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Expression of bitter taste receptor Tas2r105 in mouse kidney

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ABSTRACT

The kidney is the most important excretory organ in the body and plays an essential role in maintaining homeostasis in vivo by conserving body fluid and electrolytes and removing metabolic waste. In this study, three types of transgenic system were used to investigate the expression of the bitter taste receptor Tas2r105 in mouse renal tissue (Tas2r105-GFP/Cre, Tas2r105-GFP/Cre-DTA and Tas2r105-GFP/Cre-LacZ). The results suggest that bitter taste receptors Tas2r105 and Tas2r106 are expressed in the renal corpuscle and the renal tubule, including the proximal tubule and distal tubule. Expression of α-gustducin, an important component of taste signal transduction, was also detected in mouse kidney. Meanwhile, conditional diphtheria toxin (DTA) expression in Tas2r105+ cells caused an increase in size of the glomerulus and renal tubule, accompanied by a decrease in cell density in the glomerulus. This indicates that Tas2r105+ cells play an important role in maintaining the structure of the glomerulus and renal tubules. Overall, the current study collectively demonstrates that cells labeled by bitter taste receptor expression may play a critical role in controlling human health, and have properties far beyond the original concept of taste perception.

1. Introduction

Economic development, accelerated industrialization, urbanization and environmental pollution have resulted in an increase in the incidence of chronic diseases, including chronic kidney disease (CKD). Over 500 million people currently suffer from various kidney diseases worldwide. Every year, more than one million individuals die of cardiovascular disease associated with CKD. In parallel with cardiovascular disease, cancer and diabetes, CKD has become one of four global public health problems. The main causes of CKD originate from nephritis, hypertension and diabetes. Apart from genetic factors, diet, lifestyle and drug metabolism can also critically influence the development of CKD. CKD has significant socioeconomic burdens due to its prevalence, and a negative impact on the quality of life [1].

Few medicines can be used to treat CKD to date, such as hormones and cyclophosphamide. There is an ongoing effort to identify important gene and/or signaling pathways leading to regulation of kidney function, thereby providing new treatment strategies for CKD. Recently, bitter taste receptors and the cognate G-protein, α-gustducin, were identified in human and mouse airway smooth muscle, and regarded as new therapy targets for obstructive lung diseases [2,3].

Bitter taste receptor Tas2r is a member of the G protein-coupled receptor superfamily which contains 30 members. These proteins pass through the cell membrane seven times, forming three extracellular loops and three corresponding intracellular loops where the structure is highly conserved, and are coupled with an intracellular G-protein. The bitter taste receptor genes are distributed in clusters in mouse and human genomes. The G-protein α-gustducin, a generic protein in the process of bitter taste signal transduction, activates the ion channels or intracellular calcium stores, which leads to an increase in the concentration of calcium which produces nerve impulses inside the cell [1,8,23].

Several researchers have reported the expression of bitter taste receptors in non-taste tissues. Singh et al. detected transcripts of Tas2r104, Tas2r107 and Tas2r138 by RT-PCR in C6 glial cells cultured in vitro and in the brain stem, cerebellum, cortex, and the nucleus accumbens of mouse brain, in addition to the transcripts of Tas2r104 in primary neurons [28]. Other studies have detected Tas2r transcripts in the gastric antrum and fundus, and the duodenum of mice [25]. Xu et al. observed Tas2r transcripts in mouse testis by in situ hybridization, and functionally analyzed the

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response of mouse spermatids and spermatozoa to both naturally occurring and synthetic bitter-tasting compounds by calcium imaging [32]. In addition, the expression of bitter taste receptor Tas2r105 was observed in transgenic mouse testes in which the bitter taste receptor Tas2r105 was replaced by GFP and marked by LacZ [19].

In this study, using the established double transgenic mice generated in our previous work [5,19], Tas2r105-Cre/GFP-Rosa26-LacZ and Tas2r105-Cre/GFP-Rosa26-DTA, the expression of Tas2r105, Tas2r106 and α-gustducin was observed in the glomeruli, distal tubule and proximal tubule by immunohistochemistry and LacZ staining. Statistical analysis revealed that ablation of Tas2r105+ cells had a significant impact on renal structure, especially the glomerular structure.

