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Short communication

Activation of OR1A1 suppresses PPAR-γ expression by inducing HES-1 in cultured hepatocytes

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ABSTRACT

Olfactory receptors (ORs) comprise the largest G protein-coupled receptor gene superfamily. Recent studies indicate that ORs are also expressed in non-olfactory organs, including metabolically active tissues, although their biological functions in these tissues are largely unknown. In this study, OR1A1 expression was detected in HepG2 liver cells. OR1A1 activation by (−)-carvone, a known OR1A1 ligand, increased the cyclic adenosine monophosphate (cAMP), but not intracellular Ca^{2+} concentration, thereby inducing protein kinase A (PKA) activity with subsequent phosphorylation of cAMP response element-binding protein (CREB) and upregulation of the CREB-responsive gene hairy and enhancer of split (HES)-1, a corepressor of peroxisome proliferator-activated receptor-γ (PPAR-γ) in hepatocytes. In (−)-carvone-stimulated cells, the repression of PPAR-γ reduced the expression of the target gene, mitochondrial glycerol-3-phosphate acyltransferase, which encodes a key enzyme involved in triglyceride synthesis. Intracellular triglyceride level and lipid accumulation were reduced in cells stimulated with (−)-carvone, effects that were diminished following the loss of OR1A1 function. These results indicate that OR1A1 may function as a non-redundant receptor in hepatocytes that regulates the PKA-CREB-HES-1 signaling axis and thereby modulates hepatic triglyceride metabolism.

1. Introduction

Olfactory receptors (ORs) are classic G protein-coupled receptors that constitute the largest G protein-coupled receptor gene family (i.e., family A) in the mammalian genome (Zhang and Firestein, 2002). ORs function as chemosensors, which are involved in the recognition and distinction of a multitude of chemically diverse odorants in the environment, and trigger the perception of smell (Buck and Axel, 1991). The receptors are expressed mainly on the cilia surface of olfactory sensory neurons located in the olfactory epithelium (Zozulya et al., 2001); however, they are also expressed in a variety of non-olfactory tissues, including testis, kidney, heart, liver, and small intestine (Feldmesser et al., 2006; Ichimura et al., 2008; Pluznick et al., 2013). Moreover, several predicted mammalian OR genes are solely expressed in non-olfactory tissues, raising the possibility that the receptors have functions other than odor recognition (Parmentier et al., 1992; Zhang et al., 2007); however, these functions have yet to be described and have been difficult to study due at least in part to low levels of expression.

Nonetheless, several studies have reported OR functions in non-olfactory tissues. Mouse olfactory receptor (MOR)17-4 and MOR23 act as critical sensors involved in controlling sperm motility and chemotaxis during fertilization (Fukuda et al., 2004; Spehr et al., 2003). In vivo Olfr78 function was demonstrated in the renal juxtaglomerular apparatus, where its binding and activation by short-chain fatty acids produced by gut microbiota induced renin secretion (Pluznick et al., 2013). MOR23 promoted muscle regeneration by regulating myocyte migration and adhesion (Griffin et al., 2009); more recently, OR2AT4 expressed in human keratinocytes induced intracellular cyclic adenosine monophosphate (cAMP) concentration, which activated the extracellular-signal-regulated kinase (ERK)1/2-p38 signaling axis and modulated skin cell

Abbreviations: OR, olfactory receptor; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; CREB, cAMP response element-binding protein; HES-1, hairy and enhancer of split-1; PPAR, peroxisome proliferator-activated receptor; mtGPAT, mitochondrial glycerol-3-phosphate acyltransferase; DGAT, diacylglycerol acyltransferase; TG, triglyceride; PCR, polymerase chain reaction; siRNA, small interfering RNA.

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proliferation, migration, and regeneration (Busse et al., 2014). These results indicate that ORs are widely expressed in non-olfactory tissues and that they exert diverse physiological functions beyond odor recognition.

Hepatocytes are a major cell type in the liver that regulates lipid homeostasis. Although multiple receptor-dependent pathways modulated by insulin, glucagon, and adrenalin have been investigated, the regulation of hepatic lipid metabolism is incompletely understood, and it is possible that an as-yet unknown pathway, possibly mediated by ORs expressed in hepatocytes, controls metabolic homeostasis in the liver.

Initially, we selected several ORs that showed hepatic expression according to the public transcriptome database and performed RT-PCR and qPCR to confirm their expression in HepG2 cells. The expression of OR1A1 was the highest among the tested ORs, so we performed additional studies. Immunoblotting analysis confirmed that OR1A1 was expressed in the plasma membrane of HepG2 cells, and a previous study demonstrated that (−)-carvone was a strong ligand for OR1A1 (Saito et al., 2009), with a half-maximal effective concentration of approximately 0.5 μM (Modena et al., 2011). The widely distributed natural aroma terpenoid compound, (−)-carvone, which is especially abundant in plant essential oils such as spearmint (Fichan et al., 1999), exhibits antimicrobial, antioxidant, and anti-inflammatory activities (Aggarwal et al., 2002; Elmastas et al., 2006; Terracciano et al., 2006). OR1A1 was the first identified isoform of OR family 1 subfamily A. Although it was de-orphanized, the expression level and the physiological functions of OR1A1 in hepatocytes have not yet been described. The present study investigated OR1A1 expression in cultured hepatocytes as well as its role in hepatic lipid metabolism.

