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Bitter taste receptor mTas2r105 is expressed in small intestinal villus and crypts

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ABSTRACT

The small intestine is the most important digestion and absorption organ in the body. Taste receptors and taste signal transduction cascades were detected in a variety of non-lingual tissues including testis, kidney, nasal cavity, lung, heart and gastrointestinal (GI) tract. Though the expression of bitter taste receptors and taste signal transduction cascades has been reported in the gut for a decade, the evidence revealing the expression of Tas2rs in the gut remain unbelievable. Here, the amplification of 35 bitter taste receptors from small intestine cDNA revealed that all transcripts are present in duodenum, jejunum and ileum, except Tas2r117. In addition, Tas2Rs and taste-related signaling transduction cascades are also observed in mouse small intestine including duodenum, jejunum and ileum by RT-PCR and Western Blot. On the other hand, three types of transgenic system were used to investigate the expression of the bitter taste receptor Tas2r105 in mouse intestine (Tas2r105-GFP/Cre, Tas2r105-GFP/Cre-DTA and Tas2r105-GFP/Cre-LacZ). With the bitter taste receptor mTas2r105 transgenic mice, the expression of mTas2r105 is showed in the villus and crypts of small intestine. mTas2r105 positive cells are also observed at the connective tissue of villus. DTA expression in mTas2r105 + cells completely ablate the expression of mTas2r105 in intestinal epithelia, but did not ablate mTas1r3 expression in intestine epithelia. LacZ staining further reveals that bitter taste receptor mTas2r105 is expressed in crypt base cells.

1. Introduction

In mammals, the sense of taste has been divided into 5 different modalities: bitter, sweet, sour, salty, and umami. In particular, bitter taste has evolved a central warning signal against the ingestion of potentially toxic substances [1]. Recently, more and more evidence showed the presence of taste receptors and taste signal transduction cascades outside the tongue [2–9]. One of these sites is the gastrointestinal (GI) tract [10]. GI responds to a variety of signals originating in the lumen, including nutrient and non-nutrient chemicals, mechanical factors, microorganisms, drugs and toxins. The expression of taste receptors in GI put forward hypothesis that taste receptors functions as a chemosensor to respond to nutrients and chemicals entering the digestive tract, and trigger diverse physiological processes [11–14]. For example, sweet taste receptors are expressed in enteroendocrine cells, and have been linked to elevated glucagon like peptide-1 (GLP-1) secretion and enhanced glucose uptake [9].

The Tas2r family consists of ~30 putative functional members in rodents and ~25 members in humans [15,16]. Based on RT-PCR results and in vitro studies of enteroendocrine STC-1 cells, expression of bitter taste receptors and taste signal transduction cascades has been reported in the gut for a decade [10]. However, the cell types expressing Tas2rs in the gut remain unclear. Thus, it is very impossible to detailed elucidate Tas2r physiological functions. Even so, numerous experiments in vivo have observed bitter substance-evoked gut responses [17–19]. In addition, in vitro results in STC-1 cells have revealed a marked increase in Ca^{2+} in response to bitter stimuli, including DB, PTC and cycloheximide (CYX) [10]. More importantly, DB, PTC and CYX did not result in Ca^{2+} signaling in multiple cell lines that do not express Tas2rs [10,20,21], indicating that the effects of bitter compounds on cAMP production in enteroendocrine cell lines are mediated by taste-specific receptors that are expressed in these cells. Bitter tastants in enteroendocrine cells induce the robust Ca^{2+} increase, which

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triggers the release of GI peptides including CCK, PYY and GLP-1 [22,23].

Recently, using gene-targeted Tas2r131$^{+/BLiC/ROSA26+/tdRFP}$ knock-in mice, a subset of colonic goblet cells is shown to express Tas2r131 [13]. Curiously, cells expressing this receptor do not express molecular marker of enteroendocrine and brush cells, and taste signal transduction cascades as well, including α-gustducin and PLCβ2 [13], which is always reported to be partner of bitter taste receptors in gut before [22,23]. Here, with the bitter taste receptor Tas2r105 transgenic mice, which have been used to reveal the expression of Tas2r105 in testis [24] and kidney [8], we further show the expression of Tas2r105 in the villus and crypt of small intestine.

2. Materials and methods

2.1. Animals and transgenic mouse lines

Adult C57BL/6 mice were bought from Fudan university, mTas2r105-Cre/GFP transgenic mice were generated and genotyped, maintained on the C57BL/6 background [24]. R26: loxP-Stop-loxP LacZbpA mice were purchased from the Jackson Laboratory, Bar Harbor, USA. The R26: lacZbpAflox diphtheria toxin A (DTA) line was a gift from Brockschnieder et al. [25,26]. Mice were maintained and sacrificed in accordance with protocols approved by the Institutional Animal Care and Use Committee at the Shanghai Jiao tong University School of Medicine.

To monitor the expression of mTas2r105 gene, mouse lines that carry the fusion protein-Cre/GFP driven by mTas2r105 promoter were used in this work. PCR was used to genotyped the positive litters. To generate the double transgenic mice which were used to trace the expression of mTas2r105 gene, mTas2r105-Cre/GFP transgenic mice were crossed with R26: loxP-Stop-loxP LacZbpA transgenic mice. Moreover, R26: lacZbpAflox diphtheria toxin A (DTA) transgenic mice were crossed with mTas2r105-Cre/GFP transgenic mice to generate the double transgenic mice, which could be used to ablate the mTas2r105 positive cells.