2. Materials and methods

2.1. Animals

mTas2r105-Cre/GFP transgenic mice were generated and genotyped as described previously, maintained on the C57BL/6 background [19]. 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. [4,5]. 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.

2.2. Mouse lines and tissue collection

Mouse lines that carry the fusion protein-Cre/GFP driven by mTas2r105 promoter were used to monitor the expression of mTas2r105 gene. The positive litters were genotyped by PCR. mTas2r105-Cre/GFP transgenic mice were crossed with R26: loxP-Stop-loxP LacZbpA transgenic mice, generating the double transgenic mice which were used to further trace the expression of mTas2r105 gene. On the other hand, mTas2r105-Cre/GFP transgenic mice were crossed with R26: lacZbpAflox diphtheria toxin A (DTA) transgenic mice, generating the double transgenic mice which were used to ablate the Tas2r105 positive cells. Mice were euthanized by a slow stream of CO_2. Kidney was removed into cold, sterile phosphate buffered saline (PBS) and tissues were dissected and processed for different analyses.

2.3. Western blot analysis

Adult C57BL/6 mice were killed by CO_2 asphyxiation followed by dissection, the kidney tissue was removed and grinded in RIPA buffer (Sangon Biotech Shanghai China). 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 (1:10,000 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. Reverse transcription-PCR analysis

Total RNA was extracted from kidney tissue using TRIzol reagent (Takara technology). 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 10xLA TaqBuffer (Mg^{2+} Plus) and 8 μl dNTP Mixture. The sequences of primers used and the predicted amplification sizes are listed in Table 1. An initial denaturation step of 94 °C for 10 min was followed by 50 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s and extension at 72 °C for 30 s and finished with a final extension at 72 °C for 10 min on a thermocycler (Applied Biosystems). 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.

2.5. Immunofluorescence

Mouse kidney were dissected and fixed in 4% paraformaldehyde (PFA) in 0.1 M PBS, pH 7.4, at 4 °C for overnight, then dissected into pieces. Kidney was transferred into 70% ethanol, embedded in paraffin and serially sectioned at 5–7 μm. Polyclonal primary antibodies were specific for mTas2r106 (1:200, Goat sc-34286 Santa Cruz biotechnology); Gα-gustducin (I-20) (1:200, rabbit sc-395, Santa Cruz biotechnology); GFP (1:200, rabbit ab-6556; Abcam, Cambridge, UK). Immunosignal was visualized using an Alexa488-conjugated goat-anti-rabbit, donkey-anti-goat secondary antibodies, respectively. Images were taken using the confocal imaging system (Leica TCS SP8) For supplemental data, the different is that kidney from wild type mouse were dissected, fixed in 4% paraformaldehyde (PFA) for 2–12 h and cryoprotected in 30% sucrose in PBS at 4 °C overnight. After sectioning on a cryostat, 10–12 μm sections were collected onto superfrost plus microscope slides (Fisher Scientific, Loughborough, UK). Polyclonal primary antibodies were specific for mTas2r106 (1:200, Goat sc-34286 Santa Cruz biotechnology) and Gα-gustducin (I-20) (1:200, rabbit sc-395, Santa Cruz biotechnology). Images were taken using the microscopic imaging system (Nikon Company).

2.6. X-gal staining

Animals were perfused with 2% PFA in PBS. Renal 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 following morning, tissue was cryosectioned at 12 μm thickness. The sections were washed three times for 20 min in PBS and stained in X-gal solution (5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 2 mM $MgCl_2$, 0.02% detergent NP-40, 0.01% Na 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 a 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.)

