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REVIEW

GPR39: a Zn^{2+}-activated G protein-coupled receptor that regulates pancreatic, gastrointestinal and neuronal functions

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Abstract GPR39 is a vertebrate G protein-coupled receptor related to the ghrelin/neurotensin receptor subfamily. The receptor is expressed in a range of tissues including the pancreas, gut/gastrointestinal tract, liver, kidney and in some regions of the brain. GPR39 was initially thought to be the cognitive receptor for the peptide hormone, obestatin. However, subsequent in vitro studies have failed to demonstrate binding of this peptide to the receptor. Zn^{2+} has been shown to be a potent stimulator of GPR39 activity via the $G\alpha_q$, $G\alpha_{12/13}$ and $G\alpha_s$ pathways. The potency and specificity of Zn^{2+} in activating GPR39 suggest it to be a physiologically important agonist. GPR39 is now emerging as an important transducer of autocrine and paracrine Zn^{2+} signals, impacting upon cellular processes such as insulin secretion, gastric emptying, neurotransmission and epithelial repair. This review focuses on the molecular, structural and biological properties of GPR39 and its various physiological functions.

Keywords Cell signaling · Diabetes · Epithelial repair · Gastrointestinal tract · GPCR · Phospholipase C · Zinc receptor

Abbreviations

CRE	cAMP response element
GI	Gastrointestinal
GPCR	G protein-coupled receptor
HNF-1α	Hepatocyte nuclear factor-1α
HNF-4α	Hepatocyte nuclear factor-4α
$InsP_3$	Inositol triphosphate
IRS-2	Insulin receptor substrate-2
LYPD1	LY6/PLAUR domain-containing 1
NHE-1	Na^+/H^+ exchanger-1
PLC	Phospholipase C
SRE	Serum response element
TM	Transmembrane domain

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Introduction

GPR39 is a G protein-coupled receptor (GPCR) found in all vertebrates. It was first cloned, together with another receptor, GPR38, from human fetal brain cDNA in 1997 by McKee et al. [1]. This initial report revealed these novel GPCRs to be related to the ghrelin and neurotensin receptors. GPR38 has since been identified as the cognate receptor for motilin, a 22 amino acid peptide that regulates gastrointestinal (GI) motility [2, 3]. In 2005, Zhang et al. implicated GPR39 to be the receptor for obestatin [4], a peptide encoded by the ghrelin gene. Obestatin is proposed to have opposite roles to ghrelin in its control of food intake [4]. It is also implicated in the regulation of thirst [5], insulin secretion [6, 7] and pancreatic β-cell survival [8]. Zhang et al. postulated that the peptide activates GPR39 and stimulates adenylate cyclase activity and cAMP-dependent signaling via coupling to $G\alpha_s$. However, studies by others have failed to reproduce some of these findings, the most salient being the inability to demonstrate GPR39-binding by (or activation in response to) obestatin in vitro [9–11]. Zhang and co-workers have also had problems reproducing their original findings [12], and have since claimed these inconsistencies to be due to variation in the bioactivity of iodinated obestatin relative to the

unmodified peptide [13]. At the current time, the evidence is weighted heavily against a role for obestatin as a GPR39 agonist.

More recently, Zn^{2+} has been identified as a ligand capable of activating the receptor [9, 14]. Moreover, the potency and efficacy of Zn^{2+} as an agonist suggest it to be a physiologically relevant modulator of GPR39 in vivo [9]. Although the concept of Zn^{2+} as an activator of GPCR signaling is new it is perhaps not too surprising; it has been known for several years that the metal ion, Ca^{2+}, can act in this manner [15]. Functional studies are now beginning to yield more information regarding the physiological roles of GPR39. The molecular, structural and biological properties of GPR39 and the various processes that it is thought to regulate upon activation by Zn^{2+} are reviewed here.

GPR39 gene structure and expression

The human *GPR39* gene has been mapped to chromosome 2, position q21.2, and spans approximately 230 kb [1]. It encodes the full-length receptor (GPR39-1a) of 435 amino acids via two exons. The first exon encodes amino acids 1–285, which contain transmembrane domains (TM)-1–5. The second exon encodes the remaining 150 amino acids. This region contains TM-6 and TM-7. A splice variant of GPR39 has been identified that is essentially encoded by the first exon only (termed GPR39-1b) and is assumed to be non-functional [16]. The 3′ exon of the *GPR39* gene overlaps with *LYPD1* (LY6/PLAUR domain-containing 1), a gene transcribed on the opposite chromosomal strand (Fig. 1a). The two-exon structure and *LYPD1* gene overlap is conserved in all vertebrate *GPR39* genes characterized to date. Based upon a systematic analysis, it was indicated that the transcriptional start site is located 469 nucleotides upstream from the translation initiation site [16]. Analysis of the promoter regions using 5′-deletion constructs linked to a luciferase reporter assay revealed different minimal promoter sites in U-138 cells and HepG2 cell lines [16]. In the U-138 cells, maximal promoter activity was observed in constructs encompassing a region from −673 to −14. In the HepG2 cells, the construct corresponding to the region from −573 to −14 showed maximal activity. Disruption of hepatocyte nuclear factor (HNF)-1α, HNF-4α and Sp1 sites in the *GPR39* promoter reduces gene transcription [16]. Furthermore, it was revealed that the region between −673 and −573 contains negative regulatory elements that are utilized in Hep G2 cells and not in U-138 cells [16]. The negatively regulating elements are yet to be determined.

