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REGULAR ARTICLE

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Inhibition of connexin 43 alters *Shh* and *Bmp-2* expression patterns in embryonic mouse tongue

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Abstract The morphogenesis of fungiform papillae occurs in a stereotyped pattern on the dorsal surface of the mammalian tongue via epithelial–mesenchymal interactions. These interactions are thought to be achieved via intercellular communication. Gap junctions can be observed in many developing tissues and have been suggested to participate in a variety of functions, including the regulation of cell proliferation, differentiation, and apoptosis. Here, we demonstrate that the expression of Connexin 43 (Cx43), a gap junction protein, is correlated significantly with the development of fungiform papillae, which exhibit a pattern formation and morphogenesis similar to the development of other epithelial appendages. Antisense-oligodeoxynucleotide (AS-ODN) against Cx43 was used to assess the developmental functions of Cx43. The expression patterns of the signaling molecules were disrupted by Cx43 inhibition. Interestingly, the expression patterns of *Shh*, a key molecule in the determination of the spacing patterns of fungiform papillae, were disturbed after treatment with Cx43 AS-ODN. We have also attempted to determine the functions of *Bmp-2* by applying NOGGIN protein to tongue cultures. Our results indicate that upstream regulation via Cx43 controls the *Shh* and *Bmp-2* pathways for the morphogenesis and pattern formation of fungiform papillae.

Keywords Connexin 43 · Fungiform papilla · *Shh* · *Bmp-2* · Antisense-oligodeoxynucleotide · Mouse (ICR)

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Introduction

One of the simplest and most frequently observed embryonic patterns is the maintenance of a minimum distance between repetitive neighboring elements, also known as spacing patterns (Wolpert 1998). Spacing patterns are of fundamental importance in a variety of repeat structures, which develop at regularly spaced intervals. The mammalian tongue features three types of gustatory papillae: fungiform, foliate, and circumvallate. In mice, the fungiform papillae have been observed to form a specific patterned array via epithelial–mesenchymal interactions during early development (Hall et al. 2003; Kim et al. 2003; Mistretta et al. 2003). In particular, the development of the fungiform papillae exhibits characteristics similar to those associated with the developmental processes of other epithelial appendages, including feathers, hair, teeth, and limbs (Chuong et al. 2000). At E13 in mice, the fungiform papillae become morphologically apparent as raised areas arranged in a stereotyped pattern on the lingual surface. Previous studies have shown that *Shh*, *Ptc*, *Gli-1*, *Bmp-2*, *Bmp-4*, and *Fgf-8* transcripts are expressed in the fungiform-papillae-forming area prior to morphological changes, showing a typical pattern on the anterior portion of the tongue. This indicates that the expression of these signalling molecules is essential with regard to fungiform papillae morphogenesis (Hall et al. 2003; Jung et al. 1999; Mistretta et al. 2003).

Gap junctions can be observed in most developing tissues and have been suggested to play a variety of roles in processes, including cell proliferation, differentiation, and apoptosis (Simon and Goodenough 1998). Gap junctions are specialized areas of the cell membranes that allow for

the direct exchange of signaling molecules and metabolites between the cytoplasmic regions of neighboring cells (Bruzzone et al. 1996; Evans and Martin 2002). Signals such as hormones and growth and differentiation factors are believed to perform their functions via the modulation of the levels and activities of connexins (Huang et al. 2001). One of the major gap junction proteins in chick and mouse embryonic limbs is alpha-1 connexin or connexin 43 (Cx43). The Cx43 transcript is detected in both the apical ectodermal ridge and the posterior mesenchyme by stage 20 in the chick embryo and by embryonic day 10 in mice (Laird et al. 1992; Meyer et al. 1997). A major role of connexins in the early mesenchyme may be to enable mesenchymal cells in the polarizing region to communicate with the anterior mesenchyme (Allen et al. 1990; Dealy et al. 1994). Cx43 expression in the developing limb bud is complex, and some variations have been noted between the mouse (Meyer et al. 1997; Yancey et al. 1992) and the chick (Green et al. 1994). The expression levels of Cx43 (Warner 1999), *Shh* patterning genes (Riddle et al. 1993), and *Bmp-2* (Francis et al. 1994) have been found to be higher in the zone of polarizing activity. Interactions between the signaling molecules and junctional communication have been suggested to play an important role in developmental control (Warner 1999).

