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Expression of bone morphogenetic protein7 in developing rat tooth

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Abstract

Background: Bone morphogenetic proteins (BMPs) are secretory signal molecules which have a variety of regulatory functions during morphogenesis and cell differentiation. Teeth are typical examples of vertebrate organs in which development is controlled by sequential and reciprocal signaling between the epithelium and mesenchyme. In addition, tooth development is characterized by formation of mineralized tissues, dentin and cementum as well as epithelially derived enamel. BMp 7 plays a role with other signaling molecules in tooth development.

Aim of the study: To perform immune-histochemical analysis of the expression of BMP7starting from initiation of tooth development to completion of crown morphogenesis when dentine and enamel matrices are being deposited in rat incisor tooth **Materials and Methods:** Sixteen rat embryos were obtained from pregnant rats in gestation periods 16^{th},17^{th},18^{th},20^{th} intrauterine life and eight neonatal one day and 3 day old rat were included in this study . Each period involved 4 embryos or neonatal rats. Premaxilla (containing incisor teeth) was dissected and fixed in 10% buffered formalin and embedded in paraffin. Block sections (4 μm thickness) for Immunohistochemical localization of BMP7.

Results: Positive expression of BMP7 was recorded in ectoderm derived cells only, in developing tooth germ include oral epithelia, dental lamina and inner enamel epithelia in gestation period 16^{th},$17^{th}$and 18^{th}intrauterin life of rat embryo. Then the expression was illustrated in specialized cell of dental papilla the odontoblast and the developing dentin matrix .Ameloblast cell expressed too BMP7 in its formative and maturative stages.

Conclusion: Bone morphogenetic protein 7 may play key roles in mediating induction, differentiation events during tooth development.

Key words: bone morphogenetic protein, tooth germ, gestation period, ameloblast, odontoblast, oral epithelia

Introduction

The developing tooth is an excellent model to studyvarious aspects of developmental regulation. Teethstart as thickenings of the ectoderm covering the facialprocesses. As a result of sequential andreciprocalinteractions with the underlying neural-crest derived

mesenchymal cells the epithelium buds and undergoesfolding morphogenesis resulting in the establishmentof the form of the tooth crown [1,2]

Many works have indicated that growth factors, including endothelial growth factor (EGF), fibroblast growth factors (FGFs), and BMPs participate in epithelial-mesenchymal signaling regulating tooth morphogenesis [3,4]

A numerous growth factors normally expressed during primary odontogenesis and members of the transforming growth factor beta (TGF-beta) superfamily, including several members of the bone morphogenetic protein family (e.g. BMP-2, BMP-7), and insulin-like growth factor-1 (IGF-1) appear to play a key part in the induction of odontoblast-like cell differentiation from progenitor pulpal cells. A number of these growth factors are incorporated into the developing dentin matrix during initial tooth formation, forming a reservoir from which they can be released following dentin breakdown [5].

The roles of BMPs in embryonic development and cellular functions in postnatal and adult animals have been extensively studied in recent year. Tooth development isregulated by interactions between epithelial and mesenchyml tissues. Bone morphogenetic proteins (BMPs), together with the transcription factors play important roles in these interactions during early tooth morphogenesis [6]. To investigate the involvement of this signaling pathway in tooth development, we analyzed the expression Bmp7 in the tooth germ of rat incisor.

Materials and Methods

Sixteen rat embryos were obtained from pregnant rats in gestation periods 16^{th},17^{th},18^{th},20^{th} intrauterine life and eight neonatal one day and 3 day old rat were included in this study. Each period involved 4 embryos or neonatal rats. Premaxilla (containing incisor teeth) was dissected and fixed in 10% buffered formalin and embedded in paraffin. Block sections (4 μm thickness) for Immunohistochemical localization of BMP7. Following manufacture's protocol (Santa Cruz Biotechnology, Santa Cruz, CA) for detection and immunolocalization of anti-BMP7 (Abcam, Cambridge, MA) were used. After overnight incubation with primary antibodies in a humidity chamber, sections were rinsed with phosphate-buffered saline and incubated with a secondary antibody. Sections were then incubated with Biotin avidin–horseradish peroxidase conjugate for 30 min in humidity chamber. After washing in phosphate-buffered saline, the double linking procedure with the secondary antibody was repeated. Slides were developed with diaminobenzadine (DAB) solution and counterstained with Mayer's hematoxylin for 3–5 min. Counterstained slides were dehydrated in graded ethanol and cleared in xylene.

