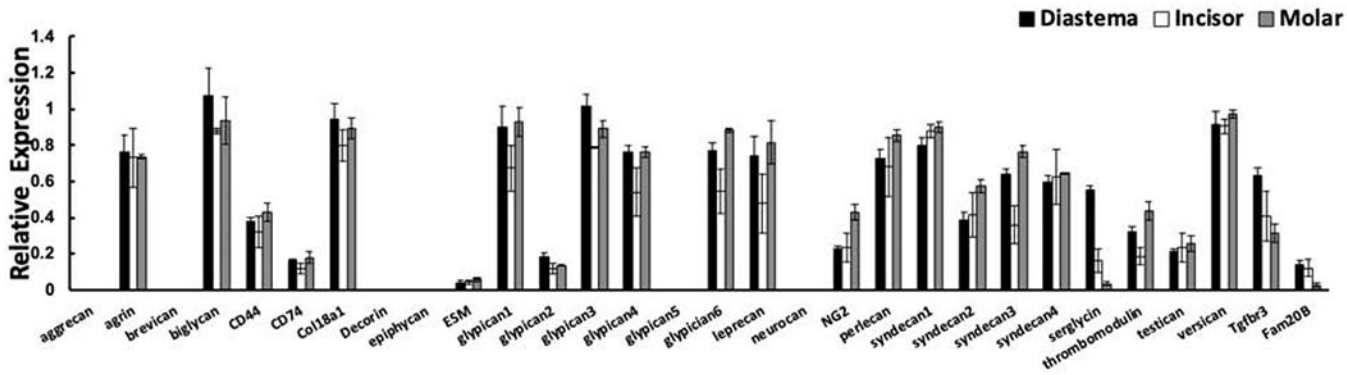

Fig.1. Semi-quantitative RT-PCR identified 23 proteoglycans and *Fam20B* in the embryonic teeth of E14.5 mice.

The tooth germs of lower incisors and molars as well as diastema grafts were isolated from the mandibles of E14.5 C57BL6/J mice. Total RNAs were extracted and reverse transcribed for semi-quantitative RT-PCR analysis. The quantity of PCR products was normalized to GAPDH (1.0 on Y-axis). Student's t tests were used to compare paired groups and one-way ANOVA for multiple comparisons.

