



# Implications of the specific localization of YAP signaling on the epithelial patterning of circumvallate papilla

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## Abstract

Circumvallate papilla (CVP) is a distinctively structured with dome-shaped apex, and the surrounding trench which contains over two hundred taste buds on the lateral walls. Although CVP was extensively studied to determine the regulatory mechanisms during organogenesis, it still remains to be elucidated the principle mechanisms of signaling regulations on morphogenesis including taste buds formation. The key role of Yes-associated protein (YAP) in the regulation of organ size and cell proliferation in vertebrates is well understood, but little is known about the role of this signaling pathway in CVP development. We aimed to determine the putative roles of YAP signaling in the epithelial patterning during CVP morphogenesis. To evaluate the precise localization patterns of YAP and other related signaling molecules, including  $\beta$ -catenin, Ki67, cytokeratins, and PGP9.5, in CVP tissue, histology and immunohistochemistry were employed at E16 and adult mice. Our results suggested that there are specific localization patterns of YAP and Wnt signaling molecules in developing and adult CVP. These concrete localization patterns would provide putative involvements of YAP and Wnt signaling for proper epithelial cell differentiation including the formation and maintenance of taste buds.

**Keywords** Differentiation · Signaling regulation · Pattern formation · Taste bud · Yes-associated protein

## Introduction

The dorsal surface of the mammalian tongue is covered with three types of specialized gustatory papillae, including fungiform, foliate, and circumvallate papillae (CVP) (Jung

et al. 2004; Adhikari et al. 2017; Zhang et al. 2020). CVP is a single and is centrally situated on the posterior third of the tongue in rodents. Development of the CVP begins with thickening of the dorsal epithelium in the middle of the posterior third of the tongue at embryonic day 11.5 (E11.5) in mice. At E13.5, the CVP forms a dome structure with invaginated epithelium and condensed mesenchyme. At E15.5, the epithelium at both ends of the domed roof generates the surrounding furrows of the CVP, eventually forming Von Ebner's gland (VEG) (Jitpukdeebodindra et al. 2002; Iwasaki et al. 2012; Adhikari et al. 2017). Concurrent with morphogenesis of the CVP, dense nerve innervations appear and penetrate the epithelium. Development of the taste bud (TB) is considered nerve-dependent (Oakley and Witt 2004; Kapsimali and Barlow 2013) and is first observed in a specific pattern in the trench wall epithelium at approximately E18 (Liu et al. 2007; Thirumangalathu and Barlow 2015). Ultimately, the CVP is distinctively structured with an apex, with the surrounding trench containing over two hundred TBs on its lateral walls and the VEG (Kim et al. 2009; Adhikari et al. 2017; Kramer et al. 2019). CVP development shares similarities with the development of various ectodermal

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appendages, including mammary glands, hair, feathers, and teeth (Jung et al. 2004). CVP formation follows a distinct series of developmental processes, but, unlike other organs of ectodermal origin, it undergoes a unique developmental stage that includes nerve innervations for final differentiation into gustatory papilla. CVP progenitor cells can differentiate into the TB and VEG. Therefore, due to the unique developmental characteristics of CVP, it has been used as an experimental model to understand cytodifferentiation and patterning during organogenesis (Jitpukdeeboontra et al. 2002; Jung et al. 2004; Liu et al. 2007; Kim et al. 2009). However, to date, most studies have only investigated the signaling pathways that are shared between development of CVP and the formation of other organs. The detailed molecular mechanisms underlying CVP differentiation have not been elucidated. In particular, studies have not investigated the early pattern formation of epithelial tissues, with or without TB, and the maintenance of physiological identification of TBs for proper sensation.

