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Original Article

Expression of Ki-67, C-Kit and FGF9 during postnatal development of the mouse submandibular gland

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Abstract: The expression patterns of Ki-67, C-Kit and FGF9 during postnatal development of the mouse submandibular gland have not been depicted in detail. In this study, the submandibular gland of mice at Postnatal 1 day (P1), P3, P5, P10, P14 and P90 were harvested, and the immunohistochemistry staining was applied to detect the expression patterns of Ki-67, C-Kit and FGF9. The results showed that Ki-67, C-Kit and FGF9 expressed both in acini cells and duct cells in submandibular gland in newborn mouse, but the expression gradually decreased and mainly localized around the ducts in adult mice. Taking these findings together, although a definite conclusion cannot be obtained, the findings implied that Ki-67, C-Kit and FGF9 may play important roles in regulating the epithelial histomorphogenesis and in ductal progenitor cells homeostasis in postnatal submandibular gland.

Keywords: Ki-67, C-Kit, FGF9, postnatal development, submandibular gland

Introduction

Radiation therapy is usually applied for treatment of oral and craniofacial malignant tumor, and salivary gland are often exposed to radiotherapy, followed by lead to irreversible hyposalivation and dry mouth, then resulted in dental caries, periodontal disease and swallowing problems [1, 2]. Treatment of hyposalivation is still a challenge, and many methods, including artificial saliva and saliva secretion stimulators, are applied to therapy it [1]. However, these methods can only partially relieve these symptoms, and recent finds revealed that submandibular gland regeneration based on the tetained stem cells in submandibular gland offers new cluse for theraping hyposalivation. Recent studies showed that human salivary gland including progenitor cells that express stem cells markers such as Sca-1, c-Kit, and CD49f, and can completely regenerate the submandibular gland after atrophy induced by duct ligation [3-6]. However, the expression pattern of these stem cell markers in postnatal mouse submandibular gland has not been described in detail.

The development of the submandibular gland, controlled by reciprocal interactions between the epithelium and the cranial neural crest derived ecto-mesenchyme, includes a dynamic process in which cellular proliferation, survival and differentiation are carefully controlled during branching morphogenesis, directed by transcriptional factor and growth factor. The c-Kit pathway plays pivotal roles in cell proliferation, survival and differentiation in mammalian gland development [1]. FGF9 is another important regulator in lung development that is similar to the development of submandibular gland, and also is a stem cell marker in nephron progenitors [7, 8]. Ki-67 was a marker indicates the proliferation ability of cells [9]. In this study, we try to detect the expression patterns of Ki-67, C-Kit and FGF9 during postnatal development of the mouse submandibular gland, and offer some clues for the existing pattern of submandibular gland stem cells during the postnatal development process.

