

Expression patterns of *tenascin-N* in the developing mandible

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Previous studies have demonstrated that *tenascin-N* belongs to the family of tenascins, which are found in the extracellular matrix of various embryonic tissues, wounds, and tumors. Tenascin is expressed in the embryonic epithelium, including the neural epithelium from which neural crest cells emerge. However, the expression pattern and role of *tenascin-N* in the craniofacial region remains unknown. In this study, expression patterns of *tenascin-N* were confirmed in the mouse craniofacial region from embryonic day 12.5 (E12.5) to postnatal 11. In the diastema region, *tenascin-N* was strongly expressed in the mesenchyme from E12.5 to E14.5. *Tenascin-N* expression was also detected in the developing tooth germ. From the bell stage to the premature stage, *tenascin-N* was expressed in the odontoblasts and ameloblasts of the molar tooth germ, and the ameloblasts of the incisor tooth germ. These findings indicate that the spatial and temporal expression of *tenascin-N* might have a role in proper mouse craniofacial development, especially tooth development.

Keywords: *Tenascin-N*, incisor, diastema, molar.

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Introduction

Tenascin family is a group of extracellular matrix components glycoproteins characterized by N-terminal globular domain and heptad repeats; epidermal growth factor like repeats; fibronectin type III domains, and C-terminal fibrinogen-related domain^{1,2}. Tenascins are found in the extracellular matrix of various embryonic tissues, wounds, and tumors. Tenascin-N protein is most recently discovered tenascin which is homologs to tenascin-W³. It was firstly identified and reported as an essential role in neurites and neurons by generating splice isoforms⁴. Previous study identified *tenascin-N* is expressed in various types of neurons in the central nervous system, medullary region in the kidney and resident macrophages of the T-cell zone in the splenic white pulp⁴. Spatial and temporal *Tenascin* contributes in different phases during morphogenesis of epithelial appendages including feather⁵. However, expression of *tenascin-N* and its role in craniofacial region was not determined. In this study, we investigated the expression pattern of *tenascin-N* in mouse craniofacial region, especially tooth forming region by *in situ* hybridization in mandible from embryonic day 12.5 (E12.5) to postnatal 11 (PN11). *Tenascin-N* was differently expressed in developing molar, incisor, diastema, Meckel's cartilage and alveolar bone region at each stage. Our findings demonstrate that spatial and temporal *tenascin-N* expression may play pivotal role during mouse craniofacial development.

Materials and Methods

All experiments were performed according to the guidelines of the Intramural Animal Use and Care Committee of the College of Dentistry, Yonsei University.

Animals

Adult ICR mice were housed in a temperature-controlled room (22°C) under artificial illumination and 55% relative humidity, with access to food and water *ad libitum*. The embryos were obtained from time-mated pregnant mice. Embryonic day 0 was designated as the day on which the presence of a vaginal plug was confirmed. Embryos at each developmental stage (E13.5, E14.5, E15.5, E16.5 and E17.5) and postnatal mice at each developmental stage (PN2 and PN11) were used in this study.

In situ hybridization

In situ hybridization on whole mouse embryos was performed as previously described⁶. Briefly, embryos were fixed in 4% paraformaldehyde (PFA), dehydrated in Methanol and stored at -20°C until use. Through the rehydration with Triton X-100 contained Phosphate-buffered saline, embryos were treated with proteinase K and fixed using 0.25% glutaraldehyde in 4% PFA. Samples were prehybridized in hybridization solution at 68°C for 1h. Digoxigenin (DIG)-labelled Ribonucleic Acid (RNA) probes were prewarmed and hybridized to samples overnight at 68°C. Mouse complementary Deoxyribonucleic Acid *tenascin-N* inserted plasmids were used as template for the synthesis of DIG-labeled RNA probes.

Results and Discussion

Tenascin-N expression pattern in mandible, tooth germ and diastema region.

