TGR5, Not Only a Metabolic Regulator

Cong Guo, Wei-Dong Chen and Yan-Dong Wang

State Key Laboratory of Chemical Resource Engineering, College of Life Science and Technology, Beijing University of Chemical Technology, Beijing, China.

Key Laboratory of Receptors-Mediated Gene Regulation and Drug Discovery, School of Medicine, Henan University, Kaifeng, China.

Key Laboratory of Molecular Pathology, School of Basic Medical Science, Inner Mongolia Medical University, Hohhot, China

G-protein-coupled bile acid receptor, Gpbar1 (TGR5), is a member of G-protein-coupled receptor (GPCR) superfamily. High levels of TGR5 mRNA were detected in several tissues such as small intestine, stomach, liver, lung, especially in placenta and spleen. TGR5 is not only the receptor for bile acids, but also the receptor for multiple selective synthetic agonists such as 6α-ethyl-23(S)-methyl-cholic acid (6-EMCA, INT-777) and a series of 4-benzofuranyloxynicotinamide derivatives to regulate different signaling pathways such as nuclear factor κB (NF-κB), AKT, and extracellular signal-regulated kinases (ERK). TGR5, as a metabolic regulator, is involved in energy homeostasis, bile acid homeostasis, as well as glucose metabolism. More recently, our group and others have extended the functions of TGR5 to more than metabolic regulation, which include inflammatory response, cancer and liver regeneration. These findings highlight TGR5 as a potential drug target for different diseases. This review summarizes the basic information of TGR5 and its new functions.

Keywords: TGR5, Gpbar1, GPCR, bile acids, receptor

INTRODUCTION

G-protein-coupled receptors (GPCRs) are large family of receptors, playing important roles in multiple pathways. They contain seven transmembrane domains. Upon binding of ligands in the extracellular space, GPCRs transduce the extracellular signal to intracellular downstream cascades through activating multiple effector pathways. Because of the important functions of GPCR in different cell signaling pathways, they have become attractive targets for treatment of many diseases.

TGR5, as a member of GPCRs, was discovered in 2002. It was classified as the founder member of the bile acid receptor subclass of GPCRs. TGR5 gene locates on chromosome position 2q35 in humans. Its open reading frame has 993 base pairs, encoding 330 amino acids. High levels of TGR5 mRNA were detected in several organs such as small intestine, stomach, liver, lung, especially placenta and spleen. TGR5 can be activated by bile acids and then it induces cAMP production. TGR5 can be activated by bile acids and then it induces cAMP production. As a membrane receptor, TGR5 can be internalized into the cytoplasm in response to its ligands. TGR5 plays important roles in cell signaling pathways such as nuclear factor κB (NF-κB), AKT, and extracellular signal-regulated kinases (ERK). Its agonists may be potential drugs for treatment of metabolic, inflammation and digestive disorders.
Activation of TGR5 has shown promise in treating various metabolic diseases such as type 2 diabetes (T2D) and obesity. Its activation also mediates novel effects on inflammation and cancer in different organs. In this review, we summarize the basic properties of TGR5 including its ligands and basic functions. Specifically, we will discuss the new findings about TGR5 in different signaling pathways and diseases.

THE LIGANDS OF TGR5

As a plasma membrane-bound GPCR, the endogenous natural agonists of TGR5 are bile acids. Taurolithocholic acid (TLCA), lithocholic acid (LCA), deoxycholic acid (DCA), chenodeoxycholic acid (CDCA), and cholic acid (CA) can dose-dependently induce cAMP production in human TGR5-transfected CHO cells. The rank order of potency (EC50) is TLCA (0.33 µM) > LCA (0.53 µM) > DCA (1.01 µM) > CDCA (4.43 µM) > CA (7.72 µM) (Kawamata et al., 2003) (Table 1). Obacunone, as a limonoid, is found in Citrus. It can dose-dependently stimulate the activity of TGR5 (Horiba et al., 2015). Some other compounds such as linolenic acid (Katsuma et al., 2005) and oleanolic acid (OA) are also identified as weak TGR5 ligands (Sato et al., 2007).