A wide range of expression studies for GPR39 have been carried out with cells/tissues isolated from humans [1, 17–22], rodents [2, 9, 16, 23–28], ruminants [29], birds [30, 31] and fish [32], as summarized in Table 1. The overall consensus is that the receptor is most highly expressed in the pancreas, GI tract, liver and kidney. Other peripheral tissues that express GPR39 include adipose tissue [16, 19, 20, 32], thyroid [1, 16], and heart [4, 16, 17]. Expression in the latter may be due to its apparent abundance in cardiomyocytes [17]. Increased GPR39 expression in adipose tissue is associated with pregnancy [19] and with individuals who are both diabetic and obese [20]. Microarray studies have identified GPR39 as a cell surface marker in Wilm's tumor-derived xenografts, which are described as being "stem-like" [21, 33]. Similarly,

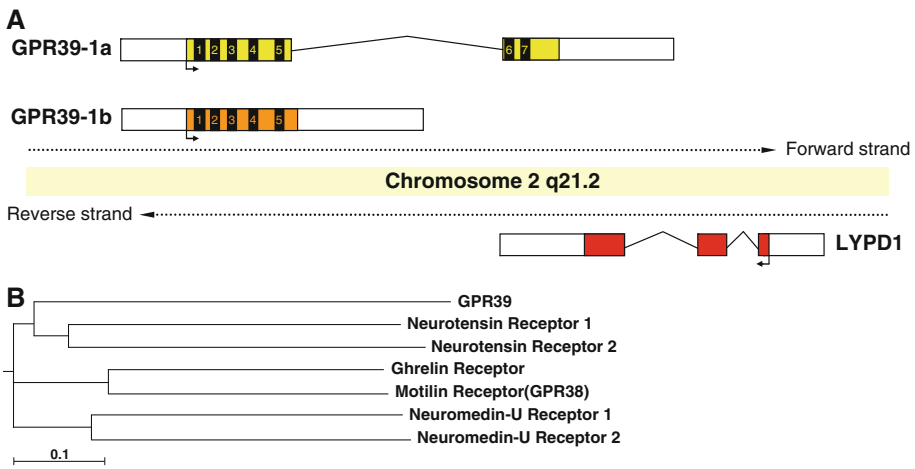

Fig. 1 **a** Diagrammatic representation of the genetic transcripts of the *GPR39* gene. *Boxes* indicate exons with colored regions representing protein coding sequence. Transcription start sites and direction of transcription are indicated by the *arrows*. GPR39-1a has a two-exon structure whilst GPR39-1b consists of only a single exon. The *LYPD1* gene is located on the opposite strand and overlaps with the 3′-end of the GPR39 gene. **b** Phylogenetic relationship between GPR39 and other receptors in the ghrelin/neurotensin GPCR subfamily. Diagram was drawn using ClustalW [70] available at the Swiss EMBnet

Table 1 Overview of GPR39 expression studies carried out with different cell and tissue types from a range of species

Species	Form	Methodology	Cell/tissue types	Expression	Reference
Human	1.8–2 kb form[a]	Northern blotting	Small intestine, stomach, amygdala, caudate nucleus, hippocampus, sustantia nigra	Yes (high)	[1]
Human	1.8–2 kb form	Northern blotting	Spleen, corpus callosum, whole brain, thalamus, cerebellum, cerebral cortex, medulla, spinal cord, occipital pole, frontal lobe, temporal lobe, colon	Yes (moderate/low)	[1]
Human	1.8–2 kb form	Northern blotting	Pancreas, adrenal medulla, thyroid, adrenal cortex, testis, thymus, prostate testis ovary, leukocytes	No	[1]
Human	3 kb form[a]	Northern blotting	Pancreas, stomach, colon	Yes (high)	[1]
Human	3 kb form	Northern blotting	Thyroid, small intestine, prostate	Yes (moderate/low)	[1]
Human	3 kb form	Northern blotting	Adrenal medulla, adrenal cortex, testis, thymus, spleen, thymus, testis, ovary, leukocytes, whole brain	No	[1]
Human		RT-PCR, IHC	Atrial tissue	Yes	[17]
Human		RT-PCR	Retinal pigment epithelial cells	Yes	[18]
Human		qPCR	Adipose tissue	Yes	[19]
Human		qPCR, western blotting, IHC	Adipose tissue	Yes	[20]
Human		IHC	Renal tubular epithelial cells	Yes (high)	[21]
Human		IHC	Nephrogenic cortex	Yes (moderate/low)	[21]
Human		qPCR	Synovium-derived mesenchymal stem cells	Yes[b]	[22]
Rat	GPR39-1a	qPCR	Liver, stomach, pancreas kidney	Yes (high)	[16]
Rat	GPR39-1a	qPCR	Small intestine, colon, adipose tissues, spleen, thyroid, lung, muscle, heart, adrenal gland, salivary gland	Yes (moderate/low)	[16]
Rat	GPR39-1a	qPCR	Thymus, pituitary gland, cerebellum, cortex, pons, hippocampus, hypothalamus, bed nucleus of stria terminalis, striatum, amygdala, septum	No	[16]
Rat	GPR39-1a	In situ hybridization	Brain (amygdala, hippocampus, hypothamlus)	No	[16]
Rat	GPR39-1b	qPCR	Stomach, small intestines	Yes (high)	[16]
Rat	GPR39-1b	qPCR	Liver, kidney, colon, adipose tissues, heart, cerebellum, pons	Yes (moderate)	[16]
Rat	GPR39-1b	qPCR	Pancreas, spleen, thyroid, lung, thymus, muscle, adrenal gland, salivary gland, pituitary gland, cortex, hippocampus, hypothalamus, bed nucleus of stria terminalis, striatum, amygdala, septum	Yes (low)	[16]
Rat	GPR39-1b	In situ hybridization	CNS	Yes (low)	[16]
Rat		qPCR	Jejunum, duodenum, stomach, pituitary	Yes (high)	[4]
Rat		qPCR	Ileum, liver, hypothalamus, heart	Yes (moderate)	[4]
Rat		qPCR	Pancreas, cerebellum, cerebrum, kidney, testis, ovary, colon, lung	Yes (low)	[4]
Rat	GPR39-1a	qPCR	Adrenal zona glomerulosa, adrenal fasciculate/reticularis, adrenal medulla	Yes (low)	[23]
Rat	GPR39-1b	qPCR	Adrenal zona glomerulosa, adrenal fasciculate/reticularis, adrenal medulla	Yes (high)	[23]
Rat		IHC	Fetal lung (primitive lung epithelium)	Yes	[24]
Mouse		RT-PCR	Septum-amygdala, parietal cells, enterocytes, neurons, pancreas	Yes	[25]
Mouse		IHC	Hippocampus (CA3 neurons)	Yes	[26]