2. Materials and methods

2.1. Cell culture and reagents

HepG2 cells were obtained from the Korean Cell Line Bank (Seoul, Korea) and were cultured in Dulbecco's modified Eagle's medium (Hyclone, Logan, UT, USA) containing 10% fetal bovine serum (Hyclone, Logan, UT, USA) and 1% penicillin/streptomycin (Welgene Inc., Seoul, Korea). Cells were maintained in a humidified atmosphere containing 5% CO_2 at 37 °C. (−)-Carvone and forskolin were acquired from Sigma (St. Louis, MO). Anti-OR1A1 antibody was purchased from Abcam (Cambridge, MA).

2.2. Polymerase chain reaction (PCR) and immunoblotting analyses

Total RNA was isolated from HepG2 cells using RNAiso Plus reagent (Takara Bio Inc., Otsu, Japan) and 2 μg of RNA from each sample was used to generate cDNA. Reverse transcription (RT)-PCR products were analyzed by agarose gel electrophoresis, and quantitative PCR was performed with the iQ™ SYBR® Green Supermix reagent and iQ5 Cycler System (Bio-Rad, Hercules, CA, USA). Primer sequences are shown in Supplemental Table 1. HepG2 cells were seeded in 100-mm plates at a density of 5×10^6/well. Protein from the plasma membrane and endosome fractions were extracted as described previously (Schenkman and Cinti, 1978; Washburn et al., 2002). Protein concentration was determined using a bicinchoninic acid protein assay kit (Pierce Biotechnology, Rockford, IL, USA). SDS-PAGE and immunoblotting were performed as described previously (Park et al., 2011).

2.3. Immunohistochemistry of OR1A1 in human liver tissue section

Human liver tissue section slide was obtained from Abnova (Taipei, Taiwan). Immunohistochemistry was performed according to the manufacturer's protocols. Briefly, the slides was deparaffinized and rehydrated before staining. Then heat-induced epitope retrieval was performed using a microwave. Next, the slide was preincubated for 30 min with 1% bovine serum albumin, and then incubated with OR1A1 antibody for 3 h. After washing, slide was incubated with secondary fluorescence rabbit antibody. Cell membrane and nuclear staining were performed with CellMask™ Orange Plasma membrane Stain (Invitrogen, CA, USA) and DAPI. The images were obtained using a confocal fluorescent microscope (LSM 700, Carl Zeiss).

2.4. Intracellular cAMP/Ca^{2+} measurement and protein kinase A (PKA) activity assay

HepG2 cells were treated with (−)-carvone (0.5, 1, or 10 μM) or 1 μM forskolin, and cAMP and PKA activities were quantified using commercial kits (Enzo Life Sciences, NY, USA) according to the manufacturer's protocols. For Ca^{2+} release, cells were seeded in 96-well plates at a density of 4×10^4/well and incubated for 24 h. An equal volume of 2× Fluo-4 Direct™ Ca^{2+} reagent loading solution (Invitrogen, CA, USA) was added to the wells and plates were incubated at 37 °C for 30–60 min. Cells were then treated with (−)-carvone (0.5, 1, or 10 μM) or 10 μM A-23187, and Ca^{2+} release was measured according to the manufacturer's protocol (Invitrogen, CA, USA).

2.5. Lipid analysis

HepG2 cells were seeded in six-well plates at a density of 10^6/well for 24 h, then lipid-loaded with free fatty acids (400 μM palmitic acid and 400 μM oleic acid) with 0.5% bovine serum albumin (Bovogen Biologicals, Melbourne, Australia) for 24 h. Lipid-loaded cells were then stimulated with (−)-carvone (0.5, 1, or 10 μM), or 1 μM forskolin for 24 h. Cells were then washed twice with PBS and treated with 1 mL hexane/isopropanol (2:1) for 30 min at room temperature. Then the extracts were dessicated using a SpeedVac™ (n-Biotek, Bucheon, Korea) and then dissolved in 95% ethanol. Cellular triglyceride (TG) and cholesterol contents were analyzed using a Cobas® C111 automated analyzer (Roche Diagnostic Systems Inc., Indianapolis, IN, USA). After the (−)-carvone or forskolin treatment, cells were stained with Oil Red O or 5 μL/mL boron-dipyrromethene (BODIPY) dye (Invitrogen, CA, USA), as described previously (Jia et al., 2013), then imaged under an ECLIPSE Ti-S microscope (Nikon, Tokyo, Japan).

2.6. OR1A1 gene knock-down

HepG2 cells were seeded in six-well plates at a density of 2×10^5/well. At 50–60% confluence, cells were transfected with 8 μL OR1A1 siRNA duplex (Santa Cruz Biotechnology, Santa Cruz, CA, USA) using FuGENE® HD reagent (Promega, Madison, WI, USA) for 24 h, and then treated with (−)-carvone (1 or 10 μM) or 1 μM forskolin for 24 h.

