Therapeutic potential of ectopic olfactory and taste receptors

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Abstract | Olfactory and taste receptors are expressed primarily in the nasal olfactory epithelium and gustatory taste bud cells, where they transmit real-time sensory signals to the brain. However, they are also expressed in multiple extra-nasal and extra-oral tissues, being implicated in diverse biological processes including sperm chemotaxis, muscle regeneration, bronchoconstriction and bronchodilatation, inflammation, appetite regulation and energy metabolism. Elucidation of the physiological roles of these ectopic receptors is revealing potential therapeutic and diagnostic applications in conditions including wounds, hair loss, asthma, obesity and cancers. This Review outlines current understanding of the diverse functions of ectopic olfactory and taste receptors and assesses their potential to be therapeutically exploited.

The olfactory and gustatory systems are the principal chemosensory systems in many organisms and detect a diverse range of environmental cues important for survival including food, toxins, predators and prey1-3. Olfactory receptors (ORs) and taste receptors are primarily expressed in olfactory and gustatory sensory cells and are activated by specific groups of odorant and tastant ligands4-6, and the nasal olfactory and oral gustatory systems transduce chemical information from odorants and tastants via electrical signals to the brain.

ORs and taste receptors are also expressed in non-olfactory and non-gustatory tissues, and this expression has often been described as ectopic. However, numerous reports have described widespread expression of ORs and taste receptors in various tissues7-9, suggesting that the extra-nasal and extra-oral expression of these receptors is normal, and their characterization as ectopically expressed appears to be inaccurate. This widespread expression of ORs and taste receptors throughout various tissues suggests that extra-nasal ORs and extra-oral taste receptors have distinct biological functions. Indeed, ORs have been reported to be involved in a variety of processes, including the regulation of sperm chemotaxis10, wound healing11,12, hair growth12, muscle regeneration13, cancer cell inhibition14-18 and adiposity19, whereas taste receptors are involved in controlling gut appetite hormone release20,21, innate immunity22 and adipogenesis23.

ORs and some taste receptors, including sweet, umami, bitter and potentially fat taste receptors, are classified as G protein-coupled receptors (GPCRs). GPCRs are major drug targets in the pharmaceutical industry, with approximately 40% of approved drugs on the market targeting GPCR proteins24. Given their variety of biological functions, ectopic ORs and taste receptors represent attractive potential novel therapeutic targets for a broad array of indications.

For example, activation of OR2AT4 in keratinocytes with a synthetic terpenoid sandalwood odorant called Sandalore induces cell proliferation and migration, thereby contributing to wound healing25. OR2AT4 is also expressed in human hair follicles, and its activation substantially increases the active growth phase of the hair follicle by inducing insulin-like growth factor 1 (IGF1) production26. Thus, Sandalore may have potential therapeutic applications in the promotion of wound healing and the stimulation of hair growth.

In addition, as OR expression is upregulated in several cancers14-16,25,26, the potential application of ORs in cancer diagnostics and therapeutics is also beginning to emerge17-19. In particular, OR51E2 is a potential marker for prostate cancer17-19, and OR2B6, OR2C3 and OR10H1 could serve as biomarkers for breast carcinoma20, melanoma21 and urinary tract cancers22, respectively.

Ectopic ORs and taste receptors may also represent potential therapeutic targets in the treatment of obesity. Indeed, azelaic acid, an agonist of mouse OLF544, has been shown to induce adipose lipolysis and hepatic fatty acid oxidation in mice23 and cultured human cells (S-J. Lee, unpublished observations). Although no human orthologue of OLF544 has been defined by phylogeny, studies are underway to further assess the potential application of azelaic acid as a treatment for obesity and subcutaneous lipid reduction. Furthermore, bitter tastants (for example, quinine and denatonium benzoate) act on TAS2Rs expressed on adipocytes and on enteroendocrine cells in the gut to reduce appetite and body weight, with increased plasma levels of glucagon-like peptide 1 (GLP1) in rodents24-27. Notably, free fatty
Taste receptors
A type of receptor that binds to tasteants dissolved in saliva to facilitate the sensation of taste. Taste receptors are widely expressed in diverse tissues and play multiple biological roles.

Sperm chemotaxis
A form of sperm guidance in which spermatids follow a concentration gradient of a chemotactic factor secreted from the oocyte and thereby reach the oocyte.

Adipogenesis
The process of cell differentiation by which pre-adipocytes become adipocytes.

Keratinocytes
Cells that are a major component of the epidermis, the outer layer of the skin, which synthesizes keratin. Keratinocytes are involved in the intricate mechanisms of initiation, maintenance and completion of wound healing.

Orthologue
A homologous gene that is related to those in different organisms by descent from the DNA of a common ancestor.

Pseudogenes
Sequences of DNA resembling a gene but usually containing mutations that alter or abolish gene function; pseudogenes thus cannot produce a normal protein.

acid receptor 4 (FFAR4), an omega-3 fatty acid receptor that has been implicated in the ability to taste fats56,59, has shown promise as a potential therapeutic target for obesity in randomized clinical trials56.

Taste receptors may also be important in asthma, as bitter tastants have been shown to act on TAS2Rs in lung epithelial cells and smooth muscle cells to increase the secretion of antibacterial peptides and to induce bronchodilatation41–43.

Although substantial advances in the understanding of the biological roles of ORs and taste receptors have been made, their potential as therapeutic targets is only beginning to be investigated. This Review summarizes the current understanding of the key biological functions of ectopic ORs and taste receptors and highlights their emerging potential therapeutic and diagnostic applications in a range of diseases. Limitations and challenges in the research of ectopic ORs and taste receptors, as well as future directions, are discussed.

Nasal olfactory system
Olfaction research began at the molecular level after Buck and Axel made the landmark discovery of a large multigene family of ORs in rats in 1991 (REF. 14). The human and mouse genomes encode approximately 370 and 1,000 functional OR genes, respectively, making the ORs the largest gene family46–53. While different studies have yielded small discrepancies in the exact number of ORs owing to slight differences in the filtering criteria and bioinformatics search methods, it is clear that these receptors constitute 5–10% of the mammalian genome, including pseudogenes46–53.

ORs have several unique characteristics. Approximately 60% of human OR genes are non-functional pseudogenes, and an individual may have either an intact or a pseudogene version of a particular OR75,77–80. A subset of OR proteins require specific chaperones such as receptor transporting proteins 1 and 2 or the heat shock protein HSC70 (also known as HSPA8) for plasma membrane targeting41–44. Each OR recognizes multiple odorants, and each odorant can be detected by different ORs; thus, a single odorant has a specific OR activation pattern, which is translated into odour perception45,46. An increase in the concentration of an odorant can also change its receptor code so that it binds to a broader spectrum of ORs45. This may explain the altered perception of odours at different concentrations. OR expression is monogenic and monoallelic and, thus, a single olfactory sensory neuron expresses only one OR of the repertoire, according to the so-called ‘one neuron, one receptor’ hypothesis48–51. The monoallelic expression of OR genes is mainly based on the molecular shape and matching electrostatics of the ligand structure and not on the vibration of chemicals, as has been shown by dynamic homology modelling50,51. With a combination of in silico structure prediction, docking analysis, homology modelling and mutagenesis studies, the selective binding of odorants has been demonstrated for several ORs49–52,55–57.

ORs have a unique molecular receptive system. Once an odour molecule is bound to an OR in the cilia of an olfactory sensory neuron where all protein components for signalling pathways are present58,59, a signal transduction cascade transforms and amplifies the chemical information into an electrical signal58,59. According to the canonical model of olfactory signal transduction, ORs generally interact with a specific stimulatory G protein, Gαolf (REF. 59), which initiates the signal transduction pathway. More specifically, when the odorant binds, ligand activation of the OR stimulates Gαolf by binding GTP and this stimulates adenylylcyclase III (ACII; also known as ADCY3). This, in turn, increases the cytoplasmic cAMP level, which causes external sodium (Na+) and calcium (Ca2+) influx by opening nonspecific cation-selective cyclic nucleotide-gated (CNG) channels. Subsequent depolarization of the plasma membrane is amplified at least in part by the Ca2+-activated chloride (Cl−) channel TMEM16B (also known as ANO2)52,53, further depolarizing the cilia by promoting Cl− efflux. The membrane depolarization triggers an action potential, which emits a frequency depending upon the intensity and duration of the olfactory message along the axons of olfactory sensory neurons towards the olfactory bulb, where the olfactory message is the first relayed for integration in the brain (reviewed previously). Interestingly, the expression of canonical olfactory signalling molecules can also be demonstrated in diverse extra-nasal tissues. However, in some non-olfactory tissues, completely different signalling components are involved, as discussed throughout the manuscript.