Tenascin-N expression was broadly detected in mandible from E7.5 to E11.5 (data not shown). At E12.5, *tenascin-N* expression was detected in incisor forming region, diastema region and molar forming region (Fig. 1A). Strong expression of *tenascin-N* was detected in epithelium of incisor forming region after frontal section (Fig. 1A¹, arrowhead). Expression of *tenascin-N* was detected in mesenchyme of diastema region at E12.5 (Fig. 1A², arrowhead). At E12.5, expression of *tenascin-N* was observed in surrounding mesenchyme of molar tooth germ, especially at buccal side (Fig. 1A³, arrowhead). At E13.5, *tenascin-N* was expressed in incisor forming region, diastema region and molar forming region (Fig. 1B). *Tenascin-N* expression was detected in underlying mesenchyme of incisor forming region and diastema region (Fig. 1B¹, B², arrowhead). Expression of *tenascin-N* was partially detected in surrounding mesenchyme of bud stage molar tooth germ and meckel's cartilage (Fig. 1B³, arrowhead). At E14.5, expression of *tenascin-N* was detected in incisor forming region and diastema region (Fig. 1C). *Tenascin-N* was expressed in incisor and diastema mesenchyme but absent in epithelium (Fig. 1C¹, C², arrowhead). Expression of *tenascin-N* was partially observed surrounding molar tooth germ and meckel's cartilage but not in molar epithelium (Fig. 1C³, arrowhead). *Tenascin-N* expressed in incisor forming region however, faintly observed in diastema and molar forming region at E15.5 (Fig. 1D). In incisor forming region, *tenascin-N* expression was detected in surrounding

mesenchyme but not in epithelium (Fig. 1D¹, arrowhead). *Tenascin-N* was weakly expressed in diastema mesenchyme at E15.5 (Fig. 1D²). In molar region, *tenascin-N* expression was observed in mesenchyme between molar tooth germ and Meckel's cartilage (Fig. D³, arrowhead). At E16.5, expression of *tenascin-N* become undetectable in incisor, diastema and molar region (Fig. 1E). *Tenascin-N* was not expressed in incisor epithelium and mesenchyme after frontal section at E16.5 (Fig. 1E¹). *Tenascin-N* was also not detectable in diastema region similar to incisor region at E16.5 (Fig. 1E²). *Tenascin-N* expression was observed between developing alveolar bone and muscle but not around molar tooth germ and Meckel's cartilage (Fig. 1E³).

