〔Original〕

Expression and activation of β-catenin in developing and denervated taste buds

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Abstract

Wnt/β-catenin signaling is critical for the development of a broad range of epithelial tissues, including taste papillae. The taste buds are also responsive to Wnt/β-catenin signaling ; however, its involvement in developing and denervated taste buds has not been explored. In the present study, the expression patterns of activated β-catenin, Wnt, and its receptor, Frizzled (Fzd), in the mouse taste buds were determined by *in situ* hybridization and immunohistochemistry. Activated β-catenin, detected as a cytoplasmic accumulation, and its translocation to the nucleus were observed in sonic hedgehog (Shh)-immunoreactive (IR) basal cells and in a few intragemmal cells. Among Fzd molecules, immunoreactive Fzd-1 and -3 were observed in the entire membranes of the taste bud cells. Also, *Wnt10b* mRNA was expressed in throughout the cytoplasm of the taste bud cells. After bilateral glossopharyngeal nerve transection, nuclear β-catenin and Fzd-1 and -3 were expressed in the entire of regenerating taste buds at 21 days. At 28 days, β-catenin positive nuclei were found in basal half of the taste buds, involving in the basal cells and Sox2-IR cells. At postnatal day (P) 2, nuclear β-catenin was expressed in the immature, developing taste buds. Immunoreactive Fzd-1 and -3 were observed in some of the β-catenin-IR cells. At P4-P10, cells with β-catenin-positive nuclei became restricted to the basal region of the taste buds ; whereas Fzd-1 and -3 became expressed in the entire taste buds. Fzd-7- and -8-immunopositive cells were observed in the taste buds at P3-P10, but these cells in the adult were negative for these receptors or weakly immunopositive for them. These data suggest that increased β-catenin transcriptional activity during early development and regeneration processes promoted the differentiation of basal cells and immature cells to become elongated taste bud cells. Moreover, the expression of several Fzd receptors was either up- or down-regulated during taste bud development.

Key words：β-catenin, *Wnt10b*, Frizzled, taste buds, *in situ* hybridization, immunohistochemistry

Introduction

β-catenin is the cytoplasmic protein in adhesions junctions and interacts with the cadherin proteins (Weis and Nelson, 2006) and it is also the key protein in the canonical Wnt signaling pathway. Wnt comprises a large family of secreted ligands that activate several receptor-mediated pathways (Cadigan and Liu, 2006). In the Wnt/β-catenin pathway, the binding of Wnt ligands to Frizzled (Fzd) receptors and low-density lipoprotein receptor-related protein (LRP) family coreceptors causes the accumulation of cytoplasmic β-catenin and its translocation to the nucleus, resulting in transcriptional activation effected by complexes of β-catenin and Lef or Tcf transcription factors. Wnt/β-catenin signaling is a key pathway in development, adult tissue homeostasis and disease (Clevers, 2006), e.g., controlling stem cell proliferation and differentiation in the nervous system

受付：平成24年9月6日　受理：平成24年10月22日

(Galceran et al., 2000 ; Hirabayashi et al., 2004), in hair follicles (Van Mater et al., 2003), in tooth germs (Obara et al., 2006 ; Liu and Millar, 2010), and in tongue epithelium (Schneider et al., 2010).

Lingual taste buds develop within 3 different types of tongue papillae : fungiform, foliate and circumvallate. In the mouse, fungiform papillae form in a stereotypical array on the dorsal lingual surface. Wnt/β-catenin signaling is known to regulate papilla induction and number during embryonic development (Liu et al., 2007), and it is also required for embryonic taste bud development (Iwatsuki et al., 2007 ; Liu et al., 2007). Among the Wnt family, which includes 19 members in mammals, *Wnt10b* is known to activate Wnt/β-catenin signaling during formation of embryonic gustatory papillae (Iwatsuki et al., 2007). However, its expression in the taste buds in the adult has not been examined. *Wnt5a* is a member known to act in the non-canonical Wnt pathway ; and in mice with its mutated form, their tongue is severely shortened, thus affecting the number of papillae (Liu et al., 2012). As regards the receptors for Wnt proteins, the Fzd family, includes 10 members in mammals ; and yet, little is known about their expression in gustatory papillae formation and in the taste buds. Mature taste buds are composed of different cell types, i.e., types-I, -II, -III, and basal cells. The basal cells are the progenitors of type-I, -II, and -III cell and express sonic hedgehog (Shh, Miura et al., 2006). Using the BAT-gal mouse line (Maretto et al., 2003), which expresses β-galactosidase (β-gal) in the presence of activated β-catenin, Gaillard and Barlow (2011) found β-catenin activity in the basal cells and in a subset of each of the type-I, -II, -III cell populations. They suggested that the presence of activated β-catenin in these cell types was for the renewal of taste bud cells.

Taste buds develop postnatally in the trench wall of circumvallate papillae of mice (Suzuki et al., 2010a ; Suzuki et al., 2011). After glossopharyngeal nerve transection, taste buds die and disappear from the trench wall and subsequently regenerate after regenerated nerve fibers appear in the trench wall (Yee et al. 2005 ; Suzuki, 2008 ; Suzuki et al., 2010b). As little is yet known about the expression of activated β-catenin in the taste buds during postnatal development or after nerve transection, in the present study we examined the expression patterns of β-catenin, Wnt10b, Wnt5a and Fzd family members in the mouse taste buds.