In developmental processes, elaborate control of cell growth and differentiation is crucial for the precise morphogenesis of tissues and organs. Recent research has suggested that Yes-Associated Protein (YAP) is a key downstream effector and transcriptional regulator of the Hippo pathway, which is primarily involved in the regulation of organ growth, regeneration, and tumorigenesis (Wang et al. 2017). Hippo pathway signaling is involved in transitioning from a proliferative to a quiescent state, ensuring precise differentiation in a biological context-specific manner (Mahoney et al. 2014; Chen et al. 2019). The nuclear/cytoplasmic localization of YAP plays a key role in regulating the specification, proliferation, self-renewal, and differentiation of an organ-specific progenitor/stem cell population (Camargo et al. 2007; Pan 2010; Habbig et al. 2011; Mahoney et al. 2014; Zhao et al. 2014; Panciera et al. 2016). Cytoplasmic phosphorylated YAP has been reported to have an important role in cell growth arrest, induced by cell to cell contact, and in modulating a variety of signaling pathways for proper tissue development (Yu et al. 2015; Davis and Tapon 2019). In particular, YAP plays an crucial role in the development of various organs, including the lung, liver, kidney, pancreas, salivary gland, mammary gland, and small intestine (Varelas 2014). Depletion of YAP results in various abnormalities in the heart, skin, and branching organs (Yu et al. 2015). These findings indicate that YAP is selectively required for development of certain, but not all, tissues. Interactions among the sonic hedgehog (shh) and canonical Wnt/ $\beta$ -catenin pathways have been widely investigated during development of papilla and TB (Iwatsuki et al. 2007; Mistretta and Kumari 2019).  $\beta$ -catenin, which mediates intracellular signal transduction in the Wnt signaling pathway, has been reported to play a critical role in the formation of TB (Barlow 2015; Thirumangalathu and

Barlow 2015). Mutations in and overexpression of  $\beta$ -catenin are associated with carcinogenesis in various organs, due to regulation of YAP activity (Wang et al. 2017; Zhang et al. 2019). However, the role of YAP in CVP development, including the formation and maintenance of TB, is not yet clearly identified. It is also unknown whether YAP regulates  $\beta$ -catenin to control proliferation and differentiation for the formation and regeneration of epithelium in developing and adult CVP. There is still an incomplete understanding of epithelial patterning, including fate determination, during TB and papilla morphogenesis.

Therefore, this study aims to determine the putative roles of YAP by examining the immunolocalization of proliferation, epithelial differentiation, and nerve innervation markers in CVP. We examine the precise localization patterns of YAP and related signaling molecules, including  $\beta$ -catenin, Ki67, cytokeratins, and PGP9.5, for pattern formation of the epithelium in developing and adult CVP.

## Materials and methods

### Animals

All experiments were carried out according to the guidance of the Kyungpook National University, School of Dentistry, Intramural Animal Use and Care Committee (KNU 2015 – 136). The adult ICR mice were housed in a room temperature of  $22 \pm 2$  °C, humidity of  $55 \pm 5\%$ , and artificial illumination with lights on from 05:00 to 17:00 h. Food and water were given *ad libitum*. Mouse embryos were obtained from time-mated pregnant mice. The day on which a vaginal plug was confirmed was designated as E0. Mouse embryos at E16 and male mice (8 weeks old) were used in this study.

### Tissue preparation

The specimens were fixed in 4% paraformaldehyde (0.1 M phosphate buffer, pH 7.4) at 4 °C for 1 day. After fixation, the specimens were routinely embedded in paraffin and sectioned at a thickness of 7  $\mu$ m in the sagittal plane along the long axis of the tongue. The sections were prepared for hematoxylin and eosin (H&E) and immunohistochemical staining.

### Immunohistochemistry

Immunohistochemistry was performed as described previously (Adhikari et al. 2018). Paraffin sections were incubated with following primary antibodies: rabbit polyclonal antibodies against Ki67 (cat. no. ab15580; Abcam, UK; 1:100), YAP (cat. no. 4912S; Cell Signaling Technology, USA; 1:500),  $\beta$ -catenin (cat. no. 9562S; Cell Signaling Technology, USA; 1:1,800), cytokeratin (CK) 8 (cat. No. ab59400; Abcam, UK;

1:200), and CK14 (cat. No. ab53115; Abcam, UK; 1:200), and rabbit monoclonal to p-YAP (cat. No. 13008S; Cell Signaling Technology, USA; 1:1250) and unphosphorylated (active)  $\beta$ -catenin (cat. no. 8814S; Cell Signaling Technology, USA; 1:800), and mouse monoclonal to PGP9.5 (cat. no. ab8189; Abcam, UK; 1:100). Sections were then incubated with a goat anti-rabbit biotinylated secondary antibody (Invitrogen, Camarillo, CA, USA) or an anti-mouse biotinylated secondary antibody (Invitrogen, Camarillo, CA, USA) at room temperature for 30 minutes, followed by the application of HRP-conjugated streptavidin (Invitrogen, Camarillo, CA, USA). The immunoreactions were visualized using a DAB substrate (Ultravision plus detection system; Lab-Vision, CA, USA). All experiments were performed a minimum of three times.