Oral taste system
Taste receptors were first characterized in 2000 (REF. 95), and six types of taste receptor and channel for sweet, umami, bitter, sour, salty and fat tastes have been identified97. Sour and salty tastes are sensed by ion channels (for example, the epithelial sodium channel (ENaC))98, whereas other tastes are detected by membrane receptors, mainly by GPCR proteins. As this Review aims to focus on olfactory and taste GPCRs, detailed discussions on sour and salty taste detection are beyond the scope of this manuscript.

Sweet and umami receptors are heteromers of the GPCR taste receptor family 1 (TAS1Rs): TAS1R2–TAS1R3 for sweet and TAS1R1–TAS1R3 for umami. Umami receptors are broadly tuned to detect several amino acids but have a higher sensitivity for glutamate in humans99. In rodents, there is evidence for another umami receptor system mediated by mGluR1 and mGluR4 in addition to TAS1R1–TAS1R3 (REF. 79). Other amino acid sensors (calcium-sensing receptor (CaSR), GPCR family C, group 6, member A (GPRC6A) and lysophosphatidic acid receptor 5 (LPA5) also known...
Box 1 | Therapeutic potential of FFAR2 and FFAR3

Free fatty acid receptor 2 (FFAR2) and FFAR3 are classified as free fatty acid receptors for short-chain fatty acids (SCFAs) (less than six carbons), most notably, acetate, propionate and butyrate, which are primarily produced by colonic fermentation of dietary fibres. FFAR2 is a promiscuous receptor that couples to G\(_{\alpha}\)o and G\(_{\alpha}\)q, whereas FFAR3 induces only G\(_{\alpha}\)q-mediated signalling\(^{111}\). A recent study demonstrated the expression of FFAR2 but not FFAR3 in taste bud tissue from human fungiform papillae\(^{112}\). However, preference and taste nerve responses to SCFAs in wild-type and knockout FFAR2 and FFAR3 mice are necessary to determine the role of these receptors in the detection of fat by taste receptor cells. Both receptors are also expressed in extra-oral tissues and show overlapping expression patterns in enteroendocrine cells and pancreatic \(\beta\)-cells\(^{108,113}\). FFAR2, but not FFAR3, is predominantly expressed in immune cells such as neutrophils, monocytes, eosinophils and regulatory T(\(\text{T}_{\text{reg}}\)) cells\(^{114,115,116}\), but dendritic cells appear to express both FFAR2 and FFAR3 (REF.\(^{117}\)). While FFAR2 is expressed in both human and mouse white adipose tissue\(^{109,118}\), FFAR3 expression in mouse adipocytes is controversial\(^{119}\). In contrast to the predominant expression of FFAR2 in adipocytes and immune cells, FFAR3, but not FFAR2, is found on sympathetic ganglia and enteric neurons\(^{120,121}\). On the basis of some of these expression patterns and consequent functional studies, FFAR2 and FFAR3 have been considered as potential drug targets for the treatment of metabolic and inflammatory disorders.

**Metabolic disorders**

Loss of FFAR2 and FFAR3 in pancreatic \(\beta\)-cells increases insulin secretion and improves glucose tolerance in high-fat diet-fed obese mice, suggesting a possible role for FFAR2 and FFAR3 antagonists in the treatment of diabetes\(^{122}\). On adipocytes, circulating SCFAs activate FFAR2 to suppress insulin-mediated fat accumulation while promoting the catabolism of lipids and glucose in liver and muscle\(^{123}\).

Long-term administration of prebiotic fibres (for example, fructans) consistently decreased weight gain in mice fed a high-fat diet, but effects on glucose intolerance have been contradictory\(^{124,125}\). In overweight adults, daily supplementation (24 weeks) with an inulin-propionate ester to deliver propionate to the colon reduced energy intake, ameliorated long-term weight gain and improved \(\beta\)-cell function with increased insulin secretion (NCT00750438)\(^{126,127}\). A placebo-controlled, randomized trial showed that daily supplementation of oligofructose-enriched inulin to overweight or obese children affected the intestinal microbiota and reduced their body weight z-scores, that daily supplementation of oligofructose-enriched inulin to overweight or obese children affected the intestinal microbiota and reduced their body weight z-scores, percent body and trunk fat, and interleukin-6 (IL-6) levels (NCT02125955)\(^{128}\).

**Inflammatory disorders**

SCFAs regulate colonic \(T_{\text{reg}}\) cell homeostasis and protect against colitis in an FFAR2-dependent manner in mice\(^{129}\). Nevertheless, studies using knockout mice failed to consistently determine whether FFAR2 is protective or exacerbates the severity of inflammatory disease\(^{130,131}\). A clinical trial with the FFAR2 antagonist GLPG0674 did not improve clinical symptoms in patients with inflammatory bowel disease after 4 weeks of treatment, although it reduced neutrophil activation and influx\(^{132}\).

It is still a matter of debate whether fat can be considered as the sixth taste in gustation because of its textual and olfactory cues. However, mounting evidence suggests that several fatty acid receptors can be involved in fat taste sensation\(^{133,134}\). Scavenger receptor CD36 and the free fatty acid GPCRs FFAR1 and FFAR4 are expressed in the taste buds, and studies in knockout mice have demonstrated the involvement of these receptors in orosensory fat perception\(^{135,136}\). In fact, there are four types of FFAR depending on the chain length of the ligand fatty acids: FFAR2 and FFAR3 for short-chain fatty acids (SCFAs) (less than 6 carbons), FFAR1 for medium-chain (6–12 carbons) to long-chain fatty acids (greater than 12 carbons) and FFAR4 for unsaturated long-chain fatty acids (for example, omega-3 fatty acids).

To date, only FFAR1 and FFAR4 have been implicated in taste perception, and further studies are required to determine any potential role of FFAR2 and FFAR3 in fat taste sensation (BOX 1). FFAR1 and FFAR4 have been intensively studied as potential drug targets for several metabolic diseases\(^{137,138}\).

Despite their diversity, TAS1Rs and TAS2Rs have a common intracellular signalling pathway and couple to several heterotrimeric G proteins such as Ga-gustducin and transducin, G\(_{\beta}\)\(_2\), G\(_{\gamma}\)\(_2\), Ga, and G\(_{\alpha}\). Upon activation by a tastant, the \(\beta\gamma\)-subunit is dissociated from the \(\alpha\)-subunit and activates the phospholipase C\(_{\beta}\) (PLC\(_{\beta}\))–inositol 1,4,5-trisphosphate (Ins(1,4,5)P\(_3\)) pathway to induce the release of Ca\(_{\text{2+}}\) into the cytoplasm\(^{139}\). Ga subunits may activate phosphodiesterase, leading to a breakdown of cAMP and inhibiting protein kinase A (PKA) activation, which otherwise inhibits the PLC\(_{\beta}\)–Ins(1,4,5)P\(_3\) pathway\(^{140}\). The increase in Ca\(_{\text{2+}}\) opens transient receptor potential (TRP) channel M5 (TRPM5), resulting in Na\(^{+}\) influx and the activation of other voltage-gated Na\(^{+}\) channels, which will induce the secretion of ATP through an atypical, non-vesicular mechanism that involves a channel composed of a heteromer of calcium homeostasis modulator 1 (CALHM1) and CALHM3 (REF.\(^{141}\)). ATP activates sensory nerves that communicate to the brain regions involved in taste perception. Each taste receptor cell is specifically tuned to detect one taste quality, which in turn relays information to the brain via single nerve fibres tuned for the same taste quality (labelled line model)\(^{142,143}\). Because taste receptor cells are constantly renewed, connections between existing ganglion processes and newly born taste receptor cells must be continuously re-established.

**Ectopic olfactory receptors**

ORs are widely expressed throughout the body in a diverse range of tissues. It should be noted that most evidence for ectopic expression of ORs is at the level of mRNA and not protein owing to the lack of receptor-specific antibodies. These extra-nasally expressed ORs have different effects on cell biological functions owing to their versatility in activating different molecular and cellular signalling mechanisms. The pathways activated depend on the cell types and the signalling components involved.