The same procedures were performed for negative controls except for the omission of the primary antibodies [7].

Results

Positive immunohistochemical localization of BMP7 was recorded in oral epithelium only in early bud stage of tooth germ at 16^{th} day of gestation period (Figure1).

At 17^{th} day intrauterine life, tooth germ shows more development in bud stage with positive expression of BMP7 in oral epithelium and dental lamina, (Figures 2 and 3).

At 18^{th} day intrauterine life, tooth germ at cap stage shows positive expression of BMP7 in oral epithelia,

dental lamina and inner enamel epithelia (Figure 4).

At 20th day intrauterine life, tooth germ at advance bell stage shows positive expression of BMP7 in odontoblast cell and predentin layer (Figure5).

Ameloblast cell in formative and maturative stages illustrate positive expression for BMP7, odontoblast, stratum intermedium were also expressed positive reaction too (Figures 6 and 7).

Discussion

The largest subgroup of the TGF-j superfamily consistsof those proteins related to the BMPs. BMP-2, -4, and -7have been shown to bind to and signal through multipleBMP receptors. This multiplicity of receptors, as well as a growing family of downstream signaling molecules, increases the spectrum of physiological responses thatcan be regulated by these proteins [(8)].

In this study the developing tooth revealed,a complex spatial and multiple expression patterns for BMP7 in dental tissue of different embryonic origin.The present results illustrate expression of BMP7in dental epithelium at early stages of tooth development, and as tooth development proceeds from bud to cap stage, BMP 7 localized in a restricted epithelial cell population,the inner enamel epithelia in the region of the forming enamel knot, which is an organizing center believed to be involved in establishing tooth form [(9)]; Therefore BMP7 may play a role with other signaling molecules in initiation, induction and differentiation of dental cells during tooth development.

As the tooth germ be in bell stage BMP7expression shifts to the mesenchymal layer that have been differentiated into odontoblasts and deposit predentin, these findings illustrate the possible roles of BMP-7 in mediating inductive events during the initiation phase of odontogenesis [(10)]. Differentiation of inner enamel epithelium into ameloblast cell which shows positive expression of BMP7 may demonstrate that BMP7 is a candidate molecule for epithelial-mesenchymal interaction signaling during initial stages of tooth formation.

The present findings also investigate positive expression of BMP7 by ameloblast in maturative stage and stratum intermedium that may reflect a role of BMP7 in mineralization process of enamel, since that ameloblast in this stage with stratum intermedium are encaged with maturation of the enamel [(11)]. The present study concludes that BMP-7 which is a member of the BMP family of signaling molecules may play key roles in mediating inductive events during embryogenesis of tooth germ.

Conclusion

Expression of BMP7 in developing rat tooth suggests a variety of regulatory functions in morphogenesis and cell differentiation of dental tissue.

References

1- Moioli EK, Clark PA, Xin X,Lal S, Mao JJ (2007). Matrices and scaffolds for drug delivery in dental, oral and craniofacial tissue engineering. *Adv Drug Deliv Rev* 59:308-324.
2- Hargreaves KM, Giesler T, Henry M, Wang Y (2008). Regeneration potential of the young permanent tooth: what does the future hold?.*J Endod* 34:S51-56.
3- Itoh F, Asao H, Sugamura K, Heldin CH, Dijke P, Itoh S (2001).Promoting bone morphogenetic protein signaling through negative regulation of inhibitory Smads. *EMBO J* 20: 4132-4142.
4- Chen D, Zhao M, Mundy GR (2004). Bone morphogenetic proteins. *Growth Factors* 22: 233-241.

