Developmental Biology 382 (2013) 82–97

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

Multiple Shh signaling centers participate in fungiform papilla and taste bud formation and maintenance

Hong Xiang. Liu [a], Alexandre Ermilov [b], Marina Grachtchouk [b], Libo Li [a], Deborah L. Gumucio [c], Andrzej A. Dlugosz [b,c], Charalotte M. Mistretta [a,*]

[a] Department of Biologic and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, MI, USA
[b] Department of Dermatology, Medical School, University of Michigan, USA
[c] Department of Cell and Developmental Biology, Medical School, University of Michigan, USA

ARTICLE INFO

Article history:
Received 18 March 2013
Received in revised form
24 July 2013
Accepted 26 July 2013
Available online 2 August 2013

Keywords:
Fungiform papilla
Papilla placode
Paracrine signaling
Stem cells
Taste cell progenitors
Ptch
Gli1
Gli2

ABSTRACT

The adult fungiform taste papilla is a complex of specialized cell types residing in the stratified squamous tongue epithelium. This unique sensory organ includes taste buds, papilla epithelium and lateral walls that extend into underlying connective tissue to surround a core of lamina propria cells. Fungiform papillae must contain long-lived, sustaining or stem cells and short-lived, maintaining or transit amplifying cells that support the papilla and specialized taste buds. Shh signaling has established roles in supporting fungiform induction, development and patterning. However, for a full understanding of how Shh transduced signals act in tongue, papilla and taste bud formation and maintenance, it is necessary to know where and when the Shh ligand and pathway components are positioned. We used immunostaining, *in situ* hybridization and mouse reporter strains for *Shh*, *Ptch1*, *Gli1* and *Gli2*-expression and proliferation markers to identify cells that participate in hedgehog signaling. Whereas there is a progressive restriction in location of Shh ligand-expressing cells, from placode and apical papilla cells to taste bud cells only, a surrounding population of *Ptch1* and *Gli1* responding cells is maintained in signaling centers throughout papilla and taste bud development and differentiation. The Shh signaling targets are in regions of active cell proliferation. Using genetic-inducible lineage tracing for *Gli1*-expression, we found that *Shh*-responding cells contribute not only to maintenance of filiform and fungiform papillae, but also to taste buds. A requirement for normal Shh signaling in fungiform papilla, taste bud and filiform papilla maintenance was shown by *Gli2* constitutive activation. We identified proliferation niches where Shh signaling is active and suggest that epithelial and mesenchymal compartments harbor potential stem and/or progenitor cell zones. In all, we report a set of *hedgehog* signaling centers that regulate development and maintenance of taste organs, the fungiform papilla and taste bud, and surrounding lingual cells. Shh signaling has roles in forming and maintaining fungiform papillae and taste buds, most likely via stage-specific autocrine and/or paracrine mechanisms, and by engaging epithelial/mesenchymal interactions.

Introduction

The adult fungiform papilla is a complex set of tissues and cell types that reside in the specialized multilayered epithelium of the tongue. This unique sensory organ includes: taste buds; a layered and keratinized surface epithelium; and, lateral epithelial walls that extend into the underlying connective tissue to surround a core of lamina propria cells (Fig. 1). The core is engorged with blood vessels and bundles of nerve fibers from geniculate and trigeminal sensory ganglia. Furthermore, the core itself is specialized into apical and more basal regions. Surrounding the fungiform papillae are other specializations of the lingual epithelium, the filiform papillae. The filiform structures do not contain taste buds, but have sharp, heavily keratinized, apical spines. Thus, to develop and maintain the fungiform papillae, many regulatory steps must be orchestrated that require close interactions between the tongue epithelium and connective tissue core.

Placement of fungiform papillae also is tightly regulated, on the anterior two thirds of the tongue (Mistretta, 1991; Mbiene et al., 1997). These patterned papillae, accessible for observation and manipulation, are an important but often over-looked model for the development of ectodermal specializations, and for understanding fundamentals of sensory cell differentiation.