**Tissue expression**

After the first report of ectopic expression of ORs in testis by Parmentier et al.\(^{114}\), which was later confirmed by others\(^{115–117}\), OR transcripts were found in various tissues, including the adipose tissue\(^{118–120}\), airway\(^{117–119}\), heart\(^{120}\), kidney\(^{121–123}\), liver\(^{124–126}\), lung\(^{126–128}\), prostate\(^{129–131}\), colon\(^{132–134}\), salivary glands\(^{125–134}\), gut\(^{135–137}\), cardiac and skeletal muscle\(^{138–140}\), pancreas\(^{141–143}\), placenta\(^{144}\), retina\(^{145–147}\) and tongue\(^{148–150}\). In addition, it has been suggested that ORs may be expressed during mammalian embryogenesis\(^{151}\).
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<th>Cell type</th>
<th>Olfactory receptors</th>
<th>Ligand*</th>
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<td>* OR1D2: induction of cell contraction and IL-8 release * OR2AG1: inhibition of histamine-induced contraction</td>
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<td>Kidney</td>
<td>Kidney tissue cells and proximal tubule HK-2 cells</td>
<td>OR51E1 and OR11H7</td>
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<td>OLFR544</td>
<td>Azelaic acid</td>
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<td>Pulmonary neuroendocrine cells and human tracheobronchial epithelial cells</td>
<td>OR2F1, OR2W1, etc.</td>
<td>Bourgeonal, bergamot oil, citronellal, nonanal and hexadecanal</td>
<td>Decrease in serotonin secretion</td>
<td>Human</td>
<td>Microarray, ISH, ICC, IHC or immunoblot</td>
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<td></td>
<td>A549 lung cancer cells</td>
<td>OR2J3</td>
<td>Helional</td>
<td>Inhibition of cell proliferation, apoptosis and migration</td>
<td>Human</td>
<td>RT-PCR, ICC, IHC or immunoblot</td>
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<td>Prostate</td>
<td>Prostate cancer tissue cells and cell lines</td>
<td>OR51E2</td>
<td>β-Ionone (antagonist α-ionone)</td>
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<td>Human</td>
<td>Microarray, RT-PCR, qPCR, IHC or immunoblot</td>
<td>17,30,131,130,161</td>
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<td></td>
<td></td>
<td>OR51E1</td>
<td>Nonanoic acid</td>
<td>Inhibition of cell proliferation and inhibition of AR-mediated cellular senescence</td>
<td>Human</td>
<td>RNA-seq, RT-PCR, ICC or IHC</td>
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and neurogenesis. Transcriptional analyses have shown that all cells and tissues investigated thus far express at least a few ORs. Recent sequencing studies demonstrated that at least some ORs, such as OR2W3 and OR51E1, were expressed in all the tissues analysed, whereas others were exclusively expressed in only one tissue, such as OR4N4 in the testis, which is in line with other transcriptional analyses. These results suggest that ORs may have a unique functionality in the expressed tissues.

### Signalling pathways

Interestingly, ectopic ORs are involved in various signalling pathways in extra-nasal tissues that require unique and diverse signalling components. For example, Ca2+ signalling pathways are activated in a tissue-specific manner by ectopic ORs in different tissues. The canonical OR signalling model involves heterotetrameric CNG channels, particularly CNGA2, and also CNGA4 and CNGB1; however, these channels were not detected in most non-nasal tissues. CNGA1, which can form exogenous functional homomeric channels and is activated by cGMP, is the most prominent channel in peripheral cells and tissues and may function as the CNG channel used by ectopic ORs. The native cone photoreceptor channel CNGA3 is expressed and functional in spermatozoa. This channel, together with the cation channel of sperm (CatSper), is responsible for OR-mediated chemotaxis in spermatozoa by controlling Ca2+ levels.

Other studies have suggested that OR-dependent intracellular Ca2+ elevation may be mediated by the PLC–Ins(1,4,5)P3–sarco/endoplasmic reticulum Ca2+–ATPase pathway in colorectal cancer cells. In addition, calcium channel inhibitor studies have suggested the involvement of TRP channels, voltage-gated L-type Ca2+ channels or Ca2+ release-activated channels. In melanocytes, the OR51E2-triggered Ca2+ signal is generated by both intracellular stores and extracellular Ca2+ influx. Interestingly, a decrease in intracellular Ca2+, mediated by the cGMP-dependent protein kinase G (PKG) was observed when OR2J3 was activated by helional in non-small-cell lung cancer.

Moreover, in prostate cancer cells, OR51E2 is activated by β-ionone, and is involved in distinct processes, including sarcoma tyrosine kinase (SRC)-dependent induction of TRPV-mediated Ca2+ influx, and the induction of various downstream signalling molecules, including p38 mitogen-activated protein kinase (MAPK) and JUN N-terminal kinase (JNK)–stress-activated protein kinase (SAPK). In advanced castration-resistant prostate cancer cells, β-ionone alternatively decreased the phosphorylation of ribosomal protein S6 kinase (p70 S6K; also known as S6K1). In addition, at lower concentrations of β-ionone, a pathway involving G, and downstream phosphoinositide 3-kinase (PI3K)–AKT signalling may be induced; this mediates different physiological processes in vitro and in vivo.

These findings demonstrate that ectopic ORs mediate intracellular signal transduction through a variety of mechanisms. Given the diverse signalling pathways regulated by ectopic ORs, these receptors have distinctive functions and may represent potential novel therapeutic targets for several disorders.

### Functions and therapeutic potential

It has been suggested that odorant phytochemicals in fruits and vegetables are sensory cues for health and nutritional value and that diverse odorant phytochemicals are bioactive substances with pharmacological activities. The physiological and pathophysiological effects of odorants may be due to the activation of ectopic ORs in extra-nasal tissues. Key physiological functions and ORs that represent the most promising

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**Table 1 (cont.) | Overview of key ectopic olfactory receptor expression patterns and their functions**

<table>
<thead>
<tr>
<th>Organ or tissue</th>
<th>Cell type</th>
<th>Olfactory receptors</th>
<th>Ligand</th>
<th>Function</th>
<th>Species</th>
<th>Detection techniques</th>
<th>Refs</th>
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<tr>
<td>Skin</td>
<td>Primary and cultured keratinocytes</td>
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<td><em>OR2A4</em> (agonist)</td>
<td>Activation of keratinocyte proliferation</td>
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<td></td>
<td>OR2A7 and OR51B5</td>
<td><em>OR2A4/OR2A7: cyclohexyl salicylate</em> OR51B5: isononyl alcohol</td>
<td>Cytokinesis and cell proliferation, migration and regeneration</td>
<td>Human</td>
<td>RNA-seq, RT-PCR, ICC and IHC</td>
<td>11</td>
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<tr>
<td></td>
<td>Primary melanocytes</td>
<td>OR51E2</td>
<td>β-ionone (agonist)</td>
<td>Inhibition of melanocyte proliferation and melanogenesis</td>
<td>Human</td>
<td>RT-PCR, immunoblot or ICC</td>
<td>25</td>
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<tr>
<td></td>
<td>Testis</td>
<td>OR1D2</td>
<td>Bourgeonal (agonist)</td>
<td>Sperm chemotaxis</td>
<td>Human</td>
<td>RT-PCR, ICC or immunoblot</td>
<td>9,316,167</td>
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<td></td>
<td>OR7A5 and/or OR4D1</td>
<td>*OR7A5: Myrac, PI-21788 and vernaldehyde OR4D1: PI-23472 and PI-23474</td>
<td>Sperm swimming speed</td>
<td>Human</td>
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AR, androgen receptor; ICC, immunocytochemistry; IHC, immunohistochemistry; IL-8, interleukin-8; ISH, in situ hybridization; MAPK, mitogen-activated protein kinase; MCFA, medium-chain fatty acid; qPCR, quantitative PCR; RNA-seq, RNA-sequencing; RT-PCR, reverse transcription PCR; SCFA, short-chain fatty acid.

*Ligands are agonists unless indicated otherwise.
Hyperventilation. Abnormal fast and deep breathing; the result of either an emotional status or a physiological condition such as asthma.

Kidney function and blood pressure regulation. Several ORs are significantly expressed in the kidney. For example, a recent study demonstrated the expression of OR51E1 and OR11H7 in the human proximal tubule cell line HK-2 (REF. 121). Activation of the two ORs by the ligands isovaleric acid and 4-methylvaleric acid led to intracellular increases in Ca²⁺ via the cAMP-mediated pathway122.

In addition, OLF78 (the mouse homologue of OR51E2) has been shown to be expressed in the renal arterioles of the juxtaglomerular apparatus122,123, which secrete the hormone renin into the bloodstream. Renin hydrolyses angiotensinogen to angiotensin I, which is further cleaved by angiotensin-converting enzyme (ACE) to angiotensin II, a potent vasoconstrictor.

The endogenous ligands that bind OLF78 are SCFAs, including acetate and propionate. These SCFAs are primarily produced by the gut microbiota following fermentation of dietary fibres and are absorbed into the bloodstream, where they play important roles in different physiological processes, including inflammation and metabolism169.

Studies involving mice lacking OLF78 or FFAR3 (another SCFA receptor) revealed that these SCFA receptors exert substantial modulatory effects on blood pressure in response to signals generated via gut microorganisms through the stimulation of renin secretion and vasorelaxation, respectively122. The authors hypothesized that the opposing responses of these receptors may protect against changes in blood pressure as a consequence of normal physiological variations in SCFA concentration. This study expands the range of physiological processes that are modulated by SCFA receptors to include blood pressure regulation and adds an OR to the SCFA receptor family.

Hypoxia sensing. Animals have an intrinsic homeostatic response to changes in oxygen availability, and ORs have been implicated in the regulation of the acute response to hypoxia.