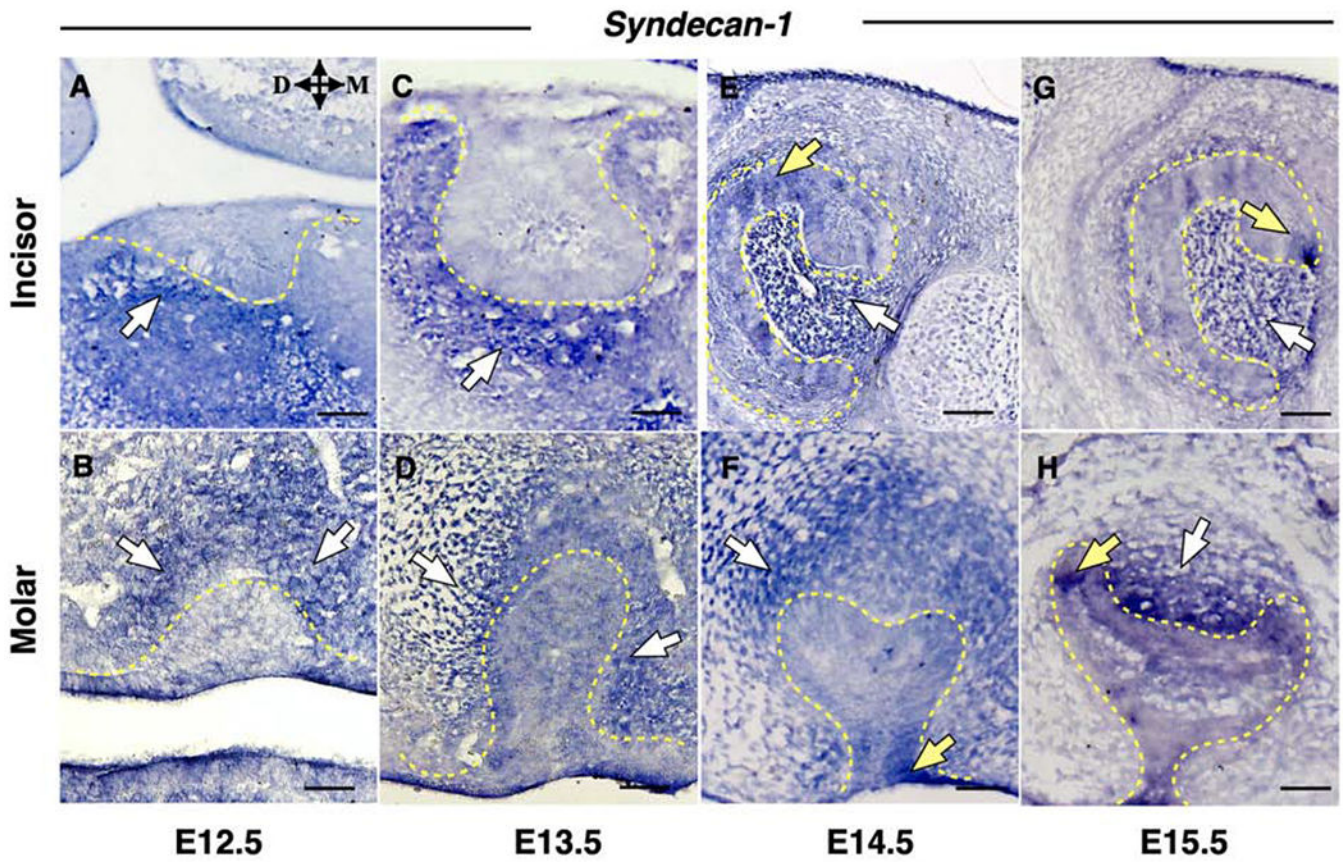

Fig.2. The expression pattern of *Syndecan-1* in murine embryonic teeth.

In situ hybridization was performed on coronal sections of lower incisors and the first lower molars from E12.5-E15.5 murine embryos. *Syndecan-1* mRNA was primarily expressed in the dental mesenchyme (arrows) at early embryonic stages (A-D). At E14.5, the expression was broader in both the dental epithelium and dental mesenchyme (E and F, arrows), and then became localized again in the dental papilla, along with a mild expression in the enamel organ. Scale bars: 200μm