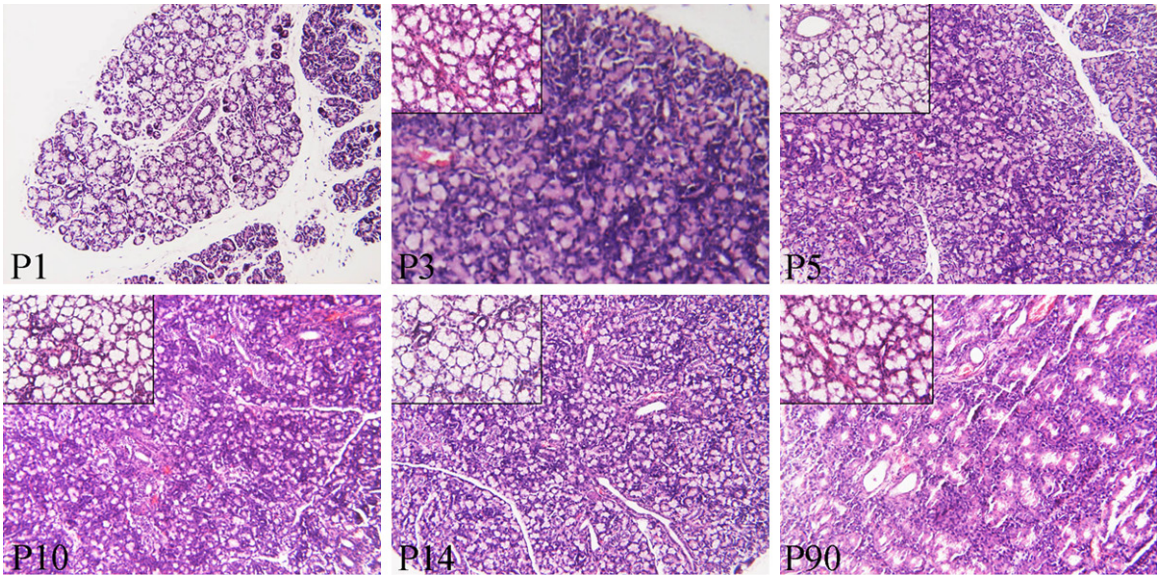

Figure 1. Histological structure of postnatal mouse submandibular gland. The mouse submandibular gland are mixed salivary glands with much serous acini and fewer mucus acini. The ducts increased and more cytoplasm was present in serous cells as the development of mice. The boxed regions are higher magnification images (scale bars = 200 μm) correspond to a region in the lower magnification images (scale bars = 200 μm).

Materials and methods

Mouse strains

The genetic background of all mice was C57BL/6J. All animal experimental procedures were approved by the Institutional Animal Care and Use Committees of our hospital.

Histological analysis

The submandibular gland of mice aged at P1, P3, P5, P10, P14 and P90 were harvested. Then it was fixated with 4% paraformaldehyde (PFA). Followed by embedding in paraffin, and the section were cut at thickness of 5 μm, and stained followed the regular H&E staining method. The stained slides were washed in running tap water, and cleared in xylene, Then mounted with resinous mounting medium.

Immunohistochemistry

Submandibular gland were embedded in paraffin and sectioned at a thickness of 5 μm. Slides were then dewaxed, followed by washing in PBS and blocked for 60 minutes with 4% BSA in PBS containing 0.3% Triton. Then sections were incubated with anti-Ki67, anti-C-Kit (Abcam, 1/100) and anti FGF9 (Santa Cruz Biotechnology, 1/300), overnight at 4°C. The secondary antibodies for FGF9 were Alexa Fluor 568 donkey anti-goat (Jackson), which were diluted 1/250 and incubated for 60 minutes at room temperature. Slides were mounted with Vectashield mounting medium containing DAPI (Invitrogen), then visualized and captured under a fluorescence microscope. For Ki-67 and C-Kit, After washing, the slides were incubated with HRP-labeled secondary antibodies at a dilution of 1:200 at room temperature for 120 minutes, followed by treating with a diaminobenzidine staining kit (Maixin, China) for 3 minutes, then counterstained with hematoxylin and mounted with permount TM mounting medium. Incubation without the primary antibody was used as a negative control.

Results

The histological structure of postnatal mouse submandibular gland

Histological observation showed the postnatal submandibular glands of C57BL/6J genetic background mice were typical mixed salivary glands with serous and mucus acini, and most of acini were serous acini. As the development of mice, there were more and more ducts, and more cytoplasm was present in serous cells (**Figure 1**).