OLF78 is expressed in several oxygen-sensitive tissues such as heart, lung and glomus cells of the carotid body7,122,170. The carotid body is a chemosensory organ at the carotid artery bifurcation that monitors blood oxygen and stimulates breathing within seconds when oxygen declines, thus functioning as a hypoxia sensor171. Chang et al.172 suggested that OLF78 is activated by lactate, a signature metabolite present during hypoxia, to induce hyperventilation under hypoxic conditions. In OLF78-deficient mice, hypoxia-induced carotid body activity was reported to be diminished, although glomus cells are structurally intact and normal171.

However, a recent study was unable to replicate these findings and reported that Olf78⁻/⁻ mice had a normal ventilatory response to hypoxia and that the physiological responses of single glomus cells to hypoxia and lactate were indistinguishable between wild-type and Olf78⁻/⁻ mice107. These discrepant findings may be related to the high sensitivity of the behavioural response to hypoxia to differences in assay conditions and genetic background. Although lactate has been reported as a partial agonist of OLF78, it has not been confirmed to be a ligand for the human homologue, OR51E2 (REF. 171). Moreover, the reported effective concentration for half-maximum response (EC₅₀) of lactate for OLF78 has varied between reports171,172,173. Therefore, there is still no clear consensus as to whether lactate is a functional ligand for OLF78 under physiological conditions.
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FFAR4, free fatty acid receptor 4; GLP1, glucagon-like peptide 1; PUFA, polyunsaturated fatty acid; TAS1R, taste receptor family 1. *Mouse receptor; studies are underway to identify the target of azelaic acid in humans.
Muscle regeneration. Several ORs are expressed in muscle, particularly during myogenesis and muscle regeneration. OLFR16 plays a critical role in muscle tissue regeneration. The signalling pathways of OLFR16 in muscle are unclear; however, activation of OLFR16 by the ligand Lyral increases intracellular cAMP levels and results in myocyte migration, myofibre branching and myogenesis, which are all associated with muscle regeneration. OLFR16 in muscle tissue regulates muscle cell migration and adhesion. These effects were...
negated in muscle cells with Olfr16 gene knockdown. Although the endogenous ligand remains uncharacterized, the treatment of myocytes with crushed muscle extracts results in a dramatic increase in myocyte migration in a cAMP-dependent manner, which is abrogated in Olfr16 small interfering RNA-treated cells. These findings suggest that muscle cells secrete a soluble ligand for OLFR16 during muscle regeneration and that ligand-dependent activation of OLFR16 is required for proper skeletal muscle regeneration.

In addition, OLFR16 regulates cell–cell adhesion and myotube formation. In vivo myogenesis and the loss of OLFR16 result in increased myofibre branching, which is commonly associated with muscular dystrophy; this involvement was further confirmed in a mouse model of muscular dystrophy (mdx). OLFR16 overexpression in mdx mice reduced myofibre branching after muscle regeneration in non-dystrophic mouse muscle and decreased the severity of myofibre branching in mdx mouse muscle. Muscles from mdx mice overexpressing OLFR16 showed significantly less damage to eccentric contractions than control mdx muscle. These results indicate that OLFR16 is necessary for proper regeneration of muscle tissue, suggesting a novel function for ORs in tissue repair. Alternatively, in human skeletal myoblasts, activation of OR2H2 with its ligand aldehyde 13–13 might lead to a reduction of myoblast fusion.

**Hepatic lipid metabolism.** Several ectopic ORs have been implicated in the regulation of hepatic lipid metabolism. For example, OR1A1 is expressed in the plasma membrane of HepG2 cultured human hepatocytes. When activated by the aromatic ligand (−)–carvone, a major compound in spearmint essential oil, the PKA signalling pathway is stimulated without affecting intracellular Ca²⁺ levels (Fig. 1); this ultimately decreases intracellular triglyceride concentrations. In addition, OR1A1 and its mouse homologue, OLFR43, have been implicated in GLP1 secretion in enteroendocrine cells. Injection of geraniol, a ligand of OLFR43, in diabetic mice improves their glucose and insulin tolerance with increased GLP1, providing another mechanism for reducing hepatic lipid accumulation in diabetes.

Furthermore, OLFR16 and OR10J5 receptors are involved in the regulation of hepatic lipid metabolism through the cAMP–PKA pathway in cultured cells. OR10J5 is activated by Lyral as well as α-cedrene, and the stimulation of cells with α-cedrene reduces hepatic lipid concentrations.

Together, these studies suggest that hepatocyte-expressed ORs may represent potential therapeutic targets for hepatic steatosis and non-alcoholic fatty liver disease. Although endogenous ligands for OR1A1, OR10J5 and OLFR16 have not been identified, these ORs can be activated by exogenous aromatic ligands such as Lyral and (−)–carvone, which may be inhaled or derived from the diet. As the regulation of lipid metabolism in hepatocytes is critical, it is possible that many regulatory pathways finely tune metabolism in a redundant manner.

**Bronchoconstriction.** Chronic inflammatory lung diseases such as asthma, chronic obstructive pulmonary disease and allergies occur owing to airway smooth muscle contraction, increased smooth muscle mass and mucus plugs. ORs expressed in the airway play a role in regulating bronchoconstriction. The expression of several ORs and of the canonical olfactory machinery, ACIII and Gαolf, was confirmed by RNA-sequencing (RNA-seq) analysis and quantitative PCR in human airway smooth muscle cells. The expression of four ORs (OR51E2, OR1J1, OR2A1 and OR6A2) in human airway smooth muscle cells, with OR51E2 representing the most highly enriched OR transcript in lung-resident cells, and further investigated the functions of OLFR78 and OR51E2 in airway smooth muscle stiffness and relaxation. Cytoskeletal remodelling was measured by beads tethered to the cytoskeleton through cell surface integrin receptors. Experiments assessing intracellular cytoskeletal remodelling by measuring spontaneous nanoscale bead motion, demonstrated that SCFAs significantly reduced cytoskeletal remodelling in both mouse and human airway smooth muscle cells, and these effects were negated in cells with OLFR78 and/or OR51E2 knockdown. Proliferation of airway smooth muscle cells was reduced by SCFAs, which may explain the reduced cytoskeletal remodelling, at least in part. SCFAs also mitigated the increased mass of asthmatic airway smooth muscle cells.

These findings suggest that OR51E2 is a functional receptor in airway smooth muscle, and its ligand could ameliorate the increased airway smooth muscle mass associated with asthma pathology.

Kalbe et al. showed that OR2AG1 and OR1D2 are also expressed in human airway smooth muscle cells. Application of OR2AG1 and OR1D2 agonists triggered transient Ca²⁺ increases in human airway smooth muscle cells via a cAMP-dependent signal transduction cascade. Interestingly, activation of OR2AG1 using amyl butyrate might inhibit the histamine-induced contraction of human airway smooth muscle cells, resulting in muscle relaxation. By contrast, stimulation of OR1D2 using bourgeonal increased cell contractility and elicited the secretion of interleukin-8 (IL-8) and granulocyte–macrophage colony-stimulating factor. These effects were inhibited by the OR1D2-specific antagonist undecanal. These findings support further investigation into the effects of OR modulation (for example, OR51E2 agonists, OR2AG1 agonists or OR1D2 antagonists) in models of early-stage chronic inflammatory lung diseases such as asthma, chronic obstructive pulmonary disease and allergies.

**Obesity and subcutaneous fat reduction.** Genetic variation of ORs has been linked with obesity. For example, copy number variations in OR7D4 and other OR7 genes on chromosome 19q13 are reportedly linked to eating behaviours and reduced levels of adiposity. In addition, a frequently duplicated region on chromosome 11q11 and three OR genes (OR4P4, OR4S2 and OR4C6) have all been associated with obesity. A genetic association between variations in the OR14C36 gene at the 1q44 locus and extreme obesity has been reported.
Melanogenesis
The production of melanin from the amino acid tyrosine via complex metabolic pathways; the process is carried out by melanocytes located in the bottom layer of the skin epidermis.

Next-generation sequencing
A high-speed nucleic acid sequencing technique, typically characterised by being highly scalable, allowing the entire genome to be sequenced at once.

also been reported10. These associations between OR gene variants and obesity may be related to an altered sense of smell and altered eating behaviour; however, it is intriguing to speculate that these ORs may have critical functions in extra-nasal tissues, where they influence adiposity. Further investigations should examine whether ligands binding to OR variants can affect food choices and eating behaviours and whether ectopic ORs found in extra-nasal tissues have critical roles in regulating adiposity.

Transcriptome profiling has indicated that many OR genes are significantly expressed in adipose tissue19,11,12, although the expressed OR genes differ depending on the study design, the type of fat tissue used and the analyses employed. Lee and colleagues11 reported that liver and adipose tissue in mice fed either normal chow or a high-fat diet showed high-level expression of an OR, OLF544, which plays a role in regulating energy and lipid metabolism (FIG. 1). When the OLF544 ligand azelaic acid was administered orally to wild-type mice fed a high-fat diet for 6 weeks or obese (ob/ob) mice, the body weight and adiposity (especially subcutaneous fat) of the mice decreased without affecting their food intake. These azelaic acid-induced metabolic alterations are associated with increased hepatic fatty acid oxidation and the stimulation of brown adipose tissue thermogenesis. Although the presence of a human homologue of mouse OLF544 currently remains unclear on the basis of sequence analysis, azelaic acid was found to regulate lipid metabolism in human primary adipocytes and cultured hepatocytes, inducing adipose lipolysis and hepatic fatty acid oxidation (S.-J. Lee, unpublished observations).