Bmp-2 and *Shh* have been identified as candidate signaling molecules that regulate the development of fungiform papillae (Hall et al. 2003; Nosrat et al. 2000) and are expressed in many regions of the developing embryo, including the developing fungiform papillae (Hogan 1996; Jung et al. 1999). They have been implicated in a variety of developmental processes, including epithelial–mesenchymal interactions in, for example, tooth and limb development (Hogan 1996; Niswander et al. 1994). Interestingly, during the development of fungiform papillae in mice, *Shh* expression levels are related to innervation, and the SHH protein is involved in the morphogenesis and pattern formation of the tongue papillae, both as a morphogen, which directs the cells toward a non-papillary fate, and as a mitogen, which induces expansion in the interplacodal epithelium (Hall et al. 1999, 2003). Moreover, disruption of *Shh* expression during the development of the rat tongue has revealed that the development and patterning of the fungiform papillae depends on this signaling pathway (Mistretta et al. 2003).

In the present study, we show that Cx43 expression is correlated significantly with the development of fungiform papillae in early mouse development. In addition, in order to define the upstream regulation of the *Shh* pathway, which was first described by Hall et al. (2003), we have examined the relationship between the expression pattern of the signaling molecules and the patterning of the fungiform papillae by utilizing in vitro organ culture. Antisense-oligodeoxynucleotide (AS-ODN) Cx43 has been used to characterize the developmental functions of Cx43. The function of *Bmp-2* during tongue development has also been investigated by using the ectopically expressing NOGGIN, a potent bone morphogenetic protein (BMP) antagonist. The expression patterns of the signaling molecules, *Shh* and *Bmp-2*, are altered by the inhibition of gap junctions, whereas that of *Shh*, which is a key molecule in the determination of spacing patterns, is disturbed by the inhibition of *Bmp-2*. These results suggest that the signaling cascade, which is regulated by Cx43, is of importance with regard to the initiation of papillary pattern formation and morphogenesis.

Materials and methods

Animals

Adult ICR mice were housed in a temperature-controlled room (22°C) under artificial illumination (lights on from 0500 to 1700 h) and at 55% relative humidity, with access to food and water ad libitum. Mouse embryos were obtained from time-mated pregnant mice. Embryonic day 0 (E0) was designated as the day on which a vaginal plug was confirmed. Embryos at each development stage (E12–E15, daily intervals) were used in this study.

Whole-mount immunostaining

The embryos were fixed overnight in freshly prepared 4% paraformaldehyde (PFA) in PBS (phosphate-buffered saline) at 4°C, washed in PBS (3×15 min), washed in PBS with 1% Triton X-100 and 10% fetal calf serum (TS-PBS; 3×l h), incubated with the primary antibody (mouse monoclonal antibody against Cx43; Zymed, no. 13-8300) for 24 h, washed again in TS-PBS (6×30 min), incubated overnight with peroxidase-conjugated goat anti-mouse immunoglobulins (diluted 1/200 in TS-PBS), and washed in PBS (6×30 min). The antibody was localized with a diaminobenzidine staining kit.

In vitro organ culture

In order to determine the regulation of the signaling molecules after micro-dissecting the tongue from an E12 mouse mandible, the tongue was separated into its aboral and oral regions (Kim et al. 2003). It was then cultured in a medium containing 10% fetal bovine serum for 2 days by using a slight modification of the Trowel-type culture method (Fig. 1d).