Table 1 continued

Species	Form	Methodology	Cell/tissue types	Expression	Reference
Mouse		IHC	Pancreas	Yes	[27]
Mouse		qPCR	Duodenum, kidney	Yes	[9]
Mouse		qPCR	Pituitary, hypothalamus	No	[9]
Mouse		In situ hybridization	Lateral, basolateral, anterior cortical, and posteromedial cortical amygdaloid nuclei, dentate gyrus, hippocampus (CA1 and CA3 neurons), auditory cortex	Yes (high)	[28]
Mouse		In situ hybridization	Basomedial amygdaloid nucleus, piriform cortex, ventral pallidum, ventral endopiriform nucleus, and inferior olive	Yes (moderate/low)	[28]
Mouse		In situ hybridization	Hypothalamus	No	[28]
Mouse		RT-PCR, IHC	Cardiomyocytes	Yes	[17]
Cow		qPCR	Abomasum (stomach), liver	Yes (high)	[29]
Cow		qPCR	Kidney, small intestine, colon, rectum, uterus and cervix[c]	Yes (moderate/low)	[29]
Chicken (adult)		qPCR	Duodenum, jejunum, ileum, cecum, uterus	Yes (high)	[30]
Chicken (adult)		qPCR	Colon and rectum[d], stomach, isthmus, vagina	Yes (moderate)	[30]
Chicken (adult)		qPCR	Gullet, gizzard, ovary, infundibulum, magnum	Yes (low/very low)	[30]
Chicken (adult)		qPCR	Crop sac	No	[30]
Chicken (chick)		qPCR	Duodenum	Yes (high)	[30]
Chicken (chick)		qPCR	Liver, kidney, stomach, oviduct	Yes (moderate)	[30]
Chicken (chick)		qPCR	Brain, pituitary, thymus, bursa of fabricius, bone marrow, ovary, testis	Yes (low/very low)	[30]
Quail		qPCR	Stomach, duodenum, jejunum, ileum, cecum, colon and rectum[d]	Yes (high)	[31]
Quail		qPCR	Gullet, crop sac, gizzard, ovary, infundibulum, magnum, isthmus, uterus	Yes (low/very low)	[31]
Seabream		Northern blotting	Intestine, stomach, liver	Yes	[32]
Seabream	GPR39-1a	RT-PCR	Midbrain, intestine, stomach, liver, testis, adipose tissue, kidney	Yes	[32]
Seabream	GPR39-1a	RT-PCR	Forebrain, hindbrain, medulla, pituitary, hypothalamus, muscle, ovary, gill filament, heart, spleen	No	[32]
Seabream	GPR39-1b	RT-PCR	Forebrain, midbrain, hindbrain, medulla, pituitary, hypothalamus, intestine, stomach, muscle, liver, testis, gill filament, heart, adipose tissue, kidney, spleen	Yes	[32]
Seabream	GPR39-1b	RT-PCR	Ovary	No	[32]

[a] McKee et al. identified two forms of GPR39 transcripts, the first ~1.8–2 kb and the second, ~3 kb in size [1]

[b] Levels of GPR39 transcript decreased following chondrogenesis

[c] Uterine and cervical tissues were pooled

[d] Colonic and rectal tissues were pooled

expression of GPR39 was detected in synovium-derived mesenchymal stem cells but decreased as the cells underwent chondrogenesis [22].

