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Olfactory receptor Olfr544 responding to azelaic acid regulates glucagon secretion in α-cells of mouse pancreatic islets

NaNa Kang [a, 1], Young Yil Bahk [b, 1], NaHye Lee [a], YoonGyu Jae [a], Yoon Hee Cho [c], Cheol Ryong Ku [c], Youngjoo Byun [d], Eun Jig Lee [c], Min-Soo Kim [e, **], JaeHyung Koo [a, *]

[a] Department of Brain Science, Daegu Gyeongbuk Institute of Science and Technology DGIST, Daegu 711-873, South Korea
[b] Department of Biotechnology, Konkuk University, Chungju 380-701, South Korea
[c] College of Medicine, Yonsei University, Seoul 120-752, South Korea
[d] College of Pharmacy, Korea University, 2511 Sejong-ro, Sejong 339-700, South Korea
[e] Department of Information and Communication Engineering, DGIST, Daegu 711-873, South Korea

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ABSTRACT

Olfactory receptors (ORs) are extensively expressed in olfactory as well as non-olfactory tissues. Although many OR transcripts are expressed in non-olfactory tissues, only a few studies demonstrate the functional role of ORs. Here, we verified that mouse pancreatic α-cells express potential OR-mediated downstream effectors. Moreover, high levels of mRNA for the olfactory receptors Olfr543, Olfr544, Olfr545, and Olfr1349 were expressed in α-cells as assessed using RNA-sequencing, microarray, and quantitative real-time RT-PCR analyses. Treatment with dicarboxylic acids (azelaic acid and sebacic acid) increased intracellular Ca^{2+} mobilization in pancreatic α-cells. The azelaic acid-induced Ca^{2+} response as well as glucagon secretion was concentration- and time-dependent manner. Olfr544 was expressed in α-cells, and the EC_{50} value of azelaic acid to Olfr544 was 19.97 μM, whereas Olfr545 did not respond to azelaic acid. Our findings demonstrate that Olfr544 responds to azelaic acid to regulate glucagon secretion through Ca^{2+} mobilization in α-cells of the mouse pancreatic islets, suggesting that Olfr544 may be an important therapeutic target for metabolic diseases.

1. Introduction

Olfactory receptors (ORs) are extensively expressed in olfactory as well as non-olfactory tissues [1,2]. The expression and functional information and the related nomenclature of non-olfactory ORs are summarized in our previous publication [1]. ORs on the cilia of the olfactory receptor neurons in the olfactory epithelium recognize volatile odorants and send a chemical signal as an action potential to the brain, resulting in the perception of smells. However, ORs are also involved in cell movement, such as that of sperm [3,4], muscle cell adhesions [5], cytokinesis [6], and another group of non-olfactory ORs are involved in hormone secretion in the gut [7] and the renal juxtaglomerular apparatus [8]. Recently, ORs were reported to play unique functional roles in brain [9,10], skin [11], and prostate cancer cells [12,13]. However, the function of ORs in non-olfactory tissues remains largely elusive.

Microarray and RNA-sequencing (RNA-seq) analyses demonstrate a robust expression of OR profiles in non-olfactory tissues [14,15]. Previous work in our laboratory noted that specific cell types in various non-olfactory tissues express ORs and their potential downstream effectors and suggested that many ORs are expressed in the neuroendocrine system [16]. The function of these ORs in the neuroendocrine system is unknown, but they may play roles in particular signaling events. The pancreas as an endocrine gland secretes hormones into the bloodstream. Although there are several examples of OR expression in non-olfactory tissues, progress in understanding their physiological functions has been slow and there are no reports on the role of the ORs expressed in the pancreas.

Therefore, in the present study, we investigated whether mouse pancreatic α-cells expressed ORs using RNA-seq and quantitative RT-PCR analyses. After we found several ORs expressed, we determined whether the OR Olfr544 stimulated by azelaic acid (AzA) resulted in the control of glucagon secretion.

* Corresponding author. Fax: +82 53 785 6109
** Corresponding author. Fax: +82 53 785 6309.
E-mail addresses: mskim@dgist.ac.kr (M.-S. Kim), jkoo001@dgist.ac.kr (J. Koo).
[1] These authors contributed equally.

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2. Materials and methods

2.1. Cell culture

The glucagon-releasing αTC1-9 cells were purchased from the American Type Cultures Collection. They were maintained in Dulbecco's Modified Eagle's medium (DMEM, Gibco, Grand Island, NY) supplemented with 2 g/L glucose, 1.5 g/L sodium bicarbonate, 10% FBS (HyClone, Logan, UT), 15 mM HEPES, 0.1 mM nonessential amino acids and 0.02% BSA.

2.2. Immunohistochemistry and immunofluorescence staining

All animal procedures were approved by the Daegu Gyeongbuk Institute of Science and Technology's Institutional Animal Care and Use Committee. Immunohistochemistry and immunofluorescence staining were performed according to a previous report [17]. The following antibodies were used: goat anti-olfactory marker protein (OMP, a gift from Frank Margolis at the University of Maryland, Baltimore, MD) along with rabbit anti-glucagon (Millipore, Temecula, CA), rabbit anti-somatostatin (Millipore), or rabbit anti-insulin (Millipore) antibodies. Goat anti-OMP antibodies were applied for double-immunostaining along with rabbit anti-glucagon, rabbit anti-type III adenylyl cyclase (ACIII, Santa Cruz Biotechnology, Santa Cruz, CA.), rabbit anti-olfactory G protein (G_{olf}, Santa Cruz Biotechnology), or rabbit anti-Olfr544 (Abcam, Cambridge, England) antibodies in αTC1-9 cells.

