Gene Expression Patterns 15 (2014) 46–51

Contents lists available at ScienceDirect

Gene Expression Patterns

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

PKA regulatory subunit expression in tooth development

Sílvia Ferreira de Sousa [a,*], Katsushige Kawasaki [b], Maiko Kawasaki [b], Ana Angelova Volponi [b], Ricardo Santiago Gomez [a], Carolina Cavaliéri Gomes [c], Paul T. Sharpe [b], Atsushi Ohazama [b,d]

[a] Department of Oral Surgery and Pathology, School of Dentistry, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
[b] Department of Craniofacial Development and Stem Cell Biology, Dental Institute, King's College London, Tower Wing Guy's Hospital, London Bridge, London SE1 9RT, UK
[c] Department of Pathology, Biological Sciences Institute, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
[d] Division of Oral Anatomy, Department of Oral Biological Science, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

ARTICLE INFO

Article history:
Received 12 December 2013
Received in revised form 7 March 2014
Accepted 10 April 2014
Available online 19 April 2014

Keywords:
PRKAR1A
PRKAR2A
Tooth development
Human
Mouse
In situ hybridization

ABSTRACT

Protein kinase A (PKA) plays critical roles in many biological processes including cell proliferation, cell differentiation, cellular metabolism and gene regulation. Mutation in PKA regulatory subunit, *PRKAR1A* has previously been identified in odontogenic myxomas, but it is unclear whether PKA is involved in tooth development. The aim of the present study was to assess the expression of alpha isoforms of PKA regulatory subunit (*Prkar1a* and *Prkar2a*) in mouse and human odontogenesis by *in situ* hybridization. *PRKAR1A* and *PRKAR2A mRNA* transcription was further confirmed in a human deciduous germ by qRT-PCR. Mouse *Prkar1a* and human *PRKAR2A* exhibited a dynamic spatio-temporal expression in tooth development, whereas neither human *PRKAR1A* nor mouse *Prkar2a* showed their expression in odontogenesis. These isoforms thus showed different expression pattern between human and mouse tooth germs.

The tooth is an organ that develops as a result of sequential and reciprocal interactions between the oral epithelium and neural crest-derived mesenchyme. The first morphological sign of tooth development is an epithelial thickening. The thickened epithelium progressively takes the form of the bud, cap and bell configurations as differentiation and morphogenesis processes. Subsequently, epithelial cells and mesenchymal cells (dental papilla) differentiate into enamel-producing ameloblasts and dentin-secreting odontoblasts, respectively. Odontogenic tumours are known to arise either from the dental epithelium, dental papilla or the dental follicle.

Protein kinase A (PKA) is a serine-threonine kinase that mediates cyclic 3′, 5′-adenosine monophosphate (cAMP) regulation for a variety of biological processes including cell proliferation, cell differentiation, cellular metabolism and gene regulation (Krebs, 1972; Roesler et al., 1988; Boynton and Whitfield, 1983; Liu, 1982; Schwartz and Rubin, 1983; Krebs and Beavo, 1979). The PKA signaling is essential in embryonic mesodermal and ectodermal differentiation, and is interacted with many other signaling pathways such as Hedgehog, Wnt and Fgf in initial morphogenesis (Jia et al., 2004; Almeida et al., 2010; Bossis and Stratakis 2004; Hammerschmidt et al., 1996; Amieux et al., 2002; Sakai et al., 2006; Kim et al., 2013).

In the inactive form, the PKA holoenzyme is localized in the cytosol as a heterotetramer composed of two regulatory (R) subunits bound to two catalytic (C) subunits (Scott, 1991; Taskén et al., 1997). PKA is activated when two molecules of cAMP bind to each R subunit, which leads to dissociation of the holoenzyme. Four R subunit isoforms (R1α, R1β, R2α and R2β) have been characterized in human and mouse, while two C subunit isoforms (Cα, Cβ) and four C subunits (Cα, Cβ, Cγ and PRKX) have been identified in mouse and human, respectively (Amieux et al., 2002; Horvath et al., 2010). Type I PKA contains either regulatory subunit R1α or R1β in its structure, and is usually associated with stimulated states of PKA; type II PKA contains either regulatory subunit R2α or R2β, and is involved in basal states (Bossis and Stratakis, 2004; Griffin et al., 2004).

