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Short communication

Immunohistochemical localization of transcription factor Sp3 during dental enamel development in rat tooth germ

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Sp3, a member of the Sp family of transcription factors, has previously been thought to be ubiquitously expressed, and its expression pattern in tooth development is not clear. This study was carried out to investigate the immunolocalization of Sp3 during the development of rat tooth germs. Sprague–Dawley rats at ages of 1, 3, 7, 10, and 14 d were used to represent different stages of tooth development. First mandibular molar tooth germs were sectioned and studied by immunohistochemistry. Sp3 was found to be localized within the nuclei of cells in developing tooth germs; however, ameloblast nuclei showed variable intensities at different developmental stages. At the same time, the positive signals in odontoblast nuclei remained stable. The results suggest that Sp3 may play a role in the development of teeth, specifically in the transcription of enamel-specific genes.

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For each gene, transcription is regulated by the binding of transcription factors to distinct promoter and enhancer elements. Many promoters and enhancers of housekeeping, tissue-specific, and viral genes contain functionally important GC-rich DNA motifs. These motifs are recognized by a large family of transcription factors that have been designated as the Sp family (1). Members of the Sp family proteins include Sp1–Sp9, which are all characterized by a highly conserved DNA-binding domain at the C terminus (2–12). Sp1, Sp3, and Sp4 are most closely related, manifested by their ability to bind the classical GC-box (GGGCGGG), as well as related motifs, with identical affinity (2, 13, 14).

Recently, targeted mutations of the mouse genes encoding Sp1, Sp3, Sp4, Sp5, and Sp8 have been reported (4, 5, 7, 15–17). SUPP *et al.* (15) found that two-thirds of Sp4 knockout animals died within the first few days after birth. The surviving animals were smaller, and the males lost their ability to reproduce. The strongest phenotypes of mutations were in Sp1 and Sp3 mutant mice. MARIN *et al.* (16) also found that Sp1-targeted embryos died at embryonic day E10. Sp3 null embryos were growth and died invariably of respiratory failure at birth. In addition, Sp3−/− mice showed pronounced defects in late tooth and bone development (4, 5). It was also reported that the enamel layer of the developing teeth was impaired owing to a lack of the ameloblast-specific gene products, amelogenin and ameloblastin (4). Together these observations suggested that Sp3 might participate and play some specific roles in tooth formation, particularly in the formation of enamel.

However, Sp3 has previously been thought to be ubiquitously expressed. Therefore, the purpose of this study was to gain a better understanding of the function of Sp3 in tooth development. An immunohistochemical technique was used to demonstrate temporal and spatial localization of Sp3 from late bell stage to mineralization stage of rat molar tooth germs.

Material and methods

Sprague–Dawley rats with litters were purchased from Vital (Beijing, China). Rats were killed in a humane manner, in accordance with the guidelines of the Peking University Animal Care Committee. For developmental studies, young postnatal (1, 3, 7, 10, and 14 d) rats were used. At each developmental stage, tissues from 6 animals were studied. For a better understanding of Sp3 localization patterns in different types of rat tooth formation, the lower incisors of 3 adult rats (2 months of age) were also studied.

Mandibles were dissected and fixed overnight in 4% paraformaldehyde (PFA) buffered with 0.1 M phosphate-buffered saline (PBS), pH 7.4, at 4°C, then decalcified in 10% ethylene diaminetetraacetic acid (EDTA)/PBS solution, dehydrated in a graded series of ethanol, and embedded in paraffin. Finally, serial sections of 5 μm were obtained.