Thus, it is possible that azelaic acid may have therapeutic applications in the treatment of obesity.

Wound healing, hair growth and skin hypopigmentation.
The skin is the outer barrier of the human body and is constantly exposed to a variety of external chemicals. Several ORs have been identified in distinct skin-specific cell types of the epidermis, including keratinocytes and melanocytes20,12,13. Basal keratinocytes, the main cell type responsible for natural wound healing, express high levels of OR2AT4 (REF. 19). Activation of OR2AT4 by Sandalore strongly increases Ca2+ via a cAMP-dependent pathway and the phosphorylation of protein kinases, promoting human keratinocyte proliferation and migration (FIG. 1). Additionally, activation of OR2AT4 might induce pannexin-mediated cell–cell communication via ATP between keratinocytes and trigeminal neurons in a co-culture system21. The effects of Sandalore on keratinocyte proliferation and migration improved cutaneous wound healing in a human ex vivo system22. The antagonist oxyphenylon inhibited this process.

Keratinocytes express at least two other ORs, OR2A4/OR2A7 and OR51B5, which participate in keratinocyte cytokinesis14. The activation of the receptor OR2A4/OR2A7 by the specific agonist cyclohexyl salicylate may increase cell proliferation and IL-1 production, whereas isononyl alcohol-dependent activation of OR51B5 may increase the migration and regeneration of keratinocytes and the secretion of cytokines, such as IL-6 (REF. 13). These findings suggest that several ectopic ORs in the skin may be involved in the regulation of keratinocyte function and may have potential applications in shortening wound-healing time.

OR2AT4 is also expressed in human scalp hair follicles15. Stimulation of OR2AT4 in organ-cultured hair follicles with Sandalore or Brahmanol, another mild woody-like odour, significantly retarded spontaneous catagen (involuting or repressing phase of hair growth) development of the hair follicle ex vivo and significantly decreased apoptosis of hair matrix keratinocytes. OR2AT4 stimulation also prolonged the anagen (active growth phase) of the hair follicle by increasing IGF1 production16. Thus, OR2AT4-mediated signaling is required for both maintaining human scalp hair follicles in anagen phase and suppressing keratinocyte apoptosis in the hair matrix. Silencing OR2AT4 by coadministering the specific OR2AT4 antagonist Phenirat significantly reversed the Sandalore-induced intrafollicular upregulation of IGF1 in organ-cultured human hair follicles17. A preliminary pilot clinical trial or hair pull test confirmed the ex vivo studies: application of a Sandalore-containing lotion for 12 weeks significantly lowered hair loss by about 20%, as compared with the control group (H. Hatt, unpublished observation). These results suggest that OR-dependent chemoreceptors may represent novel targets for the regulation of hair growth.

Cutaneous melanogenesis occurs when skin is exposed to the Sun and provides protection against the harmful effects of ultraviolet radiation; however, pathological hyperpigmentation can also occur. The activation of OR51E2 by β-ionone leads to improved melanogenesis and dendritogenesis, as well as reduced cell proliferation in primary cultures of human melanocytes18 (FIG. 2); effects which were compromised by cells with OR51E2 knockdown or antagonist treatment. These results were associated with decreases in cell proliferation and an increased capacity for differentiation among melanocytes; melanin content also increased significantly. It is possible that β-ionone, which is often found in beauty care products, may play a role in the hyperpigmentation induced by cosmetics. In addition, the activation of OR2A4/OR2A7 by cyclohexyl salicylate elevated intracellular cAMP and Ca2+ levels and might induce p38 MAPK and reduce p42 MAPK (also known as MAPK1) and/or p44 MAPK (also known as MAPK3) phosphorylation in melanocytes, in conjunction with increased melanin biosynthesis and inhibition of melanocyte growth19. Together, these finding suggest that some ORs may represent potential targets for the treatment of pigmentation disorders.

Heart rate. Next-generation sequencing analysis determined the expression pattern of more than ten different ORs in adult and fetal human heart muscle cells20,21, with OR51E1 representing the most highly expressed OR in both cardiac development stages22,23. Medium-chain fatty acids (MCFAs), consisting of 6–12 carbon atoms, were found to be potent agonists for OR51E1, with the C9-nonanoic acid showing the highest affinity, while 2-ethylhexanoic acid functioned as a competitive
**a** Prostate cancer cells

- Nonanoic acid

**b** Melanoma/melanocytes

- β-lonone
- 19-OHAD
- α-lonone

**c** Lung carcinoma cells

- Helional

**d** Colon cancer cells

- Troenan

**e** Leukaemia cells

- Sandalore
- Oxyphenylon

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Fig. 2 | **Activation of ectopic olfactory receptors regulates cancer cell growth.** Activation of olfactory receptors (ORs) OR51E1 (prostate; part a), OR51E2 (melanoma; part b), OR2J3 (lung; part c), OR51B4 (colon; part d) and OR2AT4 (leukaemia; part e) can inhibit cancer in the cells shown through the regulation of different signalling pathways, as indicated. Stimulation of OR51E2 inhibits cell proliferation but also induces invasiveness, metastasis and transdifferentiation of prostate cells. Receptor ligands are shown in green. Dashed lines indicate that these steps are postulated and were not demonstrated with experimental data. 19-OHAD, 19-hydroxyandrostenedione; AR, androgen receptor; CAMKII, Ca\(^{2+}\)/calmodulin-dependent kinase II; CREB, cAMP-response element-binding protein; DAG, diacylglycerol; ER, endoplasmic reticulum; ERK, extracellular-signal-regulated kinase; Ins(1,4,5)P\(_3\), inositol 1,4,5-trisphosphate; JNK, JUN N-terminal kinase; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; MITF, microphthalmia-associated transcription factor; ORAI, calcium release-activated calcium; p70 S6K, ribosomal protein S6 kinase; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A; PKC\(_\theta\), protein kinase C\(_\theta\); PLC, phospholipase C; PSA, prostate-specific antigen; PYK2, protein tyrosine kinase 2; RSK, ribosomal S6 kinase; SAPK, stress-activated protein kinase; SRC, sarcoma tyrosine kinase; STAT3, signal transducer and activator of transcription protein 3; TRPA1, transient receptor potential ion channel subfamily A, member 1; TRPM, TRP subfamily M; TRPV6, TRP subfamily V, member 6.
antagonist. Studies have suggested that MCFAs can be used to identify potential therapeutic targets for various metabolic and inflammatory disorders, including obesity, type 2 diabetes, cardiovascular and intestinal diseases and atherosclerosis. OR51E1-activating MCFAs are available from diets or from lipolysis products from adipose tissues. Interestingly, some MCFAs including nonanoic acid have been detected at receptor-activating concentrations in the plasma and in epicardial adipose tissue, particularly in patients with diabetes mellitus.

The activation of OR51E1 by MCFAs has been shown to induce negative inotropic effects in human explanted heart preparations (cardiac trabeculae and slice preparations of explanted ventricles) and leads to negative chronotropic effects in human stem cell-derived cardiomyocytes. These findings suggest that OR51E1 may be involved in the regulation of heart rate.

Cancer. OR expression is upregulated in several cancer tissues compared with levels in normal tissues — for example, OR51E2, OR2B6, OR2C3 and OR10H1 are upregulated in prostate cancer cells, breast carcinoma cells, melanoma cells and urinary cancer cells, respectively.

The expression of OR51E2 has been studied in particular detail (Fig. 2). The expression of OR51E2 and the closely related OR51E1 is strongly upregulated in prostate carcinomas compared with healthy prostate cells, suggesting their potential use in the diagnosis of prostate tumours and as therapeutic targets.

Overexpression of OR51E2 in phosphatase and tensin homologue (PTEN)-deficient mice accelerates prostate cancer development and progression. Intriguingly — consistent with the long-known anti-proliferative effect of the isoprenoid β-ionone on carcinoma cells — this OR51E2 agonist decreased proliferation but increased the invasiveness of human prostate cancer cells. Meanwhile, stimulation of OR51E2 with the endogenous agonist 19-hydroxyisodanshodenedione facilitated cellular transformation, resulting in neuroendocrine trans-differentiation, indicating that the activation of OR51E2 in prostate cancer may contribute to the development of non-proliferating foci. Similarly, the activation of OR51E2 in primary cultures of melanoma cells derived from metastatic growth and vertical growth phases appears to inhibit cell proliferation and migration and might induce apoptosis (Fig. 2).

