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Review

Is GPR39 the natural receptor of obestatin?

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ABSTRACT

GPR39, an orphan receptor belonging to the family of G protein-coupled receptors, was originally reported to be the receptor of obestatin. However recently, numerous reports have questioned this conclusion. In mammals, GPR39 was reported to be involved in the regulation of gastrointestinal and the metabolic functions. In this article, a latest and brief review on the receptor family, structure, distribution and physiological functions of GPR39 has been reported.

Contents

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1. Introduction

The G protein-coupled receptor 39 (GPR39) is an orphan member of a family including the receptors for ghrelin and motilin [30]. GPR39 shows a high degree of constitutive signaling through the serum response element (SRE) pathway [20]. In 2005, GPR39 was first reported to be the receptor for a peptide fragment from the ghrelin precursor named obestatin, which was proposed to be a gut hormone having the opposite effects on food intake and GI-tract function to ghrelin [52]. Thereafter, the GPR39 signaling was activated by zinc ions (Zn^{2+}) through the Gqα-PLC pathway [48]. However, Chartrel et al. [8] suggested that obestatin did not activate GPR39; therefore, the natural ligand for GPR39 is uncertain so far. In this article, we summarized the receptor family, structure, distribution and physiological functions of GPR39.

2. Receptor family of GPR39

In 1996, the growth hormone secretagogues-receptor (GHS-R) gene was cloned and shown to encode a unique G protein-