### Photography and image analysis

All slides stained for histology and immunohistochemistry were photographed using a DM2500 microscope (Leica Microsystems, Germany) equipped with a DFC310 FX digital CCD camera (Leica Microsystems, Germany). Image analysis were performed by three blind investigators using stereomicroscope. The investigators visually graded in immunohistochemical staining of Ki67, YAP, p-YAP,  $\beta$ -catenin, and non-p- $\beta$ -catenin. All grades were adopted by consensus. The developing and adult CVP were horizontally divided into three and graded semi-quantitatively according to the following score: –, none; +, exist; and ++, strong staining (Adhikari et al. 2018) (Table 1).

## Results

### Histogenesis of CVP and TB

H&E staining was used for histological analysis of the epithelial structure of the CVP at E16 and post-natal (PN) 8 weeks (Fig. 1A, B). At E16, H&E staining of developing

CVP showed specific morphogenesis, with dome-like epithelial rearrangement in the apex region of the CVP (Fig. 1A1, A2). Below the apex region, there were invaginated epithelial structures in both lateral sides of the CVP, called the lateral trench walls (Fig. 1A3). At E16, TB structures were not observed (Fig. 1A1). At PN8 weeks, the apex portion of the CVP was keratinized and multiple cell layers existed in the epithelium (Fig. 1B1, B2). In the lateral trench wall regions of the CVP epithelium, there were complex and distinct structures with patterned arrangements of TBs (Fig. 1B1, B2, B3). Particularly, the TB had an oval shaped structure with inner spindle cells and cuboidal basal cells (Fig. 1B3). Von Ebner's gland structure was examined below the CVP (Fig. 1B1).

### Localization patterns of Ki67, YAP, and p-YAP in CVP

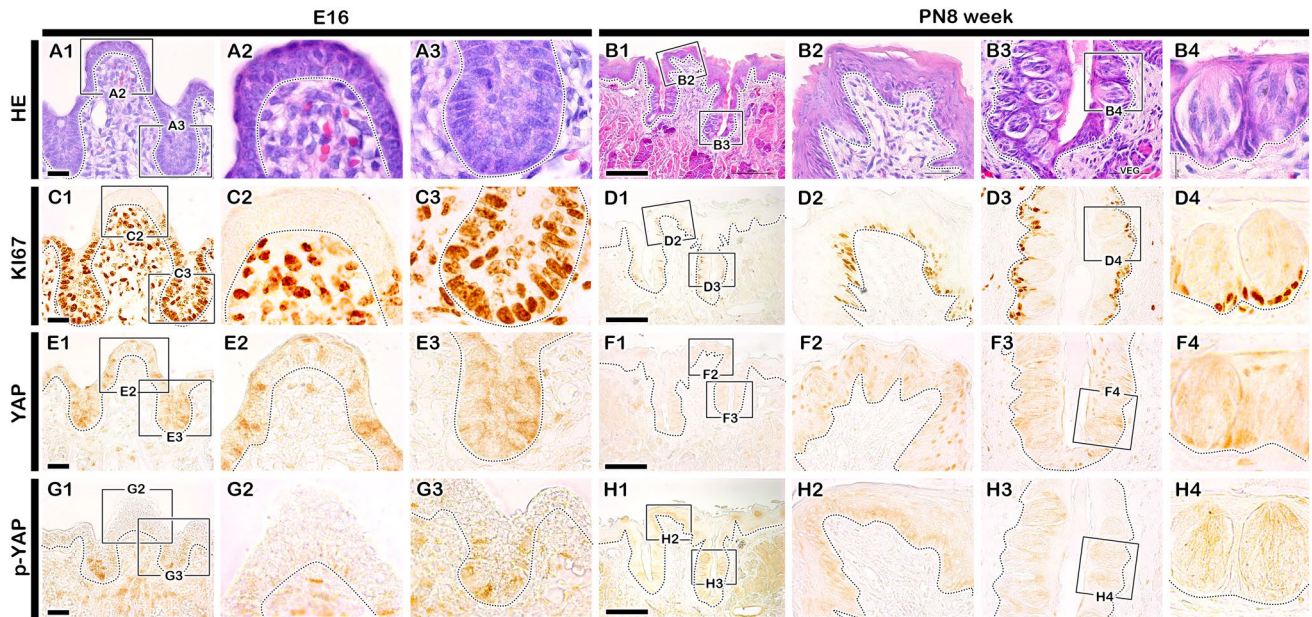
Ki67, a cell proliferation marker, was examined at E16 and PN8 weeks to define the specific proliferation pattern of CVP and TB (Fig. 1C1, D1). At E16, there was a strong positive reaction against Ki67 in the lateral trench wall and invaginating epithelium (Fig. 1C1–C3). Whereas the apex region of CVP epithelium did not show any positive staining for Ki67 (Fig. 1C1, C2). At PN8 weeks, Ki67 staining was observed in the basal layer of the entire epithelium of CVP (Fig. 1D1–D3). Particularly, Ki67 staining was detected in the basal layer of TB cells, which are supposed to be supporting cells, at PN8 weeks (Fig. 1D4). To detect the precise localization patterns of Hippo signaling molecules in development and maintenance of CVP, we examined the localization of YAP and p-YAP (Fig. 1E, F, G, H). At E16, the nuclear localization pattern of YAP, which is considered to be a signal that turns off Hippo signaling and is involved in cell proliferation, was examined in the trench wall and invaginating epithelium of developing CVP (Fig. 1E1–E3). Weak cytoplasmic localization of YAP was observed in the apex portion of the developing CVP (Fig. 1E2). The localization pattern of p-YAP, which has been reported to indicate