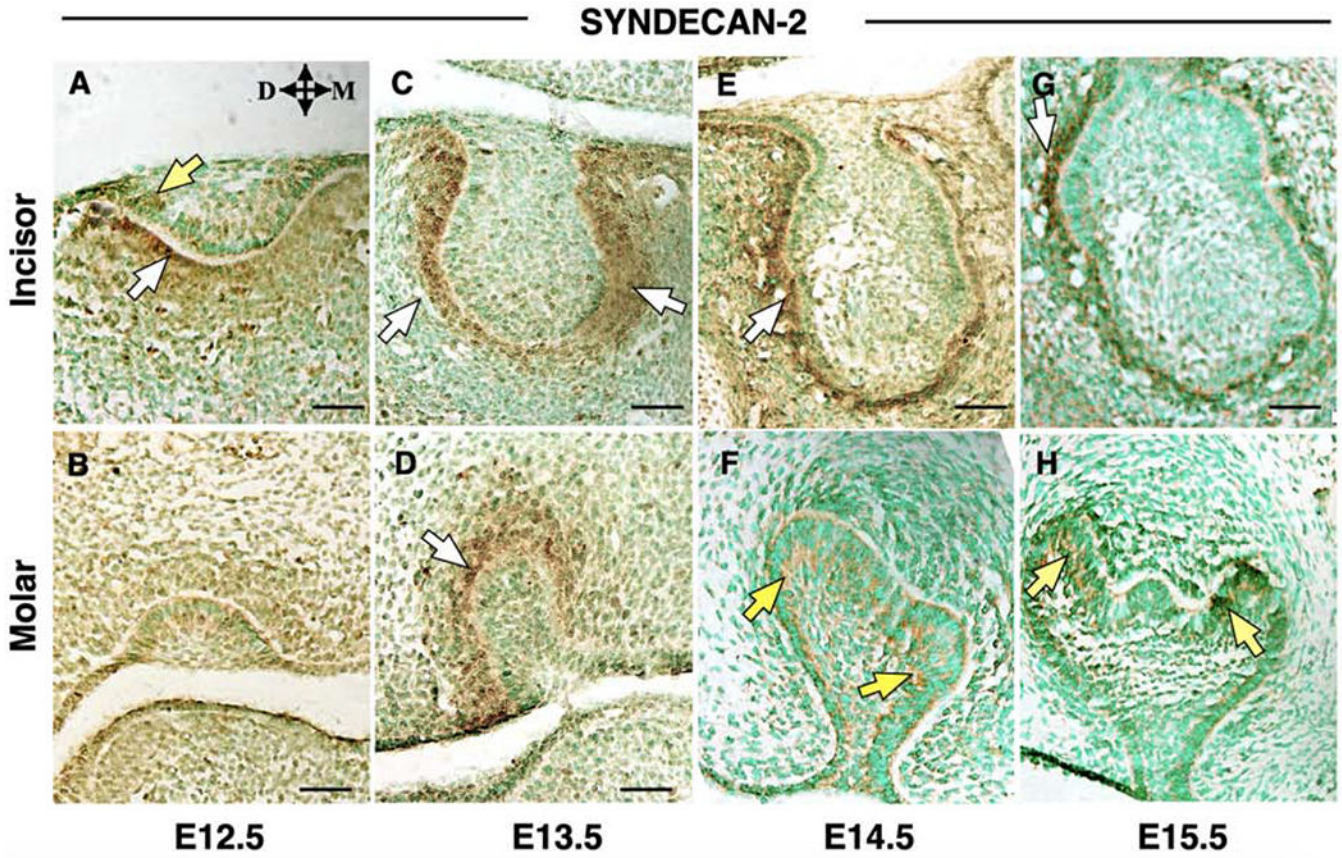

Fig. 3. The expression pattern of Syndecan-2 in murine embryonic teeth.

Immunohistochemistry staining was performed on the coronal sections of lower incisors and the first lower molars from E12.5-E15.5 murine embryos. At E12.5, Syndecan-2 was broadly expressed in both the dental epithelium and dental mesenchyme in the lower incisors and first molars (A and B, arrows). At E13.5, the expression was localized in the dental mesenchyme immediately surrounding the dental epithelium in both the incisors and molars (C and D, arrows). At E14.5, Syndecan-2 was localized in a thin layer of the dental mesenchyme immediately surrounding the enamel organ of incisors. The distal side of the dental mesenchyme showed a higher expression than the proximal side (E, arrows). In the molars, Syndecan-2 expression switched from the dental mesenchyme into the stratum intermedium and inner dental epithelium of the enamel organ (F, arrows). At E15.5, the expression pattern of Syndecan-2 was similar with that of E14.5 but more localized in the dental follicle and enamel organ (G and H). Scale bars: 200μm