5- Myers DC, Sepich DS, Solnica-Krezel L (2002). Bmp activity gradient regulates convergent extension during zebrafish gastrulation. *DevBiol* 243: 81-98.
6- Vaccaro AR, Whang PG, Patel T, Phillips FM, Anderson DG, Albert TJ, Hilibrand AS, Brower RS, Kurd MF, Appannagari A, Patel M, Fischgrund JS (2008). The safety and efficacy of OP-1 (rhBMP-7) as a replacement for iliac crest autograft for posterolateral lumbar arthrodesis: minimum 4-year follow-up. *Spine J*8:457-465.
7- Stosich MS, Bastian B, Marion NW, Clark PA, Reilly G, Mao JJ (2007). Immunohistochemical evaluation of vascularized adipose tissue grafts from human mesenchymal stem cells with bioactive cues and microchannel conduits. *Tissue Eng* 13:2881.
8- Nakashima M,Reddi AH (2003). The application of bone morphogenetic proteins to dental tissue engineering. *Nat Biotechnol* 21:1025-1032.
9- Ten AR (2007). Dentin and pulp. In: Ten Cate A.R., editor. Oral Histology. London: Mosby. pp. 246-278.
10- Jin YK, Xuejun X, Eduardo KM,Jenny C, Chang HL, Susan YF, Peter DK, Jeremy JM (2010). Regeneration of dental-pulp-like tissue by chemotaxis-induced cell homing. *Tissue Eng* Part A 16: 3023-3031.
11- Paine ML,Luo W, Wang HJ,Bringas PJ,Ngan AY, Miklus VG, Zhu DH, MacDougall M, White SN, Snead ML (2005). Dentin sialoprotein and dentin phosphoprotein overexpression during amelogenesis. J BiolChem 280:31991-31998.

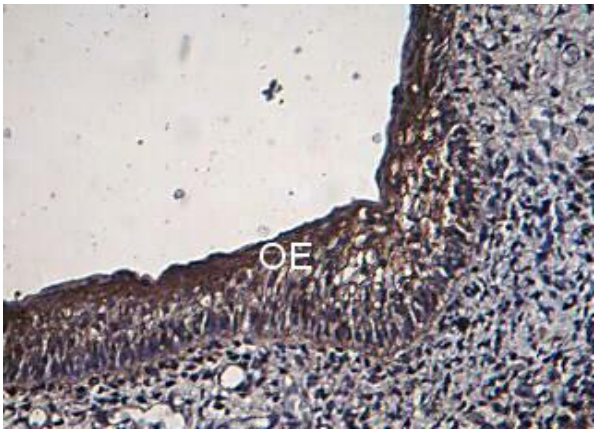

Figure 1: Tooth germ at early bud stage at (16th day IUL) shows positive expression of BMP7 in oral epithelium (OE). DAB with counter Hematoxylin stain X200

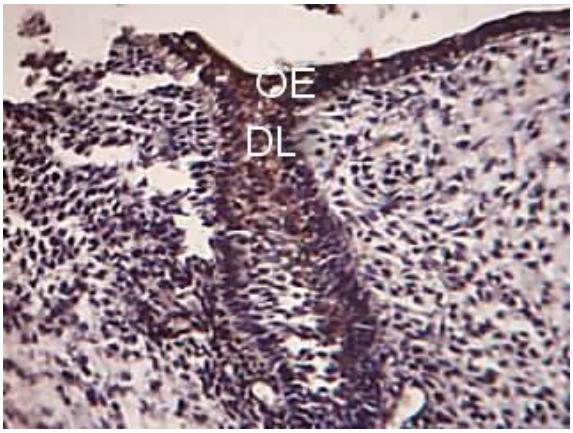

Figure 2: Tooth germ in bud stage at (17th day IUL) shows positive expression of BMP7 in oral epithelium (OE) and dental lamina (DL). DAB with counter Hematoxylin stain X200

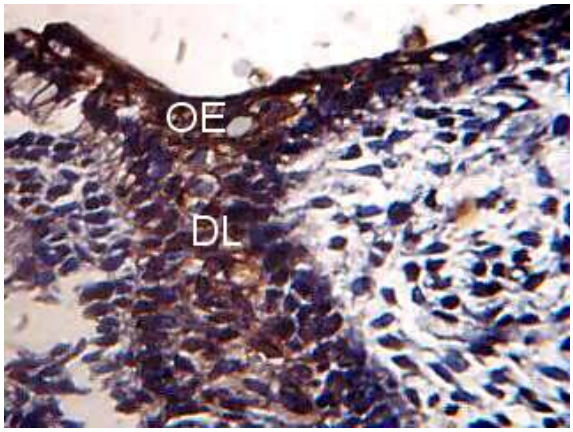

Figure 3: Magnifying view of previous figure (2) shows positive expression of BMP7 in oral epithelium(OE) and dental lamina (DL).DAB with counter Hematoxylin stain X400

