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CCAAT/Enhancer-binding protein β regulates expression of human T1R3 taste receptor gene in the bile duct carcinoma cell line, HuCCT1

Takashi Toyono *, Yuji Seta, Shinji Kataoka, Kuniaki Toyoshima

Division of Oral Histology and Neurobiology, Department of Biosciences, Science of Health Improvement, Kyushu Dental College, Kokurakita-ku, Kitakyushu 803-8580, Japan

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Abstract

The T1R family (T1R1, T1R2 and T1R3 receptors) has a role in the detection of umami and sweet tastes in taste buds. Although T1R3 is also expressed in the intrahepatic bile duct, the expression patterns of T1R1 and T1R2 in this region have not been determined. In addition, the mechanisms of transcriptional regulation of the human T1R3 gene (*Tas1r3*) have not been elucidated. In this study, we determined the expression patterns of T1R2 and T1R3 in human liver and the function of C/EBPβ in *Tas1r3* promoter activity. Immunohistochemistry showed that T1R2 and T1R3 were expressed in the intrahepatic bile duct. 5′-RACE analysis revealed that the transcriptional start sites of *Tas1r3* were located 67 bp and 176 bp upstream of the ATG. Promoter analysis of *Tas1r3* was performed using the luciferase reporter assay and EMSA in the *Tas1r3*-expressing cell line, HuCCT1. The 226-bp region upstream of the ATG had promoter activity, and C/EBPβ activated the *Tas1r3* promoter activity in HuCCT1 cells. These results show that T1R2 and T1R3 receptors, in addition to their role in taste perception, may also have a role in intrahepatic cholangiocytes. C/EBPβ was identified as the transcription factor regulating *Tas1r3* promoter activity in HuCCT1 cells.

Keywords: *Tas1r3*; C/EBPβ; Promoter; Transcription; Intrahepatic bile duct; Luciferase assay

1. Introduction

Sweet and umami (monosodium glutamate) tastes involve the T1R family (T1R1, T1R2 and T1R3 receptors), which belong to subclass 3 of the GPCR (G-protein-coupled receptor) superfamily [1,2]. T1R2 and T1R3 combine to function as a sweet receptor, which detects saccharides including sucrose and glucose, while T1R1 and T1R3 form the umami receptor, which detects L-amino acids including glutamate [2–4]. In addition to expression in taste buds, the T1R family is also expressed in the small intestine and colon [5–8]. T1R1 and T1R3 receptors are expressed in duodenal cells are possibly involved in sensing amino acids [5]. Furthermore, Taniguchi showed that T1R3 was expressed in the intrahepatic bile duct and pancreas using immunohistochemistry [8]. Thus, the T1R family is expressed in various parts of the digestive system and it is possible that these receptors may have a role in chemical sensation in these tissues. However, expression patterns of T1R1 and T1R2 in the intrahepatic bile duct have not been determined. In addition, although T1R3 is widely expressed in various parts of the digestive system, the mechanisms involved in transcriptional regulation of the T1R3 gene (*Tas1r3*) have not been elucidated.

In this study, we examined the expression pattern of the T1R family in human liver using reverse-transcription polymerase chain reaction (RT-PCR) and immunohistochemistry. We also examined the molecular mechanisms regulating the human *Tas1r3* promoter using luciferase reporter assay and electrophoretic mobility shift assay (EMSA) in a human intrahepatic bile duct carcinoma cell line, HuCCT1 [9].

2. Materials and methods

2.1. Cell Culture

HuCCT1 cells (JCRB0425; The Japan Health Sciences Foundation, Osaka, Japan) were grown in RPMI1640 (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum at 37 °C with 5% CO_2 [9].

* Corresponding author. Tel.: +81 93 582 1131x7933; fax: +81 93 582 6089.
E-mail address: toyono@kyu-dent.ac.jp (T. Toyono).

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2.2. RT-PCR

Total RNA was prepared from HuCCT1 cells using RNeasy Protect Mini (QIAGEN, Valencia, CA, USA) and incubated with DNase I to remove any contaminating genomic DNA. First-strand cDNA syntheses were performed by reverse transcription of human liver total RNA (Biochain, Hayward, CA, USA) and HuCCT1 total RNA using an Omniscript™ Reverse Transcriptase (QIAGEN). PCR amplifications were performed under the following conditions using the primers listed in Table 1: 94 °C for 30 s, 54 °C for 1 min, 72 °C for 1 min for a total of 40 cycles and an elongation step at 72 °C for 10 min for GAPDH, 94 °C for 30 s, 60 °C for 1 min, 72 °C for 30 s for a total of 40 cycles and an elongation step at 72 °C for 10 min for T1R1, 94 °C for 30 s, 57 °C for 1 min, 72 °C for 30 s for a total of 40 cycles and an elongation step at 72 °C for 10 min for T1R2, 95 °C for 15 s, 68 °C for 1 min for a total of 40 cycles for T1R3, 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min for a total of 30 cycles for C/EBPα, C/EBPβ, and C/EBPδ. The reverse transcriptase step was omitted in controls to confirm removal of all genomic DNA. All PCR products were subcloned and sequenced.

