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TAS2R expression profile in brown adipose, white adipose, skeletal muscle, small intestine, liver and common cell lines derived from mice

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1. Introduction

Taste 2 receptor (TAS2R) describes a family of one type of G-protein coupled receptor that perceives bitter taste in the oral cavity. Bitter compounds that bind to TAS2Rs expressed on gustatory cells activate these receptors, and the co-expressed G-proteins subsequently send the signal through downstream effectors. This signaling leads to the release of calcium ions from the endoplasmic reticulum to the cytosol, resulting in sodium ion influx through transient receptor potential cation channel subfamily M member 5 (TRPM5). The influx depolarizes the gustatory cells and causes the release of ATP, which transmits the bitter taste signal to the central nervous system (Roper, 2013; Travedi, 2012).

Currently, 25 TAS2Rs have been identified in humans (Shi, Zhang, Yang, & Zhang, 2003) and 35 in mice (Chandrashekar, Hoon, Ryba, & Zuker, 2006). Each TAS2R recognizes a range of compounds with different specificity, enabling the detection of a wide range of bitter compounds (Lossow et al., 2016; Meyerhof et al., 2009). For example, human TAS2R (hTAS2R)10 recognizes at least 20 bitter compounds including caffeine and quinine.

Over the last decade, much attention has been paid to the identification of TAS2R expression and function in a wide variety of extraoral tissues: brain, upper airway, heart, liver, testis (Freund & Lee, 2018); adipose tissue (Amisten et al., 2015); stomach (Liszt et al., 2017); small intestine (Xie et al., 2018); and colon (Rozengurt et al., 2006). TAS2Rs are recognized as the receptor associated with repelling, excreting and decomposing of toxins. Moreover, TAS2Rs expressed in the bordering tissues that separate the internal and external environments of the body have been shown to possess analogical functions. For example, TAS2R38 expressed in the upper airways detects acyl-homoserine lactone secreted from Gram-negative bacteria. This detection induces calcium-dependent nitric oxide production, resulting in the stimulation of mucociliary clearance function and has direct antibacterial effects (Lee et al., 2012). TAS2R43 expressed in the stomach promotes the secretion of gastric acid in response to caffeine, which presumably enhances the decomposition of toxins (Liszt et al., 2017). Furthermore, Deshpande et al. reported

that inhaled bitter tastants improved airway obstruction in mouse model of asthma (Deshpande et al. 2010). Since several members of TAS2Rs (TAS2R105, 107, 119, 121, 123, 126 and 134) are expressed in rodent airway (Tizzano, Cristofoletti, Sbarbati, & Finger, 2011), these TAS2Rs are presumed to be involved in the improvement of asthma. In contrast, TAS2Rs expressed in internal tissues are unlikely to function to repel and exclude toxic compounds; rather, these TAS2Rs would be expected to respond to bitter compounds that have already been ingested. Expression of TAS2Rs in internal tissues may have a different physiological function entirely.

Several reports suggest a role for TAS2R in metabolic functioning (Avau et al., 2015). The association of genetic variations of TAS2R38 with obesity development was implied in a cohort study (Ortega et al., 2016), and a TAS2R haplotype was shown to influence glucose homeostasis, linking it with a diagnosis of type 2 diabetes (Dotson et al., 2008). In addition, quinine, a well-known bitter compound, promotes the adipogenesis of 3T3-L1 adipocyte cells via TAS2R (Ning, He, Shi, & Yang, 2016).

On the basis of the above facts, we hypothesized that extraoral TAS2Rs may be involved in the regulation of nutrient metabolism. And as an entryway to this research, we analyzed the expression profiles of multiple TAS2R genes in extraoral tissues that are related to the metabolism of nutrients. The identification of TAS2R expression in extraoral tissues such as adipose tissue and skeletal muscle will enable the exploration of the relationship between metabolic regulation and TAS2R using specific ligands.

In this study, the gene expression profile of TAS2R in five extraoral tissues (brown adipose, white adipose, skeletal muscle, small intestine and liver) was analyzed. Analysis of guanine nucleotide-binding protein alpha subunit (Gnat) was accompanied since this protein plays an important role in bitter taste transduction. In anticipation of future in vivo experiments, we chose to use mouse tissues for this study. Furthermore, if the same TAS2Rs as mice tissues were expressed in corresponding cell lines, the cell lines would be a useful model for exploring the function of TAS2Rs. Thus, gene expression profiles of TAS2R in mouse 3T3-L1 cells and

C2C12 cells, commonly used models of adipocytes and myocytes, respectively, were analyzed to facilitate future studies on the relationship between TAS2R and nutrient metabolism.