Materials and methods

Animals and tissues

Adult and pregnant ICR mice were obtained from Sankyo Laboratories (Tokyo, Japan). They were maintained in a heat- and humidity-controlled vivarium with food and water provided *ad libitum*. Experimental protocols concerning animal handling were reviewed and approved by the Animal Ethics and Research Committee of the Health Sciences University of Hokkaido.

For immunohistochemistry and *in situ* hybridization, mice of postnatal day (P)2-P10 and those of 1.5-3 months of age were deeply anesthetized and then perfused through the left ventricle with 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS). Tongues were then removed and fixed further in the same fixative for several hours or overnight at 4°C. Each specimen was washed in PBS, cryoprotected with 25% sucrose, embedded in OCT compound (Tissue Tek, Miles Elkhart, IN), and frozen in a spray freezer (Oken, Tokyo). The tissues were sectioned sagittally or frontally at a thickness of 8-10 μm in a cryostat. The sections were then collected and placed on silane-coated slides.

Glossopharyngeal nerve transection

To assess the effects of nerve loss on the expression of β-catenin in taster bud cells, the glossopharyngeal nerves were bilaterally transected after the mice had been anesthetized by an intraperitoneal injection of Somnopentyl (Kyoritsu, Tokyo ; 50 mg/kg body weight). An incision was made in the ventral aspect of the neck. The submandibular and sublingual glands were retracted with the skin to expose the digastrics muscles. The stylohyoid muscle, which lies beneath the digastrics muscles, was pulled aside to expose the glossopharyngeal nerve. The bilateral glossopharyngeal nerves were then cut. Groups of animals were sacrificed at 11, 21, and 28 days after surgery.

Riboprobes and *in situ* hybridization

Sections were washed in PBS and treated for 10 minutes with 0.2N HCl and then for 5 minutes with protein-

ase K (1 μg/ml). They were then washed in PBS and refixed for 20 minutes in 4% PFA. Next, the sections were prehybridized for 1 hour at room temperature in a hybridization buffer containing 50% formamide, 1.3 x standard saline citrate (SSC), 5 mM EDTA, 0.5% CHAPS, 0.1% Tween-20, 1% blocking reagent, 100 μg/ml tRNA, and 50 μg/ml heparin. Hybridization was performed overnight at 65°C in a hybridization buffer containing antisense riboprobe. For the control, other sections were hybridized with sense riboprobe. The hybridized sections were washed at 65° C in 2×SSC for 1 hour and thereafter in 0.1×SSC for 1 hour. After a wash in PBS, they were next incubated with a 1% concentration of blocking reagent (Roche Diagnostics, Manheim, Germany) for 1 hour at room temperature and subsequently incubated overnight at 4° C with alkaline-phosphatase-conjugated anti-digoxigenin (DIG) Fab fragments (Roche) diluted 1 : 500 in PBS. After 3 washes in PBS, chromogenic reactions were carried out by using nitro-blue tetrazolium / 5-bromo-4-chrolo-3-indolyl-phosphate (Roche). We used the cDNA of the following genes as *in situ* hybridization probes : 5'-CCTTTCAGATGCAG CGACTAA-3' and 5'-GTCCTCGGATACAATCCGG-3' (617bp NM_007614) for β-catenin, 5'-AGGGGCTG CACATCGCCGTT-3' and 5'-GCAGCGCTCCACTCG CGTCT-3' (872bp NM_011718) for Wnt10b, and 5'-CAGTCAGACCGAACGCTGT-3' and 5'-CACCGGCT CCCCAATATCA-3' (901bp NM_009524) for Wnt5a. The PCR products were cloned into *Hind* III / *Eco* RI (Wnt10b, Wnt5a) *Hind* III/*Bam* HI (β-catenin) sites of pT7/T3 DH5α (TOYOBO, Osaka, Japan) and sequenced. DIG-labeled antisense and sense probes were produced by use of an RNA transcription kit (Roche).