AS-ODN against Cx43

The AS-ODN against Cx43 was designed as 5′ GTAATT GCGGC AGGAGGAATTGTTTCTGTC 3′ (29 mer) and has demonstrated its effectiveness in reducing Cx43 protein levels (sequence 960–989; GenBank accession no. X61576; Law et al. 2002). The control sense (CS) ODN sequence was used as the sense control for the mouse Cx43 sequence

ODN, 5′GACAGAAACAATTCCTCCTG CCGCAATTA C3′. The ODNs were purchased from GENOTECH (Korea). They were added to the culture medium at a final concentration of 30 μM. The optimal Cx43 AS-ODN concentration was determined based on the morphology of the cultured explants after tests at various concentrations (5 nM–30 μM).

Bead implantation

Affigel Blue beads (BioRad), 150 μm in diameter, were dried and then soaked in 0.5 mg/ml human recombinant NOGGIN (Regeneron). In order to characterize the gene regulation of fungiform papillae development, a NOGGIN-soaked bead was implanted in the anterior portion of the tongue at E12. After bead implantation, the tongues were used for whole-mount in situ hybridization. PBS-soaked beads were implanted as controls.

In situ hybridization

In situ hybridization was performed as described elsewhere (Kim et al. 2003). The *Bmp-2* and *Shh* plasmids were kindly provided by Dr. Y.-P. Chen. After whole-mount in situ hybridization, frozen sections of 10 μm in thickness were obtained.

Image processing and counting of *Shh* expression spots

Whole tongues were photographed and digitized with a Dimage Scan Multi-film scanner (Minolta, Osaka, Japan). Bright-field and cultured tongue images were also collected with a Spot RT digital camera (Leica, Korea). Digital images of the cultured tongues, which were analyzed via whole-mount in situ hybridization with *Shh* mRNA, were also collected. At least 30 tongue explants were used to examine *Shh*-positive papillary spots in each experiment. The tongue explants were scored as a double blind for the presence of both large and small (<10 μm diameter) *Shh*-positive papillary spots. The mean number of papillae per tongue was compared with those counted in the control group by using ANOVA (SPSS 8.0, USA). At least 30 tongue organ cultures were used in each experiment (Fig. 2b). The mean difference was considered significant at the 0.05 level for all data ($P<0.05$; Tukey HSD).

Results

Localization of Cx43 during early fungiform papillae development

In order to determine the relationship between the morphological changes and the expression pattern of Cx43 during the development of fungiform papillae, the Cx43 expression patterns in E11.5 to E14.5 mouse embryos were examined by using whole-mount immunostaining. Cx43 expression was detected ubiquitously in the epithelium and underlying mesenchyme of the tongue in the lateral swelling at E11.5 (Fig. 1a). At E12.5, Cx43 expression appeared to have become more localized and specific than at E11.5 and was restricted to the epithelium (Fig. 1b). At E13.5, Cx43 was strongly expressed in a localized spot, which appeared to be a dome-like structure in the epithelium (Fig. 1c). At E14.5, Cx43 was observed in the epithelial apex regions of individual fungiform papillae (Fig. 1e).