Immunoreactions were performed according to the instructions of the PoverVision Histostain SP kit (Zhongshan, Beijing, China). All reactions were carried out in a humidified chamber at room temperature. Briefly, sections were treated against endogenous peroxidase and 3% (v/v) hydrogen peroxide, then blocked with 10% (v/v) goat serum for 10 min, heated (autoclave 121°C, 2 atm) for

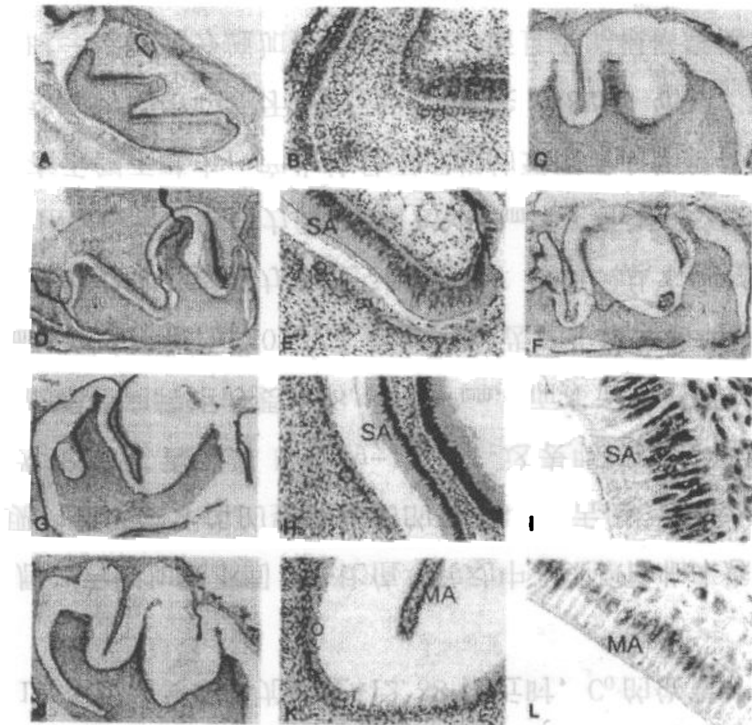

Fig. 1. Immunolocalization of Sp3 in sections of rat molar tooth germs at 1 (A, B), 3 (D, E), 7 (G, H, I), 10 (J, K, L), and 14 (C) days, and in the negative control (F). Positive signals of Sp3 were seen in cells differentiated from enamel organ cells and dental papilla. The staining intensity of Sp3 was weak in the nuclei of preameloblasts (PA) at 1 d (A, B), moderate in the nuclei of early secretory-stage ameloblasts (SA) at 3 d (D, E), strong in the secretory-stage ameloblasts (SA) at 7 d (G, H, I), very weak in the nuclei of late secretory, transition- and early maturation-stage ameloblasts (MA) at 10 d (J, K, L), and almost negative in the late maturation-stage ameloblasts at 14 d (C). A, C, D, F, G, and J, original magnification × 40; B, E, H, and K, original magnification × 200; I and L, original magnification × 1000. O, odontoblast, PO, preodontoblast.

10 min in 0.01 M citrate buffer (pH 6.0), then further incubated with a primary polyclonal rabbit anti-Sp3 immunoglobulin (Santa Cruz Biotechnologies, Santa Cruz, CA, USA; 1 : 100 dilution) for 1 h. Biotinylated goat anti-rabbit immunoglobulin G (IgG) molecules were applied as secondary antibodies for 20 min at room temperature. Sections were exposed to streptavidin–peroxidase conjugate for 10 min, then visualized by the application of diaminobenzidine (DAB) solution (Sigma, St Louis, MO, USA; 1 mg DAB/1.5 ml PBS + 1.2 ml H_2O_2) for ≈ 3 min. Finally, the sections were lightly counterstained with hematoxylin. Controls were run by omitting the primary antibody or with non-specific IgG.

Results and discussion

In the sections taken from 1 to 14 d-old rats, positive immunostaining for Sp3 was seen ubiquitously in rat tooth germs. The positive staining was localized in the nuclei of cells. Figure 1A,B shows that in the 1-d group, Sp3 was present in the nuclei of cells throughout the enamel organ. Sp3 could also be seen in the nuclei of odontoblasts and in cells of the dental papilla.

In 3-d-old rats, positive staining for Sp3 showed a similar pattern to that of 1-d-old rats, but the Sp3 signals in the nuclei of ameloblasts (representing the early secretory stage) were more intense than that in the 1-d group. (Fig. 1D,E). Positive staining was also seen in the nuclei of odontoblasts, and the intensity was similar to that of the 1-d group.