2.7. Statistical analysis

Data were expressed as the means ± standard error of the mean (SEM). All experiments were done in triplicates. One-way ANOVA followed by Tukey's HSD test was performed for multiple

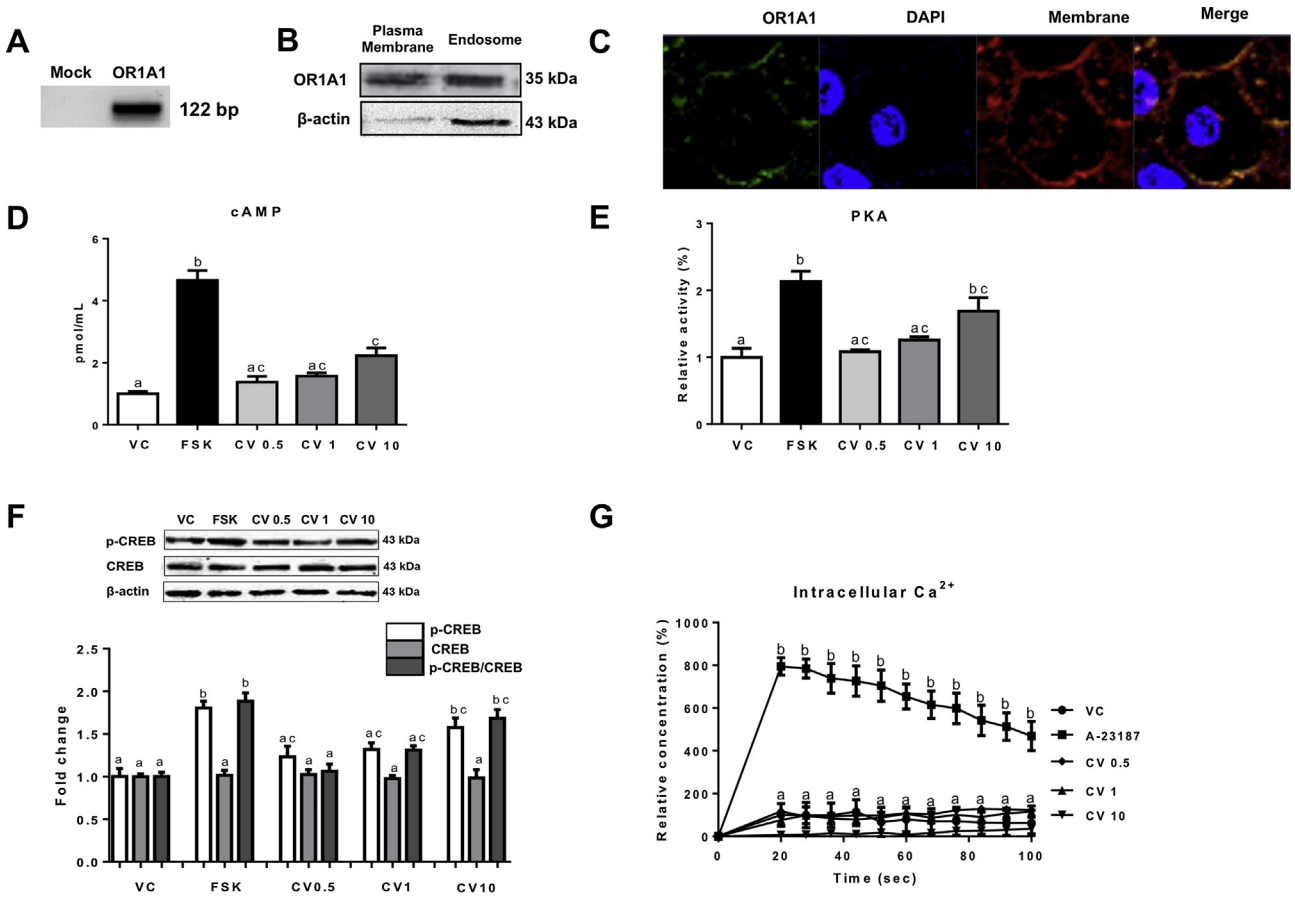

Fig. 1. Olfactory receptor (OR)1A1 activation by (−)-carvone triggers the cyclic adenosine monophosphate-protein kinase A (cAMP-PKA) pathway. (A) OR1A1 expression in HepG2 cells was detected by reverse transcription polymerase chain reaction (RT-PCR). (B) OR1A1 expression was detected in the HepG2 cell membrane protein fraction by immunoblotting. (C) Immunohistochemistry of OR1A1 in human liver tissue section (×400 magnification). (D) Intracellular cAMP levels, (E) PKA activity, and (F) cAMP response element-binding protein (CREB) and p-CREB expression, and p-CREB/CREB ratio. (G) Intracellular Ca^{2+} concentration upon (−)-carvone stimulation. A-23187 is a calcium ionophore used as a positive control. VC: vehicle treated cells; FSK: cells stimulated with forskolin (1 μM); CV: cell stimulated with (−)-carvone (0.5, 1, and 10 μM). One-way ANOVA followed by Tukey's HSD test was performed for multiple group comparisons. Different alphabets indicate statistical significance ($P < 0.05$). All experiments were done in triplicates.

group comparisons. Different letters indicate statistical significance ($P < 0.05$). Different alphabets indicate $P < 0.05$.