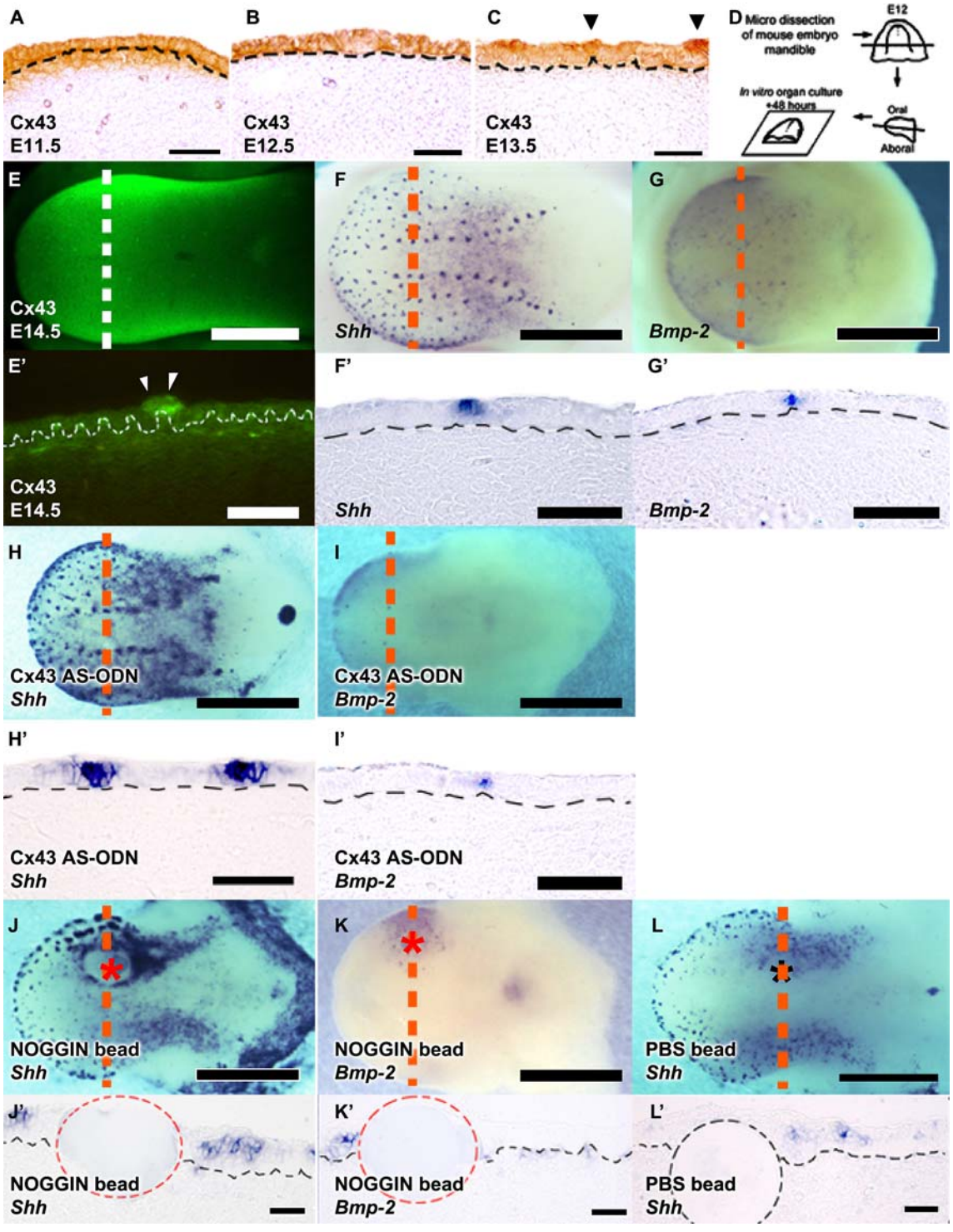
Mouse tongues express papillary markers during in vitro organ culture

In order to establish an in vitro organ culture of mouse fungiform papillae in which to study the development of the tongue, E12 tongues were micro-dissected from the mandible and separated into their aboral and oral portions. The tongues were then cultured for 2 days in a medium containing 10% fetal bovine serum by using the Trowel-type culture method with slight modifications (Fig. 1d). This study examined *Shh* expression patterns, which were detected in the dorsal surface of the tongue with a specifically patterned array, after culture for 2 days at E12 (Fig. 1f). *Bmp-2* exhibited an expression pattern similar to that of *Shh*. However, the intensity of *Bmp-2* expression was weaker than that of *Shh* expression (Fig. 1g). After whole-mount in situ hybridization, transverse sections were obtained in order to examine the *Shh* and *Bmp-2* expression patterns more precisely. Both *Shh* and *Bmp-2* were strongly expressed in localized spots in the epithelium, which manifested as dome-like structures in the epithelium (Fig. 1f′, g′).

