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Expression patterns of ABCG2, Bmi-1, Oct-3/4, and Yap in the developing mouse incisor

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ABSTRACT

Recent studies have demonstrated the existence of dental stem cells in the continuously growing tooth. However, much remains to be learned about the complex mechanism involving stem cells during tooth development. We determined the expression patterns of four stem cell markers ABCG2, Bmi-1, Oct-3/4, and Yap in the developing mouse incisors between embryonic day (E) 11 and postnatal day (PN) 20. ABCG2 was localized strongly in the perivascular region of the incisor mesenchyme from E11 to PN20, and in the odontoblasts from E18 to PN20. Bmi-1 was expressed in both the dental epithelium and mesenchyme from E11 to E14. The expression of Bmi-1 was noticeably reduced at E16, and was restricted to the apical bud from E16 to PN20. Oct-3/4 was localized in the nucleus of the cells in the superficial layer and stellate reticulum within the dental epithelium from E11 to E14 and in the apical bud from E16 to PN20. Meanwhile, once the ameloblasts and odontoblasts began to appear at E16, they expressed Oct-3/4 in the cytoplasm. Yap was expressed in most of the basal cells of the incisor dental epithelium from E11 to E14, but was expressed mainly in the transit-amplifying (TA) cells within the basal cell layer from E16 to PN20. The unique and overlapping expression patterns of ABCG2, Bmi-1, Oct-3/4, and Yap suggest the independent and interactive functions of the four stem cell markers in the developing mouse incisor.

1. Results and discussion

Stem cells are able to self-renew and to give rise to various specialized cell types (Barrilleaux et al., 2006). They therefore have potential uses in organ repair and regeneration in treatments for various diseases and disabilities. The developing mouse incisor is an ideal model with which to study stem cell biology. Stem cells are believed to be compartmentalized within the apical bud, which is the epithelial component at the end of the labial cervical loop that is responsible for continuous growth of the incisors throughout life (Harada and Ohshima, 2004; Ohshima et al., 2005; Yokohama-Tamaki et al., 2006). Within the apical bud, the stellate reticulum cells and the surrounding basal cells are known to exhibit stem cell characteristics (Harada et al., 1999; Kawano et al., 2004; Morotomi et al., 2005; Thesleff and Tummers, 2009).

Specific markers such as ABCG2, Bmi-1, Oct-3/4, and Yap (Yes-associated protein) can be used to isolate stem cells, and combinations of multiple stem cell markers can be used to identify and characterize stem cells (Jackson et al., 2001). ABCG2, a member of the ATP binding cassette transporter G family, is a transmembrane protein that plays significant roles in transporting various molecules across cell membranes (Rocchi et al., 2000). It is also thought to be involved in increasing the proliferation and retaining the phenotype of stem cells (Ding et al., 2010). Bmi-1, a member of the polycomb gene family of transcriptional repressors, is a key regulator of self-renewal and proliferation in stem cells in multiple tissues (Lessard and Sauvageau, 2003; Molofsky et al., 2005; Park et al., 2003; Wang et al., 2010). Oct-3/4, a class V POU-domain transcription factor, is a crucial regulator of pluripotency and differentiation in stem cells (Nakatake et al., 2006; Niwa et al., 2000; Pesce et al., 1998; Scholer et al., 1989; Stefanovic and Puceat, 2007; Zeineddine et al., 2006) and it has two isoforms: Oct-3/4A and Oct-3/4B (Takeda et al., 1992). Oct-3/4A is located in the nucleus, and it is considered to be related to self-renewal and pluripotency (Lee et al., 2006), while Oct-3/4B is located in the cell cytoplasm in somatic tissues (Atlasi et al., 2008; Mizuno and Kosaka, 2008). Yap is a transcriptional coactivator that regulates

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self-renewal and differentiation in stem cells (Dong et al., 2007; Lian et al., 2010). Although these stem cell markers are obviously important in understanding the biology of stem cells, their expression patterns in the developing tooth germ are yet not well described.

The present study investigated the expression patterns of four stem cell markers (ABCG2, Bmi-1, Oct-3/4, and Yap) in the developing mouse incisor. This was achieved by employing immunohistochemistry in the tooth germ at the lamina stage, bud stage, cap stage, bell stage, and eruption stage. This exploration will provide insight into the complex mechanism involving stem cells in the developing mouse incisor.

1.1. Expression pattern of ABCG2 during mouse incisor development

ABCG2 is a marker used to identify stem cells in various tissues, such as bone marrow, embryonic stem cells, limbal epithelium, skin, skeletal muscle, liver, and testis (Coomarasamy et al., 2003; de Paiva et al., 2005; Hu et al., 2008; Jackson et al., 1999; Lassalle et al., 2004; Shimano et al., 2003; Vander Borght et al., 2006; Yano et al., 2005). In addition, *ABCG2* mRNA is known to be strongly expressed in the dental pulp in human deciduous teeth (Nam and Lee, 2009). However, none of these investigations have revealed the localization of ABCG2 in the developing tooth. We analyzed the expression pattern of ABCG2 in the incisor from E11 to PN20 by immunohistochemical staining. ABCG2 was not detected in the epithelial component, including the apical bud (Fig. 1). In contrast, in the dental mesenchyme, ABCG2 staining was most prominent in the perivascular regions but was relatively weak in the rest of the dental mesenchyme. These expression patterns were noted consistently early in the lamina stage and with a relatively constant intensity throughout the process of odontogenesis (Fig. 1). Previous studies on the adult dental pulp showed that ABCG2 was expressed in the perivascular regions (Iohara et al., 2006) which are believed to be stem cell niches (Shi and Gronthos, 2003). These studies suggested the possible role of ABCG2 in the maintenance of the perivascular stem cell niche in the adult dental pulp. Since the adult dental pulp tissue originates from the dental papilla of the developing tooth germ, we suggest that ABCG2 may be involved in the maintenance of the perivascular stem cell niche in the developing mouse incisor.