Immunohistochemistry

Prior to immunostaining, the sections were reacted with a protein-blocking reagent (Dako, Glostrup, Denmark) for 20 minutes at room temperature. For double-staining, sections were co-incubated overnight at 4°C with the following commercially obtained primary antibodies at a dilution of 1 : 100 : (1) mouse anti-β-catenin antibody (BD Transduction Laboratories, San Jose, CA) +goat anti-shh antibody (R&D Systems, Minneapolis, MN), (2) mouse anti-β-catenin antibody + goat anti-Fzd-1 antibody (R&D Systems), (3) mouse anti-β-catenin antibody + goat anti-Fzd-3 antibody (R&D Systems), (4) mouse anti-β-catenin antibody + goat anti-Fzd-4 antibody (R&D Systems), (5) mouse anti-β-catenin antibody + goat anti-Fzd-6 antibody (R&D Systems), (6) mouse anti-β-catenin antibody + goat anti-Fzd-7 antibody (R&D Systems), (7) mouse anti-β-catenin antibody + goat anti-Fzd-8 antibody (R&D Systems), (8) mouse anti-β-catenin antibody + anti-PGP 9.5 polyclonal sheep antibody (Ultraclone, Wellow, UK), (9) mouse anti-β-catenin antibody + anti-Sox2 polyclonal rabbit antibody (Chemicon, Temecula, Calif.). Propidium iodide (PI, Molecular Probes, Eugene, OR) was used to stain cell nuclei. Some sections were processed for antigen retrieval with target retrieval solution (Dako) for 5 minutes at 105°C before treatment with primary antibody. After having been rinsed in PBS, the sections were incubated for 2 hours at room temperature with the following secondary antibodies : donkey anti-mouse IgG labeled with Alexa Fluor 568 + donkey anti-goat IgG labeled with Alexa Fluor 488 (Molecular Probes), donkey anti-mouse IgG labeled with Alexa Fluor 488 + donkey anti-goat IgG labeled with Alexa Fluor 568, donkey anti-mouse IgG labeled with Alexa Fluor 488 + donkey anti-rabbit IgG labeled with Alexa Fluor 568 at a dilution of 1 : 200. For fluorescence microscopic observation, the sections were washed in PBS, mounted on slides with PermaFluor (Thermo, Pittsburgh, PA), and viewed with a Leica TCS-NT confocal laser scanning microscope with a Leica Plan Apo×40 objective (NA= 1.25-0.75). As a control, PBS was used instead of primary antibodies.

Results

***In situ* hybridization**

In adult mice, *β-catenin* expression was observed in the epithelium and in the taste buds of circumvallate papillae (Fig.1A). At higher magnification of the trench wall, *β-catenin* was seen to be strongly expressed in the basal region of the taste buds (Fig.1B). *Wnt10b* was expressed throughout the taste buds (Fig.1C). At P0, strong expression of *β-catenin* was observed in elongating trenches and taste bud primordia at the papillary surface (Fig.1D). *Wnt5a* was not expressed in the taste buds of the circumvallate papillae. Sections incubated with sense riboprobes for β-*catenin* and *Wnt10b* dis-

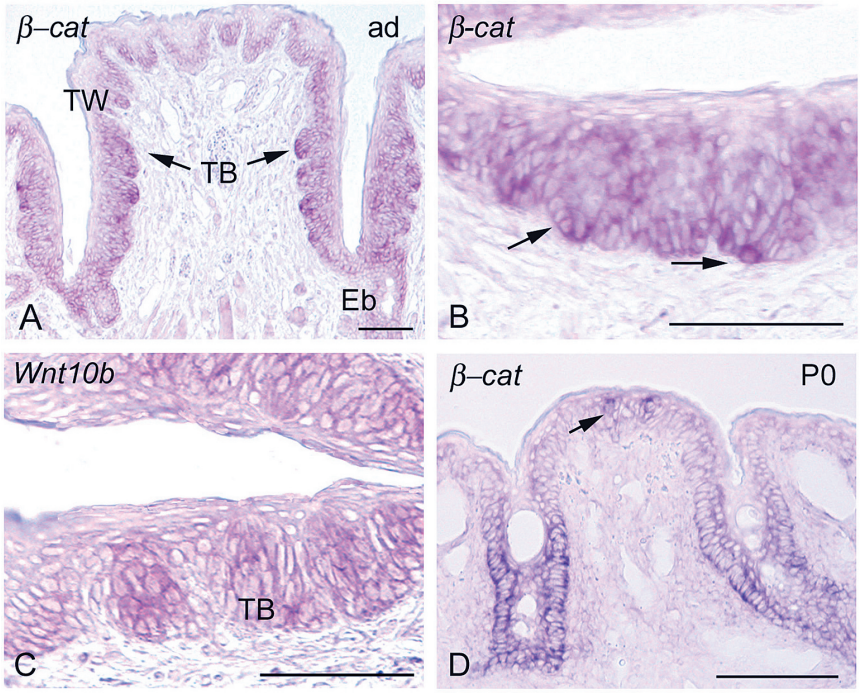

Fig.1 *In situ* hybridization with *β-catenin* and *Wnt10b* antisense probes of sections of circumvallate papillae of adult **(A-C)** and P0 mice **(D)**. **A**. Expression of *β-catenin* in the taste buds (TB) and the epithelium of a circumvallate papilla. **B**. Higher magnification of trench wall epithelium in "**A**", showing strong expression in the basal region of the taste buds (arrows). **C**. *Wnt10b* is expressed throughout the taste buds (TB). **D**. Expression of *β-catenin* at P0 is evident in the trench wall epithelium and in the taste bud primordia (arrow). Eb, von Ebner's gland. Scale bar, 100 μm

played no reactivity (not shown).