2.7. Data analysis and quantitation

Mouse kidney was embedded in paraffin and serially sectioned at 5–7 μm for hematoxylin and eosin staining. We at least count 200 glomeruli and renal tubules for each group ($n = 2$ for control group; $n = 3$ DTA group) with microscope at high magnification

Table 1
Primer sequences of 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

(×400), and the morphological measurement are carried out with Image J software including the number of cell, area and diameter. Morphometric data are presented as the mean + SEM. These data were analyzed by Graph Pad Prism5 software. Differences were considered to be significant when $P < 0.05$. Calculation formula: QA = Q/A; NV = QA/D; LV = 2 QA. (QA = contour area; NV = numerical density; LV = length density; Q = cell number; A = area; D = diameter.)

3. Results

In our previous study, a transgenic mouse line was generated in which the codon sequences of the mTas2r105 gene were completely replaced by the fusion gene Cre/GFP, disrupting the expression of mTas2r105 gene. Meanwhile, mTas2r105-Cre/GFP transgenic mice were crossed with R26: lacZbpAflox-DTA mice, in which diphtheria toxin (DTA) expression ablates mTas2r105+ cells in vivo. Immunostaining showed the expression of mTas2r106 and α-gustducin in renal tissues of C57 mice, including the proximal tubule, distal tubule, renal corpuscle and pyramid (Supplementary Fig. 1), indicating that bitter taste receptors may be expressed in renal tissues.

3.1. Expression of Tas2r receptor family genes and α-gustducin in mouse kidney

In order to investigate the expression of bitter taste receptors and signal transduction cascades, total protein and total RNA was extracted from fresh kidney tissue from adult C57 mice. Expression of seven bitter taste receptors and α-gustducin was assessed in mouse renal tissues by RT-PCR (Fig. 1A). Expression of Tas2r106 and α-gustducin were also detected by western blotting (Fig. 1B).

3.2. LacZ staining reveals expression of mTas2r105 in renal tissues

To verify the expression of bitter taste receptors in renal tissues, mTas2r105-Cre/GFP mice were crossed with R26:loxP-Stop-loxPLacZbpA transgenic mice. The renal tissues from the double transgenic mice were sectioned and stained for LacZ, and expression of Tas2r105 was assessed in epithelial cells of the medullary collecting duct, distal tubules and proximal tubules of the cortex (Fig. 2A–E). In addition, Tas2r105 expression was also observed in the parietal cells of the cortical glomeruli (Fig. 2F).

3.3. Ablation of mTas2r105+ cells in vivo suppresses GFP, Tas2r106 and α-gustducin expression in renal tissue

As shown in Fig. 3, immunofluorescent staining revealed the expression of GFP, Tas2r106 and α-gustducin in epithelial cells of the proximal tubules and distal tubules in renal tissue, as well as in the cortical glomeruli. Diphtheria toxin (DTA) expression, which ablates mTas2r105+ cells in vivo, caused suppression of GFP, Tas2r106 and α-gustducin expression in the epithelial cells of the proximal tubules, distal tubules and cortical glomeruli.

3.4. Ablation of mTas2r105+ cells significantly increases the average area and diameter of the glomerulus and proximal tubule

To investigate whether mTas2r105+ cells play an important role in maintaining normal structure in renal tissues, statistical analysis was carried out on renal tissues from control mice and from mTas2r105-Cre/GFP-DTA mice. Several parameters regarding the structure of the glomerulus and proximal tubule were investigated. As shown in Fig. 4, the statistical results demonstrate that the average diameter and area of the glomerulus significantly increased after deleting the Tas2r105 receptor, compared with normal tissue ($P < 0.05$). Conversely, the contour area (Q_A) and numerical density (N_V) of the glomerulus were significantly decreased (Fig. 4C–D; $P < 0.05$). In addition, DTA expression mTas2r105+ cells significantly increased the average diameter and area of the renal tubule ($P < 0.05$). However, there was no significant difference observed in the contour area and length density (L_V) of the renal tubule compared with control tissue.