3. Results

3.1. OR1A1 is expressed in the plasma membrane of HepG2 cells

Functional expression of ORs is generally low, and gene expression often does not guarantee protein expression in the plasma membrane. RT-PCR analysis revealed that OR1A1 was expressed in cultured HepG2 cells (Fig. 1A), and immunoblotting confirmed that the 35-kDa protein was expressed in the isolated plasma membrane and endosome fractions (Fig. 1B). These results suggest that OR1A1 is synthesized in the endoplasmic reticulum, trafficked to the cell surface, and then may be recycled between the plasma membrane and endosome in a desensitization/resensitization process, as are conventional G protein-coupled receptors (Ritter and Hall, 2009). The cell surface expression of OR1A1 was confirmed by fluorescence-activated cell sorter analysis in HepG2 cells (Supplemental Fig. 1). Furthermore, immunohistochemistry with OR1A1 antibody and human liver tissue section revealed that OR1A1 was expressed in human hepatocytes primarily in the plasma membrane (Fig. 1C). By computational modeling, we found that the (−)-carvone ligand binds in-between helices III and VI, with (−)-carvone forming a hydrogen bond with the polar Tyr258 residue in helix VI (Supplemental Fig. 2).

3.2. OR1A1 activation stimulates cAMP-PKA signaling

The ligand-dependent activation of ORs induces second messengers, of which cAMP and Ca^{2+} are two major types (Lalli and Sassonecorsi, 1994). In HepG2 cells stimulated with (−)-carvone, a known OR1A1 ligand, intracellular cAMP level increased in a concentration-dependent manner (Fig. 1D). Accordingly, OR1A1 activation stimulated the PKA-cAMP response element-binding protein (CREB) signaling axis in HepG2 cells treated with (−)-carvone (Fig. 1E and F); immunoblotting showed that CREB phosphorylation at Ser133 was also increased (Fig. 1F), whereas intracellular Ca^{2+} concentration was unaffected (Fig. 1G). Taken together, these results indicate that OR1A1 activates PKA-CREB signaling while having no effect on intracellular Ca^{2+} release.

3.3. OR1A1 activation reduces cellular TG accumulation

The PKA-CREB signaling axis plays various roles in cellular physiology, including in lipid metabolism. In HepG2 cells stimulated with (−)-carvone (10 μM), intracellular TG, but not cholesterol, concentrations were significantly reduced compared to levels in lipid-loaded, vehicle-treated controls (Fig. 2A). Lipid staining revealed a reduction in lipid accumulation in lipid-loaded HepG2 cells by (−)-carvone treatment (Fig. 2B). CREB is known to repress the expression levels of peroxisome proliferator-activated receptor (PPAR)-γ, a major lipogenic transcription factor, through

