WNT5a in Tongue and Fungiform Papilla Development

Distinct Roles Compared with WNT10b and Other Morphogenetic Proteins

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Fungiform papillae are complex taste organs that develop in a pattern on anterior tongue in rodent embryos. Several intrinsic secreted molecules are important for papilla development and patterning, including sonic hedgehog, bone morphogenetic proteins, Noggin, epidermal growth factor, and WNTs. Recent data about roles of WNTs in regulation of tongue and fungiform papilla development lead to new insights about the importance of tissue and timing contexts when studying the effects of morphogenetic proteins. WNT/β-catenin signaling is required for formation of fungiform papillae, but not for determining tongue size and shape. In contrast, WNT5a apparently is important for tongue outgrowth, but not papilla development. Preliminary data from WNT5a mutant mice separate genetic programs for papilla number from those for tongue shape and size.

Key words: **tongue; taste papilla; development; WNT; SHH; BMP; Noggin; EGF**

Fungiform papillae are taste organs with a patterned distribution on the anterior tongue and contain one taste bud each in rodents.[1,2] These papillae are highly complex sensory organs. At maturity they include: an epithelial covering with a taste bud in the apical papilla epithelium and a taste bud-free lateral papilla epithelium; a mesenchymal core of connective tissue underlying the epithelium; and an innervation from the chorda tympani nerve (to taste bud) and the lingual branch of the trigeminal nerve (to epithelium surrounding the taste bud and papilla walls).[2,3] Because of the complexity of these sensory organs, many tissue specific contexts must be considered when studying regulation of fungiform papilla development. In turn, tissues will be at different morphological stages during embryonic and postnatal development. To fully understand regulatory mechanisms and signaling factors in fungiform papillae, tissue and temporal variables must be well defined.

In rodents, the fungiform papilla develops from a homogeneous epithelium of the initial lingual swellings to a papilla placode or thickened epithelial cell cluster on the spatulate tongue, to a well defined papilla (Fig. 1A).[1,2] There are many stage-specific factors that are prominent in fungiform papilla formation: (1) formation of the tongue; (2) development of lingual epithelium and mesenchyme; (3) placode formation and invagination; (4) formation of the early papilla; (5) differentiation of papilla epithelium; (6) mesenchymal core development; (7) differentiation of inter-papilla epithelium; and (8) innervation to papilla and taste bud.

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International Symposium on Olfaction and Taste: Ann. N.Y. Acad. Sci. 1170: 11–17 (2009).
doi: 10.1111/j.1749-6632.2009.04369.x