Activated β-catenin in the taste buds

Anti-β-catenin antibody reacted with membranes of the taste bud cells and weakly with the trench wall epithelium. A strong reaction was observed in a cell population in the basal region of the taste buds (Fig.2A). Double labeling with anti-β-catenin antibody and PI, a nuclear marker (Fig.2B), revealed the presence of β-catenin in the nucleus of cells in the basal region of the taste buds, roughly 3 immunopositive cells per bud section (Fig.2C). Anti-Shh antibody was used to detect the basal cells (Fig.2E). The basal cells, and also Shh-negative, intragemmal cells were positive for nuclear β-catenin-, namely activated β-catenin (Fig.2D, F). Fzd-1 imunoreactivity was observed in the cell membranes of all taste bud cells (Fig.2H). Cells positive for nuclear β-catenin (Fig.2G) were also Fzd-1-IR ones (Fig.2I). Also, Fzd-3-immunoreactivity was observed in the cell membranes of all taste bud cells (Fig.2K). The Fzd-3-IR cells contained β-catenin positive nuclei (Fig.2J, L). Fzd -7 immunoreactivity was observed in the basal cells of the surrounding epithelium (Fig.2N), but not in the β-catenin-positive nuclei (Fig.2M, O). Antibodies against other Fzd molecules, such as Fzd-4 and -6, were not immunoreactive with the taste buds. Anti-Fzd-8 antibody stained taste buds very weakly (not shown).

Activated β-catenin in the regenerating taste buds

After bilateral transection of the glossopharyngeal nerve, cells of taste buds die and disappear progressively from the trench wall epithelium. By 11 days after transection, most of the taste buds had disappeared from the trench wall. A few regenerated taste buds appeared at 21 days, and nerve fibers had entered into the taste buds (Fig.3B). The regenerated taste buds were located from the basal to middle region of the epithelium and showed nuclear β-catenin (Fig.3A, C). Also at this stage, Fzd-1 immunoreactivity was observed in the papillary epithelium and in the regenerated taste bud cells (Fig.3E). These Fzd-1-IR cells possessed β-catenin-positive nuclei (Fig.3 D, F). Also, Fzd-3 immunoreactivity was observed to be similar to that for Fzd-1 (not shown). At this stage, other Fzd-1-IR-cells were found apically in the trench wall (Fig.3E), probably remaining, surviving taste cells (Suzuki et al. 2010b). By 28 days after transection, the regenerated taste buds had increased in number, and β-catenin-positive nuclei were

