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Analysis of expression patterns of IGF-1, caspase-3 and HSP-70 in developing human tooth germs

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ABSTRACT

Aims: To analyze expression patterns of IGF-1, caspase-3 and HSP-70 in human incisor and canine tooth germs during the late bud, cap and bell stages of odontogenesis.
Materials and methods: Head areas or parts of jaw containing teeth from 10 human fetuses aged between 9th and 20th developmental weeks were immunohistochemically analyzed using IGF-1, active caspase-3 and HSP-70 markers. Semi-quantitative analysis of each marker's expression pattern was also performed.
Results: During the analyzed period, IGF-1 and HSP-70 were mostly expressed in enamel organ. As development progressed, expression of IGF-1 and HSP-70 became more confined to differentiating tissues in the future cusp tip area, as well as in highly proliferating cervical loops. Few apoptotic bodies highly positive to active caspase-3 were observed in enamel organ and dental papilla from the cap stage onward. However, both enamel epithelia moderately expressed active caspase-3 throughout the investigated period.
Conclusions: Expression patterns of IGF-1, active caspase-3 and HSP-70 imply importance of these factors for early human tooth development. IGF-1 and HSP-70 have versatile functions in control of proliferation, differentiation and anti-apoptotic protection of epithelial parts of human enamel organ. Active caspase-3 is partially involved in formation and apoptotic removal of primary enamel knot, although present findings might reflect its ability to perform other non-death functions such as differentiation of hard dental tissues secreting cells and guidance of ingrowth of proliferating cervical loops.

1. Introduction

During odontogenesis, human tooth germs undergo several morphologically distinctive developmental stages (dental lamina, bud, cap and bell stages). In each developmental stage, basic cellular processes such as proliferation, differentiation, and apoptosis, occur simultaneously (Thesleff & Tummers, 2008). Spatio-temporal patterns of these processes are influenced by the interplay between different developmental factors (growth factors, transcription factors, extracellular matrix components, hormones), and strictly regulated in order for teeth to develop properly (Thesleff & Mikkola, 2002; Thesleff & Tummers, 2008; Thesleff, Vainio, & Jalkanen, 1989).

Insulin-like growth factor 1 (IGF-1) is a hormone with peptide sequence similar to insulin, synthesized primarily in liver, or locally in tissues in paracrine/autocrine manner. IGF-1 mediates growth hormone (GH) actions, especially during the childhood and puberty growth spurt (Welch & Dawes, 2007). IGF-1 exerts its mitogenic effects through binding to specific transmembrane receptor (IGF-1R), which is normally expressed in various tissues (Rother & Accili, 2000). Apart from IGF-1R, other members of IGF-axis, such as insulin-like growth factor 2 (IGF-2) and insulin-like growth factor binding proteins (IGFBPs), influence IGF-1 action either by competitive binding to IGF-1R (IGF-2), or by binding directly to IGF-1 in the blood stream (IGFBPs), thus causing blocking, or facilitating endocrine transport of IGF-1 to distant target tissues (Magnucki et al., 2013; Rajah, Valentinis, & Cohen, 1997). Studies on IGFs expression paterns performed on mice (Ferguson, Sharpe, Thomas, & Beck, 1992), rats (Ayer-le Lievre, Stahlbom, & Sara, 1991; Beck et al., 1987), and human embryos (Bellone, Barni, Pagni, Balboni, & Vannelli, 1990; Han, D'Ercole, & Lund, 1987) suggest that IGF-2 is the main prenatal mediator of GH-related effects on developing tissues and organs, whereas IGF-1 plays fairly limited role. However, those studies fall short on properly evaluating the importance of

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IGF-1 during fetal development, since other research (focused on particular organs) disclosed more details about its key roles in development of specialized tissues within particular organs (Tafra et al., 2014). More than just a mitogenic factor, IGF-1 takes part in epithelial-to-mesenchymal interactions (Ferguson et al., 1992), differentiation (Caton, Bringas, Zeichner-David, 2005) and maintenance of specific cell populations (Magnucki et al., 2013) within developing organs. Findings from cell culture (Delaney, Cheng, & Feldman,1999) and functional studies (Katic et al., 2007; Kondo et al., 2003; Patury, Miyata, & Gestwicki, 2009) point to the involvement of IGF-1 in the regulation of apoptosis by disclosing its participation in cell survival signaling cascades. As shown on differentiating rat incisor ameloblasts, down-regulated expression of IGF-1R, (implying cell/tissue inability to harness IGF-1 anti-apoptotic protection) makes part of these cells more susceptible to apoptotic culling (Joseph et al., 1999). So far, the expression patterns of IGF-1 are well described only in advanced developmental stages of rodent incisors (Joseph et al., 1999) and molars (Fujiwara, Tabata, Endoh, Ishizeki, & Nawa, 2005), and some data exist on IGF-1 expression in soft tissues of impacted human wisdom teeth (Magnucki et al., 2013). IGF-1 involvement in the early stages of human tooth development prior amelogenesis and dentinogenesis still needs to be investigated.

