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Osteopontin deficiency enhances parathyroid hormone/parathyroid hormone related peptide receptor (PPR) signaling-induced alteration in tooth formation and odontoblastic morphology

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Abstract

Parathyroid hormone/parathyroid hormone-related protein receptor (PPR) signaling is known to be involved in tooth development. In bone, extracellular matrix protein osteopontin (OPN) is a negative regulator of PPR signaling in bone formation. However, the role of OPN in modulation of PPR action in tooth development is not understood. Therefore, we examined the tooth in double mutant mice. Constitutively active PPR was expressed specifically in the odontoblasts and osteoblasts (caPPR-tg) in the presence or absence of OPN. Radiographic analysis indicated that the length of the third molar (M3) and the incisor was decreased in the caPPR-tg mice compared to wild type, and such reduction in molar and incisor length was further enhanced in the absence of OPN (caPPR-tg OPN-KO). With respect to histology of incisors, caPPR-tg induced high cellularity and irregularity in odontoblastic shape and this was enhanced by the absence of OPN. These morphological observations suggest that OPN modulates PPR signaling that are involved in tooth formation.

Keywords

Osteopontin; Parathyroid hormone; Receptor; Signaling; Tooth formation; Odontoblast

1. Introduction

Intracellular signaling induced by parathyroid hormone (PTH) is based on its receptor, PTH/PTH-related protein (PTHrP) receptor (PPR). Upon ligand activation, PPR accumulates cyclic AMP, and stimulates PKA-CREB pathway. These signaling lead to changes in the target gene expression. When constitutively active PPR (caPPR) was specifically expressed in type I collagen-producing cells such as odontoblasts and osteoblasts by utilizing *Col1a1*-caPPR transgenic (caPPR-tg) mice, PPR signaling pathway in these cells could be examined (Calvi et al., 2001, 2003; Adams et al., 2007).

We recently reported that osteopontin (OPN) negatively regulates PPR signaling during bone formation by investigating the mice obtained by crossing caPPR-tg mice with OPN-null mice (Ono et al., 2008). OPN is a major non-collagenous protein produced by osteoblasts (Noda et al., 1990). Although the mechanisms by which OPN exerts its inhibitory function on the bone in ca-PPR-tg mice have not been clarified yet, OPN interaction with PPR was found in not only long bone but also in spine.

In tooth, PPR signaling was reported to play a role in development. PTHrP is expressed in inner and outer enamel epithelia (Beck et al., 1995; Lee et al., 1995; Liu et al., 1998), while PPR is expressed in dental papilla, dental follicle (Lee et al., 1995; Liu et al., 1998) and odontoblasts (Calvi et al., 2004; Lundgren et al., 1998). IPThrP was shown to be required for tooth eruption (Kitahara et al., 2002; Philbrick et al., 1998). In addition, it was shown that caPPR-tg mice had delayed odontoblastic differentiation resulting in reduction of dentin matrix in the fetuses and the neonates. Although OPN is also expressed in teeth, the interaction of OPN with PPR in tooth formation is not known yet.

Here, we examined the effects of OPN deficiency on PPR signaling in tooth. To do this, we analyzed the tooth in the double mutant mice obtained by crossing caPPR-tg and the OPN deficient mice.

2. Experimental procedures

2.1. Generation of OPN-deficient *Col1a1*-caPPR transgenic mice

OPN deficient (OPN-KO) mice in C57BL/6 × 129 mixed background (Rittling et al., 1998) and caPPR-transgenic mice in FVB/N background were previously reported (Calvi et al., 2001). These caPPR-tg mice express constitutively active Jansen type PTH/PTHrP receptor (HKrk-H223R) specifically in osteoblasts and odontoblasts by a 2.3 kb fragment of the mouse *Col1a1* promoter. The OPN-deficient caPPR-tg mice were generated by crossing male hemizygous caPPR-tg OPN^{+/-} mice with female OPN^{+/-} mice as described previously (Ono et al., 2008). 10-week-old male littermates of four genotypes (wild type, OPN-KO, PPR-tg, PPR-tg/OPN-KO) were analyzed. All experiments were performed according to institutionally approved guidelines for animal welfare.

2.2. X-ray analysis of tooth

Mandibles of the mice with four genotypes were radiographically analyzed using soft X-ray apparatus (Softex-CMB; Softex Co. Ltd. Ebina, Japan). The length and the thickness of incisors were measured with cotton thread and a scale directly applied on the radiographs, respectively. For thickness, each incisor was measured at 3 points quarterly dividing the curved longitudinal axis, and the average was compared among the experimental groups.

2.3. Histomorphometric analysis of tooth

Decalcified mandibles (10% formic acid) were embedded in paraffin, and 5 μ m thick sagittal sections were made and stained with hematoxyline and eosin.