4. Discussion

The kidney is the body's most important excretory organ and plays an important role in maintaining a relatively constant environment in vivo [16]. The results of the present study suggest that bitter taste receptors, Tas2r105 and Tas2r106, are expressed in the renal corpuscle and renal tubules, including the proximal and distal

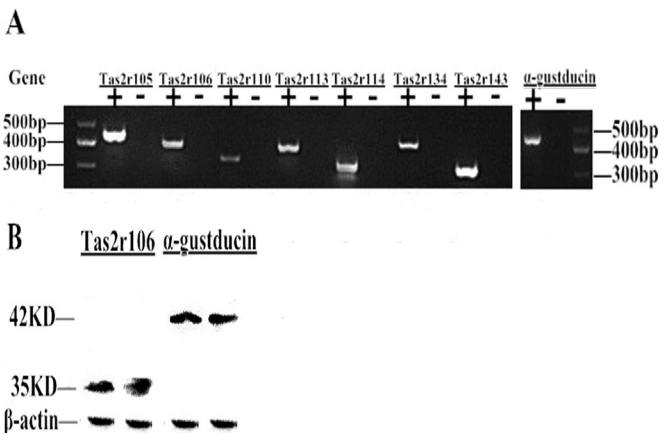

Fig. 1. (A) Expression of members of the Tas2r receptor gene family and α-gustducin in mouse kidney. RT-PCR was performed using specific primers for each of the 7 Tas2r genes and α-gustducin gene with cDNA templates reverse transcribed from mouse kidney poly(A)+ RNA in the presence (+) or absence (−) of cDNA templates. PCR products were separated on 2% agarose gel containing ethidium bromide. (B) Western blot analysis of protein lysates using antibodies against Tas2r106 and α-gustducin. The same results were obtained in two independent experiments.

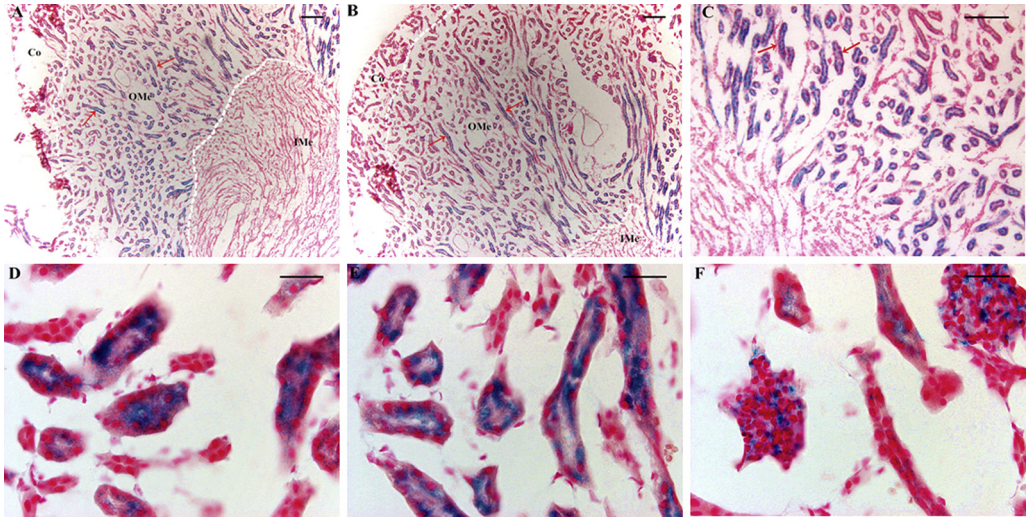

Fig. 2. Tas2r105 expression in renal tissue from Tas2r105-GFP/Cre-LacZ transgenic mice detected by LacZ staining. (A–C) Tas2r105 expression in epithelial cells of the medullary collecting duct, (D) distal tubules of the cortex, (E) proximal tubules of the cortex and (F) parietal cells of cortical glomeruli. Co = cortex; Ome = outer medulla; Ime = inner medulla. Scale bars: (A–B) 200 μm; (C) 100 μm; and (D–F) 25 μm.