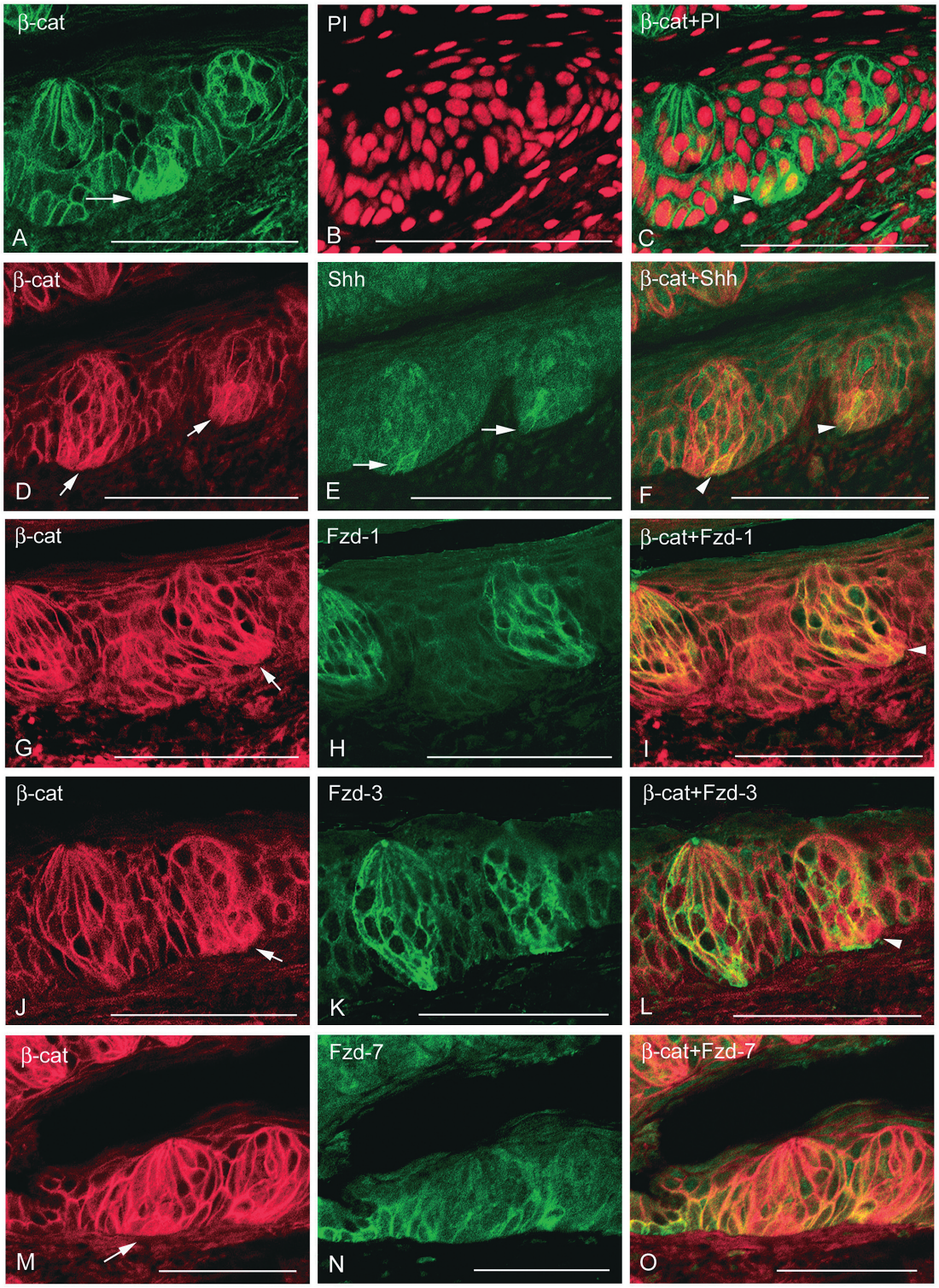

Fig.2 Confocal laser scanning microscopic images of sections of taste buds of circumvallate papillae from the adult mice. **A-C**. Double labeling with anti-β-catenin antibody **(A)** and PI, a nuclear marker **(B)**. β-catenin immunoreactivity is seen in the membrane of the taste bud cells **(A)**, strongly in a few basally located cells **(A**, arrow). Merged image **(C)** shows the presence of nuclear β-catenin in the basal region **(C**, arrowhead). **D-F**. Double labeling with anti- β-catenin **(D)** and anti-Shh **(E)** antibodies, showing activated β-catenin **(D**, arrows) and Shh-positive basal cells **(E**, arrows). Merged image **(F)** shows the basal cells possess β-catenin positive nuclei **(F**, arrowheads). **G-I**. Double labeling with anti-β-catenin **(G)** and anti-Fzd-1 **(H)** antibodies, showing activated β-catenin **(G**, arrow) and Fzd-1-IR cells in the membrane of all of the taste bud cells **(H)**. Merged image **(I)** shows Fzd-1-IR cells with nuclear β-catenin in the basal region (arrowhead). **J-L**. Double labeling with anti-β-catenin **(J)** and anti-Fzd-3 **(K)** antibodies. Nuclear β-catenin is seen **(J**, arrow). Merged image **(L)** shows Fzd-3-IR cells with nuclear β-catenin in the basal region **(L**, arrowhead). **M-O**. Double labeling with anti-β-catenin **(M)** and anti-Fzd-7 **(N)** antibodies. Merged image **(O)** shows Fzd-7-IR cells are devoid of nuclear β-catenin **(M**, arrow). Scale bar, 100 μm