OR51E2 has also been identified as a prostate tumour antigen with implications for cancer immunotherapy. Novel HLA-A2-restricted OR51E2-derived peptides, which frequently induced peptide-specific T cell responses and killed LNCaP prostate cancer cells, have been developed, with the potential to be used as diagnostic markers and immune targets for the development of anticancer vaccines.

OR51E1 has also been reported to influence prostate cancer growth, and stimulation of cells with the ligand nonanoic acid significantly reduced proliferation and induced cellular senescence in LNCaP cells (Fig. 2). Interestingly, activation of OR51E1 may interfere with the androgen receptor (AR), as the expression levels of AR target genes decrease in response to nonanoic acid.

Studies have demonstrated the overexpression and functional role of ORs in many other cancers. For example, OR2J3 is expressed in human lung carcinoma tissues and in the non-small-cell lung cancer cell line A549 (REF. 15), and its activation with the OR2J3 agonist helional triggered the release of intracellular Ca\(^{2+}\) and induced extracellular-signal-regulated kinase 1 (ERK1) and/or ERK2 phosphorylation via PI3K signalling pathways, resulting in the induction of apoptosis and the inhibition of cell proliferation and migration (Fig. 2).

OR1A2, the paralogous receptor to OR1A1, is expressed in hepatic cancer cells. The (−)-citronellal-activated olfactory receptor OR1A2 evokes a cAMP-dependent cytosolic Ca\(^{2+}\) increase in the hepatocellular carcinoma Huh7 cell line, in which the olfactory signalling components ACIII, G\(_\text{olf}\) and the CNG channel subunit CNGA1 are expressed. Moreover, (−)-citronellal mediates the phosphorylation of p38 MAPK and promotes a reduction in cell proliferation. These findings are consistent with various studies that have described the anti-carcinogenic properties of terpenes.

In addition, activation of the recently identified and deorphaned OR51B4 affects the physiology of colorectal cancer cells and native human colon cancer tissues (Fig. 2). The agonist Troenan inhibits cell proliferation and migration and actin filament formation, and it stimulates apoptosis and the release of serotonin. These inhibitory effects on cell growth may be mediated via a PLC-dependent signalling pathway, which might include a Ca\(^{2+}\) influx through calcium release from calcium release-activated calcium (ORAI) channels, increased p38 MAPK phosphorylation to induce apoptosis and decreased AKT and mTOR kinase phosphorylation for cancer inhibition. These results reveal OR51B4 as a potential novel target for the treatment of colorectal cancer. As colon cancer is accessible from the lumen, the rectal or oral application of Troenan is conceivable.

Finally, OR expression and function have also been implicated in acute and chronic myelogenous leukaemia (Fig. 2). Sandalore-dependent activation of OR2AT4 and a CAMP-dependent pathway leads to reduced proliferation and increased apoptosis in the acute and chronic myelogenous leukaemia cell line K562, by arresting the cell cycle at the G0–1 and G2–M phases. Furthermore, in the presence of an agonist, growth inhibition is accompanied by an increased number of haemoglobin-carrying erythroid cells that originate from immature myeloblasts. The inhibition of myeloid leukaemia cell proliferation can also be mediated by another OR, the isononyl alcohol-activated OR51B5 (REF. 13), which is the most highly expressed OR in K562 cells. These findings highlight novel potential targets for the treatment of acute and chronic myeloid leukemias.

It is striking that although approximately 40% of pharmacological tools target GPCRs, relatively few of these are used to treat cancer. ORs are easily the largest group within the mammalian GPCR gene superfamily. As many ORs are dysregulated in malignant tissues, they are promising targets for treating and diagnosing cancer, although further studies are necessary to further delineate the role of ectopic ORs in cancer.
Ectopic taste receptors

Tissue expression

Hofer et al. showed that the gustatory G protein α-gustducin is expressed in brush cells of the stomach and duodenum in rats, introducing the concept that taste signalling also occurs in extra-oral tissues. Following the discovery of a large family of gustducin-linked bitter taste receptors in taste receptor cells, the expression of bitter TAS2Rs was identified in mouse enteroendocrine cells. Subsequent studies showed ectopic expression of sweet, bitter and free fatty acid taste receptors in the gut but also in various other tissues, including adipose tissue, airways, blood vessels, bone, brain, breast, heart, immune cells, kidney, pancreas, skin, testis, thyroid, urethra and urinary bladder.

Signalling pathways

The signalling pathways in extra-oral tissues have been studied mainly for TAS2Rs. Cell-autonomous regulation of TAS2Rs has been demonstrated in the airway epithelia and smooth muscle cells. In human sinonasal motile cilia, a bitter-induced Ca²⁺ release regulates the ciliary beat frequency via nitric oxide and PKG, which phosphorylates ciliary proteins. In smooth muscle cells, bitter tastants induce localized Ca²⁺ release, which activates large conductance Ca²⁺-activated K⁺ channels to induce hyperpolarization and consequent muscle relaxation. Although several other mechanisms have been proposed, including a critical role for the βγ-subunits of gustducin, the relaxant properties of TAS2R agonists are confirmed in all studies.

TAS2Rs play a paracrine role in mouse olfactory chemosensory cells in the nasal cavity and in gut enteroendocrine cells, in which a bitter-induced Ca²⁺ release causes acetylcholine or cholecystokinin (CCK) release, respectively. Acetylcholine activates trigeminal afferent nerves containing nociceptors to evoke neurogenic inflammation, whereas CCK upregulates the efflux transporter ATP-binding cassette B1 to prevent further ingestion of food but are also present on parietal cells to regulate acid secretion.

After stomach emptying, food arrives in the small intestine, which is the major site of digestion and absorption. Fatty acids (via FFAR4) and amino acids (via CaSR and TAS1R1–TAS1R3) are sensed by taste receptors on I cells, which secrete CCK, a satiety hormone that delays gastric emptying and triggers the release of digestive enzymes from the pancreas and bile. In obese mice, daily gavage of denatonium benzoate or quinine over a 4-week period decreased weight gain in a α-gustducin-dependent manner.

The endocrine mechanism of TAS2Rs in the gut epithelium involves gustducin and activation of several elements of the canonical taste signalling pathway to induce the release of satiety hormones. A similar gustducin-mediated pathway is involved after the activation of sweet taste receptors by glucose to induce the release of the incretin hormone GLP1 but not the hunger hormone ghrelin. The signalling pathway activated by FFAR1 and FFAR4 involves Gαi in GLP1-containing cells and Gβγ in ghrelin-containing cells. Downstream of FFAR1 and FFAR4, the Ca²⁺ signalling pathway involves TRP channels.

Functions and therapeutic potential

Ectopic taste receptors are involved in a variety of processes, including diabetes and metabolic disease, asthma and inflammatory diseases. Their functions in the gastrointestinal tract in appetite regulation have been investigated extensively. Those functions and receptors that likely represent the most promising targets for therapeutic application are discussed below and summarized in Table 2.

Appetite regulation in the gastrointestinal tract. The gut senses meal composition and volume. Taste receptors have been described in almost all enteroendocrine cells in the gut and regulate the release of satiety hormones in response to a meal (reviewed previously). Both in vitro studies (cell lines, mucosal segments and isolated crypts) and in vivo studies of taste receptor-deficient mice have been used to demonstrate a role for taste receptors in gut hormone release.

In the stomach, the products of protein and lipid breakdown are sensed by fatty acid receptors (FFAR4), the umami receptor (TAS1R1–TAS1R3) and other amino acid sensors (CaSR and GPRC6A) on P/D1 cells, which regulate the release of the hunger hormone ghrelin. Dietary protein hydrolysates further interact with amino acid taste receptors on smooth muscle cells to prevent further ingestion of food but are also present on parietal cells to regulate acid secretion.

After stomach emptying, food arrives in the small intestine, which is the major site of digestion and absorption. Fatty acids (via FFAR4) and amino acids (via CaSR and TAS1R1–TAS1R3) are sensed by taste receptors on I cells, which secrete CCK, a satiety hormone that delays gastric emptying and triggers the release of digestive enzymes from the pancreas and bile. In obese mice, daily gavage of denatonium benzoate or quinine over a 4-week period decreased weight gain in an α-gustducin-dependent manner.