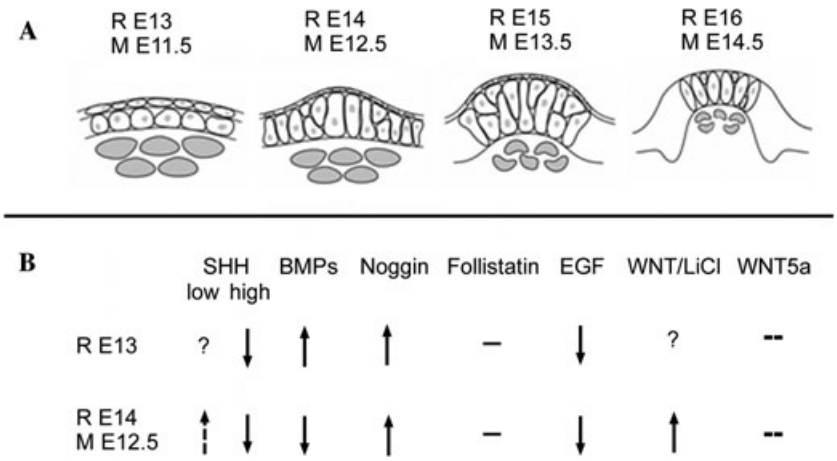

Figure 1. (**A**) Diagram for temporal progression of lingual tissue during fungiform papilla formation adapted from Ref. 19. In rodents, rat (R) and mouse (M), the fungiform papilla develops from a homogeneous epithelium of the initial lingual swellings at R E13/M E11.5, to a papilla placode or thickened epithelial cell cluster on the spatulate tongue (R E14/M E12.5), to a well defined papilla (R E15–16/M E13.5–14.5). (**B**) Summary of effects of secreted molecules sonic hedgehog (SHH), bone morphogenetic proteins (BMPs), Noggin, Follistatin, epidermal growth factor (EGF), WNT/LiCl, and WNT5a on fungiform papilla number in tongue cultures begun at R E13/M E11.5 or R E14/M E12.5 (↑ represents an increase of papilla number; ↓ decrease; – no change; ? indicates no published data; dashed lines indicate preliminary data only). SHH has dual, concentration-specific effects in E14 + 2 day rat tongue cultures, increasing papillae at low concentration and decreasing papillae at high.[14] LiCl (see WNT/LiCl) has been used to activate canonical WNT signaling in tongue cultures.

Of the many tissue and temporal factors, it has been demonstrated that intact sensory innervation is not required for tongue and papilla development.[4,5] In organ culture that excludes intact sensory ganglia, the tongue forms from mandibular swellings and papilla placodes develop from a homogeneous tongue epithelium and progress to form papillae that mirror those in the embryo in number, temporal and spatial patterns, and basic molecular markers.[5–7] Patterning and essential molecular phenotypes are, therefore, intrinsic to lingual tissues.

Intrinsic Secreted Factors in Fungiform Papilla Development

In the past decade, the *in vitro* tongue culture system[4] and loss- and gain-of-function mouse models have been increasingly used to contribute to our understanding of papilla development. Numerous secreted regulatory molecules have been identified to be important for regulating tongue and papilla development, for example, sonic hedgehog (SHH),[5,7,8] bone morphogenetic proteins (BMPs),[9] the BMP antagonist Noggin,[9] epidermal growth factor (EGF),[10] and WNTs.[11–13]

Regulatory roles for these molecules are complex and are concentration- and stage-dependent, summarized in Figure 1B. For example, in E14 + 2 day tongue cultures, exogenous SHH increases papillae at low concentration but reduces papillae at high concentration (Fig. 1B, SHH).[14] BMP2, 4, and 7 each has dual stage-specific effects on fungiform papilla formation in tongue culture, that is, increases (E13) or reduces (E14) papilla number with BMP added to culture medium or via beads (Fig. 1B, BMPs).[9] The BMP antagonist, Noggin, increases papilla number at both E13 and E14 whereas exogenous Follistatin is