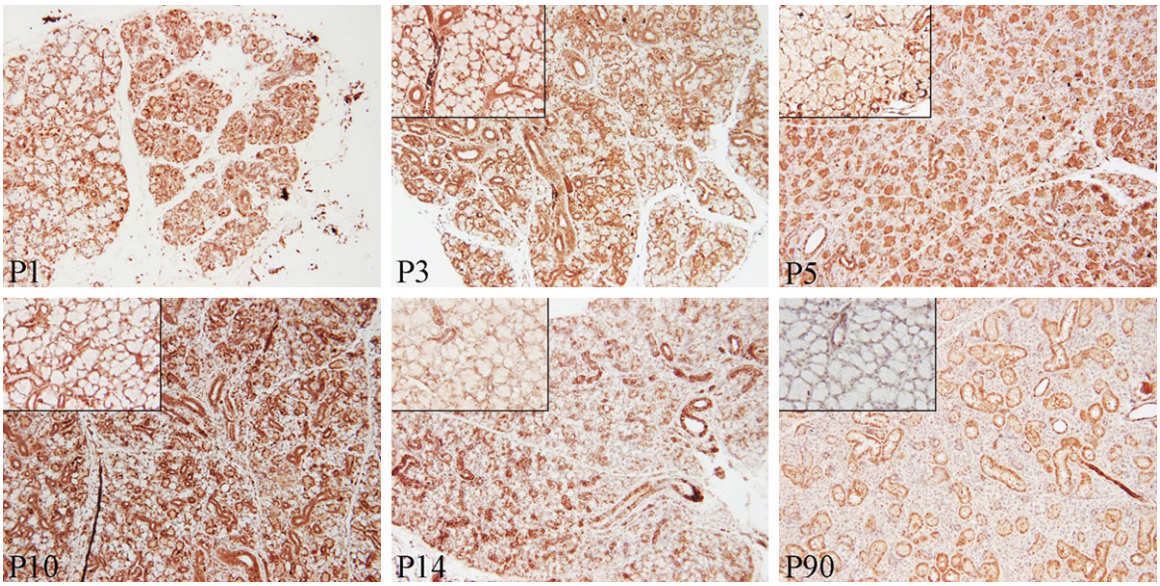

Figure 2. Expression of Ki-67 in postnatal mouse submandibular gland. Ki-67 expressed both in acini cells and duct cells, and showed stronger expression in duct cells, especially in intercalated ducts. The expression of Ki-67 decreased and mainly localized around the ducts as the development of mouse. The boxed regions are higher magnification images (scale bars = 200 μm) correspond to a region in the lower magnification images (scale bars = 200 μm).

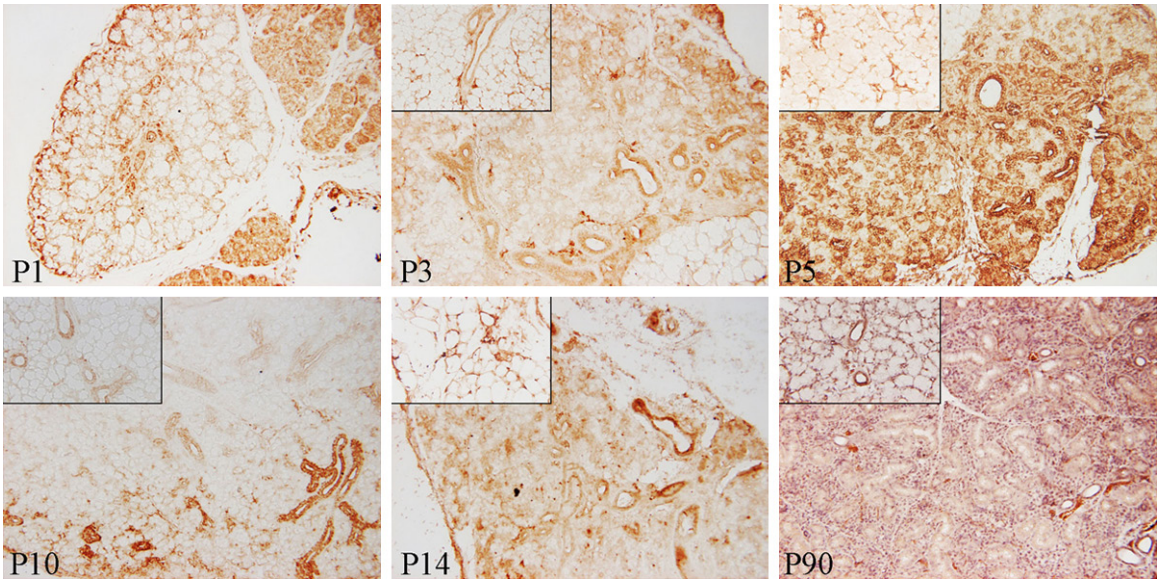

Figure 3. Expression of C-Kit in postnatal mouse submandibular gland. C-Kit expressed both in acini cells and duct cells, but more intensive in mucus acini, and gradually decreased and mainly localized around the ducts as the development of mouse. The boxed regions are higher magnification images (scale bars = 200 μm) correspond to a region in the lower magnification images (scale bars = 200 μm).

The expression of Ki-67 in postnatal mouse submandibular gland

Immunohistochemistry staining showed that Ki-67 expressed both in acini cells and duct cells, but it exhibited stronger expression in duct cells, especially in intercalated ducts, than in acini cells. In addition, the expression of Ki-67 decreased and mainly localized around the ducts from the postnatal 14 days (**Figure 2**).