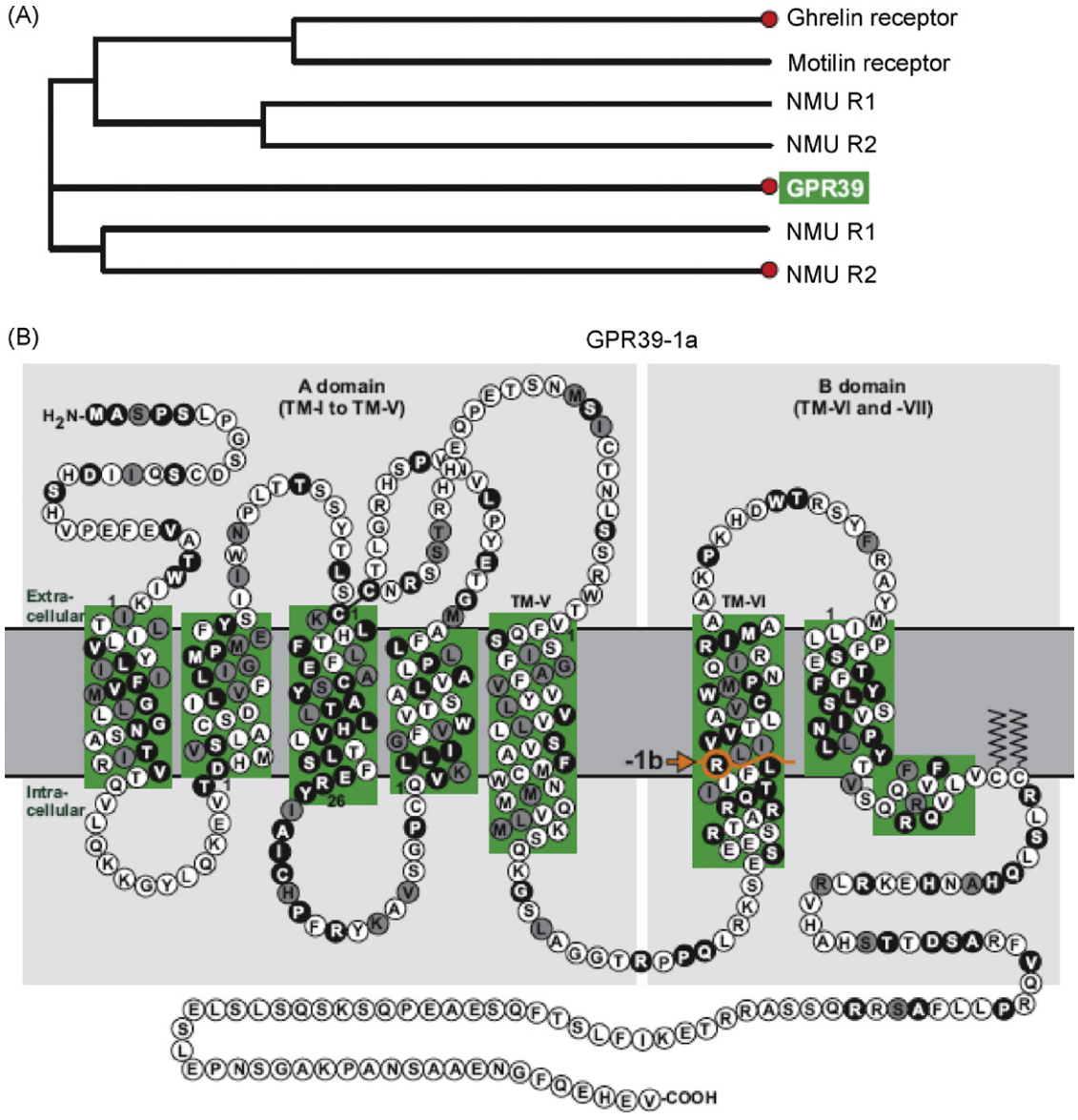

Fig. 1 – The receptor family of GPR39. (A) Schematic phylogenic tree of the receptor family of GPR39. The constitutively active receptors are highlighted with red color. (B) A model of human GPR39. GPR39-1a is the full length 7-transmenbrane (TM) receptor, and GPR39-1b is a truncated form of GPR39-1a lacking after 5-TM [12,41]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

coupled receptor with a deduced protein sequence that was 96% identical in human and rats [22]. Because of the physiological importance of the GHS-R, a search for family members was then initiated and its molecular evolution was investigated. McKee et al. originally indicated that GPR38 and GPR39 shared a significant amino acid sequence identical with the GHS-R, two neuromedin U receptors and the two neurotensin receptors (Fig. 1A). Fluorescence *in situ* hybridization demonstrated that GPR38 and GPR39 localized at separate chromosomes and were distinct from the gene encoding the GHS-R and NT-R type 1 [30].

GPR38 was encoded by a single gene expressed in the thyroid gland, stomach, and bone marrow, and it is now known to be the receptor for motilin, which mainly regulates gastrointestinal (GI) contractions and gut motility [13]. GPR39 was expressed in the brain and other peripheral tissues [30]. The GHS-R gene was later indicated to be the receptor for the GI-tract hormone ghrelin involved in a large array of