The therapeutic benefits of bitter plant extracts for treating obesity and diabetes are recognized by traditional medicine practitioners across the world and, in some cases, are supported by scientific evidence. For example, some bitter-tasting herbal medicines (for example, berberine, Hoodia gordonii and bitter melon extract) affect the release of appetite-suppressing hormones (for example, GLP1 and CCK) via TAS2Rs and have exhibited weight-reducing or antidiabetic effects in animal models. However, clinical studies on the effects of bitter melon extracts in patients with obesity and diabetes lacked an appropriate study design and were inconclusive. The bitter herbal tea yerba mate delayed gastric emptying and promoted weight loss in patients with obesity who were treated for 45 days.
Fig. 3 | Pathways activated by ectopic taste receptors in the gut and adipose tissue. a | Tastants (for example, sweet, amino acids, bitter and fat) activate specific chemosensors on enteroendocrine cells in the gut that regulate the release of gut hormones involved in appetite signalling. b | Bitter and sweet tastants inhibit the differentiation of pre-adipocytes to mature white adipocytes. Omega-3 fatty acids or free fatty acid receptor 4 (FFAR4) agonists promote adipogenesis in white and brown pre-adipocytes via FFAR4 activation. In white adipocytes, they increase insulin sensitivity and reduce inflammation by activating macrophages. FFAR4 activation promotes the browning of white fat and regulates thermogenic activation in beige and brown adipocytes to increase energy expenditure and to ameliorate insulin resistance, in part by releasing fibroblast growth factor 21 (FGF21). CCK, cholecystokinin; GIP, gastric inhibitory polypeptide; GLP1, glucagon-like peptide 1; PUFA, polyunsaturated fatty acid; PYY, peptide YY; TAS1R, taste receptor family 1.
In animal models of obesity, it decreased lipid accumulation and improved diabetes phenotypes\(^{202,203}\). Of note, given the complex and non-selective nature of bitter herbs, a direct link to the activation of TAS2Rs and the precise mechanisms mediating their health benefits still require further investigation. Recently, Kok et al.\(^{273}\) identified a pure derivative of a hops isohumulone, KDT501, that signals through TAS2R108 (also known as TAS2R4) to improve multiple features of metabolic disease in diet-obese mice. In humans with insulin resistance and prediabetes, KDT501 reduced plasma triglyceride levels\(^{276}\) (NCT02444910). Additional randomized trials of a single bitter compound are needed.

Adipogenesis. The taste-associated G protein α-gustducin is expressed in adipose tissue, and high-fat diet-induced α-gustducin\(^{-/-}\) mice show an obesity-resistant phenotype owing to increased brown adipose tissue thermogenesis\(^{150}\) (Fig. 3b). The specific taste receptor involved was not identified, but a similar obesity-resistant phenotype has been described in sweet taste receptor-deficient mice\(^{205}\). Furthermore, both α-gustducin and bitter taste receptors (TAS2R108 and TAS2R135) are expressed in white adipose tissue and mouse 3T3-F442A pre-adipocytes. In addition, a direct effect on adipocyte metabolism has been suggested, as bitter agonists inhibited 3T3-F442A differentiation into mature adipocytes, as shown by a decrease in lipid accumulation\(^{201}\).

FFAR4 is expressed in white adipose tissue, and high-fat diet-fed FFAR4\(^{-/-}\) mice have an obese phenotype with increased fatty liver and hepatic lipogenesis but decreased adipocyte differentiation and lipogenesis; this indicates that FFAR4 contributes to the process of adipogenesis\(^{203}\). A recent study showed that FFAR4 activation promotes the browning of white fat and regulates thermogenesis in beige and brown mouse adipocytes, increasing energy expenditure and ameliorating insulin resistance in part by releasing fibroblast growth factor 21 (FGF21)\(^{200}\) (Fig. 3b). Given that human adipocytes can acquire a beige phenotype after incubation with eicosapentaenoic acids\(^{207}\), further studies are needed to investigate whether FFAR4 activation also induces brown and beige adipocyte differentiation and thermogenic activation in humans. In addition, FFAR4 is highly expressed in adipose macrophages, and the administration of omega-3 fatty acids, which are ligands of FFAR4, to high-fat diet-fed obese mice reduced inflammation and increased insulin sensitivity through FFAR4\(^{160,202}\), (Fig. 3b).

Randomized clinical trials have shown that omega-3 polyunsaturated fatty acids reduce waist circumference and triglyceride levels but not body weight. A sex-specific effect was observed on insulin resistance\(^{160,278,279}\). The identification of a mutation in the FFAR4 gene, which resulted in attenuated signalling associated with an increased risk of obesity in European populations, further supports a role for FFAR4 in obesity\(^{202}\).

Finally, FFAR1 is highly expressed in pancreatic β-cells, but divergent effects have been reported on insulin secretion in mice\(^{202,203}\). In contrast to FFAR4, the development of FFAR1 agonists has experienced a serious setback owing to the failure of a phase III trial with fastigilam that induced liver injury\(^{202}\).

**Immunity, inflammation and asthma.** Bitter taste receptors are expressed in several cell types within the lungs, including epithelial, chemosensory, smooth muscle and immune cells, and play an important role in the innate immune response and bronchodilatation\(^{22}\). In the upper airways, TAS2R38 is expressed on motile cilia of human sinonasal ciliated epithelial cells, and upon activation, Ca\(^{2+}\)-dependent nitric oxide production increases ciliary beat frequency and mucus clearance\(^{221}\) (Fig. 4). Further fusion of nitric oxide in the airway surface liquid elicits direct antibacterial activity. The effect was mimicked by acyl homoserine lactones, which are quorum-sensing molecules produced by Gram-negative bacteria\(^{215,217}\). Human motile ciliated cells from the trachea and bronchi also express TAS2Rs, which increase ciliary beating\(^{216}\).

Solitary chemosensory cells (SCCs) in human primary sinonasal cultures express TAS2Rs that, upon stimulation with the bitter agonist denatonium benzoxate, induce the secretion of antimicrobial peptides to kill bacteria\(^{42}\). Sweet taste receptors in SCCs, activated by sugars in the airway lumen or sweet-tasting d-amino acids (d-Leu and d-Phe) produced by non-pathogenic Staphylococcus spp., blocked bitter-induced antimicrobial peptide secretion and inhibited biofilm formation of *Pseudomonas aeruginosa*\(^{214,277}\) (Fig. 4). Antimicrobial peptide secretion does not occur in human bronchial tissue and is thus specific for human upper airways. High glucose concentrations in the airway lining of patients with diabetes mellitus or chronic *rhinosinusitis* can promote airway infections by inhibiting bitter-induced antimicrobial production. Topical application of sweet taste receptor antagonists (for example, lactisole) may therefore be a useful therapeutic strategy to generate an antimicrobial response to bitter bacterial molecules and may represent a promising alternative to antibiotics. In mouse SCCs, activation of TAS2R38 results in a local inflammatory response and breath holding\(^{214,224}\). However, it is unknown whether a similar mechanism exists in human SCCs.

Finally, human upper airway smooth muscle cells also express TAS2Rs, which upon stimulation with bitter agonists induce airway smooth muscle relaxation\(^{216}\). In a mouse model of allergic airway inflammation and bronchial hyperresponsiveness, the TAS2R2 agonist quinine, administered as an aerosol, reduced bronchoconstriction and exceeded the therapeutic efficacy of β2-adrenergic agonists\(^{41}\). However, as quinine has multiple mechanisms of action, it is possible that these beneficial effects may not be mediated by TAS2Rs, and similar experiments using more specific bitter agonists should be performed. In mouse models challenged with allergens, (chloro) quinine, administered as an aerosol or intra-nasally, reduced hyperresponsiveness, allergen-induced airway inflammation, tissue remodelling and mucus accumulation\(^{215}\). Finally, bitter agonists inhibited the lipopolysaccharide-induced release of inflammatory mediators from the blood leukocytes of patients with asthma expressing TAS2R2 and suppressed histamine and prostaglandin D\(_2\) release from human primary mast cells\(^{217}\). These studies indicate that, in contrast to β2-adrenergic agonists, TAS2R agonists exert multiple effects that interfere with some of the key features of asthma.
Future directions and challenges

Ors and taste receptors are widely expressed in extra-nasal and extra-oral tissues, being highly expressed in several cancer cells and metabolically active tissues including gut, liver, muscle and adipose tissues (Tables 1,3). These ectopically expressed ORs and taste receptors exert diverse physiological effects depending on the tissue and cell type in which they are expressed and the signal transduction pathways they trigger (Figs 1–4). These ORs and taste receptors show substantial diversity in the activation of signalling pathways in different tissues. As the understanding of the functionalities of these receptors increases, their potential application in the diagnosis and treatment of several clinical disorders including cancer, obesity and subcutaneous fat reduction, wound healing, hair loss, metabolic disorders and upper respiratory infections is emerging (Table 2).

Regarding taste receptors, the TAS2R family represents a promising future target for the treatment of both asthma and obesity. Other promising novel targets within the taste receptor family are members of the FFAR family which influence inflammatory processes and are involved in the regulation of energy metabolism. Indeed, FFAR4 may influence metabolic disorders.

Regarding ORs, cancer represents one of the most promising therapeutic areas for further development. However, target candidates should fulfil the following criteria: good accessibility, high specificity and sensitivity, and high selectivity for a specific tissue. There are only a few cancer types that express ORs that at least partially fulfil these conditions, such as colorectal cancer (OR7C1), bladder cancer (OR10H1), melanoma (OR51E2) and leukaemia (OR51B5 and OR2AT4). In addition, some cancer tissues express OR mRNAs at very high levels, and these OR transcripts could be used as biomarkers for cancer diagnostics. Exemplary ORs include OR51E2 for prostate cancer, OR51E1 for small intestine carcinoma, OR2B6 for breast carcinoma, OR2J3 for non-small-cell lung cancer, OR7C1 for colorectal cancer and OR10H1 for bladder cancer.