* Correspondence to: Department of Biologic and Materials Sciences, Room 6228, School of Dentistry, University of Michigan, Ann Arbor, MI 48109-1078, USA.
E-mail address: chmist@umich.edu (C.M. Mistretta).

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http://dx.doi.org/10.1016/j.ydbio.2013.07.022

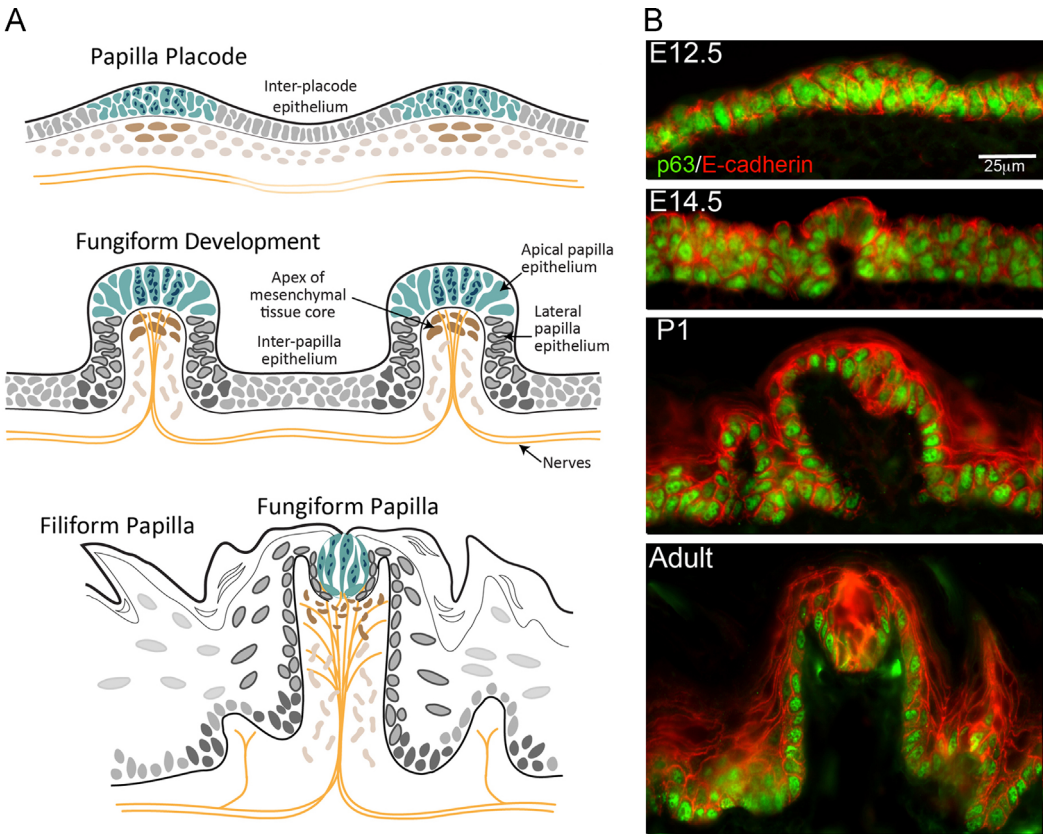

Fig. 1. Papillae and taste buds develop in a complex epithelium. (A) Schema for fungiform papilla development. The Papilla Placode (about E12.5 to E13.5) develops as a collection of epithelial cells over mesenchymal cells, with an inter-placode epithelium that is a single layer of cells. Nerves are within the tongue but not yet approaching the epithelium. With Papilla Development (about E14.5 to E17.5) cell compartments become distinctive. The fungiform papilla epithelium is differentiated at the apex and forms lateral papilla walls that delimit the papilla and extend around a papilla core of mesenchymal cells. From within the core, nerve fiber bundles distribute to the epithelium. The inter-papilla epithelium brackets fungiform papillae. In the adult, the Fungiform Papilla has a differentiated, apical collection of cells, the taste bud, in the apical epithelium. The inter-papilla epithelium has differentiated into non-gustatory, Filiform Papilla structures. In the papilla core a set of apical connective tissue, lamina propria cells are distinguished from other cells in the core. Nerves within the papilla branch to taste buds and surround, and innervate the taste bud cells. (B) Cell layers and basal cells are apparent with immunoreactions for p63 and E-cadherin. The papilla placode at E12.5 is a collection of cells within an epithelium between placodes that is essentially a single layer. Proliferating cells (p63-positive) are throughout the epithelium. At E14.5 the developing papilla has basal (p63-positive) and apical (E-cadherin-positive) cell layers. Apical papilla cells are distinguished from lateral walls. The P1 papilla has multiple cell layers (E-cadherin) over a basal cell layer (p63) and a distinct apical cell collection that is the early taste bud. Filiform papillae are seen in inter-papilla epithelium. The Adult papilla has a taste bud in the apex and interruptions in the basal cell layer under the taste bud are apparent, where nerves enter to innervate the taste bud cells. Filiform papillae surround the fungiform papillae.