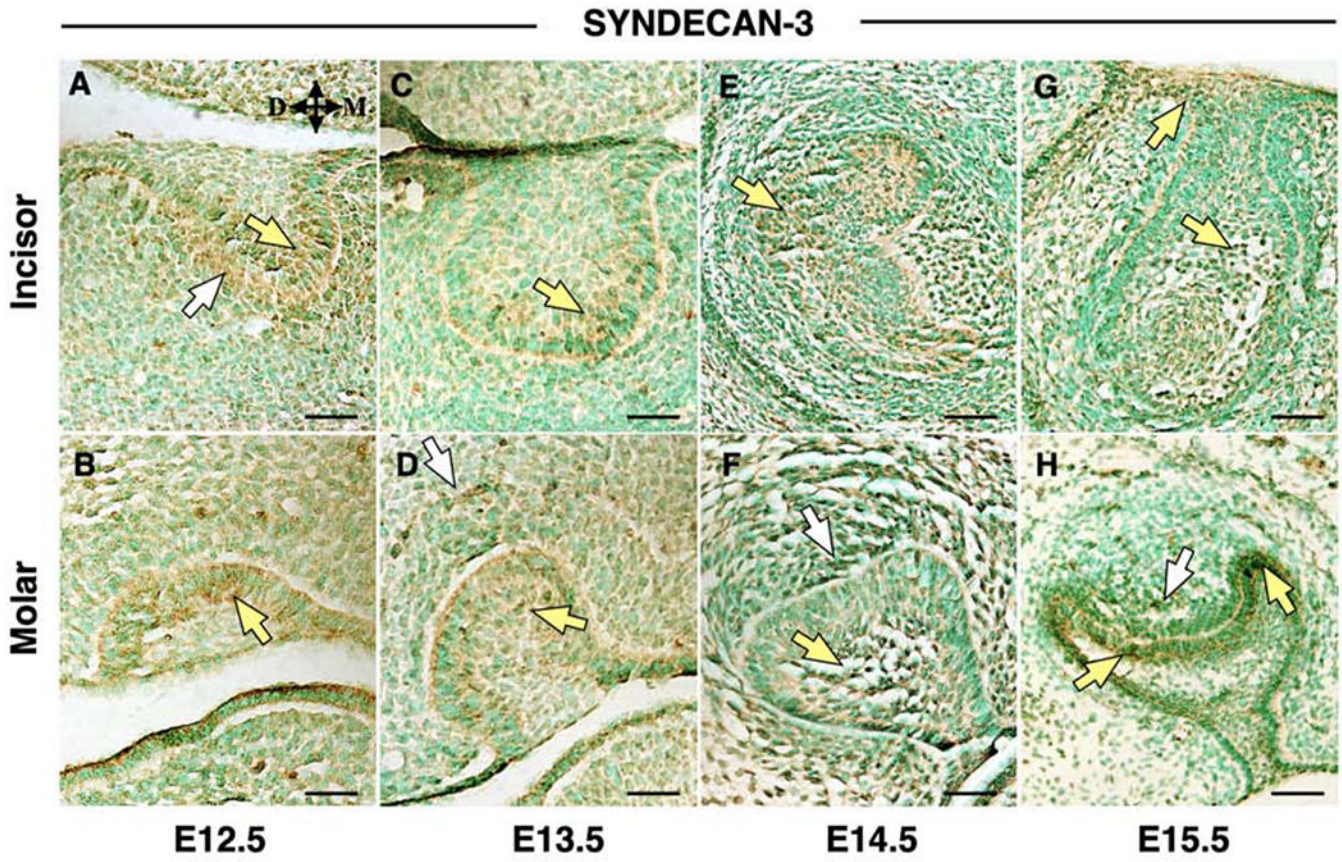

Fig. 4. The expression pattern of Syndecan-3 in murine embryonic teeth.

Immunohistochemistry staining was performed on coronal sections of lower incisors and the first lower molars from E12.5-E15.5 murine embryos. In incisors, Syndecan-3 showed a decreasing expression in the dental epithelium from the bud to cap stages (A, C, E and G, arrows). In molars, Syndecan-3 showed a dynamic expression in the dental epithelium: initially was downregulated from E12.5 to E14.5 (B, D and F), then upregulated and localized in the inner dental epithelium at E15.5 (H, arrow). Scale bars: 200μm