CDCA, DCA, LCA, ursodeoxycholic acid (UDCA) are not only the activators of TGR5 but also the activators of farnesoid X receptor (FXR) (Makishima et al., 1999; Wang et al., 2008a,b). In order to find the specific and selective TGR5 ligands, multiple TGR5 agonists were designed and synthesized. Pellicciari et al. reported 23-alkyl-substituted and 6, 23-alkyl-disubstituted derivatives of CDCA are the selective agonists of TGR5 (Pellicciari et al., 2007). 6α-ethyl-23(S)-methyl-choleic acid (6-EMCA, INT-777) had been discovered as a selective, specific agonist for TGR5 (Pellicciari et al., 2009, Table 1). Zhu et al. (2013) designed a new class of potent TGR5 agonists based on 4-phenyl pyridine scaffold. After evaluated in vitro and in vivo, three compounds showed good effects on activating TGR5. A series of 4-benzofuranyloxynicotinamide derivatives were found to be novel and potent TGR5 agonists (Zou et al., 2014, Table 1). One of them has the highest activity in vitro (hTGR5 EC50 = 0.28 nM, mTGR5 EC50 = 0.92 nM). Zambad et al. (2013) synthesized TRC210258 as a novel TGR5 agonist (Table 1). Zheng et al. found small compound WB403 could activate TGR5 and promote GLP-1 secretion (Zheng et al., 2015).

TGR5 AND CELL SIGNALING

TGR5 and AKT Pathway

AKT is a serine/threonine kinase (Faes and Dormond, 2015). It plays important roles in diverse cell processes including differentiation, proliferation, survival, and metabolism (Sasaki and Kuniyasu, 2014). AKT has pleckstrin homology (PH) domain. At the plasma membrane, the interaction between the PH domain of AKT and phosphatidylinositol trisphosphate (PIP3) induces subsequent modifications of AKT at threonine 308. AKT also can be phosphorylated at serine 473. Phosphorylated AKT inhibits pro-apoptotic members of the Bcl-2 family, contributing to cell survival (Sarbassov et al., 2005). In bovine aortic endothelial cells, treatment with TGR5 agonist TLCA enhances AKT phosphorylation and increases NO production (Kida et al., 2013, Figure 1).

Mammalian target of rapamycin (mTOR) is one of the key downstream effectors for the AKT signaling (Covarrubias et al., 2015). mTOR is required for the translation of proteins, which contribute to promoting cell survival and proliferation. TGR5 can reduce chemokine expression via AKT-mTOR pathway in macrophages (Perino et al., 2014). AKT-mTOR pathway can be enhanced through the activation of TGR5. mTOR exists as two complexes mTORC1 and mTORC2. The phosphorylation of AKT and mTORC1 affects the expression of eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP), which is involved in CCATL-enhancer-binding proteins (C/EBPβ) isoform switching. After TGR5 activation, mTORC1 increases the level of phosphorylated 4E-BP and the C/EBPβ isoform liver-inhibitory protein (LIP) expression. The link between TGR5 and AKT-mTOR-LIP reveals a new mechanism by which macrophages contribute to the antidiabetic effects of TGR5 activation (Perino et al., 2014, Figure 1).

TGR5 and NF-κB Pathway

NF-κB is a transcription factor connected with several cellular processes such as inflammation, proliferation, apoptosis and development (Wang et al., 2008c; Meng et al., 2011; Sarode et al., 2015; Papademetrio et al., 2016). NF-κB comprises of five members, RelA (p65), RelB, c-Rel, p50, and p52 (Sun et al., 2013). They are kept inactive in the plasma by binding to family members of IkB including IkBα, IkBβ, IkBγ, BCL3, IkBε, p105, and p100 (DiDonato et al., 2012). Specific IKK kinase regulates IkBα or IkBβ phosphorylation, resulting in activation of NF-κB (Verstrepen and Beyaert, 2014). Two of TGR5 agonists, DCA and LCA, can inhibit tumor necrosis factor-α production in CD14+ macrophages (Yonen et al., 2013). This inhibitory effect is mediated by the phosphorylation of c-Fos to regulate NF-κB p65 activation. Our group identified TGR5 negatively regulated hepatic inflammatory response through antagonizing NF-κB (Wang et al., 2011). We found TGR5 activation suppressed the phosphorylation of IkBα, the translocation of p65, NF-κB DNA binding activity and its transcription activity in HepG2 cells. In the same year, Pols et al. found TGR5 activation by INT-777 decreased nuclear translocation of p65 and phosphorylation ofIkBα in macrophages (Pols et al., 2011) (Table 1, Figure 1).