2.2. RT-PCR analysis

Adult C57BL/6 mice were killed by CO_2 asphyxiation followed by dissection and got the tissues, we extracted total RNA from small intestine tissue using TRIzol reagent (Takara technology), then measured concentration (NANO DROP2000 Spectrophotometer). Purification and reverse transcription RNA: 3 μg total RNA was used as template to synthesize first-strand cDNA with TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGen Biotech). The total volume is 50 μl, containing 1 μl reverse transcribed cDNA, 0.5 μl hot start Taq DNA polymerase, 3 μl of each primer in hot start buffer, 8 μl dNTP Mixture, 5 μl 10 × LA TaqBuffer (Mg^{2+} Plus) and RNA free water (Takara technology). The sequences of primers used and the predicted amplification sizes are listed in Table 1 10 min at 94 °C for initial denaturation, 50 cycles were performed at 94 °C for 30 s, 52 °C for 30 s, 72 °C for 30 s, followed by a final extension step at 72 °C for 10 min. A negative control was prepared with the omission of the cDNA template. All PCR products were separated on 2% agarose gels and stained with ethidium bromide. Gel images were photographed under UV light.

2.3. Western blot analysis

Extraction of total protein from tissues: the small intestine tissue was removed and grinded in RIPA buffer, the protein concentration in cell lysates was determined using BCA assay kit (Beyotime, Shanghai, China). Thirty micrograms of protein from each sample were separated by SDS-PAGE (10% gel) and then transferred to polyvinylidene fluoride membrane (Immobulon-P 0.45 μm, Millipore Germany) by liquid transfer. The membranes were blocked with 5% skim milk for 2 h at room temperature, and then followed by incubation overnight at 4 °C with the primary antibodies against mTas2r106 (1:200, Goat sc-34286 Santa Cruz biotechnology); Gα-gustducin (I-20) (1:500, rabbit sc-395, Santa Cruz biotechnology USA); β-actin (1:1000, rabbit, Santa Cruz biotechnology). After several washes with PBST, membranes were incubated with the horseradish peroxidase-linked anti-rabbit or anti-goat secondary antibodies (1:10000 Santa Cruz biotechnology USA) in 5% BSA in PBST for 1 h at room temperature. Immunoblots were visualized by ECL (Immobulon, Millipore Germany) and Image Develop.

2.4. Real time-PCR analysis

Total RNA was extracted from the small intestine tissue using TRIzol reagent. Purification and reverse transcription RNA: three microgram of total RNA was used as template to synthesize first-strand cDNA with TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGen Biotech). PCR was performed in a total volume of 50 μl containing 1 μl reverse transcribed cDNA, 3 μl of each primer in hot start buffer, 0.5 μl hot start Taq DNA polymerase (Takara technology), 5 μl 10 × LA TaqBuffer (Mg^{2+} Plus) and 8 μl dNTP Mixture. The sequences of primers used and the predicted amplification sizes are listed in Table 2. An initial denaturation step of 94 °C for 30 s was followed by 45 cycles of denaturation at 94 °C for 5 s, annealing at 55 °C for 15 s and extension at 72 °C for 10 s (Bio-rad).

2.5. Immunohistochemical staining

Small intestines were dissected and fixed in 4% paraformaldehyde (PFA) in the phosphate-buffered saline (PBS, pH 7.4), at 4 °C for overnight, then dissected into pieces, and preserved in 4 °C. Tissue sections were taken back to the temperature from 4 °C refrigerator for 15 min; take slice into citric acid and trisodium citrate mixed solution (PH 6.0) in the microwave repair antigen

Table 1
Primer for seven bitter taste receptors and α-gustducin.

Gene	Forward	Reverse	Annealing temperature	Size, (bp)
Tas2r105	GGCATCCTCCTTTCCATT	ACCGTCCTTCATCACCTTC	52 °C	447
Tas2r106	TCACAGGCTTGGCTATTT	TTGAGAAGAATGTGGCTTAC	52 °C	397
Tas2r110	AATCACCAACTCATCCCATA	TTTCCCAGAATCAGCACA	52 °C	345
Tas2r113	TACCCAGCATTACACGAAA	CAGGGAGAAGATGAGCAAA	52 °C	402
Tas2r114	AGTTGTTGCGAAGATGGT	CAAGTTGCTTTCTGGGAT	52 °C	340
Tas2r134	GAATAGCCGTCCTAAACAA	TCCAGATGCCGATACAGT	52 °C	433
Tas2r143	GAGGATTTCCCAGTTAGTTC	ATGGTATGTGCCTGAGTATG	52 °C	324
α-Gustducin	ATGGCTACACTGGGGATT	TTCTGTTCACCTCCTCATCT	52 °C	466

Table 2
Primer for thirty-five bitter taste receptors.