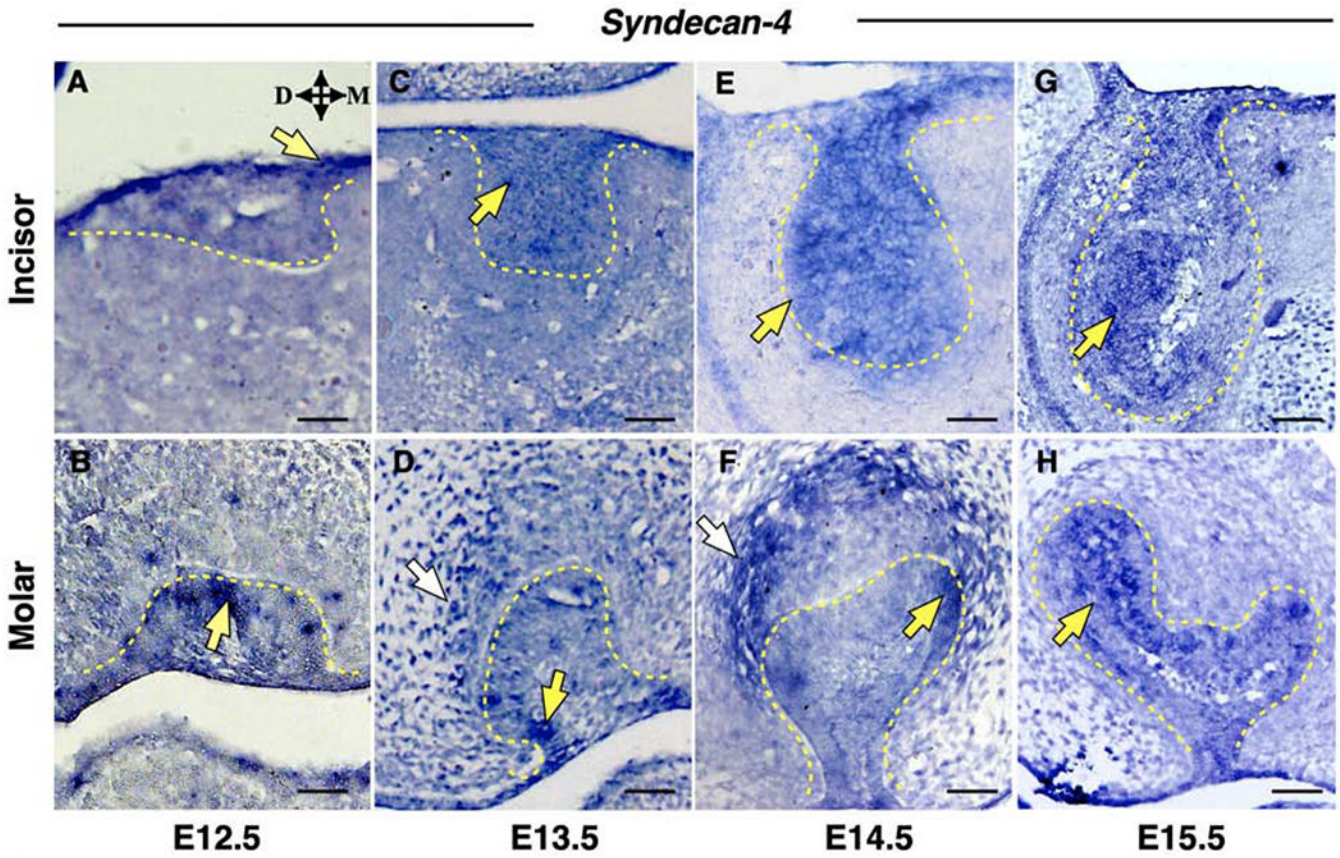

Fig.5. The expression pattern of *Syndecan-4* in murine embryonic teeth.

In situ hybridization was performed on coronal sections of lower incisors and the first lower molars from E12.5-E15.5 murine embryos. Both the incisors and molars showed dynamic expression pattern during the developmental stages. At the bud stages (E12.5-E13.5), *Syndecan-4* was mainly detected in the dental epithelium, with a weak-mild expression in the dental mesenchyme (A-D). At the early cap stage (E14.5), *Syndecan-4* was localized in the dental epithelium of incisors (E, arrow), while in molars, *Syndecan-4* was highly expressed in the dental follicle and part of outer dental epithelium (F). At the late cap stage (E15.5), and *Syndecan-4* was broadly expressed in both the dental epithelium and dental mesenchyme of incisors (G), while in molars, *Syndecan-4* was localized in the dental epithelium (H). Scale bars: 200μm

Table 1.

Summary of the previously published proteoglycan data compared to this study.