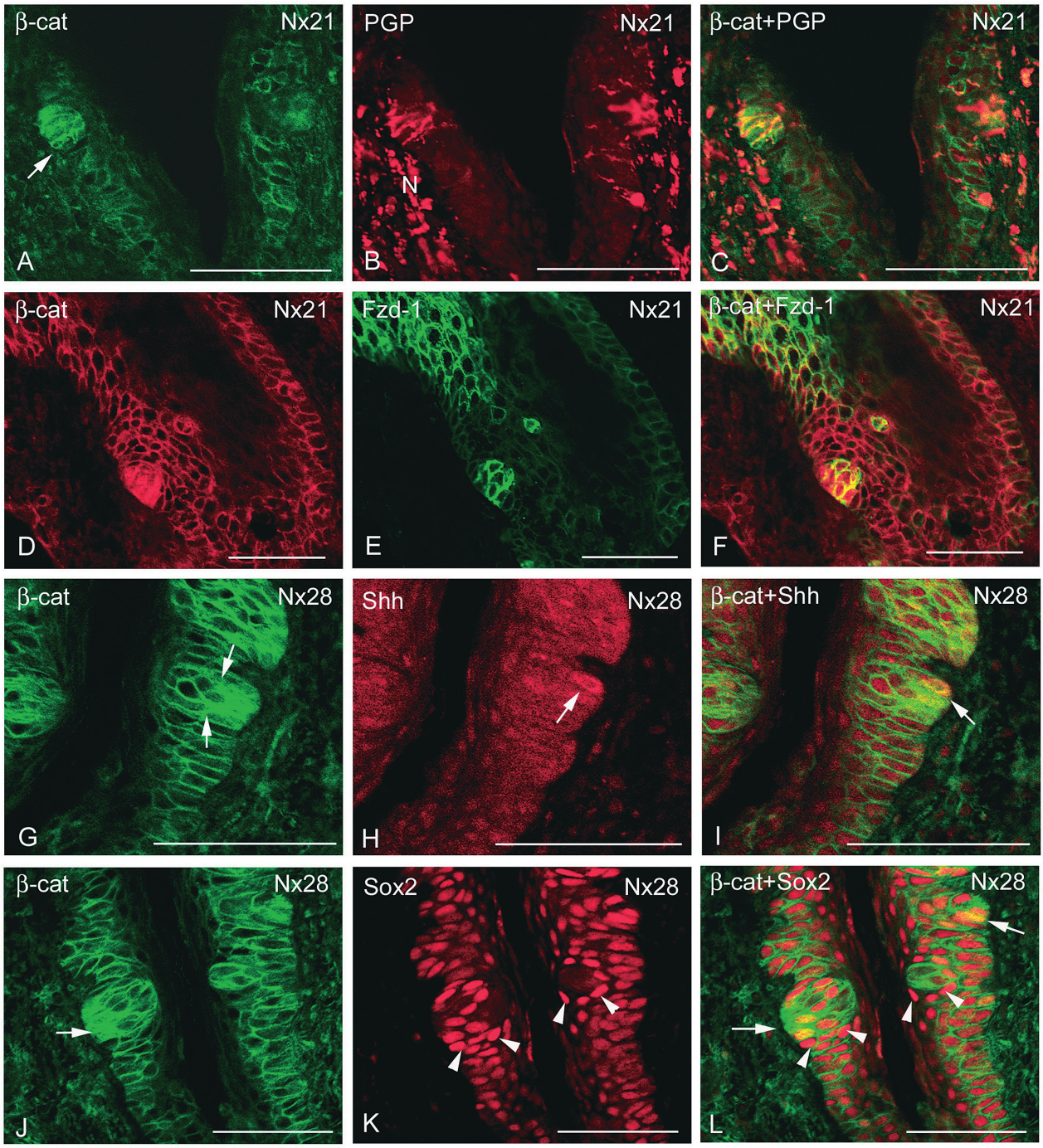

Fig.3 Confocal laser scanning microscopic images of sections of taste buds of circumvallate papillae after glossopharyngeal nerve transection (Nx). **A-F**. 21 days after transection. **A-C**. Double labeling with anti-β-catenin **(A)** and anti-PGP 9.5 **(B)** antibodies. Merged image **(C)** shows nerve fibers **(B**, N) that had entered into the regenerated taste bud cells having β-catenin-positive nuclei **(A**, arrow). **D-F**. Double labeling with anti-β-catenin **(D)** and anti-Fzd-1 **(E)** antibodies. Merged image **(F)** shows Fzd-1-immunoreactivity is seen throughout the regenerating taste buds, the cells of which possess β-catenin-positive nuclei. **G-L**. 28 days after transection. **G-I**. Double labeling with anti-β-catenin **(G)** and anti-Shh **(H)** antibodies. An Shh-positive basal cell is seen **(H**, arrow). Merged image **(I)**shows the presence of β-catenin-positive nuclei **(G**, arrows) and Shh-positive basal cells **(I**, arrow) in the basal half of the taste buds. **J-L**. Double labeling with anti-β-catenin **(J)** and anti-Sox2 **(K)** antibodies. The basal half of the taste buds contains cells positive for nuclear β-catenin **(J**, arrow). Sox2-IR cells are seen both in the perigemmal **(K**, arrowheads) and intragemmal regions. Merged image **(L)** shows intragemmal Sox2-IR cells that contain nuclear β-catenin (arrows). Sox-2-IR perigemmal cells are negative (arrowheads). Scale bar, 100 μm

observed in the middle to basal region of the taste buds (Fig.3G), which included the basal cells (Fig.3H, I). Strong Sox2 immunoreactivity was observed in the nuclei of the perigemmal epithelial cells and in some of the taste bud cells (Fig.3K). The nuclei of intragemmal Sox2-IR cells were β-catenin positive (Fig.3J, L),