Gene	Forward	Reverse	Annealing temperature	Size, (bp)
Tas2r102	GGAAGCTTGGTGTTCTTGCTTGG	AGATCAGCTCGGTCCACATTGC	55 °C	127
Tas2r103	ATTAGCACTGGGTTTACACTCACC	CCACAGGGAGAAGATGAGCAGAAG	55 °C	75
Tas2r104	AGCTTCCTTTCCGCTAGCTGTG	TGGATCAGCCAGGATGTGTTGC	55 °C	75
Tas2r105	TGCACTGGTAAACTGCATGGACTG	GCCGATGAGAAGGAAGCCAATC	55 °C	69
Tas2r106	TGCCTCTGACGCCCACATTATAG	GGCTGGTGGCAAACCATATACTTG	55 °C	79
Tas2r107	TCCCTGCGGTCACTCAATCATC	CAGTGCCTTCAAAGAGGCTTGC	55 °C	70
Tas2r108	ACAGTCGCAGAATTGCCTCTCC	AGGAATCTAGTGATGGCCAAGCTG	55 °C	60
Tas2r109	AAGGAAGAAACCTCCTTTCGTTGC	AGAGAGGCATGTCTCAGCTTTCTG	55 °C	110
Tas2r110	AGATGCAGGTCAATGCCAAACC	AGGCTTTAATGTGGGCCATGGTG	55 °C	59
Tas2r113	TCCGCACTGCTCTGGCAATTAG	TGAACAGACACCCACCAATCTAGG	55 °C	72
Tas2r114	TGCTGAGCACAATGGAAGGTGTC	TGTTCCCTACAATGCCCAGCAC	55 °C	71
Tas2r115	AGAGAAGAACGTTCCCTTCAGCAG	ACCCATATCAGAGCAAGCCTGGAG	55 °C	73
Tas2r116	AACACAGTGCCCATGGATGCAG	ATGTCTGCAAGGCTCTGATGTGG	55 °C	63
Tas2r117	GCAGGTGTTAAGCCTGCAGATTG	TGAACATGTTGCTGCATCCTCTGG	55 °C	120
Tas2r118	AAGTTGCACAACGGTTGCAGTG	TCTCCACCGGTGACAGTCTTTG	55 °C	68
Tas2r119	TCACACCCACAAGAAGGAGCAC	ACCTTAAGGATGGAGAACCTGCAC	55 °C	64
Tas2r120	TCCTTCTACCCAGCAGGTCATTC	AGCATCTCATCTGCCTCAGCAAC	55 °C	94
Tas2r121	ACACGACTGGGTCTTATGTTTAGC	CATCACCCAAAGACTGGCTTGC	55 °C	114
Tas2r122	GGTTGGCCATCTCCAGAATGTG	AGAATTTCCTGAGAGAGGCACCAG	55 °C	66
Tas2r123	TGCAGGTCAATGCCAAACAACC	CAGCAGGAAGGAGAACACAGTTTG	55 °C	83
Tas2r124	AGTCTCTGGCTTGCTACAGCTC	AGCTTCCCAGAAGCATGTGGAC	55 °C	127
Tas2r125	ATCTTCTCCCTGTGGAGACACCTG	TGGTGTCTTCGGAGCCTTTAGC	55 °C	64
Tas2r126	GCAGTGTGTGGGATTGGTCAAC	TCCCGGAGTACTCAACCAGATG	55 °C	32
Tas2r129	TTGCAGATGCCCACATCAGAGTC	TGGCACAGAGTAGGACATAGGTG	55 °C	60
Tas2r130	AGAAGTGCTCAGAGAGGTGGAC	TGTCAGCCTTTGCCATATCTTCAC	55 °C	75
Tas2r131	ATCAACATGGCTTGCCACCTG	AGCACACCTCTCAATCTCCACTTC	55 °C	105
Tas2r134	AGAGATCGGGTCCGTATTTGCTTC	TAAACCACATGGGCTGCGTTGG	55 °C	62
Tas2r135	TCAGTTCTGCCAGCAACACACC	TGAATCACCACCTGCCACATCC	55 °C	64
Tas2r136	TCTGGAGGAACCAATCCACCTG	TGCTCTCACCTGAACCATTGCC	55 °C	133
Tas2r137	AGCATACATTTGTGGCCATGCTC	AAGCAGAGGGTCCCTTAGATCCAG	55 °C	72
Tas2r138	TGCTATTCAGCTCGCCTGCTTC	TGGCTTGGTAGTTGTGGCTCAG	55 °C	62
Tas2r139	TGACAATGTTCGTCGCAACAGC	TCATGTTCAGGGTGTGTCTCCTG	55 °C	66
Tas2r140	CATGCAACACAATGCCAAAGACTC	AGGGCCTTAATATGGGCTGTGG	55 °C	60
Tas2r143	TTCCCAGGCTGCTGGTTGTATC	AGTTCCCGGTGGCTGAAATGAC	55 °C	68
Tas2r144	TGGTTTGCTGCTTGGCTCAATG	TCAGAAGGAACAGAGGGTGAGC	55 °C	73
β-Actin	ACAGCTTCTTTGCAGCTCCTTCG	ATCGTCATCCATGGCGAACTGGTG	55 °C	64

after dewaxing and hydration; 0.5% PBST soaked for 15 min, and then incubated with 3% H_2O_2 at room temperature for 15 min; 5% goat serum or donkey serum closure for 8 h at 4 °C; antibody (GFP (1:200, rabbit ab-6556; Abcam, Cambridge, UK), mTas1r3 (1: 200, Goat sc-34286 Santa Cruz biotechnology), Gα-gustducin (I-20) (1:200, rabbit sc-395, Santa Cruz biotechnology) incubated for 48 h at 4 °C; secondary antibody (1: 200) at room temperature and incubated for 1 h; ABC was incubated at room temperature for 1 h; DAB chromogenic for 6–8 min; dehydrated, mounted with neutral gum; photographed under a microscope observation. PBS instead of primary antibody was used as a negative control.