2. Materials and Methods

2.1 Cell culture

The 3T3-L1 (JCRB9014) and C2C12 (RCB0987) cell lines were obtained from the JCRB Cell Bank (Tokyo, Japan) and RIKEN BRC (Saitama, Japan), respectively. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) at 37°C in a humidified atmosphere of 10% CO_2 until reaching an adequate confluency.

2.1.1 Differentiation of 3T3-L1 cells

Adipocyte differentiation was induced 1 day after 3T3-L1 cells reached confluence (day 0) by changing the medium to 10% FBS/DMEM supplemented with 0.5 mM 3-isobutyl-1-methylxanthine, 0.25 μM dexamethasone, and 5 μg/mL insulin (differentiation medium). To enhance the differentiation, 2 days and 4 days after induction (day 2, day 4), the medium was changed to 10% FBS/DMEM supplemented with 10 μg/mL insulin (insulin medium). At day 6 and day 8, the medium was changed to 10% FBS/DMEM. Total RNA was extracted on day 0 and day 10.

2.1.2 Differentiation of C2C12 cells

Myotube differentiation was induced 1 day before C2C12 cells reached confluence (day 0) by changing the medium to 2% horse serum (HS)/DMEM. The medium was changed to 2% HS/DMEM every 2 days until sufficient differentiation was reached. Total RNA was extracted on day 0 and day 8.

2.2 RNA preparation

Male C57BL/6 mice (age 8 weeks) total RNA (three independent samples, each from

individual mice) derived from brown adipose, white adipose, muscle, small intestine and liver, was purchased from Genostaff Co. (Tokyo, Japan). For cell lines, the total RNA was extracted using the ReliaPrep™ RNA Cell Miniprep System (Promega KK, Tokyo) followed by the DNase treatment using AccuRT Genomic DNA Removal Kit (Applied Biological Materials Inc.) according to the manufacturer's protocol. The quantity of total RNA was determined spectrophotometrically. The RNA integrity was evaluated qualitatively from the intensities of the 28S and 18S ribosomal RNA bands following denaturing agarose gel electrophoresis.

2.3 Reverse transcription PCR

Reverse transcription was performed using ReverTra Ace qPCR RT Master Mix with gDNA Remover (Toyobo, Osaka, Japan) according to the manufacturer's instructions. No reverse transcription control experiments were also performed. PCR was carried out in 0.2-mL PCR tubes in a total volume of 25 μL containing 2 μM primer, 2 μL cDNA, and 12.5 μL Go Taq G2 Hot Start Polymerase Green Mastermix (Promega KK, Tokyo). Primers were designed using the National Center for Biotechnology Information primer designing tool Primer BLAST and are presented in Supplemental Table S1. Cycling conditions were as follows: 95°C for 2 min, followed by 35 cycles of 95°C for 30 s, 60°C for 30 s (or 65°C for 30 s in the case of TAS2R102, 109, 118, 138, 140 and 143), and 72°C for 60 s for TAS2R genes, or 30 cycles of 95°C for 30 s, 60°C for 30 s and 72°C for 60 s for β-actin, Gnat1, Gnat2 and Gnat3; each was followed by a final elongation step at 72°C for 5 min. β-actin expression was used as an internal control.

2.4 Electrophoresis

PCR products were examined by standard agarose gel electrophoresis (2% agarose, 40 mM Tris-acetate and 1 mM EDTA) with visualization of DNA by fluorescent dye (GelGreen™ Nucleic acid gel stain, Biotium Inc. or Midori Green Xtra, NIPPON Genetics Co.) Each DNA band was converted into numerical form and quantified by ImageJ software and rated as follows.