TGR5 and Extracellular Signal-Regulated Kinases (ERK) 1/2 Pathway

The kinases ERK1 and ERK2 are members of the mitogen-activated protein kinase family (Pascoli et al., 2014). They are involved in diverse cellular responses such as survival, differentiation, and proliferation (Cheng et al., 2013). In the recent report, Reich et al. (2016) shown that TGR5-selective agonists induced cholangiocyte proliferation through elevation of reactive oxygen species and proto-oncogene, non-receptor tyrosine kinase (cSrc)-mediated epidermal growth factor receptor transactivation and subsequent ERK1/2
| Natural agonists | Name | Structures | References |
|------------------|------|------------|------------|
| Primary bile acid | CA | ![CA structures](image) | Kawamata et al., 2003 |
|                  | CDCA | ![CDCA structures](image) | Kawamata et al., 2003 |
| Secondary bile acid | LCA | ![LCA structures](image) | Kawamata et al., 2003 |
|                  | TLCA | ![TLCA structures](image) | Kawamata et al., 2003 |
|                  | DCA | ![DCA structures](image) | Kawamata et al., 2003 |

| Natural phytochemical agonists | Linolenic acid | ![Linolenic acid structures](image) | Katsuma et al., 2005 |
| OA | ![OA structures](image) | Sato et al., 2007 |
| Obacunone | ![Obacunone structures](image) | Horiba et al., 2015 |

| Synthetic agonists | INT-777 | ![INT-777 structures](image) | Pellicciari et al., 2009 |

(Continued)
TABLE 1 | Continued

| Gene | GPBAR1, 2q35 |
|------|-------------|

**Expression in human tissues**

Placenta, Spleen, Small intestine, Stomach, Liver, Lung, Heart, Skeletal muscle, Kidney, Peripheral blood leukocytes

| Natural agonists | Name | Structures | References |
|------------------|------|------------|------------|
|                  | TRC210258 | ![Structure Image](image) | Zambad et al., 2013 |
|                  | WB403 | ![Structure Image](image) | Zheng et al., 2015 |

| Relevant diseases | Agonists | References |
|-------------------|----------|------------|
| Type 2 diabetes    | LCA, DCA, Lionelic acid, OA, INT-777, WB403 | Katsumura et al., 2005; Sato et al., 2007; Perino et al., 2014; Zheng et al., 2015 |
| Obesity           | CA, TCA, DCA, CDCA | Watanabe et al., 2006 |
| Inflammation      | Betulinic acid, 23(S)-mCDCA, TLCA, TLC, CDCA, DCA | Kawamata et al., 2003; Keitel et al., 2008; Wang et al., 2011; Mobraten et al., 2015; Guo et al., 2015b; McMillin et al., 2015 |
| Gastric cancer    | 23(S)-mCDCA, GPBARA | Guo et al., 2015a |
| Liver regeneration| CA | Péan et al., 2013 |

phosphorylation in wild type mouse cells. In the ciliated and non-ciliated cholangiocytes, TGR5 activation induces different changes in the levels of cAMP and ERK (Masyuk et al., 2013). TGR5 agonists increase cAMP level and inhibit ERK signaling, resulting in inducing proliferation in non-ciliated cholangiocytes. But in the ciliated cholangiocytes, TGR5 agonists decrease cAMP level and induce ERK signaling, resulting in inhibition of proliferation. The opposite effects of TGR5 agonists are due to the coupling of TGR5 to \( \alpha_i \) protein in ciliated cells and \( \alpha_s \) protein in non-ciliated cells (Masyuk et al., 2013, Figure 1).