2.6. LacZ staining

Animals were perfused with 2% PFA in PBS. Small intestine tissue was then fixed in 2% PFA for 1 h, after which it was cryoprotected in 30% sucrose in PBS at 4 °C overnight. The next morning, tissue was cryosectioned at 12 μm thickness, and preserved in 4 °C. Remove the slices from the freezer 4 °C and warm for 15 min; PBS washed three times, once for 20 min; stained in X-gal solution (5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 2 mM $MgCl_2$, 0.02% detergent agent NP-40, 0.01% sodium deoxycholate, 1 mg/ml X-gal) at 37 °C overnight. Stained sections were washed three times for 20 min in PBS and counterstained with nuclear fast red. Bright field images were captured using an SPOT digital camera (Diagnostic Instruments, Inc., Sterling Heights, USA) attached to a Nikon SA Micro phot microscope and minimally processed using Image Pro Plus image analysis software (Media Cybernetics, Inc).

3. Results

3.1. Tas2Rs and taste-related signaling proteins are expressed in mouse small intestine

To investigate the expression of Tas2r genes in vivo, we examined mouse small intestine. Firstly, RT-PCR using cDNA prepared from poly(A)+ RNA from duodenum, jejunum and ileum of C57BL/6 mice was performed. As expected according to previous studies [10,13,14,23], transcripts of several mouse Tas2rs were detectable (Fig. 1A). We also detected expression of α-gustducin in duodenum, jejunum and ileum as reported before [13]. We further detected the protein expression of α-gustducin and mTas2r106 by western blot. As expected, α-gustducin expression was detectable (Fig. 1B). Meanwhile, bitter receptor (mTas2r106) was detected in small intestine (Fig. 1B).

To visualize the expression of Tas2r gene in vivo, we decided to perform quantitative real-time PCR experiments (Fig. 1C). Amplification of 35 bitter taste receptors from small intestine cDNA revealed that all transcripts are present in duodenum, jejunum and ileum, except mTas2r117. Relative abundance of 35 bitter taste receptors deviated from each other, mTas2r119 was most highly expressed and mTas2r124 exhibited the lowest transcript. In addition, mTas2r110, mTas2r113, mTas2r114 and mTas2r140 mRNA were much higher compared with other receptors in duodenum, jejunum and ileum. Obviously, the mRNA level of Tas2r genes was fluctuated along small intestine and only some Tas2r genes appear to be selectively expressed within the alimentary tract.

