Molecular and Cellular Control of Palate Development in the Mouse

Akademisk avhandling

som för avläggande av filosofie doktorsexamen
kommer att offentligen försvaras i föreläsningssal 3, Odontologen,
Medicinaregatan 12, Göteborg, fredagen den 14 november, 2008 kl. 13:00

av

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Fil. lic.

Avhandlingen baseras på följande delarbeten:

I. Vaziri Sani, F., Hallberg, K., Harfe, B.D., McMahon, A.P., Linde, A., Gritli-Linde, A., 2005. Fate-mapping of the epithelial seam during palatal fusion rules out epithelial-mesenchymal transformation. *Dev. Biol.* **285**, 490-495.

II. Vaziri Sani, F., Kaartinen, V., Linde, A., Gritli-Linde. Developmental changes in cellular and extracellular structural macromolecules in the developing mouse secondary palate and nasal cavity. *Manuscript*

III. Vaziri Sani, F., Rock, J.R., Hallberg, K., Harfe, B.D., Linde A., Gritli-Linde, A., 2008. Expression patterns of the Tmem16 gene family during craniofacial development in the mouse. *Submitted*

IV. Gritli-Linde, A., Vaziri Sani, F., Hallberg, K., Kannius-Janson, M., McMahon, A.P., Linde, A. Sonic Hedgehog signaling is required for murine secondary palate development. *Manuscript*

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ABSTRACT

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The formation of a definitive secondary palate in mammals is a multistep process that comprises growth, elevation, contact and fusion of the two palatal shelves. Disruption at any step generates cleft palate, a major congenital birth defect. Therefore, it is important to understand the mechanisms governing normal palate development. This thesis analyses some of the molecular and cellular events that take place during normal (palatogenesis) and abnormal (cleft) development of the secondary palate

Disappearance of the midline epithelial seam (MES), followed by mesenchymal continuity, is a critical prerequisite for successful fusion of the palatal shelves (PS). Epithelial-mesenchymal transformation (EMT) has since long been suggested to be the fate of the MES. To investigate this, the fate of MES cells *in vivo* was determined by using the *Cre-loxP* system which enabled genetic marking of *Sonic hedgehog*- and *Keratin-14*-expressing palatal epithelial cells in mice. The results show that EMT never occurs during degeneration of the MES in mice. Evidence is also provided in support of apoptosis as a straightforward mechanism underlying regression of the MES by the use of established and novel molecular markers of apoptosis. Furthermore, identification of a highly specific and reliable molecular marker of PS peridermal cells enabled its use to provide insights into the fate of peridermal cells during fusion of the PS.

The in vivo role of Sonic hedgehog (Shh) signaling during palatogenesis was assessed in *K14-Cre;Shh*$^{c/c}$ or *K14-Cre;Shh*$^{n/c}$ mice (*Shh* mutants), which lack *Shh* activity in both the epithelial and mesenchymal cells of the PS and exhibit cleft palate. The PS of these mutants are hypotrophic and never elevate to a horizontal position. The mechanims behind this defect are unveiled following analyses with a panel of molecular markers. The abnormal small size of the *Shh* mutant PS is caused by deregulated cell proliferation. Thus, it appears that the main role of Shh signalling is to ensure normal rates of cell proliferation within the PS. The interaction between Shh signalling and other key factors expressed in the PS during palatogenesis was also analysed. Loss-of-function of Shh generates stage-dependent reduction in transcript levels of *Foxf1* and *Foxf2* in the PS mesenchyme. This indicates that Shh participates in the regulation of the expression of *Foxf1/Foxf2* during palatogenesis.

The developing palate is a heterogeneous organ containing epithelial and mesenchymal cells, blood vessels, nerves as well as a complex extracellular matrix. Furthermore, both the epithelium and mesenchyme of the PS are regionalized along the anterior-posterior and medio-lateral axes. This regionalization is crucial for successful palatogenesis. However, little is known about the molecular events underlying these regional differences. The use of a battery of molecular markers for extracellular matrix, cytoskeletal, and junctional complex components unveiled interesting dynamic changes in the distribution/expression of these components just before and during PS elevation and fusion. Epithelia that are fated to fuse with homotypic or heterotypic partners and subsequently degenerate express identical sets of molecular markers. Furthermore, some molecular changes during PS fusion are independent of Tgfβ signaling through Alk5 as indicated by in vivo and in vitro analyses.

As a first step towards unraveling the function of Tmem16 proteins, a new family of trans-membrane proteins, detailed expression patterns of transcripts of several family members were studied in the developing mouse embryo and postnatally. Interestingly, one family member displays robust expression in the medial edge epithelia and MES, indicating a crucial role for this factor during palate fusion. However, mice lacking the function of this factor have normal palate. This is likely a result of functional redundancy between Tmem16 family members. Furthermore, study of *Shh* mutants indicates that expression of these in the palate is independent of Shh signaling.

In conclusion, the mouse is in many aspects an excellent model system for the study of secondary palate development. This thesis has provided additional important information that may be of use in delineating the function of key factors of palatogenesis and the mechanisms leading to cleft palate.

Key words: cleft palate, fate mapping, Sonic hedgehog, apoptosis, extracellular matrix, mouse

ISBN: 978-91-628-7597-8