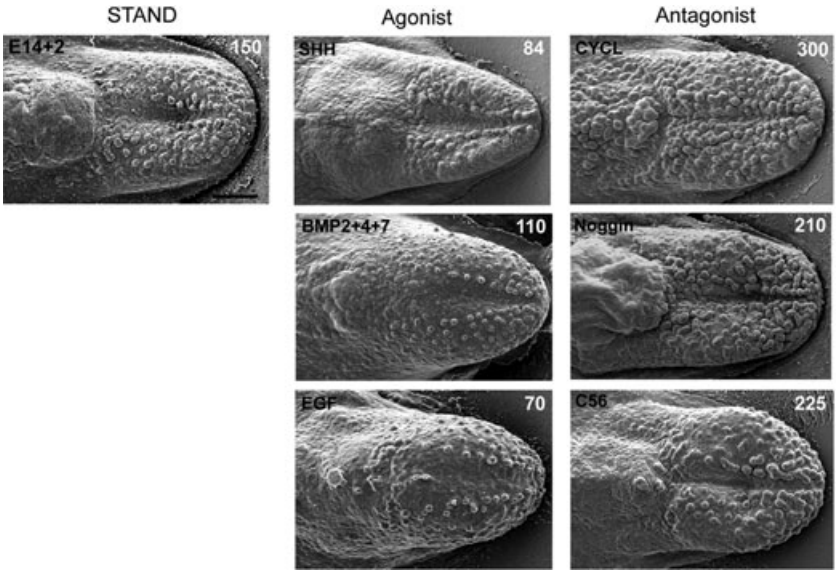

Figure 2. Scanning electron photomicrographs of E14 + 2 day rat tongue cultures with addition of sonic hedgehog (SHH), bone morphogenetic proteins (BMPs), epidermal growth factor (EGF), or antagonists in culture.[9,10,14] The number in the top right corner of each image represents the average number of fungiform papillae for each treatment. STAND: standard medium as a control; CYCL: cyclopamine, an alkaloid that disrupts SHH signaling; Noggin: BMP antagonist; C56: compound 56, a specific inhibitor of EGF receptor. With SHH (high concentration), BMPs, or EGF in the culture medium, fungiform papilla number is reduced. In contrast, with addition of antagonist to block signaling, papilla number is increased (CYCL, Noggin, C56). Formation of fungiform papillae in an atypical tongue region, the intermolar eminence, is observed with CYCL.

ineffective in culture (Fig. 1B, Noggin, Follistatin).[9] EGF reduces papilla number both at E13 and at E14 (Fig. 1B, EGF).[10] In contrast to the secreted factors SHH, BMPs, and EGF, that effect a decrease in fungiform papilla formation, activation of WNT canonical signaling with LiCl increases papillae at E12.5 in mouse (Fig. 1B, WNT/LiCl),[11–13] whereas signaling via WNT5a does not alter papilla number (Fig. 1B, WNT5a).

Organ cultures treated with antagonists or inhibitors illustrate the nature and extent of signaling effects for SHH, BMP, and EGF (Fig. 2). Exogenous SHH, BMP, and EGF each decreases papilla number compared to control cultures in standard medium. Blocking SHH signaling with cyclopamine (Fig. 2, SHH and CYCL) increases fungiform papilla number by more than double that in control organ culture and induces papilla formation on the papilla-free intermolar eminence.[5,7] When BMP signaling is antagonized with Noggin treatment (Fig. 2, BMP2, 4, 7, and Noggin), papilla number is increased by 60% compared to control cultures and large clustered papillae form.[9] Similarly, C56 (Fig. 2, EGF and C56), an EGF receptor (EGFR) inhibitor, leads to about a 40% increase of fungiform papilla number compared to cultures with standard medium.[10] We know that the EGF protein is found throughout the E13–E18 tongue epithelium whereas EGFR undergoes a progressive restriction to the epithelium *between* fungiform papillae.[10] Thus, EGF regulation of papilla formation signals through EGFR expressed in inter-papilla epithelium and results in an increase in cell proliferation between papillae.[10]

In short, to build this complex taste organ—the fungiform papilla—critical tissue and temporal contexts are imperative to the study of regulatory factors. To form and pattern the papillae, the inter-papilla lingual tissue also is