tubule. Expression of α-gustducin was also detected in these regions. It should be noted that the expression of Tas2r105 and Tas2r106 varied, dependent on the location in the renal tubule. The Tas2r family is reported to consist of approximately 30 highly divergent G-protein-coupled receptors [1,23]. Recent studies have shown wide expression of Tas2r family receptors in testes [19,31,32], upper respiratory tract [29] and lung [10,27]. Bitter tastants can activate these bitter taste receptors and increase intercellular calcium levels to induce a physiological response in these tissues. Therefore, we speculate that the majority of Tas2r receptors may be expressed in the kidney, and regulate the function of the renal corpuscle and renal tubule by detecting bitter compounds dissolved in body fluid.

Cell loss, as a consequence of either necrosis or programmed cell death, is commonly found in diseased tissues. When the affected tissue is no longer able to perform adequately, a clinically overt phenotype will be detected. For example, in the central nervous system, losing 50%–70% of specific dopaminergic striatal neurons

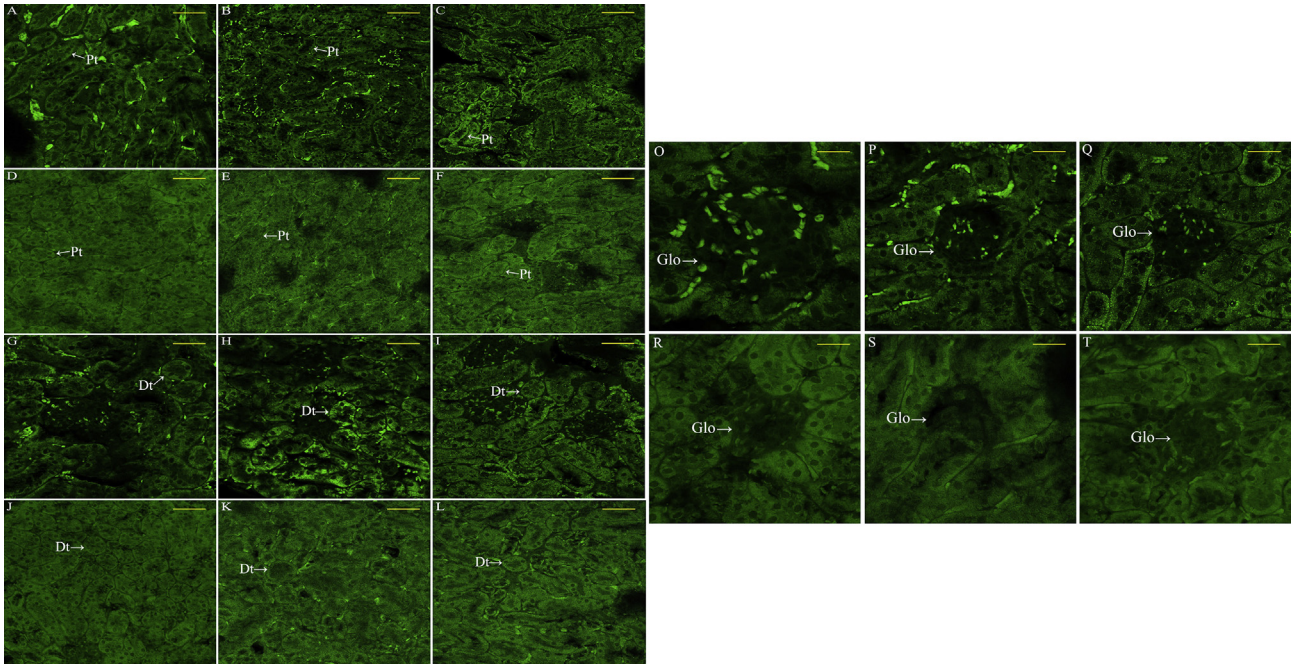

Fig. 3. Immunofluorescent staining of renal tissue from transgenic mice. Staining for expression of (A) Tas2r105, (B) Tas2r106 and (C) α-gustducin in epithelial cells from the proximal tubules in Tas2r105-GFP/Cre transgenic mice. Staining for expression of (D) Tas2r105, (E) Tas2r106 and (F) α-gustducin in epithelial cells from the proximal tubules in Tas2r105-GFP/Cre-DTA transgenic mice. Staining for expression of (G) Tas2r105, (H) Tas2r106 and (I) α-gustducin in epithelial cells from the distal tubules in Tas2r105-GFP/Cre transgenic mice. Staining for expression of (J) Tas2r105, (K) Tas2r106 and (L) α-gustducin in epithelial cells from the distal tubules in Tas2r105-GFP/Cre-DTA transgenic mice. Staining for expression