There have been conflicting views as to whether the full-length GPR39 receptor is expressed in the brain. Egerod et al. postulated that some *GPR39* gene expression studies

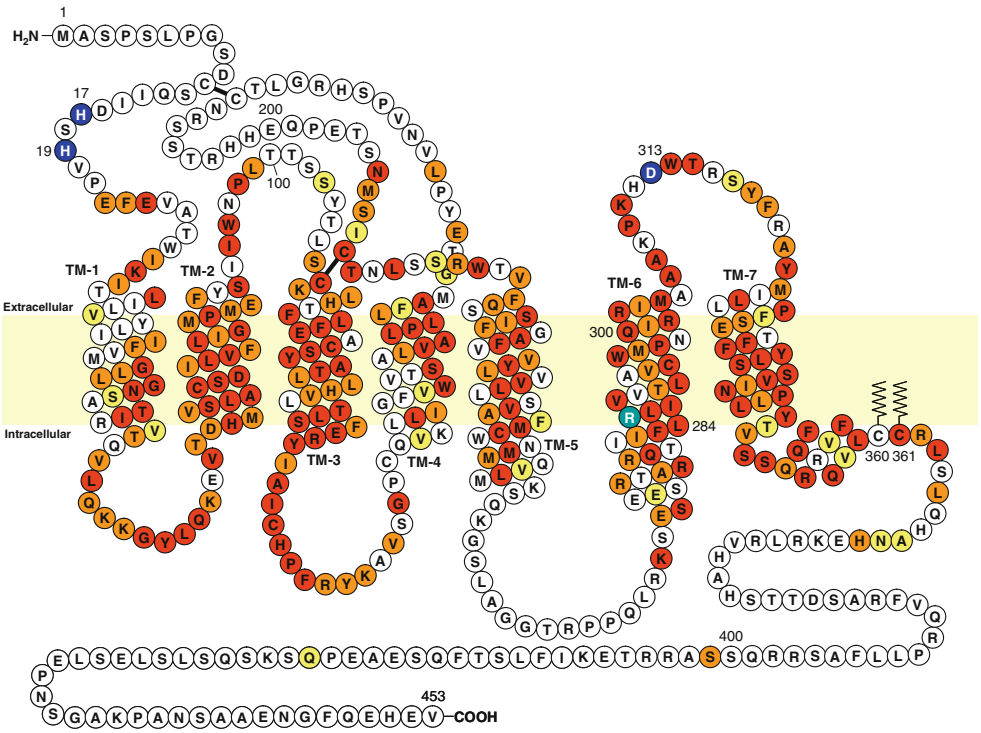

Fig. 2 Snake diagram of the full-length GPR39 receptor. Color coding is as follows: *blue* residues involved in Zn^{2+}-coordination, *green* exon1/exon2 boundary, *red* fully conserved residues, *orange* semi-conserved residues, *yellow* partially conserved residues, *white* non-conserved residues. Other structural features including disulfide brides (*bold lines*) and putative palmitoylation sites (*zig-zag lines*) are also shown

may have been complicated by the existence of the GPR39-1b variant or even by the presence of *LYPD1* at the gene locus of *GPR39* [16]. By directly measuring expression of GPR39-1a, GPR39-1b, and LYPD1 transcripts using quantitative PCR with cDNA from a range of tissue types isolated from rat, they found the full-length receptor to be highly expressed in the liver, stomach, pancreas, kidney and small intestine. Low to moderate expression of GPR39-1a was also observed in the colon, white adipose tissue, spleen, thyroid, lung and heart. No expression was observed in the brain or central nervous system. GPR39-1b was highly expressed in the stomach and small intestine and exhibited low to moderate expression in all other tissue types analyzed including those isolated from various regions of the brain. The LYPD1 transcript was highly expressed throughout the brain. Thus, it is possible that in some cases when attempting to detect GPR39-1a, transcripts corresponding to GPR39-1b or LYPD1 were inadvertently detected. It is unlikely that this is true in all cases where GPR39 expression has been detected in brain tissue [1, 4, 25, 26, 28, 30, 32]. Primers specific for the GPR39-1a transcript were used to identify expression in the midbrain of seabream, but not in the forebrain, hindbrain, medulla or hypothalamus [32]. The GPR39 expression profile in fish may of course differ from other vertebrates. A possible explanation for failure to detect GPR39-1a transcript or protein in some studies may be that the gene is expressed only in discrete regions of the brain and that the transcript is therefore diluted (or lacking) in some of the tissue samples that were used. Studies performed on tissue sections appeared to be more successful for identifying expression in the brain, with the protein particularly abundant within the murine hippocampus [26, 28]. This is presumably because these techniques allow expression in distinct regions to be observed.