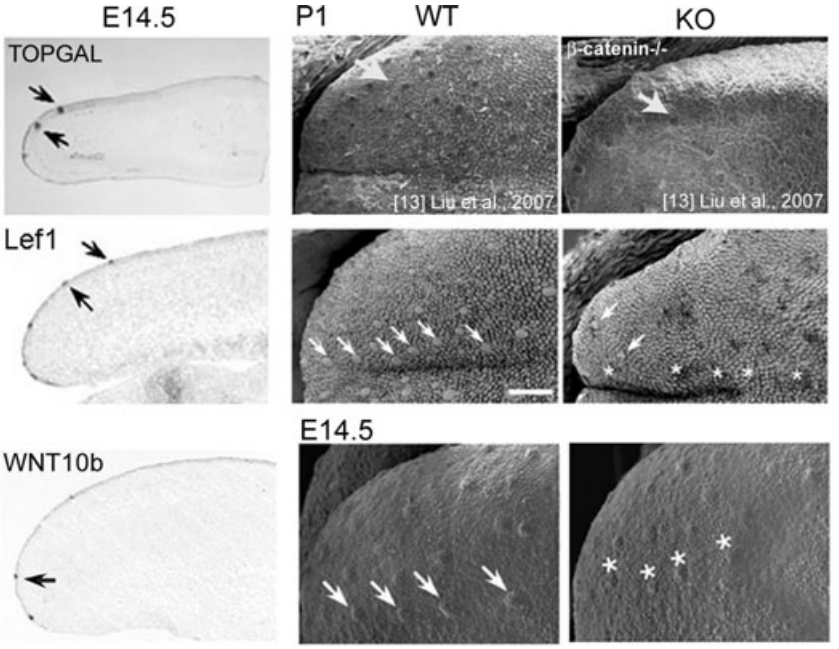

Figure 3. Requirement of WNT canonical signaling in fungiform papilla formation. (First column) Photomicrographs of E14.5 tongue sections indicate the restricted expression of TOPGAL (a β-galactosidase gene driven by a T cell factor (TCF) β-catenin responsive promoter), Lef1, and WNT10b to developing fungiform papillae (arrows).[12] (Middle and right columns) Scanning electron microscope images of tongues from wild type (WT, middle column) or null mutant (KO, right column). Arrows point to fungiform papillae. Asterisks mark missing or "atrophied" papillae. In null mutants of postnatal day 1 β-catenin,[13] or Lef1,[12] and E14.5 WNT10b,[12] fungiform papillae are dramatically reduced. It is noteworthy that the tissue positions are "held" even when papillae are missing in Lef1 knockouts.

an essential part of the puzzle. At any given time and lingual location, variations of cell fate determination, proliferation, and differentiation will contribute to cellular responsiveness. Secreted regulatory factors do not act in isolation but signaling pathways interact. Recent data about roles of WNTs in regulation of fungiform papillae lead to new insights about the importance of tissue and timing contexts in tongue and fungiform papilla development.

WNT/β-catenin Signaling Is Required for Formation of Fungiform Papillae, but not for Tongue Size and Shape

Three groups have reported that canonical WNT signaling is required for fungiform papilla formation.[11–13] Essential components for canonical WNT signaling, TOPGAL (a β-galactosidase gene driven by a T cell factor (TCF) β-catenin responsive promoter) and the transcription factor Lef1, have restricted expression in developing fungiform papillae (Fig. 3). Deletion of either β-catenin[13] or Lef1[12] leads to a striking loss of papillae, demonstrating the requirement of canonical WNT signaling for fungiform papilla development (Fig. 3). To activate canonical WNT signaling pathways, WNT10b may be an important endogenous agonist. The expression of WNT10b is restricted in developing fungiform papillae in parallel with TOPGAL and Lef1.[12] In E14.5 WNT10b null mutants, fungiform papillae are reduced in size compared to wild type (WT) (Fig. 3). Thus, WNT10b is proposed to act via canonical signaling pathways to regulate fungiform papilla development.[12]