2.4. Statistical analysis

Dates were expressed as the mean values $\pm$ S.D. Results were evaluated using Students' *t*-test or one-way analysis of variance followed by Tukey–Kramers' post hoc test. A *p* value of less than 0.05 was considered significant.

3. Results

3.1. OPN deficiency enhances constitutively active PPR signaling-induced events in tooth formation

To investigate the effects of PPR signaling and OPN deficiency in tooth formation, X-ray images of the mice (wild type, OPN-KO, caPPR-tg, and caPPR-tg/OPN-KO) were analyzed. Transgenic mice harboring the caPPR driven by 2.3 kb collagen promoter (caPPR-tg) exhibited abnormally rounded molars (Fig. 1A). Although the overall growth of the first to third molar (M1–M3) was relatively conserved, there was a significant reduction of the length (cusp-to-apex distance) in M3 in the caPPR-tg mice compared to wild type mice (Fig. 1A and B). With respect to the effect of OPN, we found that the OPN deficiency in the caPPR-tg background (caPPR-tg/OPN-KO) further decreased the length of M3 (Fig. 1A and B). No such effect of OPN deficiency alone was observed in the control background (OPN-KO vs wild type).

To examine whether the growth inhibitory effect of OPN deficiency on caPPR was observed in other tooth, the length of incisor in caPPR-tg mice was examined. CaPPR-tg shortens the length of incisors compared to wild type (caPPR-tg vs wild type) (Fig. 1C). OPN deficiency in the caPPR-tg background (caPPR-tg/OPN-KO) further reduced the length of incisor. No such effect was observed in the absence of OPN alone (Fig. 1C). In contrast to the decrease in the length of the incisors in the caPPR-tg mice, the thickness was increased in caPPR-tg mice compared to the wild type (Fig. 1D). Furthermore, OPN deficiency did not affect the levels of incisor thickness either in the absence or presence of caPPR-tg (OPN-KO and caPPR-tg/OPN-KO) (Fig. 1D), implying that the effects of OPN deficiency on the length of PPR-tg incisors was specific. Taken together, these results suggest that the constitutively active PPR signaling in odontoblasts inhibits longitudinal tooth growth and such inhibition is further enhanced by OPN deficiency.

3.2. OPN deficiency increase enhances PPR signaling-induced increase in dental pulp cells and odontoblastic cells

To obtain the morphological information on the cells, sagittal sections of incisors were analyzed. The base region of incisor is the location of undifferentiated mesenchymal cells that would affect the length of incisor. Therefore, we first focused on this region. We found that the mutant mice harboring the caPPR-tg (caPPR-tg and caPPR-tg/OPN-KO) had a larger mass in the base region compared to wild type (Fig. 2C vs A and D vs B). This cell mass formed longer and thicker basal portion of dental pulp (Fig. 2C and D). The incisors in caPPR-tg were thicker than the wild type controls (caPPR-wt/OPN^{+/+}) throughout the longitudinal axis of the tooth (Fig. 2A–D). Observation at higher magnification of the base root (green squares in A–D) indicated that the dental pulp cell-cellularity at this region was higher in the caPPR-tg than in the wild type mice (Fig. 2G,H and * vs E). In addition, OPN deficiency (OPN-KO) enhanced this enlargement in the size of base region of incisor (Fig. 2C vs D). In the double mutant mice (caPPR-tg/OPN-KO), there were small numbers of odontoblasts embedded in the dentin matrix (Fig. 2H, blue arrow heads). These observations

suggest that OPN deficiency enhances the effects of caPPR that increased cell mass at the base of incisor.

3.3. OPN deficiency exacerbated caPPR-induced irregularity in odontoblastic cells in incisor

Odontoblasts are the cells that form dentin. These cells differentiate from their precursor cells present at the base of dental pulp (Fig. 2 green square). To obtain further insight into the effects of OPN deficiency on caPPR-induced effects on odontoblasts, we next focused on the mid portion of the incisor (Fig. 2A–D, yellow square). In this region of the control mice, odontoblasts are aligned linearly in a single layer (Fig. 2I). These cells showed the typical columnar cell shape consisting with cells that are aligned to secrete dentin (Fig. 2I). Mice with OPN deficiency alone exhibited cell morphology similar to wild type (Fig. 2J). In contrast, caPPR-tg mice exhibited a thick layer of odontoblasts (Fig. 2K). In contrast to well-aligned odontoblasts in wild type, these cells are showing nuclei that aligned irregularly on the dentin matrix, (Fig. 2K vs I, yellow arrow head). These odontoblasts tended to lack linear alignment (Fig. 2K). These cells also failed to show the columnar appearance (Fig. 2K) suggesting that the attachment of odontoblasts may be impaired. OPN deficiency further enhanced these changes (Fig. 2L). The columnar odontoblasts were no longer observed and the odontoblastic layer was occupied with non-columnar cells (Fig. 2L). These observations suggest that OPN deficiency exacerbated caPPR-induced changes in the shape of odontoblastic cells in incisor.