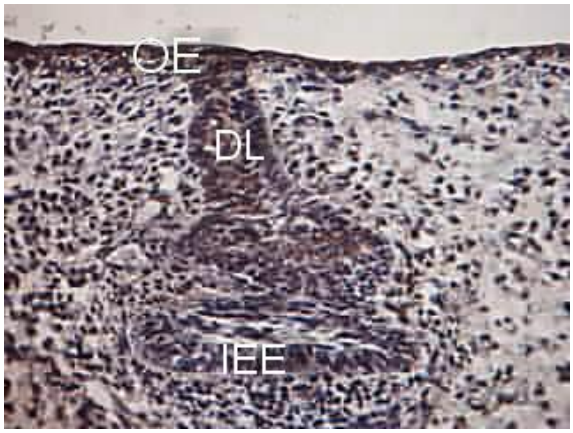

Figure 4: Tooth germ in cap stage at (18^{tn} day IUL) shows positive expression of BMP7 in oral epithelium (OE), dental lamina (DL) and inner enamel epithelia (IEE). DAB with counter Hematoxylin stain X200

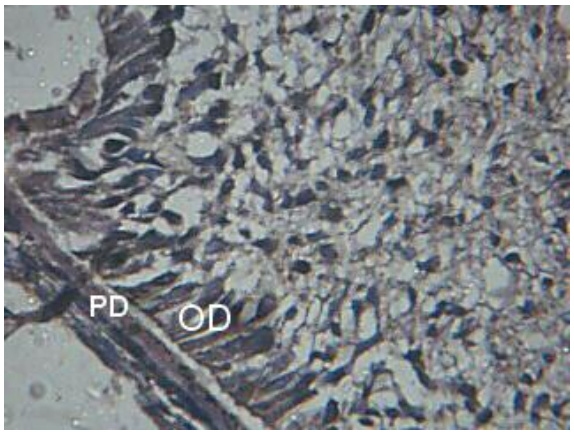

Figure 5: tooth germ in advanced bell stage at (20th day IUL) shows positive expression of BMP7 in Odontoblast (OD) and predentin (PD). DAB with counter Hematoxylin stain X200

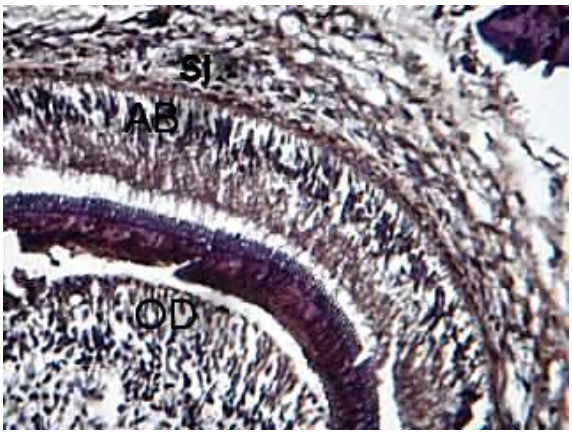

Figure 6: Tooth germ in advanced bell stage at (1 day neonatal life) shows positive expression of BMP7 in Odontoblast (OD), ameloblast (AB) in formative stage and stratum intermedium (SI). DAB with counter Hematoxylin stain X200

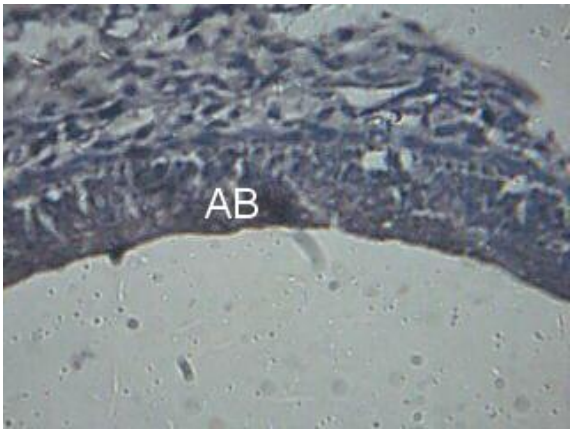

Figure 7: positive expression of BMP7 by ameloblast (AB) in maturative stage at 3 day neonatal life. DAB with counter hematoxylin stain X200