Recent studies have shown that OR transcripts (OR51E2 and OR10H1) can be measured by PCR in urine; thus, these samples can potentially be used for liquid biopsies for cancer diagnosis.

Other exciting directions involving the olfactory receptor family include the potential of activating OLFR544 in the treatment of obesity and subcutaneous fat reduction and activating OR2AT4 to promote wound healing and hair growth.

However, despite these promising findings, there are several challenges and limitations facing research on ORs and taste receptors. Deorphanization of ORs is still a major challenge. Only 10–20% of mammalian...
Table 3 | Overview of key ectopic taste receptor expression patterns and functions

<table>
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<th>Organ or tissue</th>
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<td>Airways (trachea and bronchi)</td>
<td>Ciliated epithelial cells</td>
<td>TAS2Rs</td>
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<td>FFAR1 and FFAR4</td>
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<td>impaired glucose homeostasis</td>
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<td>δ-Cells</td>
<td>FFAR4</td>
<td>Omega-3 PUFAs</td>
<td>Inhibition of somatostatin to regulate</td>
<td>Mouse and</td>
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<td>insulin secretion</td>
<td>human</td>
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CCK, cholecystokinin; FFAR, free fatty acid receptor; GIP, gastric inhibitory polypeptide; GLP1, glucagon-like peptide 1; ICC, immunocytochemistry; IHC, immunohistochemistry; ISH, in situ hybridization; PTC, phenylthiocarbamide; PUFAs, polyunsaturated fatty acid; qPCR, quantitative PCR; RNA-seq, RNA-sequencing; RT-PCR, reverse transcription PCR; TAS1R, taste receptor family 1; TAS2R, taste receptor family 2.
ORs have been deorphanized. Notably, although 25 functional TAS2R genes have been identified in humans, several receptors are yet to be deorphanized.

In addition, although ectopic functions for several OR and taste receptors have been identified, most studies have been performed in cell lines and mouse models. Confirmation of the role of these receptors in humans is therefore vital. While some orthologues have been clearly identified, for example, human OR51E2 and mouse OLF7R8, it is often that a human orthologue of a mouse receptor cannot be identified by sequence analysis. In addition, the identification of endogenous ligands is challenging, and a human orthologue may not always share ligands with a mouse receptor. Another challenge in the translation of findings in models to humans is whether, even with dietary supplementation, the levels of tastants or odorants are enough to sustain chronic agonism of the receptor or whether a potent agonist and/or antagonist with high levels of bioavailability can be identified for clinical application. Notably, it has been shown that aroma and flavour compounds can be delivered to the circulation via inhalation, percutaneous application and food intake. The concentration of particular components of essential oils, such as the monoterpenes linalool and linalyl acetate, can reach concentrations in the micromolar range.

Achieving a high level of functional expression of receptors in a heterologous system can also be difficult. Co-expression of several chaperones increases the membrane targeting of ORs and taste receptors; however, the molecular basis for the specificity and sensitivity of these chaperones to specific receptors is poorly understood. Until now, no full-length 3D structure of ORs, TAS1Rs and TAS2Rs has been elucidated. Thus, key information on the molecular mechanisms of ligand binding, which triggers signal transduction pathways, is incomplete, and the development of potent and specific agonists and antagonists is a time-intensive and labour-intensive process. However, there has been substantial progress in understanding the structure of these receptors. For example, the crystal structure of the extracellular ligand-binding domains of TAS1R2 and TAS1R3 has been recently reported. Single-particle cryo-electron microscopy has been applied to determine GPCR structures, which might also be used in the structure studies of ORs and taste receptors.

As in the case of other GPCRs, it is often difficult to develop a high-quality antibody to detect these receptors; this is especially true for ORs, which share high sequence homology (from 50% to 99%). Thus, most of the evidence for ectopic expression of ORs has been confirmed at the level of mRNAs not proteins. Therefore, nucleic acid aptamers and protein scaffolds have also been suggested to detect proteins of interest. These newer options offer opportunities to rectify problems stemming from using poorly validated antibodies in research.

Finally, when contemplating targeting ectopic ORs and taste receptors, given their often multiple functions, the potential for off-target effects must be considered. Indeed, while some TAS2Rs are narrowly tuned and detect only a limited number of bitter compounds, other TAS2Rs are broadly tuned. As a result, when targeting TAS2Rs, many off-target effects may occur and are believed to play a role in the side effects of bitter-tasting drugs in clinical use. In addition, some TAS2Rs are highly polymorphic (such as TAS2R38), which may affect the sensitivity of patients to bitter tastants. These issues are also important in OR research.

**Outlook**

More than half of the approximately 370 ORs from the nasal epithelium have been shown to be extra-nasally expressed, and many taste receptors are found in extra-oral tissues. Considering that only approximately 10% have been functionally characterized, the potential roles of unexamined ORs and taste receptors are obviously broad. ORs and taste receptors should no longer be considered as pure odour or taste receptors but rather as general chemoreceptors involved in physiological and pathophysiological processes throughout the human body, representing a largely unexplored therapeutic field.

8. This study uses next-generation sequencing to perform comprehensive RNA-seq experiments investigating the ectopic OR profiles of 16 tissues. All tissues examined express at least some ORs, and several ORs such as OR2W3 and OR51E1 are widely distributed. OR4N4 is shown to be specifically expressed in the testes.
10. Orphan designation of the role played by taste receptors in enterodendritic cells of the gut.
12. This is the first study to prove the functionality of an OR located outside the nose. In this study, OR1D2 is reported to be highly expressed in human spermatogenesis and is activated by the odorant bourgeonal, eliciting positive chemotactic and chemokinetic swimming behaviour by spermatozoa.
14. This study is the first to investigate the role of an OR in skin keratinocytes. OR2AT4 activation by Sandalore is shown to induce wound healing by stimulating keratinocyte proliferation via activation of ERK1, ERK2 and p53 in ex vivo human skin. Novel wound-healing therapeutics were developed on the basis of these findings.
17. This study demonstrates the functionality of OR2AT4 in human hair growth. OR2AT4 is expressed in the human hair folic acid, and activation by Sandalore prolongs the active growth phase of hair by inducing the expression of IGF1. Results from the preliminary test on human support the findings from ex vivo culture of human folic acid.
This study investigates the role played by the mouse OR 544 (OR544) in skeletal muscle. In this study, the receptor stimulates skeletal muscle regeneration by inducing myocyte migration and contributing to tissue repair.


In this study, transgenic mice with a targeted deletion of a taste receptor is shown to inhibit bitter taste receptor-induced antimicrobial peptide production in the upper airway and may aid in the treatment of upper respiratory infections.


In this study, genetic variation of the bitter taste receptor TAS2R38 is shown to regulate the innate defence mechanisms against quorum-sensing molecules secreted by Gram-negative bacteria in the epithelia of the human upper airway.


This paper reports the discovery of ORs in rat olfactory epithelium and suggests a breakthrough in work on olfaction. In this paper, 18 different ORs in rat olfactory epithelium are described. They are novel and show that ORs are expressed in the adult rat olfactory epithelium.


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This is a basic summary of the olfactory system, covering topics ranging from the relevant molecules to perception. For a detailed overview, the interested reader is referred to the following studies.

- **Ferrer, I. et al.** The functionality of the receptor is also implied in olfactory receptor transduction. *Channels (Austin) 11, 399–414 (2017).
- **Müller, R. et al.** Olfactory receptor family 7 subfamily C member 1 is a novel marker of colon cancer-initiating cells and is a potent target of immunotherapy. *Clin. Cancer Res. 22, 3298–3306 (2016)*.
- **Ferrer, I. et al.** Olfactory receptors modulate physiological models. *Neural Cells 1, 2059–2064 (2015)*.

This is the first study to show that ORs are functionally expressed in the human intestine and that activation thereof by odorants triggers calcium-induced serotonin release, stimulating gut motility and secretion. **Kidd, M. et al.** Luminal regulation of normal and neoplastic human EC cell proliferation, which is mediated by bile salts, amines, tannins, and polysaccharides. *Am. J. Physiol. Gastrointest. Liver Physiol. 300, G1794–G1800 (2016)*.
- **Kalbe, B. et al.** Olfactory receptor family 7 subfamily C member 1 is a novel marker of colon cancer-initiating cells and is a potent target of immunotherapy. *Clin. Cancer Res. 22, 3298–3306 (2016)*.
- **Ferrer, I. et al.** Olfactory receptors modulate physiological models. *Neural Cells 1, 2059–2064 (2015)*.

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Competing interests
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