**Table 1** Statistical analysis of immunohistochemical staining against Ki67, YAP, p-YAP,  $\beta$ -catenin, and non-p- $\beta$ -catenin in developing and adult CVP

	E16			Adult			
	Apex	Trench wall	Invaginating epithelium	Apex	TB forming region	VEG region	Taste buds
Ki67	–	++	++	+	+	+	++
YAP							
(Nuclear)	–	+	+	+	+	+	+(basal)
(cytoplasm)	+	+	–	+	+	–	+
p-YAP							
(cytoplasm)	–	+	+	+	+	+	+(pore)
$\beta$ -catenin	+	++	+	+	+	+	++(pore)
Non-p- $\beta$ -catenin	++	+	+	+	+	+	++(basal)

–: none, +: exist, ++: strong





**Fig. 1** Histogenesis of CVP and Taste buds. **A1–A3** Hematoxylin and eosin (H&E) staining of developing CVP. **B1–B4** H&E staining of adult CVP. **C1–C3** Localization pattern of Ki67 at E16, (**D1–D4**) Localization pattern of Ki67 at adult CVP. **E1–E3** At E16, localization pattern of YAP, **F1–F4** and adult CVP. **G1–G3** Localization pattern of p-YAP in developing CVP, **H1–H4** and adult CVP. Dotted line

demarcates the dome-like epithelial region of CVP. *VEG* Von Ebner's gland. Scale bars 200  $\mu$ m (**B1**, **D1**, **F1**, **H1**), 100  $\mu$ m (**A1**, **C1**, **E1**, **G1**, **B2**, **D2**, **F2**, **H2**, **B3**, **D3**, **F3**, **H3**), 50  $\mu$ m (**A2**, **C2**, **E2**, **G2**, **A3**, **C3**, **E3**, **G3**, **B4**, **D4**, **F4**, **H4**). The solid boxes depict higher magnification views (**X2**, **X3**, **X4**)

Hippo signaling, was examined in the trench wall and the invaginating epithelium regions, and cytoplasmic localization was observed at E16 (Fig. 1G1–G3). At PN8 weeks, positive nuclear staining for YAP was observed in the apex and TB forming regions of the CVP (Fig. 1F1). YAP exhibited a sporadic localization pattern in the apex epithelium of the CVP and had positive nuclear staining in the basal layer cells of TBs at PN8 weeks (Fig. 1F4). Weak cytoplasmic staining for p-YAP was detected in the basal layer of the apex region of the epithelium and the taste pore side of the TBs at PN8 weeks (Fig. 1H1–H4).