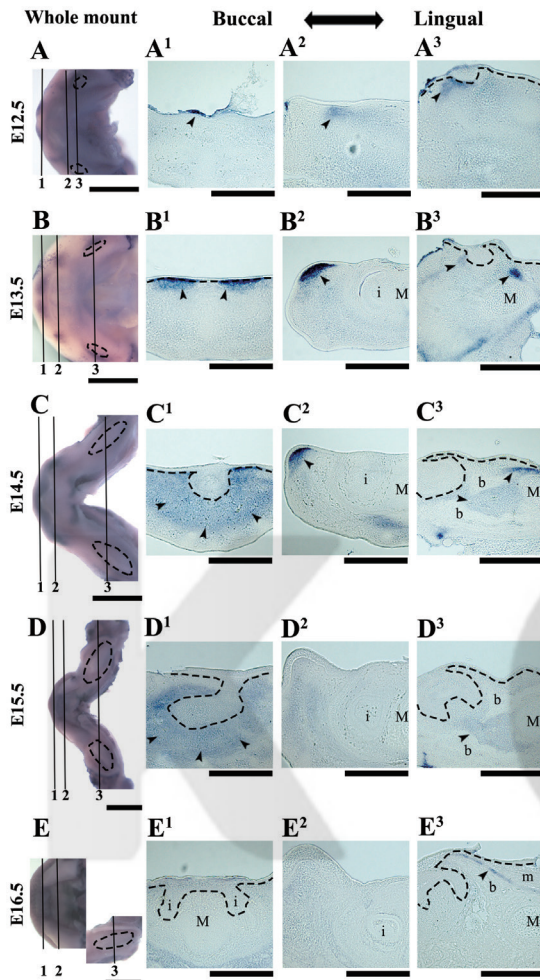


Figure 1. Expression pattern of *tenascin-N* in developing mandible

(A) *Tenascin-N* expression pattern is detected in incisor forming region, diastema region and molar forming region at E12.5. (A¹) At E12.5, specific *tenascin-N* expression is detected in epithelium of incisor forming region. (A², A³) *Tenascin-N* is expressed in adjacent mesenchyme of diastema and molar tooth germ. (B) At E13.5, expression of *tenascin-N* is detected in incisor forming region and diastema region. (B¹, B²) Strong *tenascin-N* expression is located in underlying mesenchyme of incisor and diastema region. (B³) *Tenascin-N* is expressed in mesenchyme adjacent to Meckel's cartilage. (C) At E14.5, *tenascin-N* expression is remained incisor forming region and diastema region. (C¹, C²) *Tenascin-N* is broadly detected in

mesenchyme of incisor forming region and diastema region. (C², C³) Strong expression of *tenascin-N* is partially observed in Meckel's cartilage region. (D, D¹) At E15.5, *tenascin-N* expression in incisor forming region is weaker than E14.5. (D²) In diastema region, *tenascin-N* is weakly detected in mesenchyme. (D³) *Tenascin-N* expression is detected between molar tooth germ and Meckel's cartilage. (E, E¹, E²) At E16.5, *tenascin-N* expression is undetectable in incisor and diastema region. (E³) *Tenascin-N* is expressed between developing alveolar bone and muscle at E16.5. scale bar: A~E = 500µm, A¹~A³, B¹~B³, C¹~C³, D¹~D³, E¹~E³ = 250µm, i: incisor, M: Meckel's cartilage, b: bone, m: muscle.

***Tenascin-N* expression in late bell stage to premature stage tooth germ**

To confirm the expression pattern of *tenascin-N* in molar and incisor tooth germ, *in situ* hybridization was performed. At E17.5, strong *tenascin-N* expression was observed in inner dental epithelium, outer dental epithelium and dental papillae of early bell stage molar tooth germ (Fig. 2A, A¹). *Tenascin-N* expressing mesenchymal cells were condensed surrounding Meckel's cartilage (Fig. 2A², arrowheads). At E18.5, *tenascin-N* was expressed in inner dental epithelium, stellate reticulum, stratum intermedium, dental papillae and successional dental lamina (Fig. 2B, B¹). *Tenascin-N* expression surrounding Meckel's cartilage become weaker at this stage compared to E17.5 (Fig. 2B²). At PN2, late bell stage molar tooth germ, premature hard tissue starts to form, was *tenascin-N* positive in odontoblast, ameloblast and dental papillae (Fig. 2C, C¹). *Tenascin-N* expression was strongly observed in Hertwig's epithelial root sheath at PN2 (Fig. 2C², arrowhead). At this stage, *tenascin-N* expression was detected in odontoblast, ameloblast and dental papillae in incisor (Fig. 2C³).

At PN11, *tenascin-N* was expressed in molar, incisor and alveolar bone region (Fig. 2D, D¹). Strong *tenascin-N* expression was observed in alveolar bone at PN11 (Fig. 2D², arrowheads). At PN11, *tenascin-N* expression was detected in ameloblast compared to odontoblast in incisor (Fig. 2D³, arrowhead).

In summary, this study demonstrated the unique expression pattern of *tenascin-N* in mandible, incisor, diastema and molar region from E12.5 to PN11. *Tenascin-N* is known as a member of tenascin family, which are extracellular matrix glycoprotein broadly exist in vertebrate embryo¹. *Tenascin-N* is known important in neurons and neurites development, since is detectable in all brain regions, with a characteristic staining pattern in the hippocampus⁴. *Tenascin-N* was expressed in developing incisor and diastema epithelium at E12.5. Later, *tenascin-N* expression was observed in dental and diastema mesenchyme from E13.5 to E15.5. Interestingly, *tenascin-N* expression was not detected in tooth germ and diastema region at E16.5 (Fig.1). In the late tooth development, strong expression of *tenascin-N* was found in odontoblasts and premature dentin in molar, and in ameloblasts and premature enamel in incisor. Also, *tenascin-N* expression was found in Meckel's cartilage and alveolar bone region (Fig. 2). These findings indicate that spatial and temporal expression of *tenascin-N* is necessary for proper mouse craniofacial development including incisor, molar, diastema, Meckel's cartilage and bone in mandible.