120 ○：Band intensity>0.1, ◦： Band intensity between 0.1-0.01 (The band intensity of β-actin=1).
121 Gene expression analysis was repeated at least twice to confirm the replicability. The
122 representative results are shown in the Table 1 and Supplementary figures S1-S7.
123
124 **3. Results**
125 3.1 TAS2R expression in extraoral tissues
126 The gene expression profiles of TAS2Rs in extraoral tissues were investigated by
127 reverse transcription PCR using total RNA isolated from brown adipose, white adipose, skeletal
128 muscle, small intestine and liver (Table 1). Three individually prepared samples of each tissue
129 were amplified, and the products visualized following agarose gel electrophoresis. The expression
130 of each TAS2R gene was compared with the band intensity for β-actin and rated (blank, small
131 circle, or large circle which is from a single representative sample (n=1)).
132 Individual TAS2R expression was detected in at least 2 out of 3 samples of each tissue
133 as follows: 9 members of TAS2R in brown adipose (mTAS2R108, 110, 118, 126, 134, 135, 137,
134 140, 143), 11 members in white adipose (mTAS2R108, 113, 118, 119, 126, 135, 137, 138, 140,
135 143, 144), 8 members in skeletal muscle (mTAS2R108, 126, 134, 135, 137, 140, 143, 144), 10
136 members in small intestine (mTAS2R108, 109, 119, 126, 135, 137, 138, 140, 143, 144), and 8
137 members in liver (mTAS2R108, 109, 126, 130, 135, 137, 138, 143). Similarly, Gnat1 was detected
138 in white adipose, small intestine and liver, Gnat2 was detected in all tissues we analyzed, Gnat3
139 was detected in small intestine.
140
141 3.2 TAS2R expression in cell lines
142 Adipose tissue and skeletal muscle expressed a variety of TAS2Rs. To begin exploring
143 the possible functions of TAS2R expression in these tissue types, we chose the convenience of a
144 cell line-based approach. It is possible that cell lines and tissue-derived cells express different

members of TAS2R. To minimize this concern, the expression of TAS2R was investigated in cell lines corresponding to mice adipocytes and myocytes.

Table 1. Gene expression of TAS2Rs in five extraoral tissues of mice and two common cell lines derived from mice, before and after differentiation.

	Mice extraoral tissues															Cell lines			
	Brown Adipose			White Adipose			Skeletal Muscle			Small Intestine			Liver			3T3-L1		C2C12	
Genes	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	BD	AD	BD	AD
Gnat1	○			○	◦	◦				○	◦	◦	○	○	○				
Gnat2	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	◦	◦
Gnat3										○	○	○							
mTAS2R102		◦			◦				○										
mTAS2R103	○													◦					
mTAS2R104						○			◦										
mTAS2R105						○							○						
mTAS2R106			◦					◦											
mTAS2R108	○	○	○		○	○	○	○	○	◦	○	○	◦	○	○	○	○	○	○
mTAS2R109		○			○			○			○	◦		○	○				
mTAS2R110	◦		◦			◦													
mTAS2R113	◦			◦	○	○			◦						◦				
mTAS2R115								◦						◦					
mTAS2R116				◦															
mTAS2R118	◦	○		○	○	◦	◦			◦									
mTAS2R119	◦			◦		◦	◦			◦		◦							
mTAS2R120															◦				
mTAS2R121																◦			
mTAS2R122						◦					○				◦				
mTAS2R123						◦	◦												
mTAS2R125			◦		◦														
mTAS2R126	○	○	○	○	○	○	○	○	○	○	○	○	◦	○	○	○	◦	○	○
mTAS2R130													◦	◦	◦				
mTAS2R134	○	○	○		◦		◦	○	○										
mTAS2R135	○	○	○	○	○	○	○	○	○	○	○	○		○	○	○	○	○	○
mTAS2R137	○	○	○	○	○	○	○	○	○	○	○	○		◦	◦	○	○	◦	○
mTAS2R138				○	○	◦				○	○	○	○	○	○				
mTAS2R139				○													◦		
mTAS2R140	◦	○	○	◦	○	○		○	◦		○	○		◦					
mTAS2R143	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
mTAS2R144			○	○		○		○	○		◦	◦							

BD: Before differentiation; AD: After differentiation; ○：Band intensity>0.1, Band intensity between 0.1-0.01 (The band intensity of β-actin = 1). TAS2R107, 114,117, 124, 129, 131 and 136 were not detected. Each circle is from a single representative sample (n=1).

The 3T3-L1 and C2C12 cell lines are widely used as models of white adipose cells and skeletal muscle cells, respectively. Since these cell lines can be stimulated to differentiate under established conditions, we examined the mRNA expression of TAS2R both before and after the

differentiation (Table 1). In 3T3-L1 cells, mTAS2R108, 121, 126, 135, 137, 143 were found to be expressed in undifferentiated state, mTAS2R108, 126, 135, 137, 139, and 143 were expressed in differentiated state. In C2C12 cells, mTAS2R108, 126, 135, 137 and 143 were expressed under both states. Gnat2 was detected under both states in 3T3-L1 and C2C12 cells, but Gnat1 and Gnat3 were not observed in any conditions.

