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Localization of STRO-1, BMP-2/-3/-7, BMP receptors and phosphorylated Smad-1 during the formation of mouse periodontium

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Abstract

Bone morphogenetic proteins (BMPs) and BMP receptors (BMPRs) are known to regulate the development of calcified tissues by directing mesenchymal precursor cells differentiation. However, their role in the formation of tooth-supporting tissues remains unclear. We investigated the distribution pattern of STRO-1, a marker of mesenchymal progenitor cells and several members of the BMP pathway during the development of mouse molar periodontium, from the post-natal days 6 to 23 (D6 to D23). STRO-1 was mainly localized in the dental follicle (DF) at D6 and 13 then in the periodontal ligament (PDL) at D23. BMP-2 and -7 were detected in Hertwig's epithelial root sheath (HERS) and in DF, then later in differentiated periodontal cells. BMP-3 was detected after D13 of the periodontal development. BMPRs-Ib, -II, the activin receptor-1 (ActR-1) and the phosphorylated Smad1 were detected in DF and HERS at D6 and later more diffusely in the periodontium. BMPR-Ia detection was restricted to alveolar bone. These findings were in agreement with others data obtained with mouse immortalized DF cells. These results suggest that STRO-1 positive DF cells may be target of BMPs secreted by HERS. BMP-3 might be involved in the arrest of this process by inhibiting the signaling provided by cementogenic and osteogenic BMPs.

Keywords: Bone morphogenetic protein; Mesenchymal cells; Precursor cells; Hertwig's epithelial root sheath; Dental follicle

1. Introduction

Mesenchymal cell precursors with multipotential properties have been localized in the periodontal ligament (PDL) in humans (Seo et al., 2004). These cells express STRO-1, a cell surface antigen characteristic of mesenchymal progenitor cells present in bone marrow (Simmons et al., 1994) and may differentiate into highly specialized periodontal cells (Seo et al., 2004). The dental follicle (DF) surrounding the developing tooth germ is an ectomesenchymal tissue composed of heterogeneous cell populations derived from the cranial neural crest which also include periodontal precursors (Morsczeck et al., 2005; Ivanovski et al., 2006; Kémoun et al., 2007). Although their capacity to differentiate into various cell types including osteoblasts and/or cementoblasts has also been demonstrated (Hakki et al., 2001; Zhao et al., 2002; Luan et al., 2006), expression of STRO-1 by mouse DF cells has never been investigated.

Bone morphogenetic proteins (BMPs) are involved in the development of numerous skeletal and extra-skeletal organs and tissues in vertebrates (Chen et al., 2004). BMP-2 and -7 induce rodent mesenchymal cells to differentiate into osteoblasts (Zhu et al., 2004), while BMP-3 down-regulates

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bone mineralization and density (Daluiski et al., 2001). In monkeys, BMP-2 and -7 also stimulate cementogenesis during periodontium formation (Taba et al., 2005). In-vitro experiments have shown that BMP-2 induces mouse DF cells to differentiate towards the osteo-cementoblastic phenotype (Zhao et al., 2002). Enamel matrix derivative (EMD), immature enamel extracts from porcine teeth used in periodontal regenerative therapies, contain BMP-2 and -7 (Takayama et al., 2005) and enhance the expression of CP-23, a marker of cementoblastic differentiation (Alvarez-Perez et al., 2006) by human dental follicle cells (Kémoun et al., 2007).

Several members of the BMP family have been localized during the development of mouse periodontium (Helder et al., 1998; Thomadakis et al., 1999; Yamashiro et al., 2003; Yamamoto et al., 2004). BMP-2, -3 and -7 have been detected in periodontal tissues from post-natal day 12 to 18 (Thomadakis et al., 1999) and BMP-3 transcripts were detected in DF cells, cementoblasts and alveolar osteoblasts (Yamashiro et al., 2003). However, some discrepancies remain regarding the localization of BMPs during periodontium development.

BMPs activate target cells by binding to type-Ia, -Ib and -II BMP receptors (BMPR), and to the activin receptor-1 (ActR-1) (Chen et al., 2004). These transmembrane receptors recruit and phosphorylate cytoplasmic proteins, especially the receptor-regulated signal transducers Smads 1, 5 and 8. Once phosphorylated, Smads bind to specific partners, translocate to the nucleus and up-regulate specific genes (Chen et al., 2004).

