Archives of Oral Biology 65 (2016) 82–86

Contents lists available at ScienceDirect

Archives of Oral Biology

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

Altered distribution of Ghrelin protein in mice molar development

Bo Liu[a,b], Xiuchun Han[a], Wei Feng[a], Jian Cui[a], Tomoka Hasegawa[c], Norio Amizuka[c], Xin Xu[a], Minqi Li[a,*]

[a] Department of Bone Metabolism, School of Stomatology, Shandong University, Shandong Provincial Key Laboratory of Oral Tissue Regeneration, Jinan, China
[b] Stomatology Department of Jining Medical University, China
[c] Department of Developmental Biology of Hard Tissue, Graduate School of Dental Medicine, Hokkaido University, Sapporo, Japan

ARTICLE INFO

Article history:
Received 12 May 2015
Received in revised form 25 January 2016
Accepted 31 January 2016

Keywords:
Ameloblast
Enamel epithelium
Ghrelin
Odontoblast
Odontogenesis

ABSTRACT

Objective: Ghrelin, an appetite-stimulating hormone, plays diverse regulatory functions in cell growth, proliferation, differentiation and apoptosis during mammalian development. There is limited information currently available regarding Ghrelin expression during mammalian tooth development, thus we aimed to establish the spatiotemporal expression of Ghrelin during murine molar odontogenesis.
Design: Immunohistochemistry was performed to detect the expression pattern of Ghrelin in mandible molar from E15.5 to PN7 during murine tooth development.
Results: The results showed that Ghrelin initially expressed in the inner enamel epithelium and the adjacent mesenchymal cells below, further with persistent expression in the ameloblasts and odontoblasts throughout the following developmental stages. In addition, Ghrelin was also present in Hertwig's epithelial root sheath at the beginning of tooth root formation.
Conclusions: These results suggest that Ghrelin was present in tooth organs throughout the stages of tooth development, especially in ameloblasts and odontoblasts with little spatiotemporal expression differences. However, the potential regulatory roles of this hormone in tooth development still need to be validated by functional studies.

1. Introduction

Tooth developmental processes involve sequential and reciprocal interactions between the oral ectoderm and neural-crest-derived mesenchyme. Epithelial thickening is the initial morphological manifestation of tooth development. The thickened epithelium progressively forms the bud, cap, and bell configurations within which epithelial cells and mesenchymal cells differentiate into enamel-secreting ameloblasts and dentin-secreting odontoblasts, respectively (Thesleff & Jernvall, 1997).

Ghrelin, an appetite-stimulating hormone, was first identified in extracts of rat and human stomach (Kojima et al., 1999). It has two major forms: Des-acylated and acylated; acylated Ghrelin is found to be of importance in physiological regulation via stimulating growth hormone expression by binding to the growth hormone secretagogue receptor (GHSR)-1α (Kojima & Kangawa, 2005). Ghrelin plays an essential role in body weight homeostasis through regulation of food intake and energy expenditure. It acts on hypothalamus by circulation to promote appetite and influences immune response, angiogenesis, hematopoiesis and bone formation (Fukushima et al., 2005).

Although secreted predominately by stomach tissue, Ghrelin is also expressed in many other tissues including the liver, lung, pancreas (Kojima & Kangawa, 2005), and kidney (Ghelardoni, Carnicelli, Frascarelli, Ronca-Testoni, & Zucchi, 2006). Furthermore, Ghrelin has been shown to modulate bone metabolism in an autocrine/paracrine fashion (Kim et al., 2005; Maccarinelli et al., 2005). Ghrelin is also expressed by osteoblasts, where it stimulates their proliferation and inhibits apoptosis (Fukushima et al., 2005). Ghrelin also regulates osteoclastogenesis, although whether it confers an anabolic or catabolic effect remains controversial (Costa et al., 2011; van der Velde et al., 2012). As with chondrocytes, Ghrelin protein has been detected in epiphyseal growth plate and chondrocyte cell lines and was found to modulate the metabolic activity of chondrocytes by interacting with an uncharacterized receptor (Caminos et al., 2005; Gomez, Lago, Gomez-Reino, Dieguez, & Gualillo, 2009).

Teeth and bone are mineralized tissues that share many similarities. Teeth, cartilage, and maxillofacial bone all originate embryologically from the neural crest (Gomez et al., 2009; Yoshida,

* Corresponding author at: Department of Bone Metabolism, School of Stomatology, Shandong University, Shandong Provincial Key Laboratory of Oral Tissue Regeneration, Wenhua West Road 44-1, Jinan 250012, China.
Fax: +86 531 88382095.
E-mail address: liminqi@sdu.edu.cn (M. Li).

http://dx.doi.org/10.1016/j.archoralbio.2016.01.019
0003-9969/

Vivatbutsiri, Morriss-Kay, Saga, & Iseki, 2008). The chemical compositions of teeth, especially dentin are very similar with those of bone. Particularly, many factors regulating organic substances secretion and mineral deposition are commonly shared between teeth and bone, such as bone morphogenetic proteins (BMPs), osteopontin (OPN), osteocalcin (OCN) and Cbfa1 (Runx2) (Chen et al., 2009; Chung et al., 2008; Morishita et al., 2011; Wang et al., 2012). Ghrelin is also present in murine and human teeth, being identified predominantly in odontoblasts and the pulp (Aydin et al., 2007, 2012). In this study, we assessed the immunohistochemical pattern of Ghrelin protein expression in mouse mandibular first molar tooth germs from E13.5-PN7 in an initial effort to characterize Ghrelin expression in the developing tooth.