of (O) Tas2r105, (P) Tas2r106 and (Q) α-gustducin in parietal cells of the cortical glomeruli in Tas2r105-GFP/Cre transgenic mice. Staining for expression of (R) Tas2r105, (S) Tas2r106 and (T) α-gustducin in parietal cells of cortical glomeruli in Tas2r105-GFP/Cre-DTA transgenic mice. Pt = proximal tubules; Dt = distal tubules; Glo = glomeruli. Scale bars: (A) 22 μm, (B and C) 42 μm, (D–L) 22 μm, and (O) 7 μm, (P–T) 13 μm.

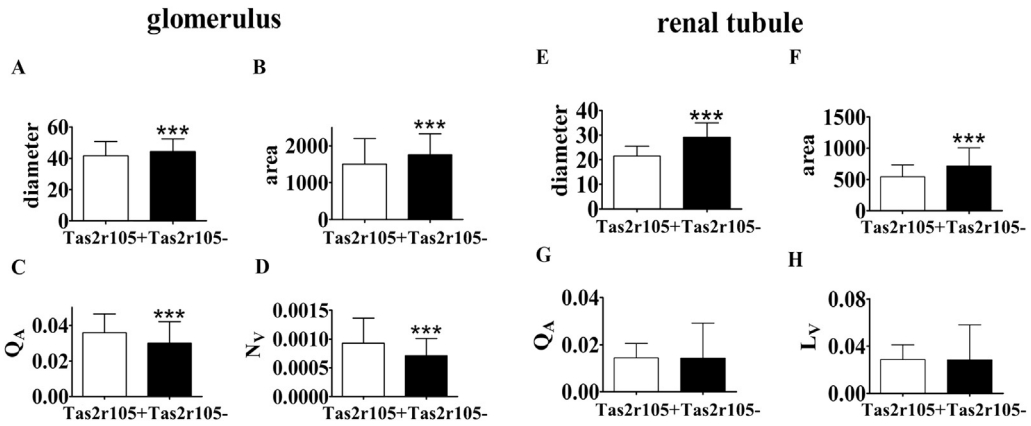

Fig. 4. Statistical analysis of the glomerulus and renal tubule structure. (A) DTA expression in mTas2r105+ cells significantly increased the average diameter and (B) area of the glomerulus ($P < 0.05$). (C) DTA expression significantly decreased the contour area (Q_A) and (D) numerical density (N_V) of the glomerulus. (E) DTA expression significantly increased the average diameter and (F) area of the renal tubule. (G) No significant difference was observed for the contour area and (H) length density (L_V) of the renal tubule compared with normal tissue.

results in Parkinson's disease [2], and loss of enteric ganglion cells leads to Hirschsprung's disease [20]. Therefore, conditional ablation of any lineage cells would be of particular help to model human diseases of various etiologies [4,17,26]. In this study, conditional DTA expression in Tas2r105+ cells caused an increase in the area and diameter of the glomerulus and renal tubule, accompanied by a decrease in cell density in the glomerulus. This indicates that Tas2r105+ cells play an important role in maintaining the structure of the glomerulus and renal tubule. In previous studies, conditional ablation of Tas2r105+ taste bud cells resulted in loss of bitter taste perception [9,19], and ablation of Tas2r105+ spermatids decreased testis size and caused male sterility [19]. Such results suggest that Tas2r105+ cells may perform important functions in vivo.