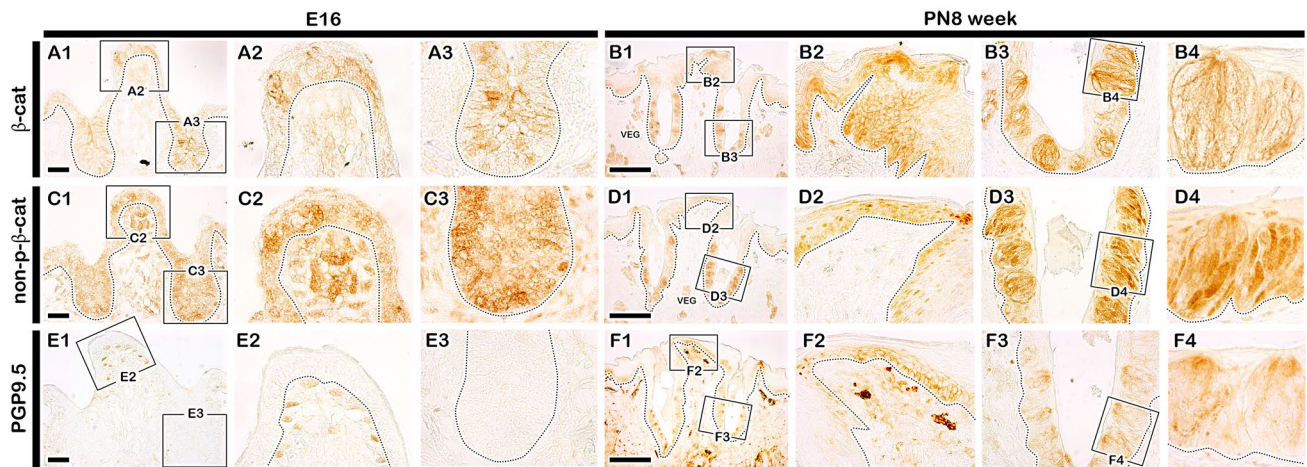
### Immunolocalization of $\beta$ -catenin, unphosphorylated $\beta$ -catenin and PGP9.5 in CVP

In order to identify the putative roles of Wnt/ $\beta$ -Catenin signaling in CVP, we examined the precise localization patterns of  $\beta$ -catenin and unphosphorylated  $\beta$ -catenin at E16 and PN8 weeks. At E16, nuclear localization of  $\beta$ -catenin was detected in the apex and invaginating regions of the CVP epithelium (Fig. 2A). Interestingly, the strongest staining for  $\beta$ -catenin was observed in both the boundary epithelium and the epithelium forming the trench wall at E16 (Fig. 2A2, A3). At PN8 weeks, strong  $\beta$ -catenin staining was observed in the apex and TB regions of the CVP (Fig. 2B1–B3). In the TBs, stronger positive staining for  $\beta$ -catenin was observed

in the taste pore side as compared to the basal layer region (Fig. 2B4). Unphosphorylated  $\beta$ -catenin was detected with a broad localization pattern throughout the entire epithelium of the CVP at E16 (Fig. 2C1). Particularly, a strong nuclear and cytoplasmic localization pattern of unphosphorylated  $\beta$ -catenin was observed in the apex and invaginating epithelium of developing CVP at E16 (Fig. 2C2, C3). At PN8 weeks, there was strong positive staining for unphosphorylated  $\beta$ -catenin in the TBs, especially on the bottom side of TBs (Fig. 2D). To understand the developmental role of nerve innervations during organogenesis and the putative involvement of peripheral nerves in the maintenance of adult CVP, we examined the detailed localization pattern of PGP9.5 in the CVP at E16 and PN8 weeks (Fig. 2E and F). PGP9.5 was localized to the mesenchymal core region underlying the apex epithelium of the CVP (Fig. 2E1, E2). At E16, there was no positive staining for PGP9.5 in the entire epithelium of the CVP (Fig. 2E). At PN8 weeks, there was positive staining for PGP9.5 below the apex epithelium and TBs (Fig. 2F1–F4).