R1α is the only isoform that is essential for early embryonic stage of mouse development (Amieux et al., 1997; Amieux et al., 2002). Mutations in the *PRKAR1A*, which encodes R1α subunit, result in many diseases including Carney Complex (CNC), an autosomal dominant disorder characterized by skin lesions, myxomas and endocrine overactivity (Horvath et al., 2010; Stratakis et al., 2001). *PRKAR1A* mutation was also identified in odontogenic myxomas (Perdigão et al., 2005). In common with *R1α*, *R2α* is widely expressed in mouse and human, and both alpha isoforms are

* Corresponding author. Address: Department of Oral Surgery and Pathology, School of Dentistry, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, Belo Horizonte, MG CEP 31270-901, Brazil. Tel.: +55 31 34092477; fax: +55 31 34092430.
E-mail address: silvinhaferreira21@yahoo.com.br (S.F. de Sousa).

http://dx.doi.org/10.1016/j.gep.2014.04.002
1567-133X/

expressed in the dental follicle during tooth eruption (Amieux et al., 1997, 2002; Yao and Wise, 2003). It is however unknown whether these isoforms are involved in embryonic tooth development. The aim of the present study was to investigate the expression of alpha isoforms of PKA regulatory subunit in mouse and human odontogenesis.

1. Results

1.1. Mouse embryos

Initiation begins before the tooth enlargement is morphologically visible. The first signals are derived from tooth presumptive epithelium at E9.5 (Ferguson et al., 2000). The first visible signs of tooth development cannot be recognized at E10.5. Whole mount *in situ* hybridization analysis showed strong *Prkar1a* expression in the maxillary and mandibular processes at E10.5, whereas *Prkar2a* expression was restricted to the posterior region of maxillary and mandible processes (Fig. 1A and A′).

Thickening of epithelium takes place from E12.5. At E12.5 whole mount *in situ* hybridization analysis showed that *Prkar1a* expression became restricted to lower incisor tooth regions, and no obvious expression could be detected in neither upper incisor, upper nor lower molar region (Fig. 1B and C). No obvious *Prkar2a* expression was observed at any tooth region (Fig. 1B′, C′). Radioactive *in situ* hybridization analysis showed that strong expression of *Prkar1a* was found in mesenchyme of both incisor and molar tooth germs, whereas weak expression was observed in tooth epithelium of both tooth types (Fig. 2A and B). *Prkar2a* showed no expression in either epithelium or mesenchyme of any tooth types (Fig. 2A′ and B′).

By E13.5 the tooth epithelium has invaginated into the underlying mesenchyme to form epithelial bud. Whole mount *in situ* hybridization analysis showed strong *Prkar1a* expression in incisor and molar regions of both upper and lower jaws, whereas no *Prkar2a* expression was observed at any tooth regions (Fig. 1D, E, D′, and E′). Radioactive *in situ* hybridization analysis showed that *Prkar1a* was strongly expressed in mesenchyme of both incisor and molar tooth germs, whereas it showed weak expression in tooth epithelium (Fig. 2C and D). *Prkar2a* was expressed in neither epithelium nor mesenchyme of any tooth types (Fig. 2C′ and D′).

By E14.5 the bud basal epithelium develops into the internal and the external (outer) enamel epithelium, and the mesenchyme forms into the dental papilla and dental follicle. Whole mount *in situ* hybridization analysis showed strong expression of *Prkar1a* in the incisor and molar regions of both upper and lower jaws (Fig. 1F and G). No *Prkar2a* expression could be detected at any tooth region (Fig. 1F′ and G′). Radioactive *in situ* hybridization analysis exhibited strong expression of *Prkar1a* in dental papillae of both incisor and molar tooth germs, and weak expression in molar tooth epithelium (Fig. 2E and F). *Prkar2a* showed no expression in either epithelium or mesenchyme of incisors or molars (Fig. 2E′ and F′).