4. Discussion

4.1 Comparison of extraoral TAS2R expression with previous studies

TAS2R expression was previously reported by Prandi et al. in mouse small intestine and liver (2018). In their report, small intestine expressed TAS2R108, 119, 126, 129, 135, 137, 138 and 143, while liver expressed TAS2R108, 126, 135, 137, 138 and 143. In contrast, in our study of small intestine, we did not detect the expression of TAS2R129, but did detect the expression of three additional TAS2R genes: 109, 140 and 144. Moreover, in the liver, we detected additional expression of TAS2R109 and 130. In addition, according to Mouse Gene Expression Database (MXD), liver, duodenum, ileum and jejunum expressed TAS2R108 and 138, jejunum expressed TAS2R119, small intestine epithelium expressed Gnat3, corresponded with the expression profile in this study. In contrast, we did not detect the expression of TAS2R118, which is confirmed the expression in the database. These differences might be explained by the mouse strain (C57BL/6 in this study and C57BL/6J in Prandi et al.), as there are different perceptions of bitter taste between individuals (Wooding, 2006). In addition, TAS2R expression is known to vary in response to environmental factors such as secretions from parasites (Luo et al., 2019) and smoking habit (Aoki, Takao, Takao, Koike, & Suganuma, 2014). Thus, the mouse strain and/or the environmental conditions for breeding mice may have influenced these differences in TAS2R expression profiles. Also, TAS2R might be transiently expressed due to these environmental factors. However, our results do not address expressional variability, thus it is still unclear how representative these data are for any related model. Since we have not confirmed the existence of

TAS2R proteins, all these points require investigation in future research.

This study is the first to report on TAS2R expression in mouse white and brown adipose tissue and skeletal muscle. TAS2R expression patterns in human adipose and airway smooth muscle have been previously reported. Amisten et al. showed that TAS2R3, 7, 14, 19, 20, 31, 43, 45 and 46 were expressed in human adipose tissue (2015), while Deshpande et al. showed that TAS2R1, 3, 4, 5, 8, 9, 10, 13, 14, 19, 20, 30, 31, 42, 45, 46 and 50 were expressed in human smooth muscle (2010). The data from Genotype-Tissue Expression (GTEx) also shows the expression of TAS2Rs in these tissues (hTAS2R3,4,5,10,13,14,40,42,43,46,50,60 in human adipose tissue, hTAS2R3,4,5,10,13,14,43,46,50 in human skeletal muscle). However, it is difficult to directly compare these expression profiles given the differences in human and mouse TAS2Rs.

mTAS2R108, 126, 135, 137, 143, which we confirmed their expression in tissues and cell lines, have corresponding orthologue in hTAS2R except for mTAS2R143 (Lossow et al., 2016). TAS2R108, 126, 135, 137 are the orthologue of hTAS2R4, 41, 60, 3. The ligands for each orthologue pairs have been previously examined, but some of the pairs have no common ligand. For example, mTAS2R108/hTAS2R4 shares 30% of the tested bitter compounds as a common ligand, and the pairs of mTAS2R126/hTAS2R41 and mTAS2R137/hTAS2R3 do not share a ligand. Thus, the orthologue of human and mouse TAS2R do not recognize same ligand making it difficult to compare.

Although difficult to compare, the common expression of TAS2Rs in adipose and muscle of both species suggests the existence of an unknown role of this receptor in those tissues. It is also noteworthy that Gnat2, one of the downstream signaling proteins of TAS2R(Liszt et al., 2017), is expressed in adipose, skeletal muscle and their corresponding cell lines, which may support that TAS2Rs function in these tissues and cells.