The aim of this study was to localize by immunohistochemistry STRO-1, BMPs, BMPRs and phosphorylated Smad1 (pSmad1) during initial, intermediate and late stages of mouse periodontium formation at day 6, 13 and 23 (D6, D13, D23). These results demonstrate, for the first time to our knowledge, the presence of STRO-1 positive cells in mouse DF, in which BMPR-Ib, -II, ActR-1 and pSmad1 were also detected. In addition, immunofluorescence and FACS analysis performed with immortalized mouse DF cells isolated from the first lower molar at D6 confirmed the expression of BMPRs by STRO-1 positive cells. CP-23 expression by DF cells was detected under EMD treatment and was partially inhibited by Noggin, a BMP antagonist. These results suggest that immortalized mouse DF cells may differentiate toward a cementoblastic phenotype, dependent on BMP-pathways. Furthermore, BMP-2 and -7 were strongly detected in HERS at early stages of tooth development. Altogether, these data suggest that STRO-1 positive DF cells are periodontal progenitor cells target of BMPs expressed by HERS.

2. Materials and methods

2.1. Materials

EMD gel was obtained from Straumann (Basel, Switzerland). Recombinant Noggin was purchased from R&D System Inc. (Minneapolis, MN, USA). The anti-STRO-1 monoclonal antibody (IgM) was purchased from the Developmental Hybridoma Study Bank (Iowa City, USA). Goat polyclonal antibodies against BMP-2 and -7 were from SantaCruz Biotechnologies Inc. (SantaCruz, USA). Goat polyclonal antibody against BMP-3 was from R and D Systems (R and D Systems, Inc., Minneapolis, MN, USA). Rabbit polyclonal antibodies that can recognize pSmad1 and both cell membrane localization and an intra-cellular expression of BMP receptors (BMPR-Ia, -Ib and -II and ActR-1) were a kind gift from Drs C.H. Heldin and P. TenDijke (Heldin et al., 1997; Rosendahl et al., 2002). The rabbit polyclonal antibody against cementum protein-23 (CP-23) was previously described (Alvarez-Perez et al., 2006). Dulbeco modified eagle medium (DMEM) was from Gibco BRL (Gaithersburg, MD, USA). Slide chambers were from Nalge Nunc Int (Rochester, NY, USA). Vectastain Elite ABC Kit, M.O.M kit and DAB Substrate Kit was from Vector Laboratories (Burlingame, CA, USA). Zenon Rabbit labeling kit Alexa Fluor 647, AlexaFluor 633 goat anti-rabbit IgG, penicillin and streptomycin were from Invitrogen (Cergy Pontoise, France). Isotype-matched control antibodies were from Serotec (Cergy Saint-Christophe, France). All other materials were from Sigma (St Louis, MO, USA).

2.2. Tissue preparation

Twelve mouse pups (breed ICR CD-1) from D6, 13 and 23 (four per time-point) were decapitated after cervical dislocation according to the European Animal Ethics Committee. Heads were fixed in 3.7% paraformaldehyde (pH 7.0) for 2 to 4 days. Hemi-mandibles were separated and decalcified in 10% EDTA for 6–10 days. Tissues were then dehydrated through graded ethanol series, embedded in paraffin and 4-μm-thick sections were performed.

2.3. Cell cultures

Mouse DF cells were obtained from first lower molars of D6 mice and cultured as described previously (Zhao et al., 2002). Briefly, tooth germs were removed under a dissecting microscope and subjected to enzymatic digestion (0.6 mg/mL of collagenase A and 0.25% (v/v) trypsin) in DMEM for 1 h, at 37 °C. The suspension was centrifuged at 1000 rpm for 5 min. Cellular pellets were resuspended and cultured in DMEM supplemented with 100 U/mL penicillin and 100 ng/mL streptomycin (i.e.: basal medium) containing 10% (v/v) FCS, at 37 °C in a humidified atmosphere of 5% CO_2. In order to obtain viable material for coverglass culture and confocal microscopy analysis, mouse DF primary cells were immortalized in the presence of pAS, a plasmid encoding the early functions of SV40 as previously described (Chatelut et al., 1998). Cells were considered immortalized when their proliferation rate was 10-fold that of non-immortalized cells. Immortalized mouse DF cells were