Protein structure and Zn^{2+}-activation of GPR39

The human GPR39 receptor is a member of the rhodopsin-like family of 7-transmembrane containing GPCRs and is closely related to the ghrelin/neurotensin receptor subfamily (Fig. 1b). The full-length receptor (GPR39-1a) is 52 kD in size. A snake representation of GPR39-1a is shown in Fig. 2, illustrating the topology and other structural features of the receptor. The GPR39-1b variant transcript encodes a protein of 32 kD that is identical in sequence to the full-length receptor between amino acids 1–285. After Leu285, the GPR39-1b transcript encodes Ser-Glu-Ser, followed by a stop codon. The GPR39-1b receptor is presumably inactive and the significance of its expression is unknown. Truncated variants of other GPCRs such as the luteinizing hormone receptor [34], calcitonin receptor [35] and α1A-adrenoceptor [36] have been demonstrated to alter the function of the native receptor when expressed together. The GPR39-1b variant may therefore play a role in regulating the activity/expression of the full-length receptor.

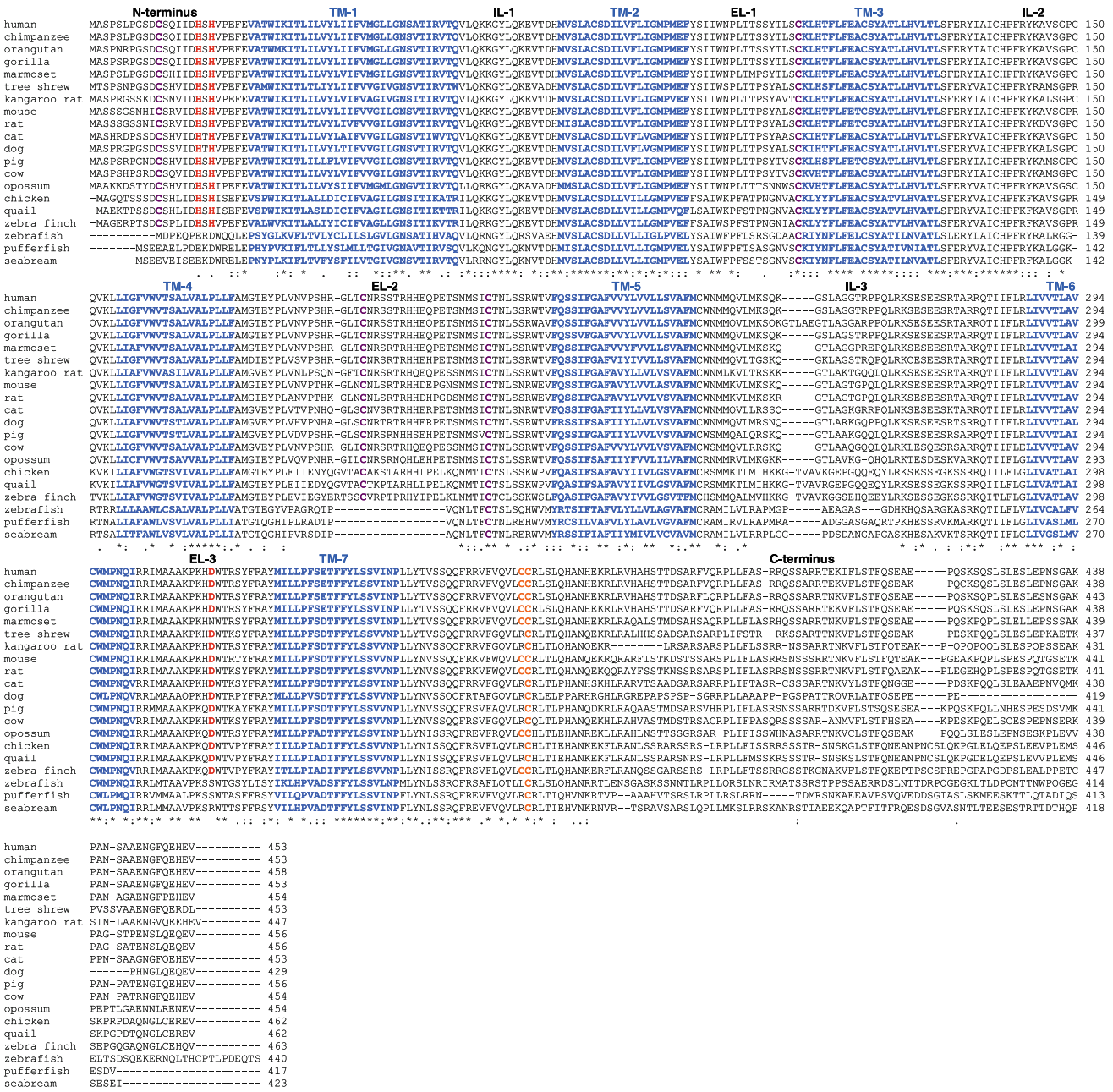

Fig. 3 Alignment of full-length vertebrate GPR39 receptor sequences drawn using ClustalW [70]. Color coding is as follows: *blue* transmembrane domains, *red* residues involved in Zn^{2+}-coordination, *purple* cysteine residues which form disulfide bridges, *orange* cysteine residues which form putative palmitoylation sites. "*" denotes fully conserved residues; ":" semi-conserved residues; and "." partially conserved residues