Interestingly, the expression of ABCG2 in the mesenchyme cells in the center of the dental papilla was down-regulated at the early bell stage (E16) (Fig. 1D, arrowhead). On the other hand, the mesenchyme cells adjacent to the dental epithelium exhibited weak expression of ABCG2 (Fig. 1E, arrowheads). Subsequently, from the middle bell stage (E18) to the eruption stage (PN20), the expression of ABCG2 in the center of the dental papilla was absent (Fig. 1G and J; arrowheads; PN10 and PN20 data not shown) and confined to odontoblasts adjacent to the dental epithelium (Fig. 1H, K, and N; PN10 data not shown). The detection of ABCG2 expression in the odontoblasts indicates that ABCG2 is also present in differentiated cells (Fig. 1H, K, and N). Therefore, together with studies showing that ABCG2 functions in the cell defense mechanism in differentiated cells (Zhou et al., 2001), we suggest that ABCG2 functions to maintain the integrity of odontoblasts by transporting various molecules across the cell membranes.

1.2. Expression pattern of Bmi-1 during mouse incisor development

Bmi-1 is another well-known stem cell marker that is essential for maintaining the ability to self-renew via proliferation in hematopoietic stem cells (Park et al., 2003), peripheral and central nervous stem cells (Molofsky et al., 2003), and leukemic stem cells (Lessard and Sauvageau, 2003). We therefore investigated the localization of Bmi-1 in the developing incisor. At the lamina stage, few cells in the dental epithelium and mesenchyme showed weak expression of Bmi-1 (Fig. 2A). Bmi-1 expression was initially abundant and stronger in the dental mesenchyme at the bud stage (Fig. 2B), extending into the epithelium during the subsequent cap stage (Fig. 2C). At the beginning of the bell stage (E16), the expression level of Bmi-1 was dramatically reduced in both the mesenchyme and epithelium, including in the apical bud (Fig. 2D). From E18 to PN20, Bmi-1-expressing epithelial cells were found mainly in the apical bud (Fig. 2E–H), and not in the ameloblasts or the odontoblasts (Fig. 2I–L). The strong expression of Bmi-1 in the dental mesenchyme in the bud stage and the cap stage may suggest that Bmi-1 expression is involved in regulating self-renewal of the stem cells in the dental papilla at the early stage of incisor development. In addition, it is important to note that at the bell stage and the eruption stage, the expression of Bmi-1 is maintained in the stellate reticulum within the apical bud (Fig. 2D–H), which has been reported to contain stem cells in the mouse incisor (Harada et al., 1999). These findings suggest that Bmi-1 may play a regulatory role in maintaining the ability of the apical bud cells to self-renew.

1.3. Expression pattern of Oct-3/4 during mouse incisor development

Oct-3/4 expression has been reported in adult pluripotent stem cells in the kidney, breast, epidermis, pancreas, mesenchyme, stomach, and liver (Tai et al., 2005). However, the expression pattern of Oct-3/4 in tooth development has yet to be described. We therefore examined the localization of Oct-3/4 during incisor development. At the lamina stage (E11), weak nuclear staining of Oct-3/4 was detected in both the dental epithelium and mesenchyme (Fig. 3A). At the late bud stage (E13), Oct-3/4 was strongly expressed in the nucleus of the cells located in the superficial layer of the dental epithelium; no expression was found in the basal cell layer (Fig. 3B). At the cap stage (E14), Oct-3/4 was localized abundantly in the nucleus of the stellate reticulum within the dental lamina region (Fig. 3C, arrow), while it was absent in the basal cells of the dental epithelium (Fig. 3C, arrowheads). At the early bell stage (E16), nuclear staining of Oct-3/4 in the apical bud which is formed by the elongation of the labial cervical loop remained mainly in the stellate reticulum and was also found in the outer dental epithelium layer (Fig. 3F, arrows), leaving the TA cells (Fig. 3F, asterisk) and the rest of the basal cells in the inner layer unstained (Fig. 3F, arrowhead). This finding is in line with that for the rest of the dental epithelium, where Oct-3/4-positive cells were found in both the stellate reticulum layer and the inner dental epithelium layer (Fig. 3E), with the number of Oct-3/4-positive cells being higher in the stellate reticulum layer than in the inner dental epithelium layer. From E18 to PN20, nuclear staining of Oct-3/4 in the apical bud was detected largely in the stellate reticulum cells and the outer dental epithelium cells, and also in the mesenchymal cells surrounding the apical bud (Fig. 3J, N, O, and S); however, no Oct-3/4 staining was detected in the TA cells (Fig. 3J, N, O, and S; asterisks) or the basal cells in the inner layer (Fig. 3J, N, O, and S; arrowheads). These distinct expression patterns of Oct-3/4 in the apical bud at the bell stage and the eruption stage suggest that Oct-3/4 may be involved in the regulation of self-renewal or pluripotency of the epithelial stem cells.

Distinct from the nuclear staining findings discussed above, Oct-3/4 was detected in the cytoplasm of the dental mesenchymal cells during incisor development. At E16, Oct-3/4 was detected mainly in the cytoplasm of the dental mesenchyme cells as they begin to align adjacent to the inner dental epithelium (Fig. 3D and E). At E18 and PN2, Oct-3/4 was detected not only in the cytoplasm of the odontoblasts but also in the subodontoblastic mesenchymal cells (Fig. 3I and M; arrows) and in most of the ameloblasts (Fig. 3I and M; Am). However, Oct-3/4 was clearly down-regulated