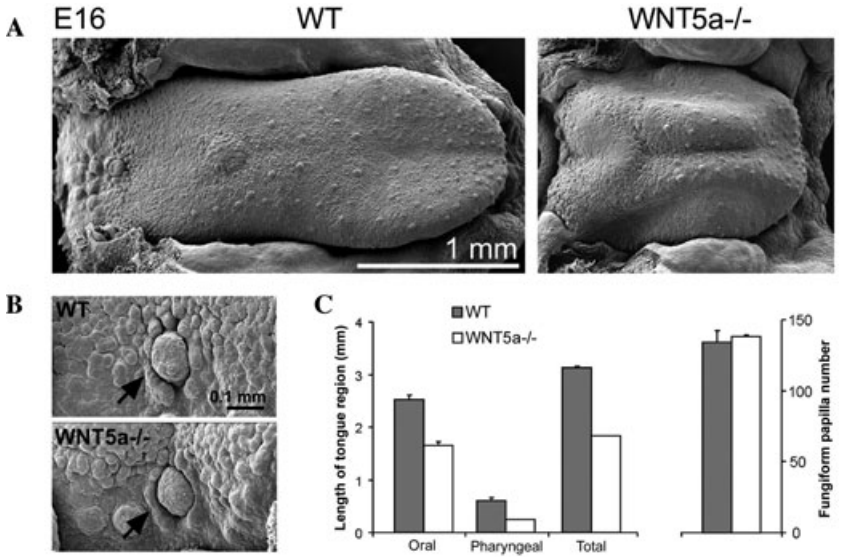

Figure 4. (**A**) Scanning photomicrographs of E16 mouse tongues from wild type (WT) and WNT5a null mutants (-/-). The tongue is shorter and misshapen in WNT5a-/- mutant compared to WT. (**B**) Scanning electron photomicrographs indicate that there is no apparent alteration of circumvallate papilla (arrows) in WNT5a-/- mutant compared to WT. (**C**) Histograms of regional tongue length (oral, pharyngeal, and total) and fungiform papilla number. Whereas the length of tongue is reduced in WNT5a-/- mutant, papilla number is not different from WT.

It is noteworthy that in postnatal day 1 Lef1-/- tongue, where fungiform papillae are "atrophied" or missing, tissue positions for papillae are maintained (Fig. 3). This suggests that there are gene programs specifically for fungiform papilla formation, separate from that for other lingual tissue development. This is supported by new data on tongue and fungiform papilla development in WNT5a null mutants.

WNT5a Is Important for Tongue Outgrowth, but not for Papilla Development

In contrast to WNT10b, WNT5a has a unique expression pattern in vertebrate embryos. Perinatal lethal WNT5a null mutant embryos have a striking phenotype with truncated face, limbs, and tail demonstrating the importance of WNT5a in proximal-distal outgrowth in vertebrate embryos.[15] Recently, there is increased interest in the roles of WNT5a in several aspects of embryonic development, for example, cell polarity and directional movement,[16] airway and vascular tubulogenesis,[17] and mammary gland development.[18]

Our laboratory has performed gene array analysis in E14 + 2 day tongue cultures. Multiple gene expression patterns were compared between anterior tongue where fungiform papillae are abundantly distributed and the intermolar eminence which is papilla-free. We found that WNT5a expression is increased by eightfold in anterior tongue compared to intermolar eminence. In our preliminary data, Western blot analysis revealed that WNT5a is expressed in embryonic (E13–E16), but not in postnatal tongue.

To learn if WNT5a has a role in development of tongue and taste papillae, in preliminary experiments we have compared embryonic tongues in WNT5a-/- mice with those of WT littermates (Fig. 4). At E16, length of oral tongue in WNT5a-/- mice was reduced to 65%

of WT length. Portions of the tongue that were anterior, or posterior, to the anterior-most border of the intermolar eminence were reduced by similar proportions. However, pharyngeal tongue in null mice was reduced to only 40% of WT. Whereas length of oral tongue was severely compromised in null mice, width was similar to that in WT. Overall, anterior tongue area was substantially reduced and shape was radically altered. However, considering the much truncated anterior tongue, preliminary analysis indicates that numbers of fungiform papillae were not different on mutant tongues relative to WT. The single circumvallate papilla on posterior tongue also was sustained without an obvious alteration in WNT5a mutant tongues at E16 (Fig. 4).

In summary, our preliminary data indicate that WNT5a is important for embryonic tongue outgrowth. This is distinct from roles for WNT10b and other regulatory factors in development of fungiform papilla numbers and pattern. Even with a much reduced anterior tongue, numbers of fungiform papillae are not different on mutant tongues relative to WT. Therefore, the WNT5a preliminary data support the novel idea that spatial constraints may not be a major factor in fungiform papilla formation. The data provide evidence of separate genetic programs for papilla number from those for tongue shape and size.

Acknowledgments

The research was supported by NIDCD, NIH Grant DC 000456 (C.M.M.) and NIDDK, NIH Grant DK065850 (D.L.G.).

Conflicts of Interest

The authors declare no conflicts of interest.

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