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

Contents lists available at ScienceDirect

Journal of Oral Biosciences

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

Original Article

Dynamic expression of nectins in enamel organs of mouse incisors

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ARTICLE INFO

Article history:
Received 2 March 2017
Received in revised form
26 May 2017
Accepted 15 June 2017

Keywords:
Ameloblast
Cell adhesion
Enamel organ
Mice
Nectin

ABSTRACT

Objectives: Nectins are immunoglobulin-like cell–cell adhesion molecules and are of four types, namely, nectin-1, nectin-2, nectin-3, and nectin-4. Cleft lip/palate-ectodermal dysplasia is caused by a mutation in the nectin-1 gene locus. However, nectin-1-deficient (KO) mice only show mild tooth defects. This study determined the intracellular localization of nectins in mouse mandibular incisors to identify their heterophilic interactions during amelogenesis.

Methods: Nectin localization was determined by performing immunohistochemical analysis with confocal microscopy. Nectin gene expression was determined by performing quantitative reverse transcription-PCR. Phenotypes of nectin-2-KO mice were examined by performing micro-computed tomography, histological analysis, and organ culture.

Results: We found that mRNA levels of nectin-1 and nectin-3 genes were higher than those of nectin-2 and nectin-4 genes in mouse enamel organs. Nectin-2 and nectin-4 were strongly expressed at the apical adherens junctions of secretory-stage ameloblasts, whereas nectin-1 and nectin-3 were distributed between the basal adherens junction of maturation-stage ameloblasts and the stratum intermedium and papillary layers. Nectin-2-KO mice showed normal mandibular tooth shape, incisor surface pigmentation, and histology. Moreover, organ cultures of the tooth organs of nectin-2-KO mice proceeded normally.

Conclusion: These results indicate that nectins show graded and overlapping distribution in mouse incisors.

1. Introduction

Interaction between neural crest-derived ectomesenchymal cells and the oral epithelium is critical for tooth development. During mouse enamel organogenesis, apical bud differentiates to form the outer enamel epithelium, stellate reticulum, stratum intermedium (SI), and inner enamel epithelium. Ameloblasts are columnar epithelial cells with tight junctions and adherens junctions (AJs) at their basal and apical ends [1]. AJs contain several AJ proteins, including E-cadherin [2] and N-cadherin [3]. Ameloblast differentiation involves apical bud, preameloblast, secretory, transition, maturation, and late-maturation stages [4]. Rodent incisors erupt continuously throughout their lives; therefore, mouse incisors contain all ameloblast developmental stages simultaneously.

During the secretory stage, ameloblasts secrete several enamel proteins that induce immature enamel formation. During the subsequent transition stage, approximately 25% ameloblasts undergo apoptosis. In the maturation stage, ameloblasts degrade and resorb organic matrix from the immature enamel to form mature enamel. Differentiation-dependent changes in adhesion apparatus composition suggest that cell adhesion molecules affect ameloblast function [5].

Nectins belong to a family of immunoglobulin-like cell–cell adhesion molecules [6]. They interact with each other through homophilic and heterophilic *trans*-interactions, with heterophilic interactions being stronger than homophilic interactions. Interaction among nectins results in the recruitment of E-cadherin to form AJs and of claudins through afadin to form tight junctions. Mutations in the nectin-1 gene are associated with cleft lip/palate-ectodermal dysplasia (CLPED1) [7]. However, nectin-1-deficient (KO) mice show a milder phenotype than human patients with

Abbreviations: AJ, adherens junction, H-E, hematoxylin and eosin; KO, deficient, micro-CT, micro-computed tomography; M_1, first molar, qRT-PCR, quantitative reverse transcription-PCR; SI, stratum intermedium, WT, wild-type

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http://dx.doi.org/10.1016/j.job.2017.06.004
1349-0079/

CLPED1 [8], which may be because of the compensatory effect of other nectins. Furthermore, nectin-1- and nectin-3-KO mice showed delayed enamel formation because of weak interactions between ameloblasts and papillary layer during the maturation stage [8,9]. Nectin-2-KO mice show male infertility [10,11], enhanced cardiac fibrosis [12], and age-related neuron loss [13]. However, nectin-2 deficiency-associated abnormal tooth phenotypes have not been reported in nectin-2-KO mice. Although above studies suggest the importance of heterophilic interactions among nectins, spatiotemporal expression pattern of nectins during enamel organogenesis should be analyzed to determine their roles in ameloblastogenesis.

2. Materials and methods

2.1. Animals

Nectin-2-KO (nomenclature: Pvrl2^{tm1Smu}) and nectin-2$^{+/-}$ mice were generated as described previously [10] and were maintained on a C57BL/6 background. Nectin-2$^{+/-}$ mice were maintained under pathogen-free conditions at the Laboratory Animal Center of Showa University. Nectin-2-KO and wild-type (WT) mice were generated by crossing nectin-2$^{+/-}$ mice. Appearance of a vaginal plug was designated as E0.

2.2. Micro-Computed Tomography

The mandibles of 1-year-old WT and nectin-2-KO mice were imaged by performing micro-computed tomography (micro-CT) with ScanXmate-L090H (Comscantecno, Kanagawa, Japan) by using the following conditions: 87 kV, 18 μA, voxel resolution of 14 μm/voxel, and 992 × 992 pixel image matrices. Three-dimensional images were generated using conneCT express software (White Rabbit, Tokyo, Japan; www.white-rabbit.jp/indexE.html). In all, 10 WT and 8 nectin-2-KO mice were used for performing this analysis.

