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Distribution of α-Gustducin and Vimentin in premature and mature taste buds in chickens

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ABSTRACT

The sensory organs for taste in chickens (*Gallus* sp.) are taste buds in the oral epithelium of the palate, base of the oral cavity, and posterior tongue. Although there is not a pan-taste cell marker that labels all chicken taste bud cells, α-Gustducin and Vimentin each label a subpopulation of taste bud cells. In the present study, we used both α-Gustducin and Vimentin to further characterize chicken taste buds at the embryonic and post-hatching stages (E17-P5). We found that both α-Gustducin and Vimentin label distinct and overlapping populations of, but not all, taste bud cells. A-Gustducin immunosignals were observed as early as E18 and were consistently distributed in early and mature taste buds in embryos and hatchlings. Vimentin immunoreactivity was initially sparse at the embryonic stages then became apparent in taste buds after hatch. In hatchlings, α-Gustducin and Vimentin immunosignals largely co-localized in taste buds. A small subset of taste bud cells were labeled by either α-Gustducin or Vimentin or were not labeled. Importantly, each of the markers was observed in all of the examined taste buds. Our data suggest that the early onset of α-Gustducin in taste buds might be important for enabling chickens to respond to taste stimuli immediately after hatch and that distinctive population of taste bud cells that are labeled by different molecular markers might represent different cell types or different phases of taste bud cells. Additionally, α-Gustducin and Vimentin can potentially be used as molecular markers of all chicken taste buds in whole mount tissue.

1. Introduction

Like mammals, chickens respond to taste stimuli [1,2]. Right after hatch, chickens exhibit aversive responses to bitter and sour tastes [2–4] and prefer umami and fat taste substances [5,6]. In chickens, the sensory organs for taste are taste buds located in the palate, base of the oral cavity, and posterior region of the tongue [7,8]. The structure and locations of the taste buds have been identified by scanning electron microscopy [7,8] and histological analyses [9,10]. At embryonic day 17 (E17) chicken taste bud primordia emerge as clusters of spherical cells and mature at E19 as ovoid-shaped cell clusters that penetrate the epithelium and have a taste pore [10].

While pan-taste cell markers for mammals are available, including K8 [11,12] and KCNQ [13], similar markers for chicken taste buds are lacking. Vimentin, an intermediate filament that is expressed in the mesenchyme, connective tissue cells, and neural precursors [14,15], labeled a population of chicken taste bud cells from embryonic to post-hatching (P) stages [16,17]. More recently, Gustducin expression was found in mature chicken taste buds at P3, suggesting a similar signaling mechanism as that in mammals [9]. However, the following questions remain: (1) when does α-Gustducin expression begin in the developing taste buds for involvement with taste signal transduction; (2) whether α-Gustducin and

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Vimentin label the same or different populations of taste bud cells; and (3) whether α-Gustducin and Vimentin together label all taste bud cells. Clarification and comparison of different molecular markers in the labeling of chicken taste bud cells will provide new insight into how chicken taste buds develop and function.

In the present study, we characterized the distribution of α-Gustducin and Vimentin immunosignals in chicken taste buds in late embryos and hatchlings. We found that α-Gustducin immunosignals emerged early in premature taste buds and were consistently distributed in a large population of mature taste bud cells. Brightly labeled Vimentin$^+$ cells were abundant in taste buds after hatch. The markers labeled distinct and overlapping taste bud cell populations. Although both markers did not label all the taste bud cells, each marker was observed in all the taste buds. These findings provide novel information regarding the development and function of chicken taste buds.

2. Materials and methods

2.1. Animals and tissue collection

Animal use was approved by The University of Georgia Institutional Animal Care and Use Committee and was in compliance with the National Institutes of Health Guidelines for care and use of animals in research.

Fertilized eggs and newly hatched (P0) male chicks were obtained from the Cobb Vantress, Inc, Cleveland Hatchery, Georgia. The chicks were housed in the animal facility until P5 with brood temperature maintained at 35 °C and room temperature at 30 °C. The chicks were continuously monitored. Animals were maintained with food (starter feed) and water *ad libitum* under a 12-12 h dark-light cycle. For tissue collected at embryonic days 17, 18 and 19 (E17, E18, and E19), the fertilized eggs were incubated in a standard egg incubator at 37.7 °C and 50–60% humidity.

Tissue from the palate, base of the oral cavity and posterior tongue was collected at E17, E18, E19, P0, P1, P3 and P5. For the embryonic tissue, timed incubated eggs were cracked and embryos were collected in 0.1 M PBS solution (pH 7.3). P0-P5 chickens were euthanized by decapitation. The tissue samples were dissected and fixed in 4% paraformaldehyde (PFA) for ~4 h at room temperature. The tissue was briefly rinsed in 0.1 M PBS followed by cryoprotection with 30% sucrose at 4 °C for ~48 h. The tissue was trimmed under a dissecting microscope to include regions that contained taste buds, and then embedded in OCT compound (Tissue Tek) at a sagittal orientation and rapidly frozen. Serial and neighboring sections were cut at 6–15 μm thickness, mounted onto gelatin-coated glass slides, and processed for different analyses as below.