Previous studies have demonstrated the wide distribution of bitter taste receptors in vivo, such as in the taste bud [8], upper respiratory tract [29], lung [10,27], brain [28], heart [13,14] and testes [19,31,32]. The current study has also demonstrated the expression of bitter taste receptors in mouse kidney. Furthermore, conditional ablation of Tas2r105+ cells resulted in pathological changes in specific tissues. This indicates that cells labeled by bitter taste receptor expression may play a critical role in controlling human health, and be far beyond the original concept of bitter taste purely as an important sensory input to warn against the ingestion of toxic and noxious substances [23]. A previous study provided evidence that allelic variations affecting the function of individual Tas2r receptors significantly impact on glucose and insulin homeostasis. A single bitter taste receptor, Tas2r109, lost normal response to its cognate ligands after one single-nucleotide polymorphism within this haplotype [11]. In German Sorbs, a genetic variation within Tas2r138 may be related to anthropometric measures and glucose homeostasis [15]. In an additional study, bitter taste receptor polymorphisms were closely related to human longevity [7]. Although the mechanism by which the bitter taste receptor associated haplotype affects glucose and insulin homeostasis remains unclear, these receptors could be involved in the modulation of glucagon-like peptide-1 secretion from gut enteroendocrine L cells [11]. Given that these receptors are expressed in a number of organs, genetic polymorphisms of bitter taste receptors may modulate food preferences, regulate food absorption processing and metabolism, and further modulate the aging process.

Many bitter substances are of genuine plant origin; however, others are derived from animals or are generated during the processing, aging or spoilage of food [12]. Bitter compounds are numerous and structurally diverse, including fatty acids, peptides, amino acids, amines, lactones, phenols and steroids [3,6,12,22,24,30]. Humans absorb a large amount of such bitter substances every day, which converge to create a dangerous cocktail that can affect kidney function by activating bitter taste receptors. We speculate that this may contribute to the cause of CKD.

Conflict of interest

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

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.01.089.

References

[1] E. Adler, M.A. Hoon, K.L. Mueller, et al., A novel family of mammalian taste receptors, Cell 100 (2000) 693–702.
[2] A.A. Bachmanov, N.P. Bosak, W.B. Floriano, et al., Genetics of sweet taste preferences, Flavour Frag. J. 26 (2011) 286–294.
[3] H.D. Belitz, Structure and taste of the sweet and sour compounds, Z Ernahrungswiss 12 (1973) 190–200.
[4] D. Brockschnieder, C. Lappe-Siefke, S. Goebbels, et al., Cell depletion due to diphtheria toxin fragment a after cre-mediated recombination, Mol. Cell. Biol. 24 (2004) 7636–7642.
[5] D. Brockschnieder, Y. Pechmann, E. Sonnenberg-Riethmacher, et al., An improved mouse line for cre-induced cell ablation due to diphtheria toxin A, expressed from the Rosa26 locus, Genesis 44 (2006) 322–327.
[6] B. Bufe, T. Hofmann, D. Krautwurst, et al., The human Tas2r16 receptor mediates bitter taste in response to beta-glucopyranosides, Nat. Genet. 32 (2002) 397–401.
[7] D. Campa, F. De Rango, M. Carrai, et al., Bitter taste receptor polymorphisms and human aging, PLoS One 7 (2012) e45232.