Alteration of gene expression patterns after treatment with Cx43 AS-ODN and NOGGIN bead implantation

The designed Cx43-specific AS-ODN was shown to be effective in knocking down Cx43 protein levels (sequence 960–989; GenBank accession no. X61576; Law et al. 2002). Control ODN sequences were used as a sense control for the mouse Cx43 ODN. Both *Bmp-2* and *Shh* expression levels were altered after treatment with 30 μM Cx43 AS-ODN. *Shh* expression levels were found to be higher than in the controls on the dorsal surface of the tongue (Fig. 1h), whereas *Bmp-2* expression was weak and was restricted to the anterior portion of the tongue (Fig. 2i). The density of the expressed cell population was disrupted by the Cx43 AS-ODN treatment (Fig. 2a, b). *Shh* and *Bmp-2* expression patterns were altered by Cx43 AS-ODN; this was confirmed in transverse sections (10 μm). The intensity of *Shh* expression was greater than that of the control (Fig. 1h′), whereas the intensity of *Bmp-2* expression was less than that of the control (Fig. 1i′).

In order to examine the gene regulation during fungiform papillae development, a NOGGIN-soaked bead was implanted in the anterior region of the right side of the tongue



at E12. A PBS bead was used as a control. Expression of the signaling molecules was assessed 2 days after bead implantation. After NOGGIN bead implantation, the *Shh* expression level was found to have been altered in the bead-implanted area (Fig. 1j). Transverse sections confirmed that the intensity of the expression of *Shh* was more pronounced than in the control (Fig. 1j′). After PBS bead implantation, no detectable changes were observed compared with the control (Fig. 1l, l′). Interestingly, *Bmp-2* expression shifted to the underlying mesenchyme near the NOGGIN bead after 2 days (Fig. 1k, k′). The number of *Shh* expression spots was examined by whole-mount in situ hybridization as a marker for the determination of the spacing pattern. The Cx43 CS-ODN-treated and PBS-bead-

◀ **Fig. 1** Whole-mount immunostaining by using Cx43 antibody (E11.5–E14.5). The frozen sections obtained after whole-mount immunostaining. **a**–**c** *Fine dotted lines* indicate basement membrane. **a** At E11.5, Cx43 expression was localized to both the epithelium and mesenchyme. **b** At E12.5, Cx43 expression was localized in the epithelium. **c** At E13.5, Cx43 was expressed in the fungiform papillae forming the epithelium (*arrowheads* strongest expression spots). **d** In vitro organ culture at E12. **e** At E14.5, expression of Cx43 showed a specific patterned array on the tongue dorsal surface (*thick white dotted line* section level). **e′** Cx43 was localized in the apex region of the fungiform papilla epithelium and in the mesenchyme region of the filiform papillae forming area (*fine white dotted line* basement membrane, *white arrowheads* epithelial expression of Cx43 in fungiform papilla forming area. *Bar* 40 μm (**a**–**c**, **e′**), 250 μm (**e**). **f**–**l** *Scarlet dotted lines* indicate section level, *black dotted lines* indicate basement membrane. **f**, **g** Expression pattern of signaling molecules after in vitro organ culture for 48 hours at E12. **f** *Shh* was expressed on the dorsal surface of the tongue in a specific array that was also observed in the E14 mouse tongue. **f′** Transverse section of the tongue showing *Shh* expression in the fungiform papillae-like structure in the epithelium. **g***Bmp-2* expression was similar to, but not as strong as, the *Shh* expression pattern. **g′** *Bmp-2* expression was also localized in the epithelium. **h**, **i** Alteration of the gene expression pattern after treatment with the AS-ODN against Cx43. **h** *Shh* expression spots increased on the dorsal surface of the tongue after treatment with Cx43 AS-ODN. The intensity of *Shh* expression in the circumvallate papilla was also stronger than that of the control. **h′** *Shh* expressed in the fungiform papillae structures in the epithelium. **i** The number of *Bmp-2* expression spots decreased on the dorsal surface of the tongue after treatment with Cx43 AS-ODN. **i′** Although *Bmp-2* was expressed in the fungiform-papillae-forming epithelium, the intensity of expression was lower than in the control. **j**–**l** Alteration of the gene expression pattern following NOGGIN bead implantation (concentration of NOGGIN protein: 0.5 mg/ml; *fine red dotted circles* NOGGIN beads). **j** NOGGIN bead was implanted on the right side of the dorsal surface of the tongue. **j′** Transverse section showing that *Shh* expression had become localized in the epithelial cells, and the size of the fungiform papillae-like structures were increased slightly, with more profound *Shh* expression level than that of the control. **k** After NOGGIN bead implantation, *Bmp-2* was expressed near the NOGGIN bead in a broad pattern. **k′** Transverse section showing that *Bmp-2* was expressed in the mesenchyme, beneath the fungiform papillae-like structure. **l** PBS beads (*black dotted circles*) were implanted as a control. There were no dramatic changes from the control. **l′** Transverse sections were also examined. *Bars* 250 μm **f**–**l**, 50 μm **f′**–**l′**