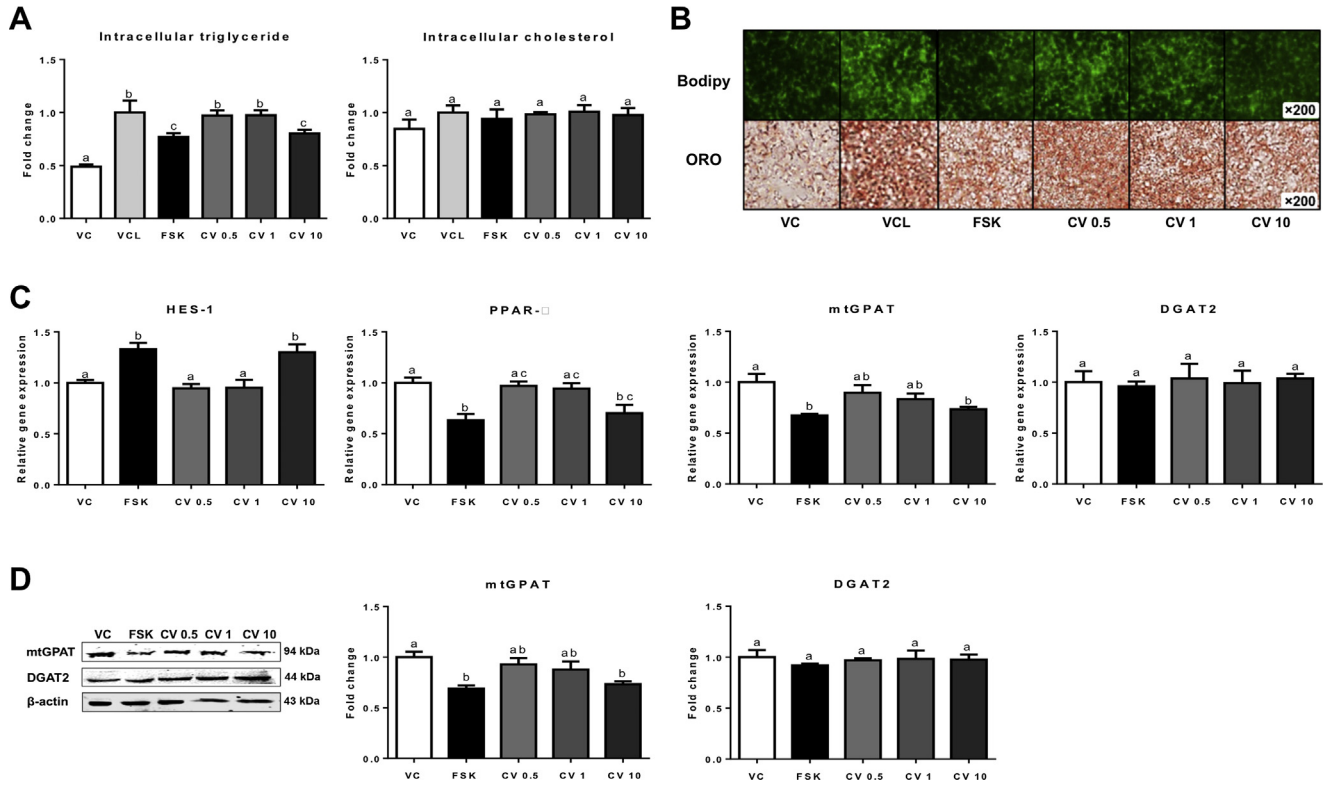

Fig. 2. OR1A1 activation induced by (−)-carvone reduces triglyceride (TG) concentration and lipid accumulation in HepG2 cells. (A) Intracellular TG and cholesterol levels were measured following (−)-carvone treatment. (B) Representative images of boron-dipyrromethene (Bodipy)- and Oil Red O (ORO)-stained lipid-loaded cells were shown (×200). (C) Hairy and enhancer of split (HES)-1, peroxisome proliferator-activated receptor (PPAR)-γ, mitochondrial glycerol-3-phosphate acyltransferase (mtGPAT), and diacylglycerol acyltransferase (DGAT2) transcript expression levels were analyzed by quantitative PCR. (D) Protein expression levels of mtGPAT and DGAT2 were analyzed by immunoblotting. VC: vehicle treated cells; VCL: vehicle treated lipid-loaded cells (Section 2); FSK: lipid-loaded cells stimulated with forskolin (1 μM); CV: lipid-loaded cells stimulated with (−)-carvone (0.5, 1, and 10 μM). Treatments were done for 24 h. One-way ANOVA followed by Tukey's HSD test was performed for multiple group comparisons. Different alphabets indicate statistical significance ($P < 0.05$). All experiments were done in triplicates.

activation of the repressor, hairy and enhancer of split (HES)-1 (Herzig et al., 2003). PPAR-γ induces the upregulation of mitochondrial glycerol-3-phosphate acyltransferase (mtGPAT) and diacylglycerol acyltransferase (DGAT), two key enzymes in TG synthesis (Cao et al., 2006; Matsusue et al., 2003). Quantitative PCR analyses revealed that HES-1 transcript expression was markedly increased, whereas that of PPAR-γ was downregulated upon stimulation with 10 μM (−)-carvone (Fig. 2C). The mtGPAT gene and protein expression levels were subsequently decreased, but those of DGAT2 were not affected (Fig. 2C and D). Potentially, DGAT2 expression is not dominantly regulated by PPAR-γ. These results indicate that OR1A1 activation suppresses hepatic TG metabolism by modulating HES-1, PPAR-γ, and mtGPAT expression.

3.4. Reduction in intracellular TG concentration by (−)-carvone is OR1A1-dependent

To evaluate the specificity of the effects of (−)-carvone on hepatic TG metabolism, OR1A1 expression was suppressed by small interfering RNA (siRNA)-mediated knockdown. In HepG2 cells transfected with the siRNA duplex targeting OR1A1, the protein expression levels was markedly reduced by 79% compared to the control group (Fig. 3A). Moreover, CREB phosphorylation, which is induced by (−)-carvone via the cAMP-PKA pathway, was abolished (Fig. 3B), as was the increase in the phospho-CREB-to-CREB ratio (Fig. 3B). Notably, the regulatory effects of (−)-carvone on hepatic TG metabolism (Fig. 3C) and the reduction in mtGPAT gene and protein expression induced by (−)-carvone (Fig. 3D and E) were also abrogated in OR1A1-deficient cells. Collectively, these results demonstrate that the effects of (−)-carvone on hepatic TG metabolism and lipid accumulation are OR1A1-dependent.

4. Discussion

ORs are chemosensors in the olfactory epithelium that transmit combined odor information to the sensory nervous system. Over the last decade, microarray and RNA sequencing transcriptome analyses identified a number of ORs expressed in non-olfactory tissues; however, little attention has been paid to the biological functions of ectopically expressed ORs in non-olfactory tissues. To date, five distinct functions have been reported (Busse et al., 2014; Fukuda et al., 2004; Griffin et al., 2009; Pluznick et al., 2013; Spehr et al., 2003; Gu et al., 2014): (1) OR17-4 and MOR23, mouse ORs, function as sensors involved in the control of sperm motility or in sperm chemotaxis during fertilization; (2) Olfr78 expresses in the kidney and regulates blood pressure-sensing short-chain fatty acids, metabolites produced by microbiota; (3) MOR23 promotes muscle regeneration by regulating myocyte migration and adhesion; (4) OR2AT4, expressed in human keratinocytes, induces intracellular cAMP concentration, which subsequently activates the ERK1/2-p38 signaling axis and thus regulates skin cell proliferation, migration and regeneration; it has been suggested that ORs function as chemosensors in human airways, which may be involved in the regulation of serotonin secretion based on the findings of OR expression and molecular orientation in solitary pulmonary neuroendocrine cells (5). In addition to these reports, we now demonstrated that OR1A1 is expressed in hepatocytes and participates in the regulation of hepatic lipid metabolism.