2.2. Histological analysis for identification of chicken taste bud structure

Frozen, 6 μm-thick sections from E17, E18, E19 and P0 tissue samples were used for hematoxylin and eosin staining following the standard procedure. The sections were examined under a Zeiss AX10 light microscope.

2.3. Immunohistochemistry and quantification

The primary antibodies used were: α-Gustducin (1:500, serum of rabbit immunized with chicken α-Gustducin, generated by Dr. Shoji Tabata's lab) [9]; Epcam (epithelial cell adhesion molecule markers) (1:200, MBS2027145, Mybioresource Inc, San Diego, CA); Vimentin (1:200, Abcam 28028, Vim3B4, Abcam, Cambridge, MA). Secondary antibodies were: Alexa Fluor 647 conjugated donkey anti-rabbit secondary antibody (1:500, Code: 711-605-152; Jackson Immuno Research Laboratories, West Grove, PA), Alexa Fluor 488 conjugated donkey anti-mouse (1:500, Code: 715-545-150, Jackson Immuno Research Laboratories, West Grove, PA). Frozen sections of the base of the oral cavity tissue at E17-P5 and palate at E19 and P0 were immunostained. In brief, sections were air dried for 1 h at room temperature then rehydrated in 0.1 M PBS. Non-specific staining was blocked using 10% normal donkey serum in 0.1 M PBS containing 0.3% Triton X-100 (PBS-X) for 30 min at room temperature. Then, the sections were incubated with primary antibody in 1% normal donkey serum in PBS-X overnight at 4 °C. Following 3 rinses in 0.1 M PBS (10 min) the sections were incubated with AF 488 (for Vimentin) and AF 647 (for α-Gustducin & Epcam) in 1% NDS in PBS-X for 1 h at room temperature. The sections were then rinsed with PBS and counterstained with DAPI (200 ng/ml in PBS) for 10 min, rinsed in 0.1 M PBS, air dried and cover slipped with ProLong® Diamond antifade mounting medium (P3697, ThermoFisher Scientific). In the negative control slide, primary antibody treatment was omitted or replaced with normal serum/IgG.

Co-localization of α-Gustducin and Vimentin immunosignals was analyzed by single plane laser-scanning confocal microscopy using a Zeiss LSM 710 microscope. Representative photomicrographs were assembled and edited using Adobe Photoshop CC 2015 software. Quantitative analysis was carried out to determine the proportion of Vimentin$^+$ and α-Gustducin$^+$ cells in taste buds at the base of the oral cavity at P5 ($n = 2$). Both Vimentin and α-Gustducin immunosignals, together with the structural organization of DAPI stained nuclei, were used to mark the boundaries of the taste buds on each section. The total number of DAPI stained nuclei within the boundary was quantified and described as the total number of cell profiles in a taste bud. The numbers of Vimentin$^+$, α-Gustducin$^+$, and Vimentin$^+$α-Gustducin$^+$ cells with clear DAPI staining were counted.

3. Results

3.1. Distribution of α-Gustducin and Vimentin in early taste buds in chicken embryos

Based on a previous report stating that chicken taste buds emerge at E17 and mature by E19, we examined the immunosignals of α-Gustducin and Vimentin in E17-19 chicken embryos. At E17, taste bud structures were identified in the H & E stained sections. However, α-Gustducin and Vimentin immunoproducts were not detected in neighboring sections of those with taste bud structures (data not shown). At E18, α-Gustducin signals were observed in the specified cell clusters in the epithelium of the base of the oral cavity (Fig. 1A). Vimentin immunosignals, although sparse, were also observed in the α-Gustducin$^+$ cell cluster region (white dotted outlines, Fig. 1A) and underlying mesenchyme (arrows, Fig. 1A). At E19, α-Gustducin$^+$ cell clusters were apparent and larger in size (Fig. 1B, C) as compared to those observed at E18. Vimentin immunosignals were sparse in the α-Gustducin$^+$ cell cluster region at E19, in addition to being distributed in the underlying mesenchyme. Cells double labeled with both α-Gustducin and Vimentin were observed infrequently (Fig. 1B, arrowhead).

No signals were detected in the specified cell clusters (presumably taste buds) (white dotted outlines, Fig. 1D) in control slides without primary antibody treatment. However, solid labeling of whole cells was observed in negative control sections when omitting primary antibodies (asterisks, Fig. 1D), which suggests that the pattern of labeling outside of taste buds in the sections was non-specific (asterisks, Fig. 1A–C).