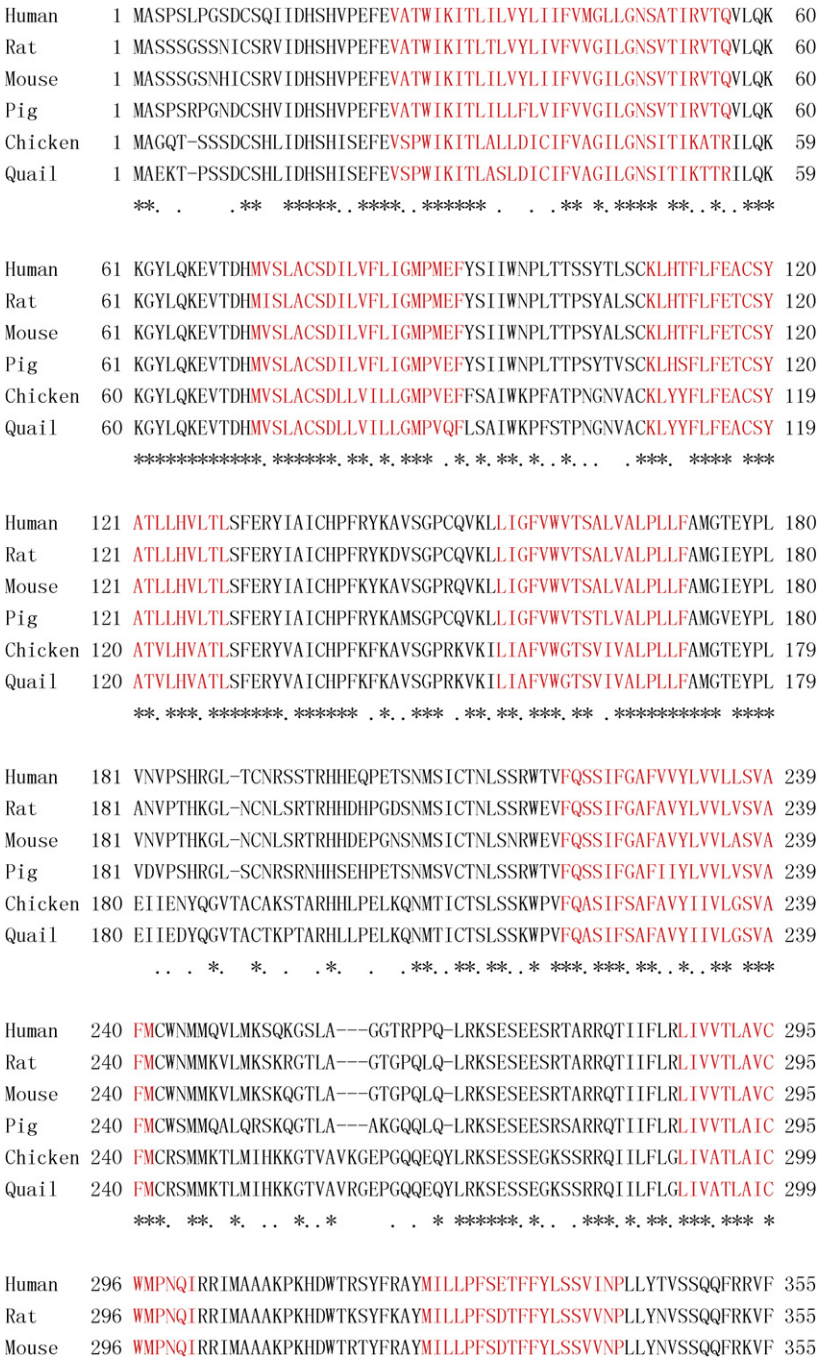

Fig. 2 – Alignment of amino acid sequences of human, mouse, rat, chicken, quail and pig GPR39. Transmembrane regions were represented as red letters; the gene sequences are quoted from GenBank accession (nos. NM001508, NM001114392, ENSRNOG00000021586, NM001080105, EF375709, and EU669821). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

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Pig      296 WMPNQIRRMMAAAKPKQDWTKAYFKAYMILLPFSDTFFYLSSVVNPLLYNVSSQQFRSVF 355
Chicken  300 WMPNQIRRIMAAAKPKQDWTVPYFRAYIILLPIADIFFYLSSVVNPLLYNISSQQFRSVF 359
Quail    300 WMPNQIRRIMAAAKPKQDWTVPYFRAYIILLPIADIFFYLSSVVNPLLYNISSQQFRSVF 359
             ********.******* ***  **.**.****....*******.*****..****** **

Human    356 VQVLCCRLSLQHANHEKRLRVHAHSTTDSARFVQRPLLF-AS-RRQSSARRTEKIFLSTF 413
Rat      356 WQVLCCRLTLQHANQEKQQRAYFSSTKNSSRSARSPLIFLASRRSNSSSRRTNKVFLSTF 415
Mouse    356 WQVLCCRLTLQHANQEKRQRARFISTKDSTSSARSPLIFLASRRSNSSSRRTNKVFLSTF 415
Pig      356 AQVLRCRLTLPHANQDKRLRAQAASTMDSARSVHRPLIFLAS-RSNSSARRTDKVFLSTS 414
Chicken  360 LQVLRCHLTIEHANKEKFLR-ANLSSRARSSRSLRPLLFISSRR-SSSTRSNSK-GLSTF 416
Quail    360 LQVLRCHLTIEHANKEKFLR-ANLSSRARSNRSLRPLLFMSSKR-SSSTRSNSK-SLSTF 416
              *** *.*.. *** .* .*    *.  .     .** * .* *  ** *.. * .***.

Human    414 QSEAE---P-QSKSQSL---SLESLEPNSGAKPANSAAENGFQEHEV              453
Rat      416 Q--AEAK-PLEGEHQPL---SPESPQTGSETKPAGSATENSLQEQEV              456
Mouse    416 Q--TEAK-PGEAKPQPL---SPESPQTGSETKPAGSTTENSLQEQEV              456
Pig      415 QSESEAK-P-QSKPQLL---NHESPESDSVMKPANPATENGIQEHEV              456
Chicken  417 QNEANPNCSLQKPGLELQEPSLEEVPLEMSSKPRPDAQNGLC-EREV              462
Quail    417 QNEANPNCSLQKPGDELQEPSLEVVPLEMSSKPGPDTQNGLC-EREV              462
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Fig. 2. (Continued).

physiological functions including the regulation of food intake, body weight, GI motility and hypothalamic and hypophyseal hormone secretion [18,27,33,49]. Other members of the GPR39 receptor family are neuromedin U receptors and neurotensin receptors. Neuromedin U and neurotensin both have been implicated in the control of food intake and GI functions [21,54].