Human GPR39 contains four cysteine residues in its extracellular domains. These have been shown to form two disulfide bridges: the first between Cys11 (N-terminal tail) and Cys191 (extracellular loop-2) and the second between Cys108 (extracellular loop-1) and Cys210 (extracellular loop-2) [44]. These are conserved within all vertebrate GPR39 sequences with the exception that Cys11 and Cys191 (which form the first bridge) are absent from the fish receptors. An alignment of GPR39 amino acid sequences from a range of vertebrate species is presented in Fig. 3. The Cys11-Cys191 disulfide bond has been shown to be important for cell surface expression and agonist-induced and constitutive activity [44]. Two other cysteine residues in the C-terminal domain (Cys360 and Cys361) are putative palmitoylation sites. Only the latter cysteine residue is conserved in all vertebrate GPR39 receptors, with the former absent from fish as well as a range of mammalian and avian species. GPR39 also contains a

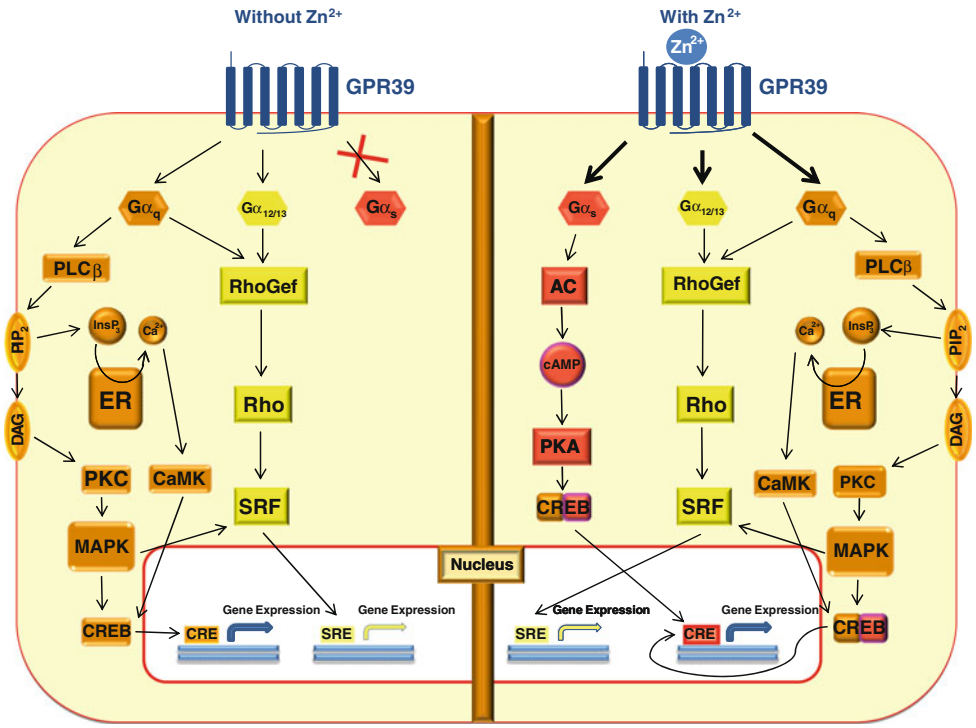

Fig. 4 Constitutive and Zn^{2+}-activated signaling pathways of GPR39. GPR39 has a high degree of constitutive activity mediated through $G\alpha_q$ and $G\alpha_{12/13}$ pathways. Following Zn^{2+}-activation of the receptor, stimulation of these pathways is further enhanced with signaling also occurring through the $G\alpha_s$ pathway. This figure is based upon data from ref. [9]. *CaMK* Ca^{2+}/calmodulin dependent kinases, *CRE* cAMP responsive element, *CREB* cAMP responsive element binding protein, *DAG* 1,2-diacylglycerol, *ER* endoplasmic reticulum, *$InsP_3$* inositol 1,4,5-triphosphate, *MAPK* mitogen activated protein kinase, *PIP_2* phosphatidylinositol 4,5-bisphosphate, *PLCβ* phospholipase C-*β*, *PKC* protein kinase C, *RhoGef* Rho guanosine exchange factor

number of phosphorylation sites and is heavily phosphorylated under basal conditions [45]. The functional significance of GPR39 phosphorylation is unknown but it has been demonstrated that it does not stimulate *β*-arrestin-2 recruitment or internalization [45]. The receptor also contains a (D/E)RY motif within intracellular loop-2. This motif is found in many GPCRs and has putative roles in regulating constitutive activity, agonist affinity, G protein-coupling and receptor conformation [46].

Like the other GPCRs in the ghrelin/neurotensin subfamily, GPR39 has been shown to have a relatively high level of constitutive activity [37, 38]. This appears to be mediated through $G\alpha_q$, which stimulates inositol 1,4,5-triphosphate ($InsP_3$) turnover and cAMP response element (CRE)-mediated transcription (via MAPK activation) and $G\alpha_{12/13}$ which stimulates serum response element (SRE)-mediated transcription via Rho, but not the $G\alpha_s$ pathway [9, 39]. Mutational analyses showed that the constitutive activity of GPR39 is controlled, at least in part, by residues residing on the inner surfaces of TM-5 and TM-6. Constitutive activity can systematically be either increased or decreased by substitutions at Asn299, which resides in TM-6 [39], or decreased by mutating Tyr231 (in TM-5) to alanine [40].