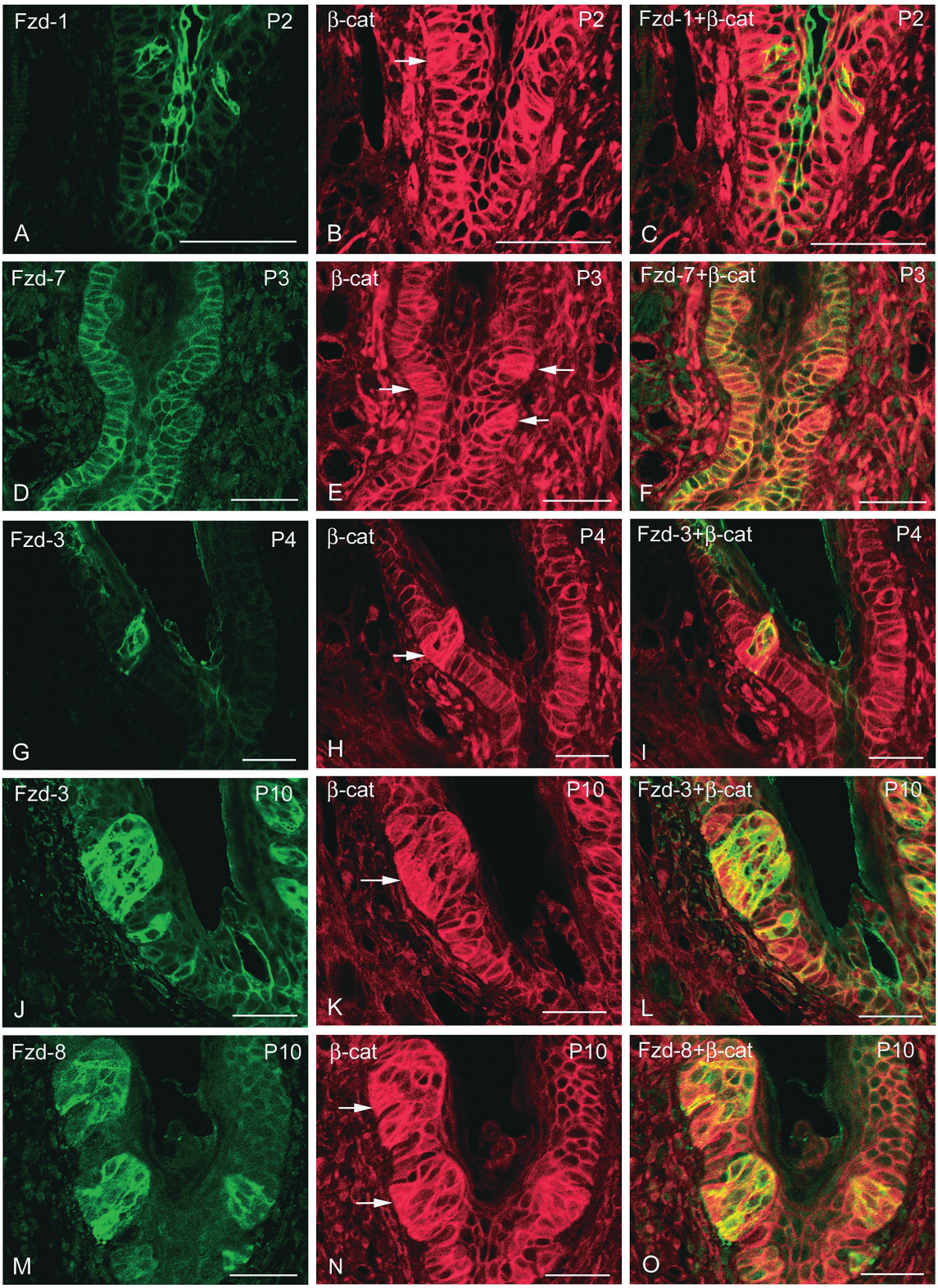

Fig.4 Confocal laser scanning microscopic images of sections of taste buds of circumvallate papillae of developing mice. **A-C**. P2. Double labeling with anti-Fzd-1 (**A**) and anti-β-catenin (**B**) antibodies. Merged image (**C**) shows Fzd-1-IR cells with β-catenin-positive nuclei (arrow in **B** and **C**). **D-F**. P3. Double labeling with anti-Fzd-7 (**D**) and anti-β-catenin (**E**) antibodies. Merged image (**F**) shows Fzd-7-IR cells contain β-catenin positive nuclei (**E**, arrows). **G-I**. P4. Double labeling with anti-Fzd-3 (**G**) and anti-β-catenin (**H**) antibodies. Merged image (**I**) shows nuclear β-catenin in cells in the basal region (**H**, arrow), which cells are partly devoid of Fzd-3-IR. **J-L**. P10. Double labeling with anti-Fzd-3 (**J**) and anti-β-catenin (**K**) antibodies. Merged image (**L**) shows β-catenin positive nuclei (**K**, arrow) within Fzd-3-IR cells. **M-O**. P10. Double labeling with anti-Fzd-8 (**M**) and anti-β-catenin (**N**) antibodies. Merged image (**O**) shows β-catenin-positive nuclei (**N**, arrows) within Fzd-8-IR cells. Scale bar, 50 μm

whereas those of perigemmal Sox2-IR cells were negative for nuclear β-catenin (Fig.3L).

Activated β-catenin and Frizzled in the developing taste buds

At P0, weak immunoreactivity indicating Fzd-1 appeared in the epithelia of papillae and the trench wall as well as that for β-catenin. At this stage, *β-catenin* mRNA was strongly expressed in the trench wall epithelium (Fig.1D), but nuclear β-catenin was not observed in it (not shown). The taste bud primordia were present at the papillary surface, but soon disappeared from that region. At P2, a few taste buds appeared in the trench wall. Strong immunoreactivity for Fzd-1 was observed in the trench wall (Fig.4A). Also, staining for nuclear β-catenin was positive probably in the entire region of developing taste buds (Fig.4B). Double labeling revealed that Fzd-1 immunoreactivity was present in some of the cells with β-catenin-positive nuclei (Fig.4C). At P3, Fzd -7 immunoreactivity was observed in the epithelium including taste buds (Fig.4D). β-catenin-positive nuclei were found in Fzd-7-IR cells (Fig.4E, F). Fzd-7 immunoreactivity disappeared from the taste buds progressively into the adult stage. At P4, expression of nuclear β-catenin was observed mainly in the basal region of the taste buds (Fig.4H). Fzd-3 immunoreactivity was also observed at that time (Fig.4G), but some were devoid of nuclear β-catenin (Fig.4I). At P10, β-catenin-positive nuclei were reduced in number and restricted to the basal region of the taste buds (Fig.4K). Fzd-3 immunoreactivity was observed in all of the taste buds (Fig.4J), and these cells were positive for nuclear β-catenin (Fig.4L). Also, Fzd-1 immunoreactivity was observed in similar manner to Fzd-3 at P4-P10. Fzd-8 immunoreactivity was evident at P4 (not shown) and at P 10 (Fig.4M), and progressively disappeared up to the adult stage. At P10, Fzd-8-IR cells contained nuclear β-catenin (Fig.4N, O).