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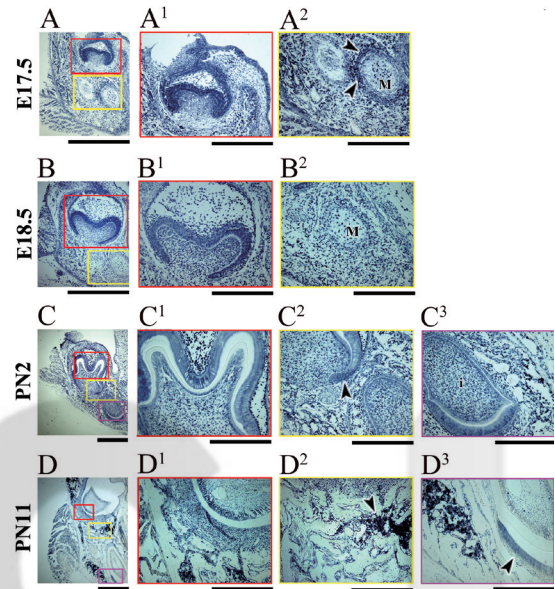


Figure 2. Expression pattern of *tenascin-N* in developing tooth germ

(A) At E17.5, *tenascin-N* strongly expressed in dental epithelium of early bell stage tooth germ. (A¹) *Tenascin-N* expression is strongly observed in inner and outer dental epithelium. (A²) Meckel's cartilage is surrounded by *tenascin-N* expressing mesenchymal cells. (B, B¹) At E18.5, *tenascin-N* is expressed in dental epithelium and dental papilla. (B²) *Tenascin-N* expression surrounding bone and meckel's cartilage is weaker than E17.5. (C, C¹) At PN2, strong expression of *tenascin-N* is observed in odontoblast and ameloblast layer. (C²) *Tenascin-N* expression is detected in Hertwig's epithelial root sheath. (C³) Ameloblast, odontoblast and dental papilla are marked by strong *tenascin-N* expression in incisor. (D, D¹, D²) At PN11, *tenascin-N* is expressed in odontoblast and surrounding alveolar bone. (D³) *Tenascin-N* is expressed in ameloblast of developing incisor. scale bar; A~D 500µm, A¹~A², B¹~B², C¹~C³, D¹~D³ 250µm, i: incisor, M: Meckel's cartilage.

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한글초록

발생중인 생쥐 하악에서 *tenascin-N*의 발현 양상장수산¹, 박성호², 오상빈¹, 정영수², 이종민¹, 정한성¹¹연세대학교 치과대학 구강생물학교실, BK21 플러스 통합구강생명과학단.²연세대학교 치과대학 구강악안면외과학교실

기존 연구를 통해 *tenascin-N*은 다양한 배아 조직, 상처 및 종양의 세포외 기질에서 발견되는 *tenascin* 패밀리에 속하는 것임이 알려져 있다. *Tenascin*은 신경능선 세포가 출현함에 따라 신경 상피를 포함하는 배아 상피에 존재한다는 사실 또한 알려져 있다. 그러나, 두개안면 영역에서 *tenascin-N*의 발현양상 및 역할은 알려져 있지 않다. 이 연구에서 배아 12.5 (E12.5) 일 부터 출생 후 11 일까지 생쥐 두개악안면 영역에서 *tenascin-N*의 발현 양상을 확인하였다. 치아간극(diastema) 영역에서 *tenascin-N*은 E12.5부터 E14.5까지 중간엽에 강하게 발현되는 것이 확인되었다. *Tenascin-N*의 발현은 또한 치아씩 발생과정 중에도 관찰되었다. 종 시기에서 조속단계까지, *tenascin-N* 발현은 앞니와 어금니의 상아질모세포, 법랑질모세포에서 관찰되었다. 이러한 발견은 *tenascin-N*의 시간적 및 공간적 발현이 적절한 생쥐 두개악안면 발생, 특히 치아 발생에 중요한 역할을 할 수 있음을 시사하는 것으로 판단된다.

주제어: *Tenascin-N*, 앞니, 치아간극, 어금니