PG \ Stage	Molar					Incisor				
	Lamina	**Bud**	**Cap**	**Bell**	**PN**	**Lamina**	**Bud**	**Cap**	**Bell**	**PN**
HS (Thesleff , 1981; Bai, 1994;)	NR	NR	NR	Basement membrane	Basement membrane, predentin	NR	Dental epithelium and dental mesenchyme	IEE, dental mesenchyme and dental sac	IEE, OEE, dental mesenchyme and dental sac	NR
Decorin (Hikake, 2003)	NR	NR	NR	IEE, preodontoblast, odontoblast	IEE, preodontoblast, odontoblast	NR	NR	NR	NR	NR
Biglycan (Hikake, 2003)	NR	NR	NR	Preodontoblast, odontoblast	preodontoblast, odontoblast	NR	NR	NR	NR	NR
Unclassified Syndecan (Thesleff, 1988; Vainio, 1989; 1992)	Oral & dental epithelium, dental mesenchyme	Strong in oral & dental epithelium, and dental mesenchyme	Strong in dental mesenchyme, dental sac & oral epithelium. Weak in dental epithelium	Moderate in oral & dental epithelium. Strong in dental mesenchyme	Strong in SI & SR	NR	NR	NR	NR	NR
Syndecan 1 (Bai, 1994; Hikake, 2003)	NR	NR	NR	IEE, OEE, SI, dental mesenchyme	OEE, SR, SI, preameloblast, dental pulp	NR	Weak in IEE, strong in dental mesenchyme	Weak in IEE and OEE, strong in dental mesenchyme and dental sac	SR, SI, distal dental mesenchyme	NR
Syndecan 1 (Fig. 2 of this study)	Weak in dental epithelium, strong in dental mesenchyme	Moderate in dental epithelium, strong in dental mesenchyme	Strong in the oral-dental epithelium junction & dental mesenchyme	Strong in IEE, cervical loop, SI, and dental mesenchyme	NR	Weak in dental epithelium, strong in dental mesenchyme	No Expression in dental epithelium, strong in dental mesenchyme	Strong in SR and mesenchyme	Cervical loop, SR, dental mesenchyme	NR
Syndecan 2 (Bai, 1994;)	NR	NR	NR	NR	NR	NR	Strong in dental mesenchyme	Moderate in dental mesenchyme, strong in dental sac	Ameloblast, odontoblast, dental sac	NR
Syndecan 2 (Fig. 3 of this study)	Weak in dental epithelium & mesenchyme	Strong in dental mesenchyme.	Strong in IEE and SI	Strong in cervical loop	NR	Moderate in dental epithelium, strong in dental mesenchyme	Strong in dental mesenchyme	Strong in dental mesenchyme & dental sac	Strong in dental sac	NR
Syndecan 3 (Hikake, 2003)	NR	NR	NR	IEE, dental mesenchyme	Odontoblast, preodontoblast, dental pulp	NR	NR	NR	NR	NR

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PG \ Stage	Molar					Incisor				
	Lamina	**Bud**	**Cap**	**Bell**	**PN**	**Lamina**	**Bud**	**Cap**	**Bell**	**PN**
Syndecan 3 (Fig. 4 of this study)	Moderate in dental epithelium	Weak in dental epithelium	Weak in SR	Strong in IEE & cervical loop	NR	Strong in dental epithelium	Moderate in dental epithelium	Strong in SR, IEE	Weak in SR, moderate in oral lamina	NR
Syndecan 4 (Fig. 5 of this study)	Strong in dental epithelium	Strong in both dental epithelium & mesenchyme	Strong in both dental epithelium & mesenchyme	Strong in SR & SI	NR	Strong in dental epithelium	Strong in dental epithelium	Strong in dental epithelium	Strong in SR, SI	NR
Tenascin (Vainio, 1989, 1992)	No expression	Strong in dental mesenchyme & basement membrane	Strong in dental mesenchyme, dental sac, basement membrane	NR	NR	NR	NR	NR	NR	NR
Perlecan (Yonemochi, 2006)	NR	Dental epithelium	NR	IEE, SR, dental mesenchyme	SR, preameloblast	NR	NR	NR	NR	NR
Versican (Jiang, 2010)	Dental epithelium, basement membrane	Dental epithelium, dental mesenchyme	IEE, SR, dental mesenchyme	Cervical loop, dental papilla	NR	NR	NR	NR	NR	NR

PG, proteoglycan; PN, postnatal; HS, heparan sulfate; Basement membrane, the membrane between the dental epithelium and dental mesenchyme; IEE, inner enamel epithelium; OEE, outer enamel epithelium; SI, stratum intermedium; SR, stellate reticulum; NR, no report.