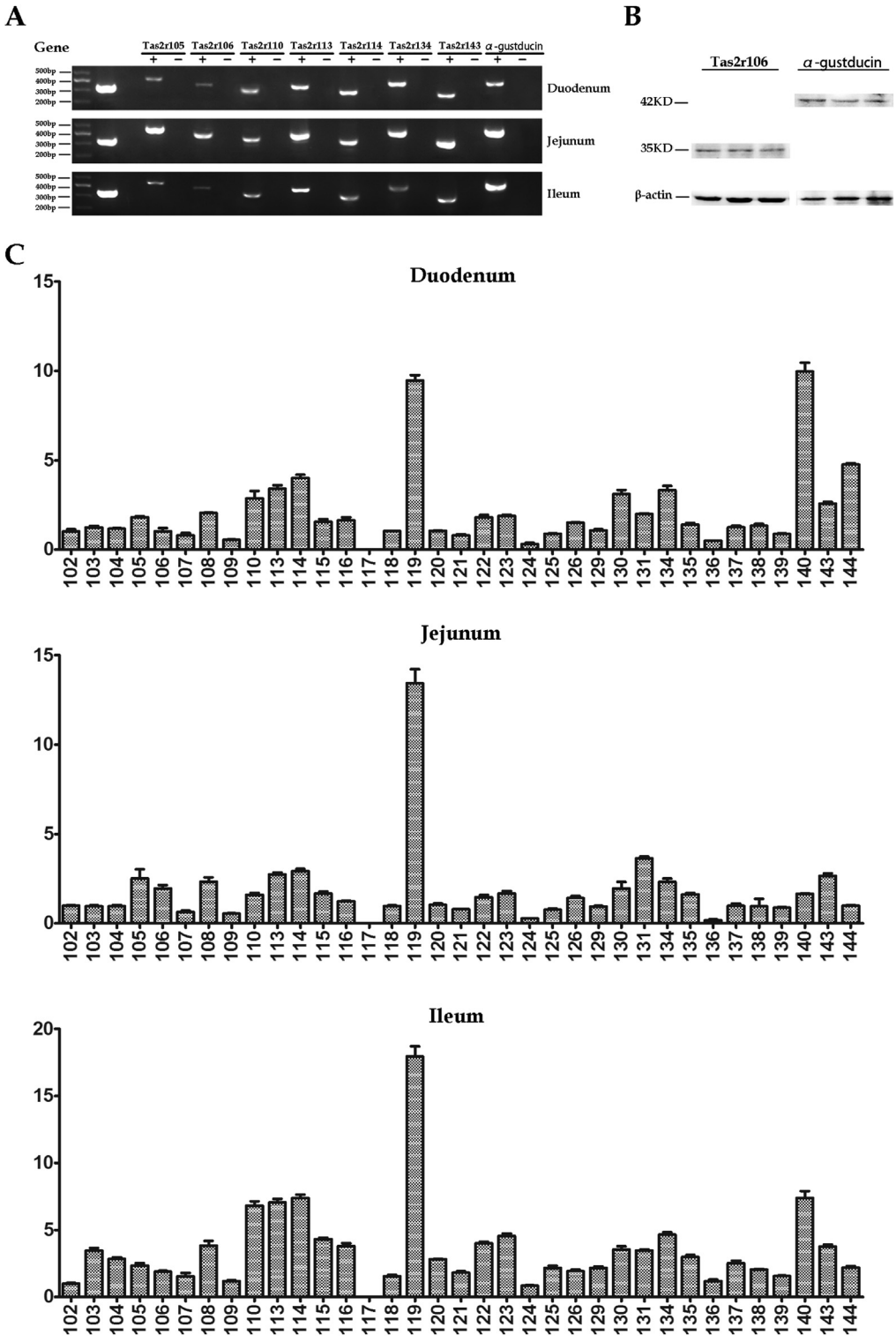

Fig. 1. Expression of Tas2rs and taste-related signaling transduction cascades in mouse small intestine. (A) RT-PCR was performed using specific primers for each of the 7 Tas2r genes and α-gustducin gene with cDNA templates reverse transcribed from mouse intestinal poly(A)+ RNA in the presence (+) or absence (−) of cDNA templates. (B) Western Blot analysis for mTas2r106 and α-gustducin was performed on total protein extracts prepared from small intestine of C57BL/6 mice. The same results were obtained in three independent experiments. (C) Quantitative real-time PCR analysis for 35 bitter taste receptors shows that all transcripts are present in duodenum, jejunum and ileum, except mTas2r117.

3.2. DTA expression in mTas2r105 + cells ablated GFP expression in small intestine

To further investigate the expression of bitter taste receptor, we sought to determine the Tas2r expression pattern in the intestines of mTas2r105-Cre/GFP transgenic mice. In previous study, we have showed the expression of mTas2r105 in testis [24] and kidney [8] from mTas2r105-Cre/GFP transgenic mice. These mice contain an engineered mTas2r105 allele driving expression of Cre/GFP fusion protein in mTas2r105-expressing cells. Immunohistochemical analysis with anti-GFP revealed the GFP expression in taste bud of CV papilla from mTas2r105-Cre/GFP transgenic mice (Fig. 2A). DTA expression in mTas2r105 + cells ablated GFP expression in taste bud from mTas2r105-Cre/GFP-DTA double transgenic mice (Fig. 2B). Furthermore, immunohistochemical analyses of intestine tissue sections showed GFP expressing cells in the luminal mucosa. As previously reported [13], the 2 types of cells can be detected in intestine epithelia: 1) cells having a goblet cell-like shape; 2) elongated cells with a columnar shape (Fig. 2E). Occasionally, we encountered a large number of cells expressing GFP in small intestine epithelia (Fig. 2C–G). It should be noted that positive cells were also observed in connective tissues (Fig. 2D–G). In mTas2r105-Cre/GFP-DTA double transgenic mice, no labeled cells were detected, indicating that DTA expression in mTas2r105 + cells ablated GFP expression in intestine epithelia (Fig. 2H).

In previous study, mTas1r3 expression has been shown in intestine epithelia [9]. mTas1r3 and mTas2r105 is separately expressed in a subset of taste bud cells [15]. As previously reported [9], mTas1r3 is detected in intestine epithelia from mTas2r105-Cre/GFP transgenic mice (Fig. 3A). In mTas2r105-Cre/GFP-DTA double transgenic mice, many labeled cells were still detected, indicating that DTA expression in mTas2r105 + cells did not ablate mTas1r3 expression in intestine epithelia (Fig. 3B–D).

3.3. Bitter taste receptor mTas2r105 is expressed in crypt base cells

After confirming activity of the mTas2r105 transgenic locus, we analyzed the in situ localization of mTas2r105-expressing cells. We