Results of this study revealed for the first time that an OR is expressed in hepatocytes and regulates lipid metabolism. OR1A1 expression was detected in HepG2 cells and human liver section, demonstrating that OR1A1 is expressed and concentrated at the cell

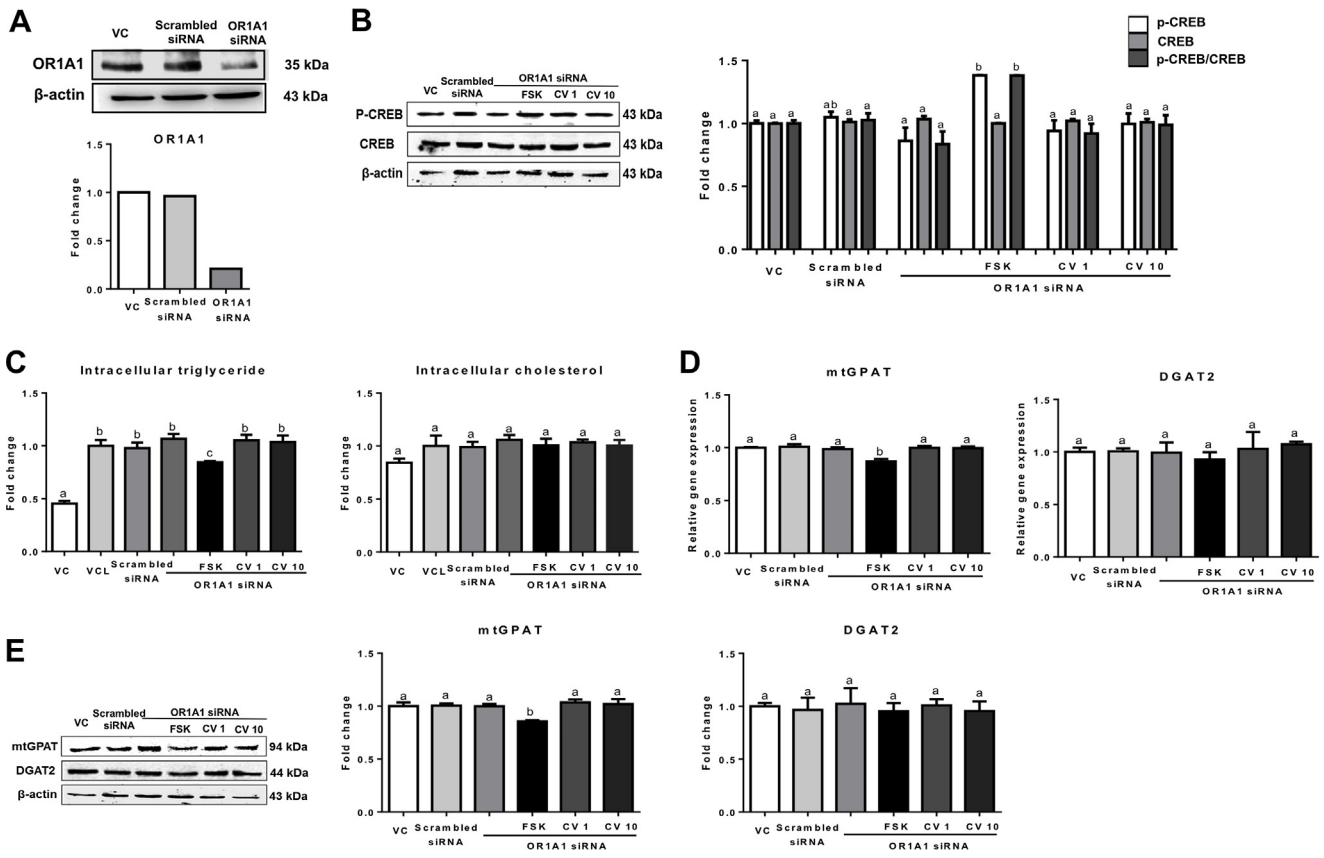

Fig. 3. The cAMP-PKA signaling pathway and intracellular lipid concentration are unaltered by (−)-carvone treatment in OR1A1-deficient HepG2 cells. (A) OR1A1 protein expression in cells transfected with OR1A1 siRNA was determined by immunoblotting. (B) Protein expression levels of CREB and phosphorylated CREB following transfection with OR1A1 siRNA were determined by immunoblotting. (C) Intracellular TG and cholesterol levels, as well as (D) mtGPAT and DGAT2 transcript and (E) protein levels were determined. VC: vehicle treated cells; VCL: vehicle treated lipid loaded cells; FSK: lipid-loaded cells stimulated with forskolin (1 μM); CV: lipid-loaded cells stimulated with (−)-carvone (1, and 10 μM). Treatments were done for 24 h. One-way ANOVA followed by Tukey's HSD test was performed for multiple group comparisons. Different alphabets indicate statistical significance ($P < 0.05$). All experiments were done in triplicates.

membrane surface and acts as a functional transmembrane receptor. OR1A1 activation, mediated by (−)-carvone, a known OR1A1 ligand, resulted in cAMP-PKA pathway activation. CREB was then activated by phosphorylation, which stimulated the expression of HES-1, thereby suppressing PPAR-γ and its target gene, mtGPAT, to reduce TG level and lipid accumulation in cultured hepatocytes. Thus, the CREB-HES1-PPAR-γ signaling axis (Herzig et al., 2003) was regulated by OR1A1 activation. No changes were observed in the intracellular calcium concentration following (−)-carvone stimulation. Thus, OR1A1 activation may specifically stimulate Gαs in hepatocytes.