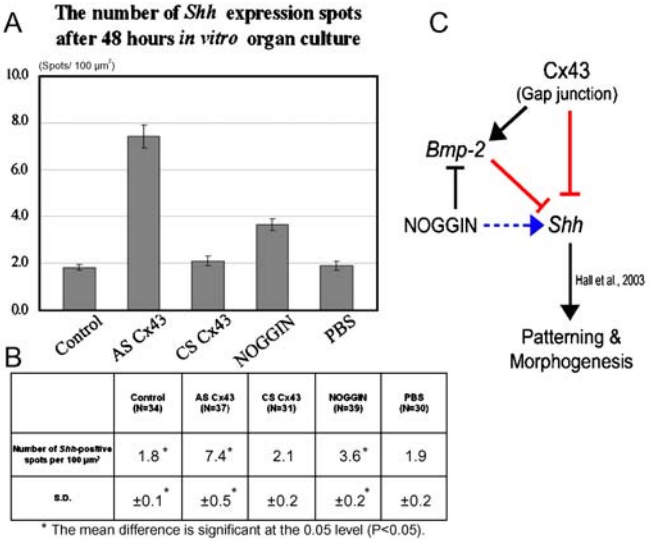


Fig. 2 **a**, **b** Number of *Shh* expression spots on the dorsal surface of the tongue. Alteration of the *Shh* expression pattern was examined by counting the expression spots after Cx43 AS-ODN treatment and NOGGIN bead implantation (spots/100 μm^2). The number of spots was assessed in at least 30 samples. Data are expressed as mean±SD. **c** Representation of regulation of signaling molecules during development of fungiform papillae (*red bar* inhibition, *blue arrow* activation). *Shh* is a key molecule in the patterning of fungiform papillae (Hall et al. 2003). Cx43 regulates *Bmp-2* and *Shh* in different ways. NOGGIN might also play an important role in the patterning and morphogenesis of fungiform papillae

implanted tongues exhibited similar numbers of *Shh* expression spots of the control culture, with 1.8 spots/100 μm^2 being counted in the anterior part of the dorsal tongue area. However, after treatment with Cx43 AS-ODN and NOGGIN bead implantation, the number of *Shh* expression spots increased, to 7.4 and 3.6 spots/100 μm^2, respectively, in the anterior area of the dorsal tongue (Fig. 2a, b).

Discussion

Gap junction during mouse tongue development

Gap junctions are intercellular channels commonly involved in cell-to-cell communication (Richard 2000). Small molecules, including ions and second messengers with molecular weights less than 1 kDa, are able to pass through gap junction channels (Saez et al. 1989). Gap junctions are believed to be important for the maintenance of tissue homeostasis and for the communication of certain intracellular signals (Simon and Goodenough 1998). Alpha-1 connexin, also known as Cx43, is one of the major gap junction proteins in chick and mouse embryonic limbs (Laird et al. 1992; Meyer et al. 1997). The major role of connexins in the early mesenchyme may be to enable mesenchymal cells in the polarizing region to communicate with the anterior mesenchyme (Allen et al. 1990; Dealy et al. 1994).