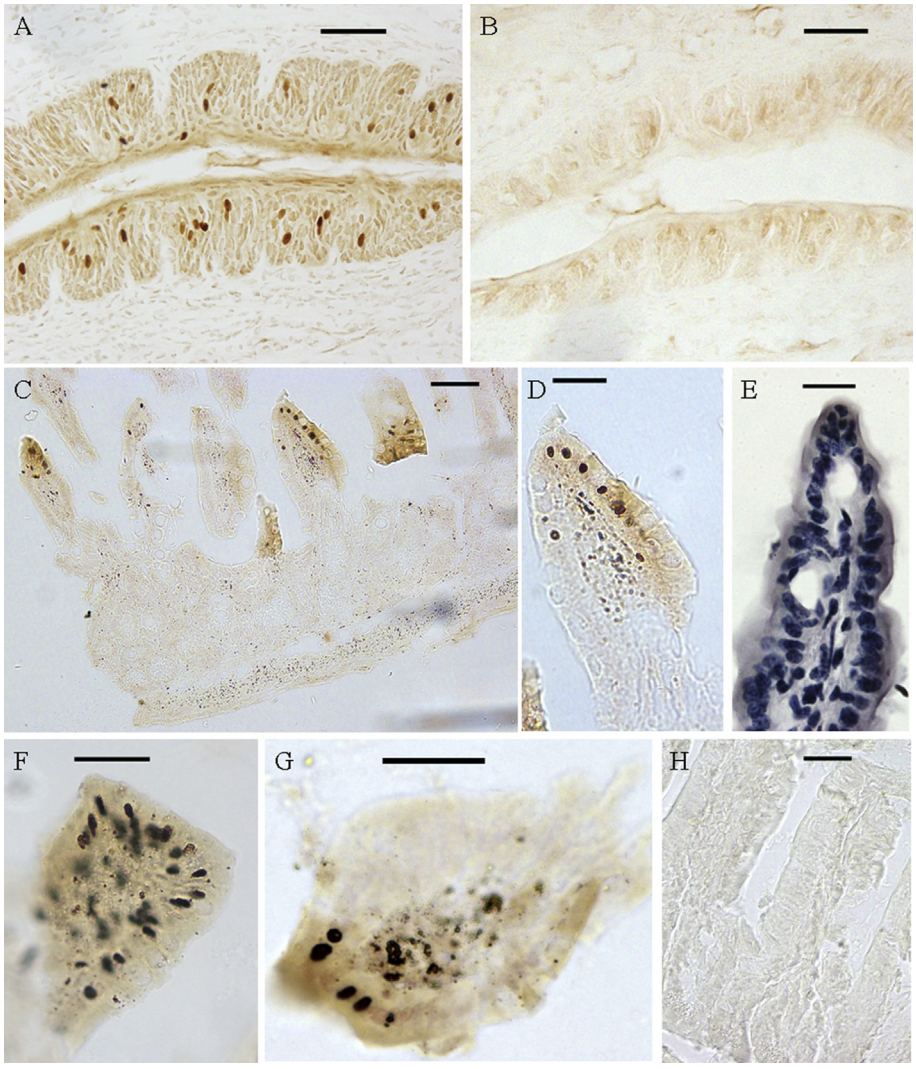

Fig. 2. Immunostaining of bitter taste receptor in the intestines of mTas2r105-Cre/GFP transgenic mice and mTas2r105-Cre/GFP-DTA double transgenic mice. (A) Immunohistochemical analysis reveals GFP expression in taste bud of CV papilla from mTas2r105-Cre/GFP transgenic mice. (B) DTA expression in mTas2r105 + cells ablates GFP expression in taste bud from mTas2r105-Cre/GFP-DTA double transgenic mice. (C) mTas2r105 expression in small intestine of mTas2r105-Cre/GFP transgenic mice (lower resolution). (D) mTas2r105 expression in villus (higher resolution). (E) Hematoxylin staining shows the structure of villus in intestinal epithelia. (F and G) the horizontal section shows that mTas2r105 is expressed in both epithelia cell and connective tissues. (H) DTA expression ablates the GFP expression in small intestinal epithelia Scale bar: (A–C) 50 μm; (D–G) 25 μm.