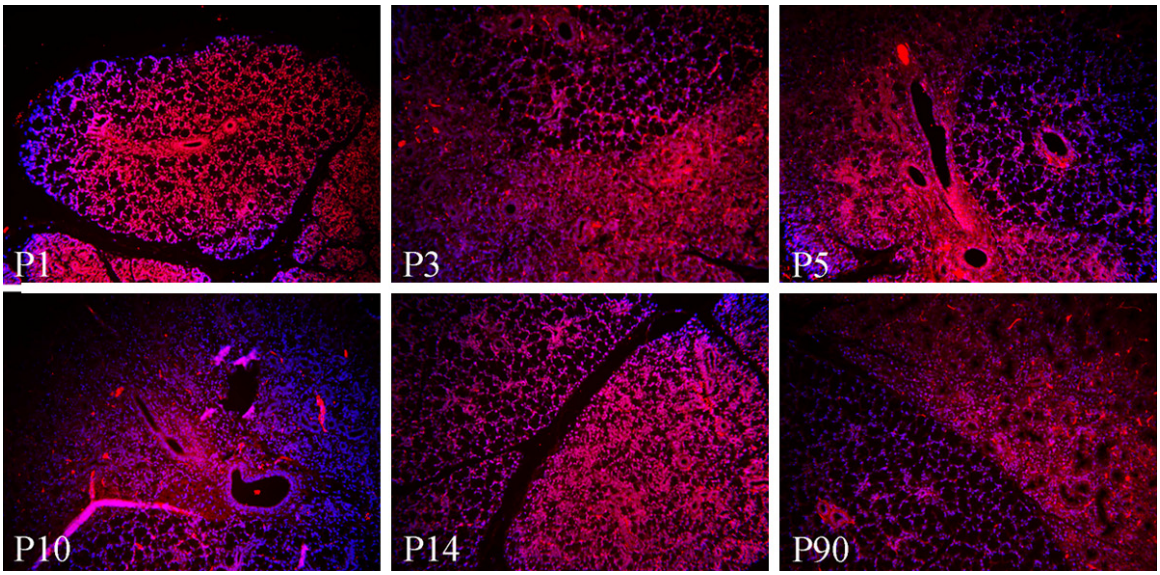

Figure 4. Expression of FGF9 in postnatal mouse submandibular gland. FGF9 strongly expressed both in acini cells and duct cells, but gradually decreased and mainly expressed in duct cells in adult submandibular gland. The boxed regions are higher magnification images (scale bars = 200 μm) correspond to a region in the lower magnification images (scale bars = 200 μm).

The expression of C-Kit in postnatal mouse submandibular gland

Immunohistochemistry staining also showed that C-Kit expressed both in acini cells and duct cells, but showed intensive expression in mucus acini. As the development of submandibular gland, especially in adult mice, the expression of C-Kit gradually decreased and mainly localized around the ducts (**Figure 3**).

The expression of FGF9 in postnatal mouse submandibular gland

The immunofluorescence staining results demonstrated that the FGF9 strongly expressed both in acini cells and duct cells, both in serous and mucus acini tissues in new born submandibular gland. However, the expression of FGF9 gradually decreased and mainly expressed in duct cells, and almost only expressed in partial duct cells in adult submandibular gland (**Figure 4**).

Discussion

The c-Kit pathway plays crucial roles in embryonic development. Previous studies demonstrated that c-Kit is expressed in many developing tissues, such as lung cells, pancreas islet cells, and cells originate from the first branchial arch, and was used as the stem cell marker of epithelial stem or progenitor cells isolated from salivary gland, especially in embryonic development stage [1, 10-12]. Ki-67 is a cell-cycle-associated marker, and has proven to be of value as a marker of cell proliferation [13]. Previous study also showed that Ki-67 may be a early markers of submandibular gland regeneration following ductal ligation [1, 4]. In addition, it also may be a prognostic marker in submandibular gland carcinomas [14]. In this study, our finds showed that C-kit and Ki-67 mainly expressed in duct cells, especially in intercalated ducts in mouse submandibular gland as the postnatal development. Previous findings also suggested that salivary gland stem cells reside in the ductal compartment, and express stem cells markers such as c-Kit, Sca-1, and CD49f. Recent report showed that duct originated c-Kit salivary gland stem cells can be isolated and expanded, and differentiated to both ductal and acinar structures both in vitro and in vivo. Taking these finds together, it implied that submandibular gland cells may mainly existed around the duct region.

Fibroblast growth factor 9 (FGF9) is a secreted signaling molecule that plays crucial roles in the development of lung, a similar organ of submandibular gland [15]. Previous finds suggest-

ed that increased signaling through the FGF9/FGFR axis may be involved in "transforming" lung stem cells [7, 15]. In addition, FGF9 acts on the epithelium to control its proliferation during the development of lung and submandibular gland [5, 15]. In this study, FGF9 mainly expressed in partial duct cells in adult submandibular gland. It implied that FGF9 also acts n the epithelium of submandibular gland, and contributes to regulate the biological process of ductal stem cells of submandibular gland.

In summary, this study showed that Ki-67, C-Kit and FGF9 expressed both in acini cells and duct cells in submandibular gland in newborn mouse, but the expression gradually decreased and mainly localized around the ducts in adult mice. Although definite conclusion cannot be obtained, our finds suggested that Ki-67, C-Kit and FGF9 may play crucial roles in regulating the epithelial histomorphogenesis and in ductal progenitor cells homeostasis in submandibular gland.

Disclosure of conflict of interest

None.

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