3. Structure and distribution of GPR39

3.1. *Structure of the GPR39 receptor*

The GRP39 receptor belongs to the class of rhodopsin-like receptor family including GHS-R and motilin receptor (GPR38) [20,30]. The amino acid sequences of GPR39 in human, rat, mouse, quail, chicken and pig are shown in Fig. 2.

The molecular weight of human GPR39 is 52 kDa [14]. The human GPR39 gene consists of two exons separated by a very large intron of approximately 200 kb [36]. PCR analysis verified the notion that GPR39 was expressed by two splice variants, namely GPR39-1a, corresponding to the full length 7-transmenbrane (TM) receptor, and GPR39-1b, corresponding to a truncated form of GPR39-1a lacking after 5-TM (Fig. 1B) [12]. Yamamoto et al. [46,47] reported the amino acid sequences and gene structures of chicken and quail GPR39. Chicken and quail GPR39 both encode a 462-amino acid protein, with high sequence homology to human, rat and mouse GPR39. The quail GPR39 cDNA consisted of 354 bp of 5′-UTR, 1484 bp of 3′-UTR and 1389 bp of coding region [47]. The chicken GPR39 gene is composed of two exons separated by an intron, HNF-1, GC box and CCAAT box, but no canonical TATA box was found in the chicken GPR39 gene [46]. Recently, we determined the pig GPR39 cDNA encoding a 465-amino acid protein (Fig. 2).

3.2. *Distribution of GPR39 receptor*

Distribution of GPR39 mRNA or protein in the vertebrates examined so far is summarized in Table 1. Analysis on GPR39 distribution indicated that GPR39 mRNA was detected mainly in the digestive tracts, and also observed in adipose tissue, liver, spleen, thyroid, lung, heart, reproductive tissues and various brain regions.

In human, consistent with the role in the regulation of energy balance [2,7], GPR39 had been mapped to human chromosome 2 and expressed in multiple tissues including the stomach, intestine and hypothalamus [30]. GPR39 expression was detected in atrial tissue and retinal pigment epithelium cells (hRPE) by RT-PCR and immunocytochemistry [5,23]. GPR39 mRNA was expressed in adipose tissue and reduced in obese T2DM patients [7]. It was observed that the GPR39 receptor protein was localized on the adipocyte cell membrane in obese and in pregnant women by fluorescent immunohistochemistry [14].

In rats, a wide range of tissues expressed GPR39 mRNA at a relatively higher level in digestive tissues such as the jejunum, duodenum, stomach and ileum, and at a moderate level in the brain regions including the hypothalamus [52]. On the other hand, a recent study reported that no mRNA of GPR39 could be found in the hypothalamus of rats [35]. Quantitative RT-PCR (Q-PCR) analysis demonstrated that GPR39-1a was highly expressed in liver, GI-tract from the stomach to colon, pancreas, kidney, as well as in adipose tissue. Lower expression was observed in the spleen, thyroid, lung and heart, and no significant expression was detected in the central nervous system. GPR39-1b expressed more widely but especially in GI-tract, liver and pancreas as well as in kidney and adipose tissue. Furthermore, the GPR39-1b form was a broader expression pattern including the CNS, and the highest expression was observed in stomach and small intestine. A

Table 1 – Distribution of the GPR39 receptor.