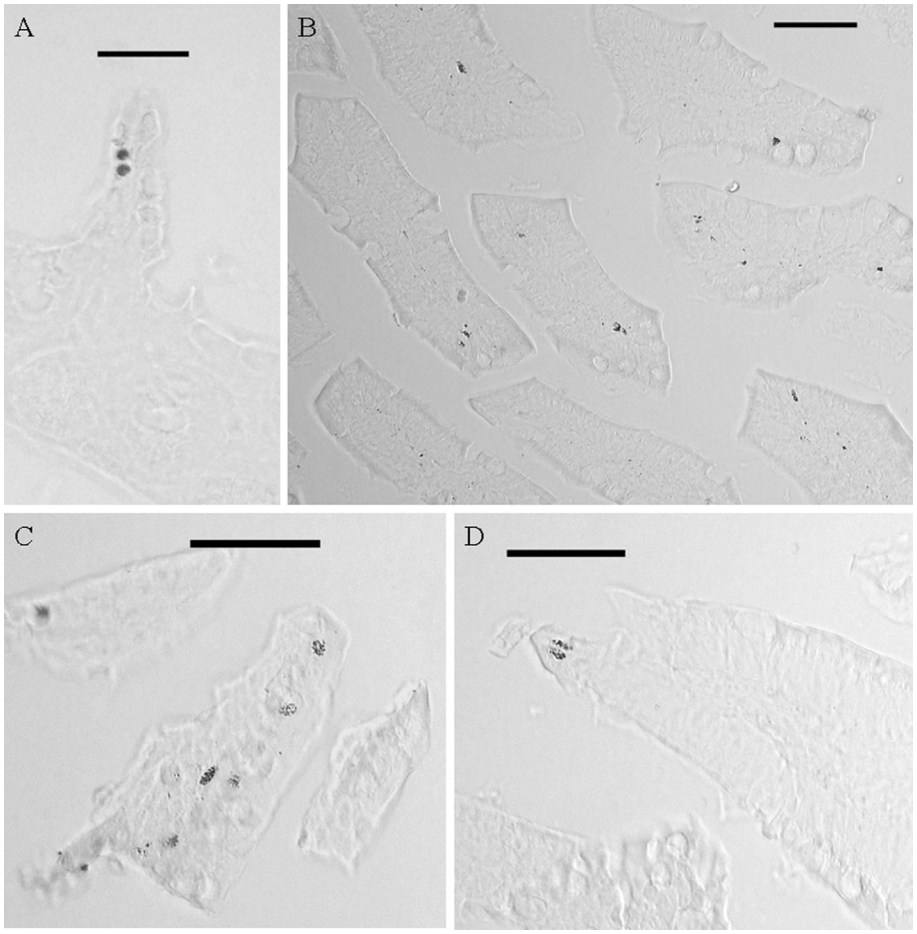

Fig. 3. DTA expression in mTas2r105 + cells fail to ablate mTas1r3+ cells in the intestinal epithelia. (A) Expression of mTas1r3 in the intestinal epithelia of mTas2r105-Cre/GFP transgenic mice. (B–D) mTas1r3 expression in intestinal epithelia of mTas2r105-Cre/GFP-DTA double transgenic mice. (B) After DTA expression in mTas2r105 + cells, a large number of mTas1r3+ cells are detected in intestinal epithelia (lower resolution). (C) Several mTas1r3+ cells are observed in villus (higher resolution). (D) two mTas1r3+ cells are detected in tip of villus. Scale bar: (A, C and D) 25 μm; (B) 50 μm.

crossed the mTas2r105-Cre/GFP transgenic mice with the Cre-activatable Rosa26-lacZ reporter [24]. After microscopical analyses of tissue section, it is revealed that blue LacZ signals appeared at the base of the crypt (Fig. 4A–F), which is thought as the putative stem cell zone in small intestine. In previous study with Tas2r131$^{+/BLiC/ROSA26+/tdRFP}$, it is revealed that bitter taste receptor Tas2r131 is expressed in entire crypts [13]. After screening the tissue section, it is found that blue LacZ signals covered the intestinal stem cell niche, extending from a position 0 the very base of the crypt to +4 (Fig. 4B–E). In addition, blue LacZ signals were also found in villus (Fig. 4B–D).

4. Discussion

A decade after the first report about Tas2rs expression in the gut has gone [10], but the evidence on cell types expressing Tas2rs in situ is scarce [14,23]. This is explained by a considerable lack of experimental tools for the investigation of Tas2rs, which include a lack of sufficiently specific antisera and mouse models genetically modified. Recently, using gene-targeted Tas2r131$^{+/BLiC/ROSA26+/tdRFP}$ mice, Tas2r131 gene expression is obviously identified in distal part of the GI tract [13]. Furthermore, immunostaining with specific antisera reveal that Tas2r131 do not co-express with several molecular markers, indicating that Tas2r131 positive cells do not belong to enteroendocrine and brush cell types, but goblet cells. In addition, Tas2r131 positive cells do not express α-gustducin and PLCβ2, widely used molecular markers for taste-like cells [13]. In the present study, our results further reveal the expression of mTas2r105 in the villus. On the other hand, mTas2r105 positive cells distribute at the muscoa and connective tissue of villus. Furthermore, mTas2r105 expression is localized at so-called putative stem cell niche, extending from a position +1 at the very base of the crypt to +4, where is particularly enriched for nucleotide label-retaining cells (LRCs) [27].

The small intestine go through a rapidly and continuously regeneration in its life. At the base of the crypt is the putative stem cell zone [28,29]. The relatively undifferentiated crypt at the base continuously divides and the generated progeny immigrate into villus, composed of differentiated epithelial cells. Long-term DNA-label cells locate at “position +4” [29]. Lgr5 is expressed within the stem cell zone that spans position +1 to +4. Lgr5-positive crypt cells generate all epithelial lineages, suggesting that it represents the stem cell of the small intestine [30]. Curiously, recent study further shows that Lgr5+ cell can generate all taste bud cell lineages and lingual epithelial cells [31–33]. In addition, a large number of study gradually reveals the highly restricted Lgr5 expression in a variety of other tissue, suggesting that adult stem cells may be identified on the basis of the expression of a single gene [30,31,34,35]. Bitter taste receptor mTas2r105 is initially found in taste bud [15]. With the mTas2r105-Cre/GFP transgenic mice,