[8] J. Chandrashekar, K.L. Mueller, M.A. Hoon, et al., T2Rs function as bitter taste receptors, Cell 100 (2000) 703–711.
[9] J. Chandrashekar, M.A. Hoon, N.J. Ryba, et al., The receptors and cells for mammalian taste, Nature 444 (2006) 288–294.
[10] D.A. Deshpande, W.C. Wang, E.L. McIlmoyle, et al., Bitter taste receptors on airway smooth muscle bronchodilate by localized calcium signaling and reverse obstruction, Nat. Med. 16 (2010) 1299–1304.
[11] C.D. Dotson, L. Zhang, H. Xu, et al., Bitter taste receptors influence glucose homeostasis, PLoS One 3 (2008) e3974.
[12] A. Drewnowski, C. Gomez-Carneros, Bitter taste, phytonutrients, and the consumer: a review, Am. J. Clin. Nutr. 72 (2000) 1424–1435.
[13] S.R. Foster, E.R. Porrello, B. Purdue Chan, et al., Expression, regulation and putative nutrient-sensing function of taste GPCRs in the heart, PLoS One 8 (2013) e64579.
[14] S.R. Foster, K. Blank, L.E. See Hoe, et al., Bitter taste receptor agonists elicit G-protein-dependent negative inotropy in the murine heart, FASEB J. 28 (2014) 4497–4508.
[15] M. Keller, X. Liu, T. Wohland, et al., Tas2r38 and its influence on smoking behavior and glucose homeostasis in the German Sorbs, PLoS One 8 (2013) e80512.
[16] K. Kudo, J. Shiraishi, S. Nishimura, et al., The number of taste buds is related to bitter taste sensitivity in layer and broiler chickens, Anim. Sci. J. 81 (2010) 240–244.
[17] P. Lee, G. Morley, Q. Huang, et al., Conditional lineage ablation to model human diseases, Proc. Natl. Acad. Sci. U S A 95 (1998) 11371–11376.
[19] F. Li, M. Zhou, Depletion of bitter taste transduction leads to massive spermatid loss in transgenic mice, Mol. Hum. Reprod. 18 (6) (2012) 289–297.
[20] X. Li, A.A. Bachmanov, K. Maehashi, et al., Sweet taste receptor gene variation and aspartame taste in primates and other species, Chem. Senses 36 (2011) 453–475.
[22] W. Meyerhof, C. Batram, A. Kuhn Brockhoff, et al., The molecular receptive ranges of human Tas2r bitter taste receptors, Chem. Senses 35 (2010) 157–170.
[23] K.L. Mueller, M.A. Hoon, I. Erlenbach, et al., The receptors and coding logic for bitter taste, Nature 434 (2005) 225–229.
[24] S. Rodgers, J. Busch, H. Peters, et al., Building a tree of knowledge: analysis of bitter molecules, Chem. Senses 30 (2005) 547–557.
[25] E. Rozengurt, Taste receptors in the gastrointestinal tract. I. Bitter taste receptors and alpha-gustducin in the mammalian gut, Am. J. Physiol. Gastrointest. Liver Physiol. 291 (2006) G171–G177.
[26] M. Saito, T. Iwawaki, C. Taya, et al., Diphtheria toxin receptor-mediated conditional and targeted cell ablation in transgenic mice, Nat. Biotechnol. 19 (2001) 746–750.
[27] A.S. Shah, Y. Ben-Shahar, T.O. Moninger, et al., Motile cilia of human airway epithelia are chemosensory, Science 325 (2009) 1131–1134.
[28] N. Singh, M. Vrontakis, F. Parkinson, et al., Functional bitter taste receptors are expressed in brain cells, Biochem. Biophys. Res. Commun. 406 (2011) 146–151.
[29] M. Tizzano, B.D. Gulbransen, A. Vandenbeuch, et al., Nasal chemosensory cells use bitter taste signaling to detect irritants and bacterial signals, Proc. Natl. Acad. Sci. U S A 107 (2010) 3210–3215.
[30] M.G. Tordoff, L.K. Alarcon, S. Valmeki, et al., T1R3: a human calcium taste receptor, Sci. Rep. 2 (2012) 496.
[31] A. Voigt, S. Hubner, K. Lossow, et al., Genetic labeling of tas1r1 and Tas2r131 taste receptor cells in mice, Chem. Senses 37 (2012) 897–911.
[32] J. Xu, J. Cao, N. Iguchi, et al., Functional characterization of bitter-taste receptors expressed in mammalian testis, Mol. Hum. Reprod. 19 (1) (2012) 17–28.