PPAR-γ regulates the expression of mtGPAT, the mitochondrial isoform of a key enzyme in hepatic TG synthesis that is associated with the outer mitochondrial membrane, in contrast to microsomal GPAT in the endoplasmic reticulum (Bell and Coleman, 1980; Takeuchi and Reue, 2009). GPAT catalyzes the first step of glycerol-3-phosphate acylation in TG synthesis (Takeuchi and Reue, 2009). In general, mtGPAT accounts for only ~10% of the total GPAT activity; however, this value is around 50% in the liver, implying a major role in hepatic TG synthesis (Bell and Coleman, 1980; Linden et al., 2004). Recent studies indicate that mtGPAT is involved in dyslipidemia associated with obesity (Jamdar and Cao, 1995; Linden et al., 2004), and treatment of *ob*/*ob*-PPAR-$\gamma^{fl/fl}$AlbCre^{-} mice with PPAR-γ-agonist-induced GPAT expression (Matsusue et al., 2003). HepG2 cells stimulated with (−)-carvone had reduced levels of mtGPAT transcript and protein compared to controls, suggesting that CREB regulates TG metabolism by stimulating HES-1 expression, resulting in the repression of PPAR-γ and its target, mtGPAT. DGAT2 catalyzes the formation of TG from fatty acyl-CoA and diacylglycerol (Ganji et al., 2004). However, DGAT2 mRNA and protein expression were unaltered by (−)-carvone treatment, suggesting a mechanism other than one that involves PPAR-γ.

The OR1A1-dependent reduction of (−)-carvone on hepatic TG and lipid accumulation was confirmed with OR1A1 siRNA. The successful knockdown of OR1A1 was confirmed by RT-PCR. OR1A1 knockdown revealed that the downstream cAMP-PKA signaling axis was not stimulated by (−)-carvone, including p-CREB induction and decreased mtGPAT expression. Thus, reduced hepatic TG levels were abolished in cells transfected with OR1A1 siRNA. These results indicate that (−)-carvone-induced regulation of hepatic TG metabolism was an OR1A1-dependent process.

(−)-Carvone, which is widely distributed in the diet and in essential oils, including caraway and mandarin seeds, is a strong ligand for OR1A1 (Saito et al., 2009). Schmiedeberga et al. (2007) also reported several aromatic ligands of OR1A1. G protein-coupled receptor ligands could show differential biological effects depending on whether they are functioning as a balanced or biased ligand. The role of each ligand in cell function should be studied individually; thus, the potential effects of ligands on OR1A1 and hepatic TG metabolism should be investigated individually in future studies.

(−)-Carvone is a terpenoid that is widely distributed in plant foods (e.g., caraway, dill, and peppermint). The (−)-carvone detected in human blood and other tissues is likely of dietary origin. The acceptable daily intake value for carvone is 1 mg/kg of body weight (Council of Europe, 1992); thus, (−)-carvone in the diet could regulate OR1A1 in the liver after intake.

Our study demonstrated that OR1A1 is expressed in hepatocytes, where it may function as a non-redundant receptor that regulates the cAMP-PKA signaling axis and thereby modulates hepatic TG metabolism. The present study extends knowledge of the physiological functions of ORs in non-olfactory tissues to include hepatic TG metabolism.

Authors' contributions

C.W. and S.-J.L. designed the experiments. C.W., Y.J., J.H.L., Y.K. and S.S. performed the experiments. C.W., Y.J., J.H.L., S.S., V.S.B and S.-J.L. interpreted the results. C.W. and S.-J.L. wrote and reviewed the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biocel.2015.03.008.

References

Aggarwal KK, Khanuja SPS, Ahmad A, Kumar TRS, Gupta VK, Kumar S. Antimicrobial activity profiles of the two enantiomers of limonene and carvone isolated from the oils of *Mentha spicata* and *Anethum sowa*. Flavour Frag J 2002;17:59–63.

Bell RM, Coleman RA. Enzymes of glycerolipid synthesis in eukaryotes. Annu Rev Biochem 1980;49:459–87.

Buck L, Axel R. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell 1991;65:175–87.

Busse D, Kudella P, Gruning NM, Gisselmann G, Stander S, Luger T, et al. A synthetic sandalwood odorant induces wound-healing processes in human keratinocytes via the olfactory receptor OR2AT4. J Invest Dermatol 2014;134:2823–32.

Cao JS, Li JL, Li DM, Tobin JF, Gimeno RE. Molecular identification of microsomal acyl-CoA: glycerol-3-phosphate acyltransferase, a key enzyme in de novo triacylglycerol synthesis. Proc Natl Acad Sci U S A 2006;103:19695–700.

Council of Europe. Flavouring substances and natural sources of flavourings. Part I. 4th ed. Strasbourg: Maisonneuve; 1992.

Elmastas M, Dermirtas I, Isildak O, Aboul-Enein HY. Antioxidant activity of S-carvone isolated from spearmint (*Mentha spicata* L-Fam Lamiaceae). J Liq Chromatogr Relat Technol 2006;29:1465–75.

Feldmesser E, Olender T, Khen M, Yanai I, Ophir R, Lancet D. Widespread ectopic expression of olfactory receptor genes. BMC Genomics 2006;7:121–39.

Fichan I, Larroche C, Gros JB. Water solubility, vapor pressure, and activity coefficients of terpenes and terpenoids. J Chem Eng Data 1999;44:56–62.

Fukuda N, Yomogida K, Okabe M, Touhara K. Functional characterization of a mouse testicular olfactory receptor and its role in chemosensing and in regulation of sperm motility. J Cell Sci 2004;117:5835–45.

Ganji SH, Tavintharan S, Zhu DM, Xing YD, Kamanna VS, Kashyap ML. Niacin noncompetitively inhibits DGAT2 but not DGAT1 activity in HepG2 cells. J Lipid Res 2004;45:1835–45.