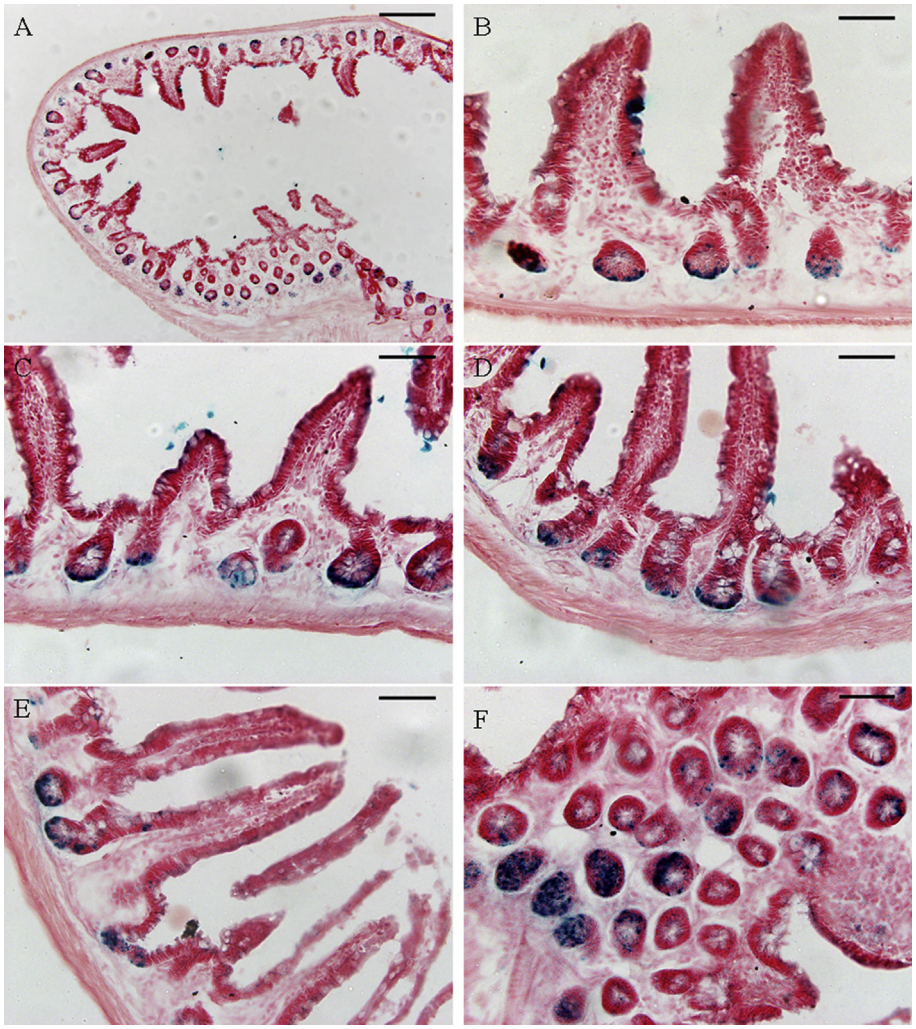

Fig. 4. LacZ staining reveals the expression of bitter receptor mTas2r105 in the small intestine of Tas2r105-GFP/Cre-LacZ transgenic mice. (A) mTas2r105 expression in the small intestines (lower resolution). (B–E) Bitter receptor mTas2r105 is expressed in crypt base cells and villus. (F) mTas2r105 expression in intestinal gland. Scale bar: (A) 100 μm; (B–F) 25 μm.

mTas2r105 expression is obviously detected in testis [7,24] and kidney [8]. Furthermore, mTas2r105 expression is observed during spermatogensis from spermatocyte to spermatid [7]. Similarly, mTas2r131 expression is also detected in testis [36]. The current study further reveals the common expression pattern of both genes in crypt. Although it remains uncertain for the function of bitter receptor in crypt, mTas2r131 expression is not co-localized with several marker proteins of differentiated cell types [13], suggesting that these bitter taste receptor-expressing cells may represent a population of stem cells or immature cells in crypt.

The current study collectively reveals that bitter taste receptors may play a critical role in small intestine, based on several following facts. Firstly, compared to Tas1rs [9], there is more wide distribution. Members of the Tas2r family of bitter taste receptors has been reported in the gastric and intestinal mucosa in human and rodents as well as in enteroendocrine cell lines in culture by RT-PCR and real-time PCR analysis for a decade [10,14,23]. Recently, with transgenic mice model of mTas2r105 and mTas2r131 [13], two receptors located at different gene cluster are clearly showed to express in the gastric and intestinal tract. Moreover, mTas2r105 positive cells widely distributes at the villus and crypt. Secondly, compared to mTas1rs, there is a large family for bitter taste receptors, for example 35 Tas2rs in the mouse genome and 25 Tas2rs in the human genome [15,37]. Thirdly, bitter compounds are not only numerous but also structurally diverse. Many bitter substances are of genuine plant origin, and estimates are in tens of thousands with the small molecular compound of the chemical synthesis [38–40].

In conclusion, the use of Tas2r105-Cre/GFP mice enabled us to reveal Tas2r105 expression in villus and crypts. Future studies should be dedicated to reveal molecular and physiological characteristics of intestinal bitter-sensing cells. The expression and function of bitter taste receptors in specific cells of the small intestine and elucidating of the signal-transduction pathways that mediate their biological effects in these cells has a number of important implications including the design of novel molecules, and open a new windows for exploring molecular sensing in the small intestine.

Conflict of interest

There is no financial or other potential conflict of interest for Fu Gu, Xin Liu, Jie Liang, Jiaying Chen, Fuxue Chen, Feng Li.

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