Our study has demonstrated alterations in the Cx43 expression patterns that occur during the development of fungiform papillae in mice. We have assessed Cx43 expression patterns via a whole-mount immunostaining method. Cx43 has been found to be expressed on the dorsal surface of the mouse tongue from E11.5. At E12.5, Cx43 expression becomes localized to the epithelium. From E13.5, Cx43 expression has been examined specifically in the areas in which fungiform papillae are formed. These expression patterns are similar to those associated with signaling molecules such as *Shh* and *Bmps* suggesting that a relationship exists between gap junctions and signaling molecules, as has also been observed in limb development (Green et al. 1994; Warner 1999).

Expression patterns of signaling molecules after in vitro organ culture

Recent studies of fungiform papillae in developing mouse embryos have demonstrated that *Shh*, *Ptc*, *Gli-1*, *Bmp-2*, *Bmp-4*, and *Fgf-8* transcripts are expressed in the areas of

developing fungiform papillae, prior to morphogenesis, thereby indicating that signaling molecules are of great importance in papillae morphogenesis (Hall et al. 1999; Jung et al. 1999). *Shh*, in particular, plays a critical role in the patterning of the lingual epithelium and is also apparently involved in several fundamental developmental processes (Chuong et al. 2000). *Shh* may be an active factor in the establishment of papillary spacing patterns and in epithelial mesenchymal interactions (Hall et al. 2003) and is essential during the initiation, determination, and differentiation of fungiform papillae (Kim et al. 2003). In mice, *Shh* is expressed broadly and ubiquitously in the epithelium at E11. However, by E12-13, *Shh* expression no longer occupies the entire tongue region and is instead restricted to individual spots. Similarly, *Ptc*, *Gli-1*, *Bmp-2*, and *Bmp-4* are expressed in the dorsal epithelium and share similar expression patterns at regular intervals located in characteristic rows on both sides of the median sulcus, prior to the development of morphological changes. *Shh* continues to be expressed in developing fungiform papillae in discrete spot patterns at E14 and E15. BMP-2 has been reported to be involved in a host of developmental processes, including epithelial mesenchymal interactions during the development of teeth and limbs (Hogan 1996; Niswander et al. 1994; Niswander and Martin 1992).

Interestingly, in vitro organ culture of mouse tongue has allowed us to observe the regulation of the signaling molecules. Thus, the expression patterns of the signaling molecules, *Bmp-2* and *Shh*, have been examined during in vitro organ culture at E12. After 2 days of culturing, we have observed the expression of *Shh* with a stereotyped pattern on the dorsal surface of the tongue by using whole-mount in situ hybridization. The expression pattern of *Bmp-2* has also been examined in the areas in which fungiform papillae are formed on the dorsal surface. Both signaling molecules are expressed in the fungiform papillae formation area in the epithelium. These expression patterns are similar to those it E14 controls in vivo (Jung et al. 1999). This in vitro organ culture method has proved to be beneficial to our understanding of the signaling pathway during the early development of fungiform papillae. Regulation of the activation and inhibition of these signaling molecules can be examined by using a variety of other techniques, including AS-ODN treatment and protein bead implantation.

Altered gene expression patterns in fungiform papillae following Cx43 AS-ODN treatment

Interactions between signaling molecules and junctional communication have been previously suggested to play important roles in the control of development (Meyer et al. 1997; Warner 1999). Our study has examined the way that communication through Cx43 channels influences the cascade of gene expression involved in the patterning of fungiform papillae. Cx43 protein expression has been found to be correlated significantly with fungiform papillae development by using Cx43 AS-ODN treatment. The designed Cx43-specific AS-ODN has been shown to be effective in the transient knocking down of Cx43 protein levels (Law et al. 2002). A CS-ODN sequence has been used as a sense control for the mouse Cx43 sequence ODN.