The terminal differentiation of dentin-forming odontoblasts from dental papilla cells and the enamel-forming ameloblasts from the internal epithelium occurs between E18 and P0. Strong expression of *Prkar1a* was observed in the odontoblasts layer of molars and incisors, and stratum intermedia of incisors (Fig. 2G and H). No expression of *Prkar2a* could be detected in any tooth germs (Fig. 2G′ and H′).

1.2. Human embryos

PRKAR2A expression was observed in both epithelium and mesenchyme at bud and cap stages, whereas no obvious expression of *PRKAR1A* could be detected in either bud or cap stage tooth germs (Fig. 3A–B′). At the bell stage, *PRKAR2A expression* became restricted to stratum intermedia, odontoblasts and dental papillae, whereas no obvious expression of *PRKAR1A* could be detected at bell stage (Fig. 3C and C′). The qRT-PCR showed similar mRNA transcriptional levels of *PRKAR1A* and *PRKAR2A* in the human germ, with RQ (relative quantification) values of 0.020 and 0.025, respectively (data not shown).

The expression of human *PRKAR2A* and mouse *Prkar1a* during tooth development is summarized diagrammatically in Fig. 4.

2. Discussion

We show here dynamic spatio-temporal expression of alpha isoforms of PKA regulatory subunit in odontogenesis. In mice, *Prkar1a* showed strong expression in tooth germs, whereas no expression of *Prkar2a* could be detected. Conversely, in human tooth development, *PRKAR2A* was strongly expressed, but *PRKAR1A* showed no obvious expression in tooth germs in the *in situ* hybridization analysis. Many genes such as *Shh*, *Pax9* and *Fgf8* have been shown different expression pattern in tooth development between mouse and human (Lin et al., 2007; Hu et al., 2013). The different expression pattern is likely to be established by different evolutional processes in each animal. It is possible that different expression pattern contribute the different shape between human and mouse molar tooth. On the other hand, we examined *PRKAR2A* and *PRKAR1A* expression on human deciduous tooth germs, whereas mouse dentitions we examined were permanent tooth dentition. It is also possible that molecular mechanisms of tooth development are significantly different between permanent and deciduous tooth dentition. Furthermore it is difficult to identify the tooth type in human embryos, while we showed *Prkar1a* and *Prkar2a* expression on murine first molar tooth germ. Even in mouse permanent tooth dentition, slightly different expression pattern of *Prkar1a* was observed between incisors and molars. It is conceivable that human tooth germs we examined are not molar tooth germs.

PKA is known to be involved in several signaling pathways which play critical roles in regulating tooth development. The stimulation of cementoblasts with parathyroid hormone-related protein (PTHrP), a regulator of cementogenesis, was described to occur via the cAMP/PKApathway (Ouyang et al., 2000). In addition, PKA has been shown to antagonize Sonic hedgehog (SHH) signaling which is important factor for tooth root formation (Amieux et al., 2002; Hammerschmidt et al., 1996; Makinodan and Marneros, 2012; Nakatomi et al., 2006; Niewiadomski et al., 2013). The expression of R1α and R2α are observed in the dental follicle during tooth eruption when cement and tooth root also start to form (Yao and Wise, 2003). PKA is thus likely to play a critical role in cement/root formation through Shh and PTHrP signaling. *PRKAR1A* mutation has been shown in human odontogenic myxomas which is a tumor probably derived from the tooth germ ectomesenchyme (Perdigão et al., 2005). It is also possible that mutation of *PRKAR1A* leads to the changes of Shh or PTHrP signaling activity which induce odontogenic myxomas formation, since aberrant activity of both signaling pathway is known to result in tumor formation (Bijlsma and Roelink 2010; Kremer et al., 2011), including odontogenic tumors (Gomes et al., 2009; Gomes and Gomez, 2011; Farias et al., 2012).

3. Experimental procedures

3.1. Mouse and human embryos

Human embryos at different stages were collected by The Human Developmental Biology Resource (HDBR), which has been