

IDENTIFICATION AND CHARACTERIZATION OF EPITHELIAL STEM CELLS DURING MOUSE INCISOR DEVELOPMENT

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INTRODUCTION: The apical bud, an epithelial protrusion at the apical end of the rodent incisor, has been reported to contain epithelial stem cells that enable continuous growth of the incisor^[1]. Recently, there have been studies on the apical bud region and the relation with epithelial stem cells^[2]. However, the epithelial stem cell during incisor development is not completely studied. Bromodeoxyuridine (BrdU) is a thymidine analog that incorporates DNA of dividing cells during the synthetic phase of the cell cycle. Therefore BrdU incorporation method can be useful for localizing stem cells which are slow-cycling cells. Here, we identified and characterized the epithelial stem cells during mouse incisor development.

METHODS: Incisor tooth germs of the mouse embryos from BrdU-injected mice were dissected after different lengths of post-injection chasing period. They were examined by BrdU staining in order to localize label-retaining cells (LRCs; slow-cycling cells) in the incisor tooth germ. The developing incisor tooth germs were analyzed via immunohistochemistry to label stem cells within the apical bud. Stem cell markers were used as primary antibodies. After dissecting the developing incisor tooth germs, they were divided into the apical bud, inner enamel epithelium, and the mesenchyme surrounding the apical bud. In order to investigate the mRNA expression of these tissues, real-time PCR was performed. Relative abundance of stem cell markers such as *ABCG2*, *Bmi-1*, *CD133*, *p63*, *c-kit*, *Oct4*, *Sca-1*, and *SSEA1* in the apical bud, inner enamel epithelium, and the mesenchyme surrounding the apical bud were verified.

RESULTS: BrdU label-retaining cells which indicate slow cycling cells within the developing tooth germ were localized in the apico-labial part of the apical bud, including the stellate reticulum layer and the surrounding basal layer of the epithelium, and also in the apical and labial part of the mesenchyme tissue surrounding the apical bud. *Bmi-1* was detected in the stellate reticulum layer of the apical bud. *Sca-1* and *ABCG2* was detected in the mesenchyme surrounding the apical bud.

Bmi-1 and *Oct4* showed increased mRNA expression in the apical bud.

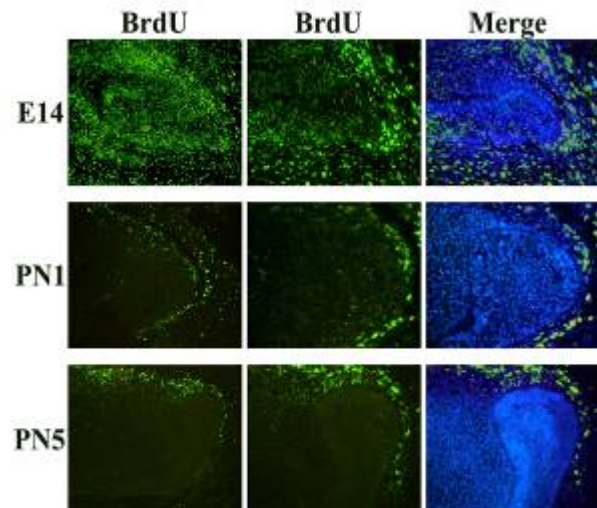


Fig 1: BrdU Label-retaining cells within the developing incisor tooth germs from different developmental stages

DISCUSSION & CONCLUSIONS: The location and markers of epithelial stem cells in the developing mouse incisor tooth germ were confirmed by BrdU pulse-chase analysis, immunohistochemistry, and real-time PCR. The results correspond with the current researches on stem cells of rodent incisors and suggest specific markers of epithelial stem cells.

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