A tridentate Zn^{2+}-binding site on GPR39 has been identified that consists of residues His17, His19 and Asp313 [41]. Upon binding of Zn^{2+}, human GPR39-1a stimulates both PLC activity and cAMP production in a dose-dependent manner, displaying EC_{50} values of 22 and 7 μM, respectively [9]. However, the affinity constant for Zn^{2+} binding to GPR39 is yet to be determined. CRE- and SRE-mediated transcription and arrestin mobilization to the plasma membrane (but not internalization) were also increased upon Zn^{2+}-induced activation [9]. Zn^{2+}-induced stimulation of PLC activity has also been observed with mouse and rat GPR39 receptors [14]. It is thought that Zn^{2+} stimulates activation of the receptor by diverting Asp313 from functioning as a tethered inverse agonist through binding to this residue. The tridentate Zn^{2+} site is conserved in all vertebrate sequences to date with the exception of fish. In the marmoset protein, Asp313 is replaced by asparagine which would likely still bind Zn^{2+} but with weaker affinity. Constitutive and Zn^{2+}-stimulated signaling pathways downstream from GPR39 are summarized in Fig. 4.

Collectively, the available data suggest that the receptor functions as a receptor for Zn^{2+} in mammals and birds but not fish. The Zn^{2+}-co-ordinating aspartic acid residue in

the human receptor is replaced in the zebrafish and pufferfish sequences by a serine residue and in the seabream sequence by an arginine. The three fish sequences also contain glutamic acid and aspartic acid residues in place of the first and second conserved histidines, respectively. Serine, glutamic acid and aspartic acid (as found in zebrafish and pufferfish sequences at the corresponding positions) could potentially form a divalent metal ion site similar to that observed in other proteins [42, 43]. However, such a site would likely favor a metal ion such as Ca^{2+}, rather than Zn^{2+}. The amino acid sequences of vertebrate GPR39 proteins share ~30% identity and ~50% similarity (including conserved and semi-conserved differences). Given that Zn^{2+} does not appear to be an agonist for fish GPR39 receptors, it is possible that another naturally occurring agonist or inverse agonist may exist that is able to bind to and activate fish (and perhaps other vertebrate) receptors.

Functional effects of GPR39 activation

Insulin release and pancreatic function

GPR39 is localized within the insulin-storing β-cells of the pancreatic islets and in the duct cells of the exocrine pancreas [27]. Key information relating to the function of GPR39 in the pancreas has come from knockout mouse studies. Disruption of the *GPR39* gene is associated with impaired insulin secretion [27, 47]. Tremblay et al. reported insulin secretion to be normal in both $GPR39^{-/-}$ and wild-type mice as they aged from 12 to 52 weeks. However, insulin levels decreased in 52-week-old $GPR39^{-/-}$ mice, relative to wild-type mice, when fed a sucrose-rich diet. This impairment was attributed to a reduction in levels of insulin receptor substrate 2 (IRS-2) expression in β-cells [47]. Holst et al. carried out glucose tolerance tests using mice aged 11–12 weeks and found moderately reduced insulin levels the in $GPR39^{-/-}$ mice. They also found that expression of the transcription factors, insulin promoting factor-1 and HNF-1α in the pancreatic islets was lower in the $GPR39^{-/-}$ mice [27].

The observed differences in gene expression in $GPR39^{-/-}$ mice relative to wild-type mice highlight an important role for GPR39 within the insulin-secreting cells. It is known that autocrine effects are important for maintaining normal exocrine function in these cells. For example, insulin following its release from β-cells, binds to and activates insulin receptors on the β-cell surface. This process requires the insulin receptor substrates, IRS-1 and IRS-2 [48–50], and is important for efficient regulation of glucose-stimulated insulin secretion and β-cell development. Zn^{2+} is enriched in insulin-containing vesicles through the action of the Zn^{2+} transporter, ZnT5 [51] and is released together with insulin. Thus, Zn^{2+} may also contribute to autocrine (and paracrine) signaling in the pancreas via activation of GPR39.

Gastrointestinal and metabolic function

GPR39 is widely expressed in the stomach and GI tract and has been implicated as a regulator of GI and general metabolic function. One of the key studies to support this was carried out by Moechars et al. [25]. Here, they found that the gastric emptying time of $GPR39^{-/-}$ mice to be almost half that of the wild-type mice. They also found that gastric fluid secretion (but not gastric acid secretion) was significantly increased in the $GPR39^{-/-}$ mice. From this, it appears that GPR39 plays a role in regulating normal GI function. These observations are interesting as diarrhea is a common symptom of zinc deficiency. In fact, zinc tablets are used clinically to treat diarrhea [52]. The presence of a Zn^{2+}-sensing receptor has been identified in colonocytes [53]. Hershfinkel et al. have proposed that activation of this receptor in these cells may ameliorate the erosion of the mucosal lining of the colon to prevent or reduce diarrhea [54].