In sections from 7-d-old rats, Sp3 was strongly stained in the nuclei of secretory ameloblasts (Fig. 1G–I), while the nuclear staining in odontoblasts and pulp cells remained moderate, as in the previous stages.

At 10 d, the staining intensity of Sp3 in the nuclei of ameloblasts (representing the late secretory, transition and early maturation stages) was very weak (Fig. 1J–L). The staining of Sp3 in odontoblasts was still of moderate intensity, similar to that in the previous groups.

In sections from 14-d-old rats, staining of Sp3 had decreased and was no longer detectable in the nuclei of ameloblasts in the late-maturation stage (Fig. 1C), while it was still of moderate intensity in odontoblasts and pulp cells. Negative controls showed no signals (Fig. 1F).

In sections taken from rat incisors, staining of Sp3 was similar to that of molars. Staining in nuclei was weak in preameloblasts, stronger in secretory ameloblasts, and almost undetectable in maturation-stage ameloblasts (Fig. 2A–D). Odontoblast nuclear staining was moderate throughout.

Taken together, this study demonstrated that Sp3 is extensively distributed (as seen by immunolocalization) in the nuclei of many cells in tooth germs, including enamel

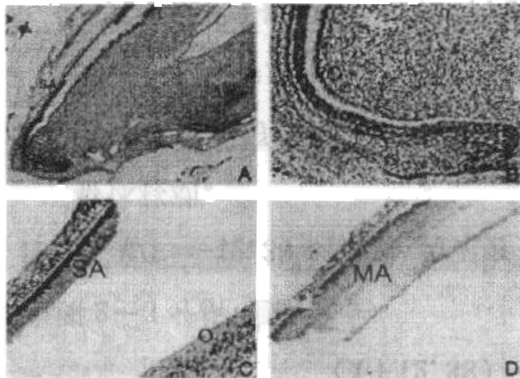

Fig. 2. Immunolocalization of Sp3 in the sections from rat incisors: sections at a low-power view (A). The staining of Sp3 is weaker in preameloblasts (PA) than in secretory ameloblasts (SA) (B and C). The staining is almost negative in maturation-stage ameloblasts (MA) (D). *A*, original magnification × 40; B, C, and D, original magnification × 200. O, odontoblast; PO, preodontoblast.

organ cells and dental papilla expression lasts until late tooth developmental stages. Sp3 is an important transcription factor for many genes and is essential for normal mouse embryogenesis (2,4), and Sp3 may also play some special role in odontogenesis.

In contrast to what was previously thought (i.e. that Sp3 is ubiquitously expressed), we found that the staining intensity of Sp3 in the nuclei of ameloblasts differed between different developmental stages. At the stage when the inner enamel epithelium differentiates from preameloblasts to early secretory ameloblasts (1–3-d tooth germs in the rat), staining of Sp3 in the ameloblast nuclei was weak. At a later stage (7-d tooth germs), when those ameloblasts have differentiated into secretory ameloblasts that secrete huge amounts of enamel matrix proteins, the Sp3 signals had their strongest intensity in the nuclei of ameloblasts. These extracellular enamel matrix molecules, which include amelogenin, tuftelin, ameloblastin, enamelin, and proteases, are thought to guide the unique size, shape, and orientation of the enamel crystallites (18). This may be taken to indicate that Sp3 participates in regulating their gene transcription. This assumption is further supported by the observation that Sp3 staining in the nuclei of maturation ameloblasts was remarkably decreased at 10 d and virtually negative at 14 d. During days 10–14, rat enamel is in a rapidly mineralizing stage. The secretion of enamel matrix proteins has essentially come to a halt and, instead, enamel proteins are degraded and removed in parallel with the increasing degree of mineralization. At this later stage, we thus found the staining intensity of Sp3 to have been sharply decreased. The results from rat incisors showed that the immunolocalization pattern of Sp3 in incisors was similar to that in molars.

In conclusion, the results of this study suggest that Sp3 may participate in regulating the transcription of enamel-specific genes.

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