Discussion

Using *in situ* hybridization and immunohistochemistry, we showed in the present study the expression of activated β-catenin, *Wnt10b* and Fzd family members in the taste buds of mouse circumvallate papillae. In the adult, non-operated mice, the localization of nuclear β-catenin in the basal region of the taste buds was consistent with that found in the previous study using BAT-gal reporter mice (Gaillard and Barlow, 2011). However, cells with nuclear β-catenin were fewer in number than β-gal-positive cells (roughly 3 versus 7 per bud section). Also, by *in situ* hybridization, strong expression of β-catenin mRNA was restricted to a few basally located cells. Wider expression of signals of reporter proteins was observed in nestin/GFP-positive cells in the central nervous system (Yamaguchi et al., 2000), and in Six1/GFP- and Six4/β-gal-positive cells in the taste buds (Suzuki et al., 2010b). The half-life of β-gal is reported to be approximately 13 hours (Jacobson and Willumsen, 1995), but the life-span of taste bud cells including basal cells is from a day to over 3 weeks (Hamamichi et al., 2006 ; Miura et al., 2006). The undetectable level of β-catenin expression might have been amplified to a detectable one in the BAT-gal mice. In the present study, the cell population including Shh-positive basal cells was also positive for nuclear β-catenin. Also, double-labeling for β-catenin and Sox2, the latter being expressed in basal cells and in immature type-I cells (Suzuki, 2008), revealed that β-catenin-positive nuclei might have been those of immature type-I cells. The present study clarified that β-catenin was activated throughout the taste buds in the early stages of regeneration and postnatal development, which activation likely triggered the transcription of target genes in immature taste cells to induce their differentiation. In cultured oligodendroglial cells, insulin-like growth factor (IGF)-I increases the expression of β-catenin protein (Ye et al., 2010). It is likely that β-catenin mediates IGF-I action, which is essential to the growth and development of the taste buds, since *IGF-I receptor* mRNA is expressed in the taste buds at P6 (Suzuki et al., 2005). Moreover, in later stages of development and during the regeneration process, β-catenin showed reduced expression. When its expression ceased, the protein would have been degraded as the cells completed their differentiation.

The present study clarified that *Wnt10b* mRNA was expressed in all of the taste bud cells. *Wnt5a*, which is a non-canonical family member (Grumolato et al., 2010), was not detected. We did not examine other Wnts ; however, Iwatsuki et al (2007) examined the expression

of 15 Wnts (*Wnt1, 2b, 3, 3a, 4, 5a, 5b, 6, 7a, 7b, 9a, 10a, 10b, 11,* and *16*) during formation of embryonic taste papillae. Only *Wnt10b* showed expression in embryonic fungiform papillae (Iwatsuki et al., 2007). Wnt proteins bind to Fzd proteins, which are seven transmembrane G-protein-coupled receptors ; and the canonical Wnt/β-catenin pathway activates β-catenin. Various combinations of Wnt and Fzd families are reported to act in canonical and non-canonical Wnt pathways : Fzd-1, -3, -7, and -8 are coupled to the β-catenin canonical signaling pathway (e.g., Zaghetto et al., 2007). The expression of Fzd-1 and -3 and that of *Wnt10b* in all of the taste bud cells revealed that these proteins comprised Wnt/β-catenin pathway in the adult taste buds. In early postnatal days, a few cells were immmunoreactive for Fzd-1 and -3 and came to be expressed in all of the taste bud membranes as development proceeded. In contrast, Fzd-8 immunoreactivity was found in the taste buds up to P10, and then became weak in the adult. Also, Fzd-7 immunoreactivity was found in the taste buds at P3, and disappeared from the taste buds until adult stage. Fzd-7 and -8 may activate β-catenin in the taste buds together with Fzd-1 and -3 during postnatal development. A change in Fzd expression during development was also reported to occur in the olfactory neurons and olfactory bulb in relation to synapse formation between them (Rodriguez-Gil and Greer, 2008). Moreover, Fzd-7 immunoreactivity was found in both the taste buds and the epithelium in early postnatal days, and only that in the latter remained in the adult. Fzd-7 may be associated with the proliferative/stem cell compartment, as revealed to be the case in the mouse gut epithelium (Gregorief et al., 2005).

In summary, *Wnt10b* and its receptors, Fzd-1 and -3, comprised the Wnt/β-catenin pathway in mouse taste bud cells, which pathway would activate β-catenin to up -regulate the expression of genes involved in early stages of postnatal development and regeneration.

Acknowledgements

This study was supported by the following grants ; 1) HAITEKU Research Center Project for Private Universities : matching fund subsidy from MEXT, 2007-2011 ; 2) Grant-in-Aid for Scientific Research (No.22592044) from Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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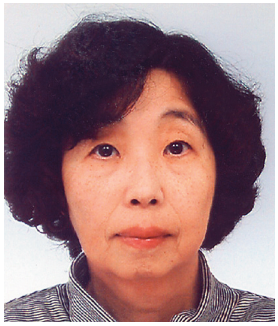

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