Moechars et al. also reported that mature body weight, body fat, and circulatory cholesterol levels were significantly increased in the $GPR39^{-/-}$ mice [25]. Contrary to this, another study failed to identify any significant differences in body weight, adiposity or food intake between $GPR39^{-/-}$ and wild-type mice [55]. A putative role in metabolic regulation or satiety control is therefore less clear. Studies examining whether GPR39 signaling impacts upon satiety and appetite in particular were initially complicated by the belief that obestatin may be a cognate ligand for GPR39. Tremblay et al. examined whether obestatin actions are altered in the $GPR39^{-/-}$ mice relative to wild-type in order to gain evidence for obestatin-induced activation of the receptor. They found no difference in food intake between $GPR39^{-/-}$ and wild-type mice following injection of the peptide [47]. A host of other studies have also failed to identify a role for obestatin in regulating satiety or food intake [9, 56–58]. The mechanism by which GPR39 directs its apparent effects on GI function is unclear; a direct role for Zn^{2+} (via activation of GPR39) is credible but remains to be investigated.

Neuronal function

A role has been proposed for GPR39 in facilitating neurotransmission via synaptic release of Zn^{2+} in the hippocampus [26]. Zn^{2+}-containing neurons are a subclass of glutaminergic neurons found in various brain regions

[59]. Within these cells, Zn^{2+} accumulates in synaptic vesicles through the action of the Zn^{2+} transporter, ZnT3 [60], and is released along with glutamate during synaptic transmission [61, 62]. Besser et al. reported that addition of 200 μM Zn^{2+} to mouse hippocampal slices induced an intracellular Ca^{2+} response that was inhibited by both U73122 (PLC inhibitor) and YM-254890 ($G\alpha_q$ inhibitor) [26]. This suggests that the Ca^{2+} response is mediated by $G\alpha_q$-dependent PLC activation and $InsP_3$ release [26]. A similar response to Zn^{2+} was also observed in the neuronal SH-SY5Y cell line, which endogenously express GPR39. Inhibition of GPR39 expression using RNAi resulted in significant attenuation of the Ca^{2+} response, highlighting involvement of the receptor in neuronal Zn^{2+} signaling [26]. To determine whether synaptically released Zn^{2+} is capable of activating the receptor, Zn^{2+} release in hippocampal slices was induced by electrical stimulation. This triggered an increase in intracellular Ca^{2+} which could be inhibited by chelation of Zn^{2+} with CaEDTA. Similarly, the response was significantly attenuated in hippocampal slices taken from *ZnT3*$^{-/-}$ mice (which totally lack synaptic Zn^{2+}) relative to wild-type mice [27]. Direct involvement of GPR39 in mediating the Zn^{2+}-dependent Ca^{2+} response in hippocampal slices was not determined but the evidence for its involvement is nevertheless compelling. To date, no phenotype related to a disruption in neural functioning has been reported in *GPR39*$^{-/-}$ mice. Defects in cognitive behavior [63], neurogenesis [64] and an increased predisposition to seizures [65] are associated with *ZnT3*$^{-/-}$ mice.

Wound healing

Zinc deficiency has long been known to be associated with poor wound healing [66, 67]. This has largely been presumed to be due to the essential role Zn^{2+} plays in DNA and protein synthesis [67]. Recently, a role for Zn^{2+} in "triggering" wound healing has been proposed, whereby Zn^{2+} activates GPR39 following its release from injured cells [68]. Addition of extracellular Zn^{2+} to human HaCaT keratinocytes was found to induce a metabotropic Ca^{2+} response that was abolished by silencing the expression of GPR39. Similarly, using a scratch closure assay, it was found that Zn^{2+}-stimulated cell growth and was GPR39-dependent. It was demonstrated that this occurs via stimulation of the MAPK pathway and its subsequent activation of Na^+/H^+ exchanger-1 (NHE-1) [68]. NHE-1 is an ion exchanger known to be important for cell migration and cell polarization [69]. Interestingly, NHE-1 is also activated by Zn^{2+} in colonocytes [53]. Chelation of released Zn^{2+} inhibited the response suggesting that it may be sufficient to trigger epithelial repair via activation of GPR39 [68]. One surprising finding from this study was that the EC_{50} value for Zn^{2+}-induced Ca^{2+} mobilization was in the sub-nanomolar range. This concentration is approximately 100,000-fold lower than the EC_{50} previously observed for InsP and cAMP accumulation in COS-7 cells transiently transfected with human GPR39 [9]. The molecular basis for the extremely enhanced sensitivity of this pathway in keratinocytes is unknown.

Conclusion

Early work concerning the GPR39 was complicated by the belief that the receptor is the cognitive receptor for obestatin. However, identification of Zn^{2+} as an endogenous ligand for the receptor has driven research into many emerging physiological roles. These studies suggest the receptor to be an important transducer of paracrine and autocrine signals. However, there are a number of areas of GPR39 research that necessitate further investigation. The dynamics of Zn^{2+} binding to the receptor are largely unknown; the affinity of the receptor toward Zn^{2+} needs to be measured to better understand the molecular and pharmacological properties of the receptor and the pathways that it stimulates. The physiological significance of the GPR39-1b truncated variant also remains to be determined. Comparative analysis of vertebrate receptors suggests that the fish receptors are not Zn^{2+} sensitive. This highlights the possibility that GPR39 in these species is activated via a different unidentified endogenous ligand(s). If novel ligands for GPR39 were to be identified in fish, it would be interesting to determine whether they may also have actions upon GPR39 receptors from other vertebrates including the human form.

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