4. Discussion

We have shown that OPN deficiency modulates caPPR signaling-induced morphological changes in tooth formation. We have also observed that the OPN deficiency enhances caPPR-induced increase in odontoblasts on the dentin in the incisor. OPN deficiency enhanced the caPPR-induced increase in the cellularity of odontoblastic cells, but also cell morphology. Our observations on OPN deficiency for the first time identified that OPN is a modulator of PPR-regulation of odontoblastic cells.

Causative relationship between OPN and caPPR-tg in odontoblasts regarding incisor formation is still not known. Following possibilities for OPN-targets in PPR-induced events may be thought based on our observations. Firstly, the inhibition of odontoblast maturation by PPR signaling might have resulted in retention of premature odontoblasts, increasing in their numbers. Secondly, the caPPR-tg by itself might have increased the number of the premature odontoblastic cells by promotion of proliferation. This is reminiscent to its function hypothesized in osteoblastic precursor cells in bone marrow. Thirdly, the recruitment of the odontoblastic precursor cells might have been increased by PPR signaling. These possibilities of OPN target in PPR signaling-induced events are not mutually exclusive, and combination of these might be also the case.

Our observations on OPN deficient mice with the caPPR-tg background (caPPR-tg/OPN-KO) suggest that OPN may suppress proliferation of odontoblast progenitor cells. OPN may also suppress maturation of the odontoblasts in the background of caPPR-tg. Thus, OPN-deficiency may affect both cell proliferation and differentiation in odontoblasts in caPPR background. The effects of OPN deficiency may be exerted in relatively immature odontoblasts that were increased by caPPR signaling.

We have identified OPN as a negative modulator of PPR signaling. However, this notion is not sufficiently substantiated by the current data. The additive effects of OPN deficiency on top of the phenotype of caPPR-tg mice could be mediated by an independent pathway, and not necessarily by modulation of PPR signaling. Although the additive contribution of both

pathways to the phenotype is clearly observed the possible interactions between OPN and PPR remain to be elucidated.

OPN has been shown to exert diverse functions including bone resorption (Yoshitake et al., 1999), mechanical stress (Ishijima et al., 2001) cell adhesion (Reinholt et al., 1990), apoptosis, inflammatory responses and tumor metastasis (Denhardt et al., 2001; Ohyama et al., 2004). In addition OPN suppresses bone marrow niches for hematopoietic stem cells. Such bone niche is also regulated by PPR signaling. Mesenchymal stem cells expressing nestin in bone marrow can support the hematopoietic stem cells as the niche cells (Méndez-Ferrer et al., 2010). If this would be similar in tooth, expression of both OPN and *Pthr1* may play a role in the stem cells control in the tooth formation. OPN expression in osteoblasts is increased by PPR signaling (Stier et al., 2005). If this is the case in odontoblasts, OPN may be a part of negative feedback system for PPR signaling in tooth.

In summary, we have shown that OPN deficiency enhances caPPR-induced phenotype in tooth. Our results would help understand new aspects of biology in tooth formation.

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Fig. 1. Morphometric analysis of the effects of OPN deficiency on the tooth of caPPR-tg mice. (A) Representative X-ray images of the mutant mouse mandibles. (B) The cusp-to-apex distance of the third molar (mm) was compared among the groups. The arrowheads (in B) indicate measured lengths. (C) The length of the incisor (mm) as indicated by curved red line indicated by arrowheads. (D) The thickness of the incisor (mm) was measured at 3 points equally dividing the red curve drawn in (D). The average values were compared among the groups. $*p < 0.01$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Fig. 2.

Histological analysis of incisors in mutant mice. Saggital sections of the periapical region of incisors from wild type (A), OPN-deficient (OPN-KO) (B), Col1a1-caPPRTg (C) and OPN-deficient Col1a1-caPPRTg (PPR-tg/OPN-KO) (D). Sections were stained with hematoxylin and eosin. Root apex is to the right. (E–H) Higher magnification images of the apical region indicated by green squares in A–D. (I–L) Higher magnification images of the mid portion indicated by yellow squares in A–D. (G and H) Asterisks (*) indicate high cell density of dental pulp cells observed in caPPR or caPPR-tg/OPN-KO mice. (H) Blue arrowheads indicate the odontoblasts present in the dentin matrix. (K) Yellow arrowheads indicate

odontoblastic cells with aberrant alignment. OD: odontoblasts (layer), PD: predentin, D: dentin matrix. Scalebars indicate 500 μm in A–D, and 30 μm in E–L.