Griffin CA, Kafadar KA, Pavlath GK. MOR23 promotes muscle regeneration and regulates cell adhesion and migration. Dev Cell 2009;17:649–61.

Gu X, Karp PH, Brody SL, Pierce RA, Welsh MJ, Holtzman MJ, et al. Chemosensory functions for pulmonary neuroendocrine cells. Am J Respir Cell Mol Biol 2014;50:637–46.

Herzig S, Hedrick S, Morantte I, Koo SH, Galimi F, Montminy M. CREB controls hepatic lipid metabolism through nuclear hormone receptor PPAR-gamma. Nature 2003;426:190–3.

Ichimura A, Kadowaki T, Narukawa K, Togiya K, Hirasawa A, Tsujimoto G. In silico approach to identify the expression of the undiscovered molecules from microarray public database: identification of odorant receptors expressed in non-olfactory tissues. Naunyn-Schmied Arch Pharmacol 2008;377:159–65.

Jamdar SC, Cao WF. Triacylglycerol biosynthetic-enzymes in lean and obese Zucker rats. Biochim Biophys Acta – Lipids Lipid Metab 1995;1255:237–43.

Jia YY, Kim JY, Jun HJ, Kim SJ, Lee JH, Hoang MH, et al. Cyanidin is an agonistic ligand for peroxisome proliferator-activated receptor-alpha reducing hepatic lipid. Biochim Biophys Acta – Mol Cell Biol Lipids 2013;1831:698–708.

Lalli E, Sassonecorsi P. Signal-transduction and gene-regulation – the nuclear response to camp. J Biol Chem 1994;269:17359–62.

Linden D, William-Olsson L, Rhedin M, Asztely AK, Clapham JC, Schreyer S. Overexpression of mitochondrial GPAT in rat hepatocytes leads to decreased fatty acid oxidation and increased glycerolipid biosynthesis. J Lipid Res 2004;45:1279–88.

Matsusue K, Haluzik M, Lambert G, Yim SH, Gavrilova O, Ward JM, et al. Liver-specific disruption of PPAR gamma in leptin-deficient mice improves fatty liver but aggravates diabetic phenotypes. J Clin Invest 2003;111:737–47.

Modena D, Trentini M, Corsini M, Bombaci A, Giorgetti A. OlfactionDB: a database of olfactory receptors and their ligands. Adv Life Sci 2011;1:1–5.

Park HM, Lee JH, Yaoyao J, Jun HJ, Lee SJ. Limonene, a natural cyclic terpene, is an agonistic ligand for adenosine A(2A) receptors. Biochem Biophys Res Commun 2011;404:345–8.

Parmentier M, Libert F, Schurmans S, Schiffmann S, Lefort A, Eggerickx D, et al. Expression of members of the putative olfactory receptor gene family in mammalian germ cells. Nature 1992;355:453–5.

Pluznick JL, Protzko RJ, Gevorgyan H, Peterlin Z, Sipos A, Han J, et al. Olfactory receptor responding to gut microbiota-derived signals plays a role in renin secretion and blood pressure regulation. Proc Natl Acad Sci U S A 2013;110:4410–5.

Ritter SL, Hall RA. Fine-tuning of GPCR activity by receptor-interacting proteins. Nat Rev Mol Cell Biol 2009;10:819–30.

Saito H, Chi QY, Zhuang HY, Matsunami H, Mainland JD. Odor coding by a mammalian receptor repertoire. Sci Signal 2009;2:ra6–29.

Schenkman JB, Cinti DL. Preparation of microsomes with calcium. Methods Enzymol 1978;52:83–9.

Schmiedeberga K, Shirokovaa E, Weberb HP, Schillingb B, Meyerhofa W, Krautwurst D. Structural determinants of odorant recognition by the human olfactory receptors OR1A1 and OR1A2. J Struct Biol 2007;159:400–12.

Spehr M, Gisselmann G, Poplawski A, Riffell JA, Wetzel CH, Zimmer RK, et al. Identification of a testicular odorant receptor mediating human sperm chemotaxis. Science 2003;299:2054–8.

Takeuchi K, Reue K. Biochemistry, physiology, and genetics of GPAT, AGPAT, and lipin enzymes in triglyceride synthesis. Am J Physiol Endocrinol Metab 2009;296:E1195–209.

Terracciano S, Aquino M, Rodriquez M, Monti MC, Casapullo A, Riccio R, et al. Chemistry and biology of anti-inflammatory marine natural products: molecules interfering with cyclooxygenase, NF-kappa B and other unidentified targets. Curr Med Chem 2006;13:1947–69.

Washburn KB, Turner TJ, Talamo BR. Comparison of mechanical agitation and calcium shock methods for preparation of a membrane fraction enriched in olfactory cilia. Chem Senses 2002;27:635–42.

Zhang X, Firestein S. The olfactory receptor gene superfamily of the mouse. Nat Neurosci 2002;5:124–33.

Zhang XH, De la Cruz O, Pinto JM, Nicolae D, Firestein S, Gilad Y. Characterizing the expression of the human olfactory receptor gene family using a novel DNA microarray. Genome Biol 2007;8:R86–95.

Zozulya S, Echeverri F, Nguyen T. The human olfactory receptor repertoire. Genome Biol 2001;2, 0018.1–12.