2.3. Immunohistochemistry

Paraffin sections of human liver (Super Bio Chips, Seoul, Korea) were processed using the Tyramide Signal Amplification (TSA) method. The TSA method was performed with an antibody to T1R2 (1:300 dilution) (LS-A5073) or T1R3 (1:300 dilution) (LS-A5073) (LifeSpan Biosciences, Seattle, WA, USA), as a primary antibody using TSA™ Kit with HRP–goat anti-rabbit IgG and Alexa Fluor488 tyramide (green; Invitrogen). The specificity of T1R2 or T1R3 immunoreactivity was determined by substitution of phosphate-buffered saline (PBS) for the primary antibody. Nuclei were stained with DAPI (Invitrogen) (blue).

Indirect single-immunofluorescence staining was performed on HuCCT1 cells plated onto culture slides using an anti-CCAAT/enhancer-binding protein β (C/EBPβ) antibody (SC-7962) (1:500 dilution) or an anti-C/EBPδ antibody (SC-636) (1:1000 dilution) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) as the primary antibody and Alexa™ 488-conjugated goat anti-mouse IgG (1:400 dilution; Invitrogen) or Alexa™ 488-conjugated goat anti-rabbit IgG (1:400 dilution; Invitrogen) as the secondary antibody, respectively. The specificities of C/EBPβ and C/EBPδ immunoreactivities were determined by substitution of PBS for the primary antibody.

2.4. Rapid amplification of 5′ cDNA ends

Poly(A)$^+$-RNA was prepared from the total RNA of HuCCT1 cells using a GenElute™ mRNA Miniprep Kit (Sigma-Aldrich). Two micrograms of poly (A)$^+$-RNA from HuCCT1 cells was reverse transcribed and amplified using the SMART RACE cDNA Amplification Kit (Takara bio, Otsu, Japan) with the adaptor primer and gene-specific primer (Table 1). The PCR products were subcloned and sequenced.

2.5. Construction of plasmid

The 5′-flanking regions of human *Tas1r3* from −1394 bp to +67 bp (positions based on the nucleotide number shown in Fig. 2) were amplified by PCR using the human *Tas1r3* BAC genomic DNA (RZPDB737E0229D; RZPD, Berlin, Germany). PCR was performed using the primers (Table 1). The amplified fragments were gel purified, inserted into pGEM-T easy vector (Promega, Madison, WI, USA), digested with *Eco*RI, and inserted into *Eco*RI-linearized pBluescript II SK(−). The plasmids were then digested with *Kpn*I/*Sac*I, and inserted into *Kpn*I/*Sac*I-linearized pGL4.10 to form pGL-1394/+67, pGL-896/+67, and pGL-465/+67. The resulting plasmids were analysed by DNA sequencing to ensure the fidelity of amplification and the correct orientation.

To construct pGL-310/+67, pGL-465/+67 was digested with *Kpn*I/*Bsa*I, and was blunted with the Blunting high (Toyobo, Osaka, Japan), then self-ligated and transformed. To construct pGL-59/+67, pGL-465/+67 was digested with *Kpn*I/*Van*91I, and was blunted, then self-ligated and transformed. The construction of pGL-159/+67 was as follows: the plasmid was constructed by inserting the 5′-flanking region of human *Tas1r3* from −456 bp to +67 bp into pBluescript II SK(−), digested with *Hinc*II, then self-ligated and transformed.

Table 1
Primers used for RT-PCR, 5′-RACE, construction of luciferase assay plasmids, and site-directed mutagenesis

Project/Primer name	Sequence (5′–3′)	PCR product size (bp)
RT-PCR/		
T1R1-F	CTACCACCTCTTCCAGGCTA	428
T1R1-R	CAACCAGAGAGATCCAGGTC	
T1R2-F	CAGAACATGACGTCAGAGGA	442
T1R2-R	CATAGACCGCAGAGTACACG	
T1R3-F	CGTGAGCGCAGGGCTAAA	100
T1R3-R	TGAGGC GTTGCA CTGAAGAG	
C/EBPα-F	GAACACGAAGCACGATCAG	268
C/EBPα-R	CCAAAACCAAAA GGAAAGGGAG	
C/EBPβ-F	TGATAAACTCTCTGCTCTCCC	191
C/EBPβ-R	AAACATCAACAG CAACAAGCC	
C/EBPδ-F	AACGACCCATACCTCAGAC	240
C/EBPδ-R	ACAAATGTACCTTAG CTGCATC	
GAPDH-F	TGAAGGTCGGAGTCAACGGA	983
GAPDH-R	CATGTGGGCCATGAGGTCCA	
5′-RACE/		
T1R3-specific primer	GCTGACCTGGGGCATGAGGAAGAAG	
Luciferase assay plasmid/		
pGL-1394/+67-F	GGGCACACCTCCTGGTATCT	1461
pGL-1394/+67-R	GGCAGAGGCAACTTCCAACA	
pGL-896/+67-F	ACTGGTCTGCAGGCCTAGGG	963
pGL-896/+67-R	GGCAGAGGCAACTTCCAACA	
pGL-465/+67-F	GCGTGTGCCACACGTCGTTT	532
pGL-465/+67-R	GGCAGAGGCAACTTCCAACA	
Site-directed mutagenesis/		
T1R3-C/EBP mut.-F	CTGGGGCCCCAGGGTGCCACAAGTGAGGATGGCAA	
T1R3-C/EBP mut.-R	TTGCCATCCTCACTTGTGGCACCCTGGGGCCCCAG	