### Localization patterns of cytokeratins in CVP

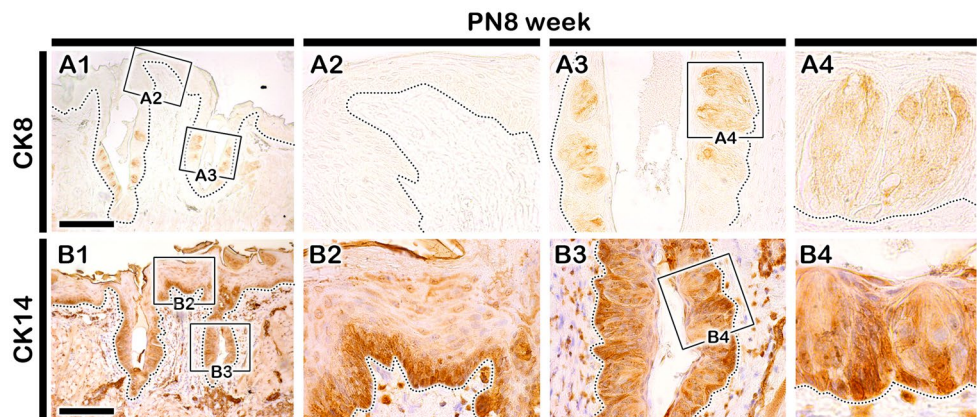
We examined the precise localization patterns of CKs, which are critical markers of epithelial differentiation, in order to reveal the relationship between signaling regulation and



**Fig. 2** Immunolocalization of  $\beta$ -catenin and PGP9.5 in CVP. **A1–A3** Localization pattern of  $\beta$ -catenin in developing, **B1–B4** and adult CVP. **C1–C3** At E16, localization pattern of non-phospho  $\beta$ -catenin, **D1–D4** and adult CVP. **E1–E3** Localization pattern of PGP9.5 at E16 and **F1–F4** adult CVP. Dotted line demarcates the dome-like

epithelial region of CVP. VEG Von Ebner's gland. Scale bars 200  $\mu$ m (**B1**, **D1**, **F1**), 100  $\mu$ m (**A1**, **C1**, **E1**, **B2**, **D2**, **F2**, **B3**, **D3**, **F3**), 50  $\mu$ m (**A2**, **C2**, **E2**, **A3**, **C3**, **E3**, **B4**, **D4**, **F4**). The solid boxes depict higher magnification views (**X2**, **X3**, **X4**)

**Fig. 3** Immunolocalization of CK8 and CK14 in CVP. **A1–A4** Localization pattern of CK8 in adult CVP. **B1–B4** At adult, localization pattern of CK14. Dotted line demarcates the dome-like epithelial region of CVP. Scale bars 200  $\mu$ m (**A1**, **C1**), 100  $\mu$ m (**X2**, **X3**), 50  $\mu$ m (**X4**). The solid boxes depict higher magnification views (**X2**, **X3**, **X4**)



the morphological changes of CVP, including TBs, at PN8 weeks (Fig. 3). Weak CK8 localization was observed in the TBs, especially in the TB pore site (Fig. 3A). However, there was strong positive staining for CK14 in the entire epithelium of the apex and lateral regions of the CVP, particularly in the basal layer (Fig. 3B).

## Discussion

### Specific localization of TB in the CVP epithelium

The key role of YAP in the regulation of organ size and cell proliferation in vertebrates is now well understood (Zhao et al. 2007; Davis and Tapon 2019). However, little is known about the role of this signaling pathway in the regulation of cell state transitions between proliferation and differentiation, which are critical for development.

CVP, which is an ideal model for studying epithelial pattern formation, has a distinctive structure, with patterned arrangement of TB in the lateral trench wall region and the non-TB bearing and keratinized apex region (Fig. 1). In this study, we identified the localization pattern of YAP, which is the key effector of the Hippo pathway, in the CVP of developing and adult mice. To evaluate the precise localization patterns of YAP and other related molecules, immunohistochemistry was used in mice at E16 and in adult mice. The mice undergoing development had epithelial patterning of the TB and mice in the adult stage had TB that function as a gustatory organ. Histogenesis, with specific localization patterns of CKs, also provided rational evidence for the selection of the developmental stages. We aimed to assess two stages in which we could investigate the epithelial patterning of TBs in development and the maintenance of TBs for gustatory function (Fig. 3). In this study, immunohistochemistry revealed that



CK14 was localized on the basal cells of the TBs with distinctively strong intensity (Fig. 3B).