The *Shh* expression pattern is disrupted following treatment with Cx43 AS-ODN. The number of *Shh* expression spots in the treated tongue cultures is markedly higher than those of the controls. The number of *Shh* expression spots in the control specimen is 1.8 spots/100 μm^2 in the anterior part of the dorsal tongue. In contrast, whereas the numbers of *Shh* expression spots in the Cx43 CS-ODN-treated specimens are similar to those of the control, the Cx43 AS-ODN treated specimens exhibit much higher spot density, at 7.4 spots/100 μm^2. These results demonstrate that the Cx43-mediated regulation of fungiform papillae development is crucial with regard to fungiform papillae patterning, and that the transient knockdown of Cx43 expression leads to a disruption of fungiform papillae patterning. The *Bmp-2* expression pattern is altered and decreases following treatment with Cx43 AS-ODN. After whole-mount in situ hybridization, histological sections have shown that the intensity of *Bmp-2* expression decreases from control levels. In addition, *Bmp-2* expression levels are lower, and *Shh* expression levels are higher, after treatment with Cx43 AS-ODN. This implies that *Shh* and *Bmp-2* are downstream of Cx43-mediated signaling in this particular cascade of fungiform papillae development. This signaling cascade in the development of fungiform papillae is similar to that observed in limb development. In the case of limb development, as has been reported elsewhere, Cx43 activates *Bmp-2* expression, and *Bmp-2* in turn down-regulates the expression of *Shh* (Allen et al. 1990; Green et al. 1994).

Gene expression patterns are altered in fungiform papillae following NOGGIN bead treatment

In order to characterize the regulation of signaling during early stages of fungiform papillae development, applied NOGGIN (a potent BMP antagonist) was added to the in vitro organ culture via bead implantation. The BMP-inhibitor NOGGIN protein blocked BMP-2 expression in the epithelium. Interestingly, *Bmp-2* expression was found to have relocated to the mesenchyme. *Shh* expression was slightly altered by exposure to NOGGIN protein. The density of *Shh* expression spots increased to 3.6 spots/ 100 μm^2 in the anterior portion of the dorsal tongue.

After implantation of the NOGGIN bead, morphological disturbance in the fungiform papillae has been observed in transverse sections taken during fungiform papillae development. These fungiform papillae are found to be larger than those seen in the control. This difference in size might be attributable to the effects of the regulation of the signaling molecules, including *Bmp-2*, *Shh*, and Cx43, during the early development of fungiform papillae, prior to nerve innervation. The shifts of expression of *Bmp-2* from epithelium to mesenchyme after the NOGGIN bead implantation are difficult to explain (Yung et al. 2002). This might be the result of inhibitory influences by nerves or possibly

other direct unknown effects of NOGGIN. The roles of BMP-2 in fungiform papilla development need to be further elucidated.

Overall, Cx43 plays an important role during the development of fungiform papillae. Treatment with Cx43 AS-ODN results in the down-regulation of *Bmp-2* expression and the up-regulation of *Shh* expression. The expression pattern of *Shh*, which is the most important molecule in the pattern formation of fungiform papillae, is disrupted by treatment with AS-ODN against Cx43. This result is consistent with previous reports of cytokeratin differentiation on the dorsal surface of the tongue. The late onset of epithelial differentiation is indicated by reduced Cx43 expression (Dahl et al. 1995). Because the apex epithelial cells of the filiform papillae, which are orthokeratinized structures on the dorsal surface of the anterior region of the tongue, begin to keratinize at E16, those of the fungiform papillae will differentiate into taste buds. The pattern of Cx43 expression, then, plays an important role in the patterning of fungiform papillae. NOGGIN bead implantation results in alterations in the size of the fungiform papillae and in an up-regulation of *Shh* expression. These treatments result in the development of abnormal fungiform papillae morphology. Our results indicate that, by regulating *Shh*, *Bmp-2*, and Cx43-mediated signaling, one should be able to regulate the pattern formation and morphogenesis of fungiform papillae (Fig. 2c). Further investigations are required to analyze the roles of cellular communication in organogenesis.

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