2.3. Histological analysis

The mandibles of 3- and 8-week-old WT and nectin-2-KO mice were fixed overnight in 4% paraformaldehyde in PBS and were decalcified using 10% EDTA in PBS (pH 7.4) for 14 days at 4 °C. After decalcification, each sample was dehydrated using a graded ethanol series at room temperature, cleared using xylene at 4 °C (30 min, 3 times), infiltrated overnight in a xylene paraffin (dilution, 1:1) solution at 60 °C and then in paraffin at 60 °C (60 min, 3 times), and embedded in paraffin. The paraffin-embedded samples were cut into 5-μm-thick sections, placed on silane-coated glass slides (Muto Pure Chemicals, Tokyo, Japan), and stained with hematoxylin and eosin (H-E) and TUNEL (In Situ Cell Death Detection Kit, POD; Roche, Basel, Switzerland). In all, 13 WT and 14 nectin-2-KO mice were used for performing histological analysis.

2.4. Immunohistochemical analysis

For this, the mandibles of WT and nectin-2-KO mice were fixed in 4% paraformaldehyde in PBS on ice for 2 h; decalcified using 10% EDTA in PBS (pH 7.4) for 7 days; immersed in 5%, 15%, and 30% sucrose; embedded in Tissue-Tek OCT Compound (Sakura Finetek USA, Torrance, CA, USA); and snap-frozen in a mixture of acetone and dry ice. The frozen embedded tissue samples were cut into 8-μm-thick sections and were placed on silane-coated glass slides. The cryosections were air dried for 1 h and were washed in PBS. For performing the immunohistochemical staining of nectin-1, the sections were incubated with 5% fetal bovine serum and 0.1% Triton X-100 in PBS for 1 h, followed by overnight incubation at 4 °C with an anti-nectin-1 antibody in Can Get Signal Immunostain Solution A (Toyobo, Osaka, Japan). After rinsing with

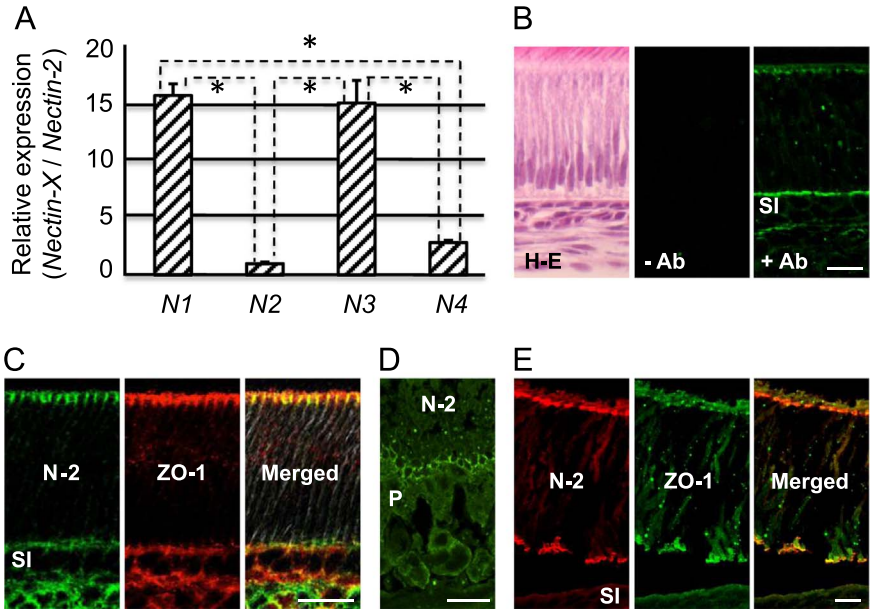

Fig. 1. Nectin expression in ameloblasts. (A) The mRNA expression of nectin genes in ameloblast-rich tissues obtained from the mouse mandibular incisors of 4-week-old WT mice. The mRNA expression levels of genes encoding nectin-1, nectin-3, and nectin-4 were normalized to that of the gene encoding nectin-2. *N1*, *N2*, *N3*, and *N4* denote genes encoding nectin-1, nectin-2, nectin-3, and nectin-4, respectively. Results are expressed as mean ± SD. Significant differences among four groups were evaluated using ANOVA; *$P < 0.05$; nectin-1 vs nectin-2, nectin-1 vs nectin-4, nectin-2 vs nectin-3, and nectin-3 vs nectin-4; n = 3. (B) Localization of nectin-2 in the mandibular incisors of 8-week-old WT mice. Left, enamel organs in the secretory stage stained with H-E stain. Right, the apical end of ameloblasts and the interface between ameloblasts and SI layer stained with anti-nectin-2 antibody. Center, negative control. (C) Colocalization of nectin-2 with ZO-1. Enamel organs in the secretory stage were stained with anti-nectin-2 and anti-ZO-1 antibodies. (D) Nectin-2 expression in ameloblasts but not in papillary layer. Enamel organs in the maturation stage were stained with anti-nectin-2 antibody. P, papillary layer. (E) Ameloblasts detached from the SI layer showed the localization of nectin-2 with ZO-1. Enamel organs in the secretory stage were stained with anti-nectin-2 and anti-ZO-1 antibodies. Scale bar = 20 μm.

Please cite this article as: Kawashima T, et al. Dynamic expression of nectins in enamel organs of mouse incisors. J Oral Biosci (2017), http://dx.doi.org/10.1016/j.job.2017.06.004