Species	Form of GPR39	Analysis methods	Distribution sites	Expression levels	References
Human	GPR39	Northern blot	Stomach, intestine hypothalamus	High	[30]
		RT-PCR and immunocytochemistry	Atrial tissue	High	[23]
		RT-PCR	Human retinal pigment epithelium cells (hRPE)	High	[5]
		Real-time RT-PCR	Adipose tissue	High	[7]
		Fluorescent immunohistochemistry	Adipocyte cell membrane	High	[14]
Rat	GPR39	RT-PCR	Jejunum, duodenum, stomach and ileum	High	[52]
			Liver, hypothalamus, pancreas, cerebellum, cerebrum	Moderate	[52]
			Testis, ovary, colon and lung	Low	[52]
			Hypothalamus	No	[35]
	GPR39-1a	Q-PCR	GI-tract, kidney and white adipose tissue	High	[12]
			Spleen, thyroid, lung and heart	Low	
			Brown adipose tissue	No	
		In situ hybridization	Hypothalamus	No	[12]
	GPR39-1b	Q-PCR	Stomach and small intestine	High	[12]
		In situ hybridization	CNS	Low	[12]
Mouse	GPR39	RT-PCR	Septum-amygdala Parietal cells, enterocytes, neurons, and pancreas	High	[31]
		Q-PCR	Duodenum and kidney	High	[19]
			Pituitary and hypothalamus	No	
		In situ hybridization	Amygdala, hippocampus and auditory cortex	High	[24]
			Hypothalamus	No	
		RT-PCR and immunocytochemistry	Cardiomyocytes	High	[23]
Quail	GPR39	RT-PCR	Stomach, uodenum, jejunum, ileum, cecum, colon and rectum	High	[47]
			Infundibulum, magnum, isthmus, and uterus	Moderate	
Chicken	GPR39	Q-PCR	Duodenum	High	[46]
			Liver, kidney, stomach and oviduct	Moderate	
			Brain, pituitary, thymus, bursa of fabricius, bone marrow, ovary and testis	Low	
Hen	GPR39	Q-PCR	Duodenum, jejunum, ileum, and cecum	High	[46]
			Colon, rectum, oviduct, vagina and uterus	Moderate	
			Gullet, crop sac and gizzard	Low	

functional analysis of the GPR39 promoter region identified that HNF-1α, HNF-4α, and SP1 were involved in the control of GPR39 expression [12].

In mice, GPR39 mRNA expression was detected in septum-amygdala, parietal cells, enterocytes, neurons and pancreas [31], in peripheral organs such as the duodenum and kidney but not in the pituitary and hypothalamus by Q-PCR [19] and in various brain regions except the hypothalamus by *in situ* hybridization [24]. By RT-PCR and immunocytochemistry, Iglesias et al. [23] reported that GPR39 mRNA was expressed in murine cardiomyocytes cultured *in vitro*.

In birds, Yamamoto et al. reported a detail distribution of GPR39 mRNA in chickens, where a wide range of tissues distribution was observed with the highest level in the duodenum, and moderate levels in the liver, kidney, stomach and oviduct. The expression levels were low in the brain pituitary, thymus, bursa of fabricius, bone marrow, ovary and testis. Expression levels of GPR39 mRNA were also measured by Q-PCR in digestive and reproductive tissues in 1-year-old hen. The highest expression was observed in the duodenum, jejunum, ileum and cecum. Colon, rectum, oviduct, vagina and the uterus showed a moderate expression level, and the expression levels in gullet, crop sac and gizzard were low [46]. In Japanese quail, GPR39 mRNA was expressed at high levels in digestive tracts such as stomach, duodenum, jejunum, ileum, cecum, colon and rectum, and at moderate levels in the oviduct including infundibulum, magnum, isthmus, and uterus [47]. These findings indicated that GPR39 could be involved in the regulation of GI and the reproductive functions in birds.

4. Endogenous ligand for GPR39

4.1. *Obestatin*

Obestatin, a 23-amino acid peptide derived from the ghrelin precursor protein, was reported as an endogenous ligand for

GPR39 [52]. Moechars et al. [31] and Zhang et al. [50] suggested that obestatin was a hormone capable of binding to GPR39 to regulate the functions of diverse gastrointestinal and adipose tissues. Further studies indicated that obestatin was involved in inhibiting thirst and anxiety [37], improving memory [6], affecting cell proliferation [5,53], controlling fluid homeostasis [38] and increasing the secretion of pancreatic juice enzymes [25]. But the effects of obestatin on food intake and body weight [4,15,26,28,32,39,40,43,52,55], gastric motility [1,3,9,10,15,52], hormone secretion and energy balance [4,16,17,34,45,52] are still controversial. The latest studies indicated that obestatin was present at very low levels in the rat and human compared with ghrelin [32], and that obestatin was a hormone without metabolic actions [44].

A series of subsequent studies from a number of independent groups have not been able to demonstrate any binding of obestatin to GPR39 or any stimulatory function of the obestatin on GPR39 [8,29,43]. In 2007, in the article responded to the comment of Chartrel et al. [8], Zhang et al. [51] proved again that obestatin really had effects on food intake, body weight and GI motility in mice, but they also confirmed that their original result of obestatin for GPR39 was unreproductive. Thus, it is concluded that GPR39 was not the receptor for obestatin.