At E16, the epithelium of the CVP invaginated into the mesenchyme to form a dome-shaped structure with a mesenchymal core (Fig. 1A). The epithelial layer of the CVP began to differentiate into stratified squamous epithelium, without showing TB structures. As previously reported, morphologically, the TBs of the CVP first appear at approximately E18 and develop only in the lateral trench epithelium at birth (Uchida et al. 2003; Barlow 2015). Adult CVP (8-weeks-old) had well-developed stratified squamous epithelium, with apex and lateral trench epithelium containing numerous TBs (Fig. 3). Each of TB displayed the specified cell-type components with specific localization patterns (Asano-Miyoshi et al. 2008). The localization patterns of CKs, including CK8 and CK14, which are epithelial-specific indicators, were investigated in the epithelium of the adult CVP (Fig. 3). One of the simple epithelial keratins, CK8, is a marker for mature and maturing TB cells (Asano-Miyoshi et al. 2008). Our results also showed the localization of CK8 in the TBs of the adult CVP (Fig. 3A). In addition, we examined CK14 (Fig. 3), which known to be localized to mitotically active basal cells of the stratified squamous epithelium and is a well-known marker for keratinization (Lee et al. 2006; Asano-Miyoshi et al. 2008). As previously reported, CK14 was highly localized to the basal layer of the stratified epithelium of the adult CVP (Asano-Miyoshi et al. 2008; Iwasaki et al. 2011). Interestingly, the specific localization patterns of CK8 and CK14 revealed that CK8 was localized in the pore region and that CK14 was localized in the bottom region of TBs (Fig. 3A4, B4). PGP9.5 staining, marking the peripheral nerve patterns (Chou et al. 2001), was observed in the basal lamina of the developing CVP at E16 and in the densely innervate TB cells of the adult CVP (Fig. 2E3, 2F4). These specific localization patterns of CKs and PGP9.5 suggest that the stages of CVP selected in this study, including E16 and PN8 weeks, are ideal time points for examination of CVP histogenesis, particularly for understanding the formation and maintenance of TBs in the CVP.

### The Hippo signaling pathway in CVP

The Hippo signaling pathway is composed of a core kinase cascade terminating with phosphorylation of YAP. Phosphorylation of YAP leads to its retention in the cytoplasm, while unphosphorylated YAP, which is transported to the nucleus, acts as a transcriptional activator (Hansen et al. 2015). In mammals, YAP, the key downstream effector of the Hippo pathway, activity is generally high during the embryonic stage, but drastically declines to a basal level soon after birth (Wang et al. 2017). Based on our findings, in developing CVP at E16, YAP is primarily localized in the cytoplasm of the apical epithelium and the nucleus of the

invaginating lateral epithelium and deep trench epithelium, which will form the VEG (Fig. 1E). The localization of YAP was restricted to the basal keratinocytes in the adult CVP (Fig. 1F). Previous studies have reported that YAP regulates organ size and controls cell proliferation in response to cell density (Zhao et al. 2007; Davis and Tapon 2019). Our results also suggest that there is a strong correlation between the nuclear localization of YAP and staining with the proliferation marker Ki67 (Fig. 1C-F). Ki67 staining was also intensively observed in the lateral epithelium and VEG-forming region, in the region with the deep trench of invaginated epithelium (Fig. 1D1). The nuclear localization pattern of YAP in epithelial cells of the lateral and VEG-forming regions suggests that YAP may be involved in pattern formation and specification of the TB by controlling proliferation in developing CVP (Fig. 1C1). Subcellular localization and phosphorylation of YAP may also play a key role in modulating differentiation status (Lian et al. 2010; Mahoney et al. 2014; Wang et al. 2017). At E16, phosphorylated YAP was localized to the cytoplasm in the trench wall epithelium of the developing CVP, suggesting that the localization of phosphorylated YAP is related with the differentiation of the TB (Fig. 1G). In addition, the activity of YAP was highly conserved in several mature organs, including the small intestine, liver, and skin, which have been well-characterized and have a high turnover rate of parenchymal cells (Lange et al. 2015; Wang et al. 2017). Our study also revealed that nuclear YAP was localized in the undifferentiated basal cells of the TB, while cytoplasmic phosphorylated YAP was more distinctively localized in the differentiated cells of the taste pore side of TBs in the adult CVP (Fig. 1F and H). Basal cells also had localization of YAP and Ki-67, a proliferation marker (Fig. 1F4). Distinctive spatial localization patterns for YAP and Ki-67 indicate that the proliferative activity was exclusively enhanced in basal cells below the TB. YAP could be intimately involved in this proliferative activity of basal cells (Fig. 1D4). Therefore, YAP was considered to play an important role in development by controlling the proliferation and differentiation of progenitor cells (Lange et al. 2015; Wang et al. 2017; Chen et al. 2019). The YAP localization pattern in the TBs of adult CVP suggests that it is involved in TB maintenance, as well as renewal of the papilla epithelium.