Caspases are evolutionarily preserved proteases that play essential roles in apoptosis, necrosis and inflammation (Danial & Korsmeyer, 2004; Sirois et al., 2011). There are two types of caspases—initiators and executioners among which caspase-3 is considered as a chief executioner caspase and is located at the "point of no return". Both extrinsic apoptotic pathways (initiated by specific ligands binding to cell surface death-receptors) and intrinsic apoptotic pathways (involving factors from mitochondria) converge on activation of caspase-3, a penultimate step from which apoptosis cannot be arrested (Abdul-Ghani & Megeney, 2008). Since apoptosis normally occurs during organogenesis, this implies that caspase-3 is required for normal development of tissues and organs (Vukojevic et al., 2012). Developing tooth germs proved to be appropriate models for research on involvement of caspase-3 (and apoptosis) in pre- and post-natal development. Namely, apoptosis in developing tooth germs seems to be caspase-3-dependent (Matalova, Kovaru, & Misek, 2006a; Setkova et al., 2006; Shigemura et al., 2001), it occurs in all developmental stages, whereas temporo-spatial patterns of caspase-3 expression strongly suggest its importance for normal completion of odontogenesis (Nakagawa et al., 2012; Vaahtokari, Aberg, & Thesleff, 1996). However, some studies on caspase-3-knockout mice showed that lack of caspase-3 (and therefore omission of apoptosis) had none or very little adverse effects on normal tooth development (Matalova et al., 2006b). Furthermore, stage-specific suppression of apoptotic activity in developing tooth germs by pancaspase inhibitors yielded contradictory results considering the impact of apoptosis on tooth crown size and shape (Coin, Kieffer, Lesot, Vonesch, & Ruch, 2000; Kim et al., 2006). On the other hand, data on involvement of caspase-3 during human tooth development are scarce. It is known that caspase-3-mediated apoptosis is important for the regulation of life span of odontoblasts, thus affecting maturation of hard dental tissues in permanent human teeth with incomplete root formation, and remodeling of dental pulp chamber in normal and decayed permanent teeth (Mitsiadis, De Bari, & About, 2008). Therefore, investigation of caspase-3 expression patterns during early stages of human tooth development should improve our present knowledge about the roles of caspase-3 in odontogenesis and the impact it exerts on particular human tooth germ tissues.

Heat shock proteins (HSPs) are a group of highly conserved and functionally related proteins that generally operate as intracellular chaperones (Santoro, 2000). Based on molecular weight they are divided into large (HSP-110, -90, -70, -60), and small HSPs (Nakasone, Yoshie, & Ohshima, 2006a). HSPs expression is induced by environmental and physiological stress caused by factors such as elevated temperatures, desiccation, exposure to toxic chemicals, infection and hypoxia (Santoro, 2000). Their expression is also needed in normal conditions since HSPs participate in maintenance of cellular structure and tissue homeostasis (Borges & Ramos, 2005; Lavoie, Hickey, Weber, & Landry, 1993). They can also act as powerful anti-apoptotic agents, and when overexpressed HSPs can prevent apoptosis, while their absence makes cells more susceptible to pro-apoptotic stimuli (Lanneau, de Thonel, Maurel, Didelot, & Garrido, 2007; Stankiewicz, Lachapelle, Foo, Radicioni, & Mosser, 2005). Likewise, increase or decrease of HSPs expression can initiate cell differentiation, meaning that their expression levels must be strictly regulated (Nakasone, Yoshie, & Ohshima, 2006b). The involvement of HSPs in tooth development was investigated on rats (Du, Gu, Gong, Yang, & Ling, 2009; Nakasone et al., 2006a, 2006b; Ohshima, Nakakura-Ohshima, & Maeda, 2002; Onishi et al., 2002Nakasone et al., 2006a; Nakasone et al., 2006b; Ohshima, Nakakura-Ohshima and Maeda, 2002 Onishi et al., 2002), mice (Wada et al., 2002) and humans (Leonardi, Barbato, Paganelli, & Lo Muzio, 2004) in the early and advanced stages of odontogenesis. Those studies mostly investigated small HSPs, showing that their appearance is confined to epithelial parts of tooth germ with transient expression in its mesenchymal tissues, later to coincide with differentiation and maturation of ameloblasts and odontoblasts in advanced stages of odontogenesis. Similar expression patterns were described for some large HSPs as well (Wada et al., 2002). It is known that HSP-70 is expressed during the early embryonic development and is involved in apoptosis and differentiation of embryonic tissues (Bevilacqua, Fiorenza, & Mangia, 1997; Lanneau et al., 2007). However, data on potential roles of HSP-70 in human tooth development are still missing.

In the present study, we analyzed expression patterns of IGF-1, caspase-3 and HSP-70 in developing human incisor and canine tooth germs. Our findings are compared with data from other developmental and functional studies on these factors, and discussed regarding proliferation, apoptosis and differentiation of odontogenic tissues.

2. Materials and methods

2.1. Tissue acquirement and processing

For the present study, ten human fetuses were collected after spontaneous abortions or tubal pregnancies from the Department of Pathology, University Hospital in Split, Croatia. Permission for tissue processing was given by the Ethical and Drug Committee of the University Hospital in Split (Class: 033-081/11-03/0005, No: 2181-198-03-04/10-11-0024) in accordance with Helsinki Declaration (Williams, 2008). External measurements were used for assessment of gestational age of human fetuses (O'Rahilly, 1972) and were matching the period between the 9th and 20th developmental weeks. Analyses of fetal tissues were performed only on head areas or parts of jaws containing teeth. After fixation with 4% paraformaldehyde in phosphate-buffered saline (PBS) and dehydration in graded ethanol dilutions, fetal tissues were paraffin-embedded and cut in transverse or frontal plane (serial 7 μm sections). Tissue sections were mounted on glass slides and microscopically examined using Olympus BX51 light microscope (Olympus, Tokyo, Japan). Once the examination of control sections stained by hamatoxylin and eosin confirmed proper tissue preservation, immunohistochemistry and double immunofluorescence were applied for further processing.

2.1.1. Immunohistochemical staining

Tissue sections were deparaffinized in xylene and rehydrated in water, which was followed by 30 min incubation in 0.1% H_2O_2 for