2.3. RNA isolation, RNA-seq, and gene array analyses

To identify ORs expressed in α-cells by RNA-seq and microarray analyses, total RNA and a sequencing library were prepared using a previously described method [18]. For the gene array, total RNA was labeled and amplified using a Low Input Quick Amp labeling kit (Agilent Technologies, Santa Clara, CA). After labeling, the RNA was used with Agilent's SurePrint Mouse Genome 4 × 44K v2 Microarray kit (Agilent Technologies) and analyzed using an Agilent scanner with its associated software.

2.4. RT-PCR and quantitative real-time RT-PCR

Conventional RT-PCR and real-time RT-qPCR were performed with primer sets (Table S1) according to a previous report [18]. The ORs presenting a clear band were used for real-time qRT-PCR and their relative expression values were calculated using a standard concentration curve generated with the pCI-Rho–*olfr544* construct.

2.5. Intracellular Ca^{2+} mobilization measurement

For initial screening of candidate odorants responding in αTC1-9 cells, the concentration of intracellular calcium ($[Ca^{2+}]_i$) in αTC1-9 cells (5×10^5 cells) was determined with the Grynkiewicz method using Fura-2/AM [18]. The various amounts of odorants were tested for Ca^{2+} mobilization. The changes in fluorescence at the dual excitation wavelengths of 340 nm and 380 nm and the emission wavelength of 500 nm were measured using a spectrofluorometer.

2.6. Luciferase reporter assay

The Dual-Glo Luciferase Assay System (Promega, Madison, WI) was used for the luciferase reporter assay. For pairing of Olfr544 or Olfr545 with AzA, CRE-Luc (Stratagene, La Jolla, CA) was used to measure receptor activation using the method described previously [19]. AzA and sebacic acid (SeA) were purchased from Sigma–Aldrich.

2.7. Glucagon measurements

The levels of glucagon secreted from αTC1-9 cells were monitored with a DuoSet ELISA Development System according to the manufacturer's protocol (R&D systems, Minneapolis, MN). The αTC1-9 cells were cultured for 2 days in DMEM. The cells were then incubated at 37 °C in 0.5 mL modified Krebs–Ringer bicarbonate HEPES (mKRBH) buffer (130 mM NaCl, 3.6 mM KCl, 1.5 mM $CaCl_2$, 0.5 mM $MgSO_4$, 0.5 mM KH_2PO_4, 2 mM $NaHCO_3$, 10 mM HEPES, 0.1% BSA and 10 mM glucose, pH 7.4) for 2 h. Next, the cells were incubated for 30 min in 400 μL of mKRBH buffer (without glucose) with the samples in a 24-well plate. At the end of the incubation, 350 μL of medium was collected from each well for measurement of the secreted glucagon.

2.8. Small interfering RNA (siRNA) treatment and western blot analysis

To test whether the effects of Olfr544 activation on intracellular Ca^{2+} mobilization and glucagon secretion could be inhibited, RNA interference was performed according to the method provided in a previous report [20]. Briefly, αTC1-9 cells grown in 6-well plates were transfected with an appropriate amount of olfr544 siRNA (#5: GCUCAGAUGUGAUCCGCCA; #10: GAGCAAUGGCACCUACAAU) or non-targeting siRNA (UGGUUUACAUGUCGACUAA, Thermo Fisher Scientific, Waltham, MA) using Lipofectamine 2000, twice sequentially (separated by 24 h). Three days after the transfection, western blot analysis and intercellular Ca^{2+} concentration and glucagon measurements were conducted using standard procedures [18].

2.9. Data analysis

Data are shown as mean ± SEM. Student's *t*-test or one-way ANOVA with Dunnett's multiple comparisons test were performed using Microsoft Excel or GraphPad Prism software (GraphPad Software, La Jolla, CA).

3. Results and discussion

3.1. Expression of olfactory signaling downstream effectors in mouse pancreatic α-cells

Many ORs have been identified in non-olfactory systems since OR genes were found in the testis and sperm [1]. Recently, microarray and RNA-seq analyses showed that the principal olfactory signaling components OR, G_{olf}, ACIII, and OMP are also expressed in many non-olfactory tissues [14,15]. Previous work in our laboratory suggested that OMP expression is an indicator of potential OR-associated events in non-olfactory systems [16]. To determine whether ORs and OR-mediated chemoreception existed and functioned, respectively, in the pancreas, we verified the existence of ORs in the islets of Langerhans. In the present study, we first assessed the expression of OMP in the mouse pancreas. We found that OMP was significantly detected in pancreatic tissue (Fig. 1). This staining was most likely specific because blocking the antibody with the recombinant OMP protein completely abrogated the immunopositivity (data not shown). To determine the identity of the cells expressing OMP, double immunofluorescence for OMP with glucagon, insulin, or somatostatin was performed. OMP co-localized with the glucagon-producing α-cells, but not with insulin or somatostatin (Fig. 1 left panel). These results demonstrate that OMP expression is limited to a subset of cells within the islet, mostly likely α-cells, a result supported by the quantitative analysis (Fig. S1). The islets of Langerhans, the main endocrine unit controlling glucose homeostasis, are composed of clusters of four cell