Table 2.

Primers used in semi-quantitative RT-PCR analyses of proteoglycans in E14.5 embryonic teeth.

Gene name	Primer Sequence (5'-3')	Gene name	Primer Sequence (5'-3')
aggcecan	F: GACGTCCCCTGCAATTACCA R: GAAAGGCACGGTGGGAAATC	*glypican5*	F: ATGAATTGGCTGCCCCTGAA R: GACTCAGTTCCTGACGCACA
agrin	F: TCCACTGCTACCTATGGCCT R: AGACACACTGGGCAAAACCA	*glypican6*	F: GCTGCTGGAAAACGCAGAAA R: AGCCCTTCATGACGTTGAGG
bcevican	F: GGAACCCGCACAGAGAAGG R: CGATCCTCCGAGCTGTCTTC	*leprecan*	F: CGGCACACACCTTCTTTGTG R: CTCCGTGACACAGTTCTGCT
biglycan	F: CCTCCCCAGGAACATTGACC R: GAGTTTTTGCAGCTTCCGCA	*neurocan*	F: GTGGCTGCTTCTCCTAGTGG R: ATACAGCCCAGAGTCGCTTG
CD44	F: ATCCTCGTCACGTCCAACAC R: GTGGTCACTCCACTGTCCTG	*NG2*	F: AGCTAGAGGTGGTCCAGGAG R: AGCTAGCAGAACAAAGGCGT
CD74	F: GGCTAGAGCCATGGATGACC R: CAGGCCCAAGGAGCATGTTA	*peclecan*	F: GCTCTCGTCTACGGCTACAC R: GAGGAGGAAGTCTCGATGCG
Col18a1	F: CGCGTTAGAAATGGCTTCCG R: ATCCTGCAGCCTAGAGGACA	*syndecan1*	F: CAACTTCTCTGGCTCTGGCA R: GGCAGTTCCATCCTCGACAA
Decocin	F: GAGAAGGGGGCCGATAAAGTT R: TAGCAAGGTTGTGTCGGGTG	*syndecan2*	F: TAGTGCTGCTTCCCCCAAAG R: AGGGCCAGTATGGCTCTGTA
epiphycan	F: TGGAAGATGACAGTTGACCAGA R: GGGGAAGAGCCATCGATCAG	*syndecan3*	F: AGGCTGGTCAACACAGCTAC R: ACCTCCTTCCGCTCCAGTAT
endocan	F: GCAAGAGGACAGTGCTGGAT R: CCATCTCCGGATGCTGAGTC	*syndecan4*	F: CTCAGAGCCCAAGGAACTGG R: CTGCCAGAGCAACTGAGACA
glypican1	F: CTGAGCTGCGCCTCTACTAC R: GTCCTTGTTGTCCTGGAGGG	*serglycin*	F: GTTATCCTGCTCGGAGAGCC R: ATGGAGGTGGGGAGACAGAG
glypican2	F: TCTCGCATTCCTATGGTCGC R: ATGGACTCAGCAGCCAGTTC	*Thrombomodulin*	F: TGTCGAAACTTCCCTGGCTC R: TCAAGTCCTCCCTACCCTCG
glypican3	F: TGATGGTTAAGCCTTGCGGT R: TCTTCATCCCGTTCCTTGCC	*testican*	F: GCTCACAAACACTGGCTTGG R: TGGATCAGAGCTGGTAGGCT
glypican4	F: GCAAAGTGTCCGTGGTGAAC R: TCATTGCAAACGGTGCTTGG	*vecsican*	F: CCGCGCTTCCTATGTGATCT R: TATTTCCGTTCTGGGCCACC
Fam20B	F: GTAGAAGGGGAGCCATACGC R: CAGCATCGCAGTAGCTCTCA	*Tgfbc3*	F: CGTGCTGGAGTGGTAGTGTT R: CATCTAGAGAGGTGCAGGCG
GAPDH	F: GCAAAGTGGAGATTGTTGCCA R: GGAAGAGTGGGAGTTGCTGTTG		