4.2. *Zinc ions*

A new study showed GPR39 could not be activated by obestatin but by the high concentration of zinc ions (Zn^{2+}) [19]. Yasuda et al. [48] implemented further study on GPR39 agonists in fetal bovine serum (FBS) and suggested that GPR39 was a Gq-coupled Zn^{2+}-sensing receptor. In addition, Zn^{2+} also activated mouse and rat GPR39 through Gqα-PLC pathway. This suggested that Zn^{2+} could be a physiologically relevant agonist or modulator of GPR39, and that the function of GPR39 as a Zn^{2+} modulated receptor was conserved across species. Storjohann et al. [42] revealed that Zn^{2+} acted as an agonist for GPR39 through binding to His17 and His19 in the extracellular domain and potentially by diverting Asp313 from functioning as a tethered inverse agonist through engaging this residue in a tridentate metal-ion binding site.

5. Functions of GPR39

It is likely that GPR39 receptor is important for the functions of numerous metabolic organs such as the liver, gastrointestinal tracts, pancreas and adipose tissue [12]. In fact, there is some evidence that GPR39 modulates food intake, gastric mobility and cell death.

5.1. *Food intake and gastrointestinal activity*

To gain further insights into the role of GPR39 in food intake, gastrointestinal motility and body weight homeostasis, GPR39 gene knockout (KO) mice have been used in different studies. Tremblay et al. found that body weight, adiposity, and food intake were similar between GPR39(+/+) and GPR39(−/−) mice. Furthermore, fasting glucose and insulin levels were found to be similar between both genotypes [43]. Moechars and his coworkers focused on the gastrointestinal functions of GPR39 and gained more precise results with the same animal models. They presented evidences that gastric emptying, GI-tract passage, cholesterol levels and the volume of gastric secretion were enhanced significantly, but gastric acid secretion was unchanged after 4 h pylorus ligation in the GPR39(−/−) mice, and that the mature body weight and body fat composition of GPR39(−/−) mice were significantly higher. Food intake was reduced after fasting in GPR39(−/−) mice compared with that in GPR39(+/+) mice [31].

5.2. *Inhibition of cell apoptosis*

Dittmer et al. found that a hippocampal cell line over-expressed GPR39 resisted against diverse stimulators of cell death and protected against oxidative and endoplasmic reticulum stress by coupling to Gα13 and induction of SRE mediated transcription by the small GTPase RhoA [11]. This result suggested that GPR39 was a novel inhibitor of cell death, which might represent a therapeutic target with implications for processes involving apoptosis and ER stress like cancer, ischemia/reperfusion injury, and neurodegenerative disease. Another observation revealed that GPR39 was up-regulated in adipose tissue in fasting and 14 diabetic rats. Furthermore, GPR39 was expressed in mouse embryonic fibroblasts with its expression changed during adipocyte differentiation [12]. These results showed that GPR39 expressed specifically on pre-adipocytes and possibly played a role in adipocyte differentiation, which indicated that this receptor was a metabolically interesting target. It was speculated that GPR39 could possibly play a similar role in the liver, adipose, endocrine pancreas and GI-tract tissue regeneration and differentiation.

In the case of GPR39-1b, Egerod et al. [12] concluded that this ‘‘5TM’’ protein could serve as a function in the *in vivo* setting. If GPR39-1b does affect the signaling of the full length GPR39, it could be easily indicated that the 1b form would be a novel, ligand-independent way of fine-tuning the level of constitutive activity of the receptor. For GPR39, the stomach and small intestine would then be the tissues where GPR39-1b could be expected to serve a physiological purpose.

6. Conclusion

GPR39 has already been described as being widely expressed in human and other animals, especially with the highest expression in gut and adipose tissues. The expression of GPR39 mRNA in a wide range of tissues has driven the researchers to gain insight into the biological function of the GPR39. The studies show that GPR39 has exerted physiological functions involved in regulation of body weight, gastrointestinal mobility, hormone secretion and cell death, but more functions of GPR39 that seem rather useful and interesting are still unknown. Therefore, further study is still required concerning the roles of GPR39 in normal physiology, which should be conducted independently to the function of obestatin. The endogenous ligand of GPR39 is still a new area for further investigation.

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