### Hippo and Wnt signalings in CVP

The complex relationship between the Hippo and Wnt pathways has been relatively well studied, mainly in the intestine (Hansen et al. 2015). Previous studies reported that phosphorylated YAP inhibits the Wnt signaling pathway through interactions with disheveled and/or the cytoplasmic sequestration of  $\beta$ -catenin (Heallen et al. 2011; Imajo et al. 2012; Hansen et al. 2015; Kim et al. 2016).

Other studies found that nuclear YAP activates expression of  $\beta$ -catenin, which mediates T cell factor-dependent transcription and facilitates the nuclear translocation of SHP2, ultimately activating Wnt target gene transcription (Kim and Jho 2014; Hansen et al. 2015). In our study, nuclear unphosphorylated  $\beta$ -catenin was localized in the apical and lateral portion of developing CVP epithelium, with overlapping nuclear YAP localization at E16 (Figs. 1E and 2C). In adults, nuclear unphosphorylated  $\beta$ -catenin was exclusively localized in the basal side, rather than the taste pore side, of TBs (Fig. 2D). This result suggests that active  $\beta$ -catenin localization in the apical and lateral epithelium may be involved in CVP epithelial cell arrangement and TB differentiation in developing CVP (Fig. 2A). Interactions between YAP and  $\beta$ -catenin are important for organogenesis and homeostasis in various organs, in order to regulate organ size and differentiation (Kim et al. 2016; Pan et al. 2018). Wnt signaling pathway is important for differentiation and maintenance of epithelial cells, however, it is not elucidated the upstream regulation of its expressions and interactions among other signaling pathways in a range of organogenesis (Pinto 2003; Fevr et al. 2007; Grigoryan et al. 2008; Choi et al. 2013). Our results suggest that there are specific localization patterns of Hippo and Wnt signaling molecules in CVP, which provide a putative model for Wnt signaling regulation and epithelial cell differentiation. Overall, our findings suggest that there are possible interactions between the Hippo and Wnt signaling pathways in epithelial patterning in embryonic development and renewal of TB in the adult CVP. Although further studies are needed to identify the more precise role of YAP in CVP development and TB renewal, we have presented the localization pattern of YAP, and identified that it has a role in controlling cell fate, such as pattern formation and specification. We demonstrate the morphological trends regulating the early pattern formation of epithelium in the developing CVP and maintenance of TBs for gustatory function of mouse. Clarification for these distinct morphogenetic events and the accompanying specific localization patterns of YAP in developing and adult CVP, will serve a crucial step towards an understanding the principle mechanisms on patterning and maintaining of CVP.

## Conclusions

This study provides principle information about dynamic epithelial morphogenesis during embryonic stages and the maintenance of TB in CVP, a specialized and complex structure and working as a sensory organ. Based on our results, we suggest that early CVP development and TB

turnover throughout adult life is regulated by cell proliferation, differentiation, and quiescence, which is mediated by YAP and its interactions with other signaling pathways, including Wnt/ $\beta$ -catenin.

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**Author contributions** JYK and TYK contributed to conception, data acquisition, analysis and interpretation, drafted and revised the manuscript. ESL and YPA contributed to the analysis and interpretation of data. EP, SS, and WJS contributed to the analysis and quantification of data. JYK and JKJ contributed to conception, design, analysis and data interpretation and critically revised the manuscript.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflicts of interest.

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