2. Materials and methods

2.1. Tissue and section preparations

Embryonic and postnatal ICR mice were maintained and all experimental procedures were approved by the Committee on the Ethics of Animal Experiments of Shandong University and Hokkaido University. All surgeries were performed under chloral hydrate anesthesia, and all efforts were made to minimize suffering. The mice were obtained by mating male and female adult mice. The embryonic age was determined by the day when the vaginal plug was found and considered as embryonic day (E) 0.5 (E0.5), whereas the date of birth was regarded as postnatal day (PN) 0.5 (PN0.5). Three pregnant mice from each period (E13.5, E15.5 and E17.5) and four postnatal mice at series development periods (PN1, PN3, PN5, PN7) from different mothers were chosen and sacrificed for histological analysis. The head (E13.5, E15.5 and E17.5) and mandibles (PN1–PN7) of embryonic and postnatal mice were dissected and fixed in 4% paraformaldehyde buffer at 4 °C for 24 h. The mandibles were then decalcified in 10% ethylene diamine tetraacetic acid/phosphate-buffered saline (PBS) solution. Then, the tissue was dehydrated through a graded ethanol, vitrification by dimethylbenzene and embedded in paraffin. Frontal serial sections for heads and sagittal serial sections for mandibles with 5 μm in thickness were prepared.

2.2. Immunohistochemistry staining

Prepared 5 μm thick paraffin sections were examined for Ghrelin immunolabeling. In brief, antigen retrieval was achieved by heat treatment at 98 °C in PT module buffer 1 (citrate buffer, pH 6.0) for 20 min. Endogenous peroxidase activity was blocked with 0.3% H_2O_2 then preincubated with 1% bovine serum albumin in phosphate-buffedred saline (BSA-PBS) for 20 min at room temperature to reduce nonspecific binding. The sections were then incubated with anti-Ghrelin (rabbit polyclonal, 1:200, Abcam Ltd., Hong Kong) for 2 h at 37 °C. After washing with PBS, the sections were then immersed in secondary antibodies of Polyclonal Swine Anti-rabbit Immunoglobulins/HRP (DakoCytomation, Denmark) for Ghrelin at a dilution of 1:100 for 1 h at room temperature. Visualization was achieved with the aid of a diaminobenzidine (DAB) substrate. Negative controls were prepared from adjacent sections omitting the primary antibody, and no positive immunostaining was detected. Positive controls were performed with normal mouse stomach tissue (Fig. 1A and B). All sections were faintly counterstained with hematoxylin and staining results were assessed under light microscopy (BX53, Olympus Corp., Japan).

3. Results

Hematoxylin-eosin staining was used to determine the morphogenesis and structure of the mandibular first molar from E13.5-PN7. The tooth germ underwent the typical developmental stages, including the bud, cap, bell stages and initial tooth root formation. Immunohistochemical staining showed that spatial and temporal expression of Ghrelin was mainly confined to enamel-secreting ameloblasts and dentin-secreting odontoblasts during the tooth germ development. In this study, we detected on the acylated isoform of ghrelin, as the des-acylated isoform is believed to be biologically inactive and does not signal through GHSR (Delhanty, Neggers, & van der Lely, 2012). As shown in Fig. 1A, Ghrelin was strongly expressed in the region from the neck to the base of the oxyntic gland of mice stomach, consistent with previous findings (Kojima et al., 1999).

3.1. Expression of Ghrelin protein at the bud stage

At the bud stage (E13.5), the thickened dental lamina epithelium invaginated into the underlying mesenchyme to form the epithelial bud. The bud epithelium, also named enamel organ, is composed of epithelium and stellate reticulum. Ghrelin was present predominantly in the epithelium and weakly expressed in the mesenchyme surrounding the enamel organ (Fig. 1C).

3.2. Expression of Ghrelin protein at the cap stage

At the cap stage (E15.5), the bud basal epithelium differentiated into the internal (inner) and external (outer) enamel epithelia, while the mesenchyme differentiated into the dental papillae and the dental follicle. Positive immunostaining for Ghrelin was observed in the inner and outer enamel epithelia. The protein was also weakly detected in the dental papilla especially the part beneath the inner enamel epithelium but was absent from the stellate reticulum and dental follicle (Fig. 1D).

3.3. Expression of Ghrelin protein at the bell stage

E16.5 and E18.5 are commonly considered as the early bell stage and late bell stage respectively, during which the differentiation of dentin-forming odontoblasts from dental papilla and the enamel-forming ameloblasts from the internal epithelium occurs. We selected E17.5 as a representative time point of the entire bell stage. Intense staining for Ghrelin was evident in the polarizing preameloblasts of the inner enamel epithelium. The outer enamel epithelium and the stratum intermedium exhibited moderate Ghrelin staining, whereas the stellate reticulum had no detectable staining. A relatively strong Ghrelin signal was also observed in the polarizing and elongating preodontoblasts underlying the inner enamel epithelium of the cusp, with a gradient of expression increasing with the degree of preodontoblast differentiation (Fig. 1E and F).

3.4. Expression of Ghrelin protein in the postnatal murine molar

During the bell stage and after birth, polarizing ameloblasts and odontoblasts differentiated into secretory and mature phenotypes in order, performing extracellular matrix secretion followed with consequential matrix mineralization. At PN1, Ghrelin was positively expressed in both secretory and mature ameloblasts and odontoblasts. Compared with the bell stage, the staining intensity for Ghrelin at this stage was slightly weaker (Fig. 2a A–C). Substantial immature enamel and dentin were produced by ameoblasts and odontoblasts, respectively, as they matured from PN3–PN7 and simultaneously moved in opposite directions. Here, secretory and mature ameloblasts and odontoblasts persistently express Ghrelin, while odontoblastic processes in the dentinal tubules did not. Notably, the immunostaining was relatively stronger in ameloblasts than in odontoblasts (Fig. 2a D–F, b). Tooth