208 4.2 Speculation of the TAS2R function in liver, muscle and adipose tissues

209 A glimpse of the possible receptor functions of TAS2Rs in liver, muscle and adipose
210 tissues is given by the biological effects of their respective ligands. Several ligands have been
211 identified for receptors TAS2R108, 126, 135, 137 and 143, which are commonly expressed in
212 liver, muscle and adipose tissues (Lossow et al., 2016). Among these ligands, the effects of
213 quinine (ligand for TAS2R108, 126, 137), denatonium benzoate (TAS2R135), allyl isothiocyanate
214 (TAS2R135), salicylic acid (TAS2R135), and epicatechin (TAS2R126) may offer clues as to the
215 functions of TAS2Rs in these tissues. Oral administration of quinine is reported to decrease body
216 weight without a change in energy intake in obese mice (Avau et al., 2015). In the same report,
217 denatonium benzoate also reduced body weight but was accompanied by decreased energy intake.
218 Allyl isothiocyanate, the organosulfur compound in radish and wasabi, increased glucose uptake
219 in insulin-resistant C2C12 myotubes and reduced diet-induced obesity in mice fed a high-fat diet
220 (Ahn, Lee, Im, Jung, & Ha, 2014). Salicylic acid, an active metabolite of aspirin with bitter taste,
221 is known to lower blood glucose levels in diabetic patients (Rumore & Kim, 2010). Epicatechin,
222 the flavonoid contained in green tea and cocoa, reduces body weight gain and improves insulin
223 sensitivity in mice fed a high-fat diet (Cremonini, Bettaieb, Haj, Fraga, & Oteiza, 2016; Hoek-
224 van den Hil et al., 2015). Additionally, epigallocatechin 3-*O*-gallate, the major flavonoid in green
225 tea, activated TAS2R144 expressed in white adipose tissue and skeletal muscle, and has been
226 widely explored for its anti-obesity or anti-diabetes effects (Eng, Thanikachalam, & Ramamurthy,
227 2018; Suzuki, Pervin, Goto, Isemura, & Nakamura, 2016). All of these effects are related to
228 metabolic function. Although some of the molecular mechanisms for these metabolic effects have
229 been revealed, the direct target of the above bitter compounds is often unclear. Therefore, as
230 hypothesized earlier, the expression of TAS2R in metabolically important tissues including the
231 liver, skeletal muscle and adipose tissues, may imply a connection between TAS2R and
232 metabolism. It is also noteworthy that human adipose tissue is known to express several members
233 of TAS2R that recognize quinine (TAS2R7, 14, 43 and 46) or denatonium benzoate (TAS2R43

and 46) as a ligand, implying that human TAS2Rs may possess metabolic functions.

4.3 Cell lines as a model to study TAS2R functions

Comparing the expression profile of TAS2R in tissues and cell lines, Gnat2, mTAS2R108, 126, 135, 137, and 143 were expressed at relatively higher levels in both tissues and cell lines. Whereas, in 3T3-L1 cells, we did not detect Gnat1, mTAS2R113, 118, 119, 138, 140 and 144, which are expressed in white adipose tissues. In C2C12 cells, we did not detect mTAS2R134, 140 and 144, which are expressed in skeletal muscle tissues.

Environmental factors might have influenced this result, similarly to that described for the difference between the Prandi et al. (2018) report and our study regarding TAS2R expression in small intestine and liver tissue. Cell lines are cultured in media that are largely different from the conditions of the mouse tissues showing differential expression of TAS2Rs. Another consideration is the result of immortalization. Martin et al. reported that TAS2Rs are expressed in human epithelial ovarian and prostate cancer cells, influencing cell survival (2019). Genetic variations in TAS2R3 and 4 have been reported to modify papillary carcinoma risk (Choi et al., 2018). Abnormal cell growth is a characteristic of cancer cells; thus TAS2R expression might have been influenced during the construction of these cell lines.

Although the profiles and levels of TAS2R gene expression in cell lines and mouse tissues are somewhat different, 3T3-L1 and C2C12 cells should be a useful tool to explore the functions of TAS2Rs.

5. Conclusions

We have investigated the expression profile of TAS2R in multiple extraoral tissues that are closely related to metabolism, namely brown and white adipose, skeletal muscle, small intestine and liver. Several Tas2Rs are expressed in these five metabolically meaningful tissues and previous studies imply that TAS2Rs might possess functions to regulate metabolism.

However, the connection of TAS2Rs to metabolism is still unclear and further considerations are required to clarify the involvement of TAS2Rs in metabolism. Additionally, we discovered that 8 members of TAS2Rs express in 3T3-L1 and 6 members of TAS2Rs express in C2C12 cells which expressions are observed in corresponding tissues. The TAS2R expression profile in these cell lines support their use as model cells for exploring the function of TAS2R expression in white adipose and skeletal muscle.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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