After embryonic formation and development, the taste papillae and non-gustatory filiform papillae in the postnatal tongue grow, differentiate and turn over in cell renewal cycles (Mistretta, 1991; Mistretta and Hill, 1995, 2003). Importantly, the taste buds make a perinatal emergence and continue to differentiate and turn over postnatally, as taste bud cells are renewed throughout the life span (Beidler and Smallman, 1965; Hendricks et al., 2004; Krimm and Hill, 2000). Increased extent and complexity of taste bud innervation coincides with development of taste function and papilla and taste bud cell turn over.

Among the growing cast of identified molecules that regulate the fungiform papillae, Shh is a key player (reviewed in Mistretta and Liu, 2006). Shh signaling has roles in fungiform induction, development and patterning (Hall et al., 2003; Liu et al., 2004; Mistretta et al., 2003) and is proposed to function in taste bud formation (Thirumangalathu et al., 2009). However, there is no deep grasp of where the Shh signal originates and where signaling targets are positioned in the embryonic, postnatal and adult papilla and taste bud. Whereas there is demonstrated tight control of hedgehog signals that talk across epithelium and mesenchyme in formation of hair follicle, tooth and intestinal villus (Chuong et al., 2000; Dassule et al., 2000; St Jacques et al., 1998; Walton et al., 2012), we have scarce data about locales and timing for expression of *Hh* signaling components in papilla development.

The Hh pathway is well defined. When Hh ligands bind to the Patched (Ptch) transmembrane receptor, Ptch repression of a second transmembrane protein, Smoothened (Smo), is relieved (Robbins et al., 2012). Smo then initiates intracellular signaling, ultimately activating Gli transcription factors. This leads to induction of Shh target genes. Effectors of Hh signaling in vertebrates are Gli proteins, Gli1, Gli2, Gli3. Diffusible morphogens such as Shh can be strong activators at short range and continue activation at longer range, of 200 μm or more (Saha and Schaffer, 2006). At the same time, a surrounding zone of lateral inhibition can act to pattern tissues in coordination with other pathways (Liu et al., 2004; Zhou et al., 2006).

To understand how Shh signals in tongue, papilla and taste bud formation and maintenance, it is necessary to know where and when Shh ligand and pathway components are positioned. We identified Shh signaling centers in the context of defined cell and tissue compartments in fungiform papillae with reporter mouse lines. By mapping expression of the hedgehog targets *Ptch1* and *Gli1*, which provide a direct indication of *Shh* responsiveness (Ahn and Joyner, 2004; Marigo et al., 1996), spatial and temporal changes in signaling centers were demonstrated and responding cells shown to bracket the restricted location of Shh protein and message. With lineage tracing for *Gli1* we found that Shh-responding cells contribute progeny not only for maintenance of