**TGR5 and Signal Transducer and Activator of Transcription 3 (STAT3) Pathway**

STAT3 was at first found as a DNA-binding factor in interleukin-6 (IL-6) stimulated hepatocytes. It is an enhancer element in the promoter region of acute-phase genes (Akira et al., 1994). As a transcription factor, STAT3 controls several cellular processes including development, differentiation, immunity, invasion, and metabolism (Kane et al., 2014; Teng et al., 2014; Poli and Camporeale, 2015). It is overexpressed in pathological conditions such as cancer (Yamanaka et al., 1996). Many reports showed STAT3 is activated in various tumor cell lines such as colon, gastric, lung, skin, and breast cancer cells (Levy and Lee, 2002; Yin et al., 2006; Sansone et al., 2007; Yoshimura et al., 2007). Our group found that TGR5 is a suppressor of gastric cancer cell proliferation and migration through antagonizing STAT3 signaling pathway (Guo et al., 2015b). TGR5 activation antagonized STAT3 signaling pathway through suppressing the phosphorylation of STAT3 and its transcription activity induced by lipopolysaccharide (LPS) or IL-6. It suggests that TGR5 antagonizes gastric cancer proliferation and migration at least in part by inhibiting STAT3 signaling. These findings identify TGR5 as an attractive therapeutic target for treatment of gastric cancer (Guo et al., 2015a,b, Figure 1).

**TGR5 and Exchange Protein Directly Activated by cAMP (Epac) Pathway**

Epac is a member of guanine nucleotide exchange factor family and an essential cAMP effector (Gloerich and Bos, 2010). It has multiple binding factors, and is involved in several cellular events (Breckler et al., 2011). In pancreatic \( \beta \) cells, the activation of TGR5 by OA and INT-777 selectively activates \( \alpha_s \). And then the levels of intracellular cAMP and \( \text{Ca}^{2+} \) will be increased. Epac but not protein kinase A (PKA) can be activated by 8-pCT-2′-O-Me-cAMP, a cAMP analog, and stimulates phosphoinositide (PI) hydrolysis. As the result of the effect, insulin releases from pancreatic \( \beta \) cells (Kumar et al., 2012). In enteroendocrine cells, TGR5 ligand OA can also stimulate \( \alpha_s \) and cAMP formation, and activate Epac increasing PI hydrolysis, glucagon-likepeptide1 (GLP-1) and Peptide YY (PYY) release (Figure 1).
**TGR5 and T2D**

Diabetes is one of the fastest growing diseases in the world. T2D is the most common type of diabetes (Zarrinpar and Loomba, 2012). The development of T2D is commonly related to obesity, hypertension, and dyslipidemia (Goedecke and Mckliesfield, 2014; Maki and Phillips, 2015). These latter complications promote the development of cardiovascular disease (Johnston et al., 2014). And they are the most common mortality linked to T2D. T2D is classically described as a heterogeneous group of disorders, characterized by a decline in insulin-producing pancreatic β cells, an increase in peripheral insulin resistance, an increase in hepatic glucose production, or a combination of all the factors (Alejandro et al., 2015). Therapies for T2D are made based on reducing hepatic glucose production, increasing insulin secretion, and improving insulin sensitivity (Zarrinpar and Loomba, 2012).

Several studies show the importance of bile acids in glucose homeostasis. Bile acids can improve glycemic control (Zarrinpar and Loomba, 2012). TGR5 as a receptor of bile acids has effect on the regulation of glucose metabolism. In 2005, the study of Katsuma et al. shown the activation of TGR5 could promote GLP-1 secretion in a murine enteroendocrine cell line STC-1 (Katsuma et al., 2005). GLP-1, as the incretin hormone, has the incretin effect, which is the augmentation of insulin secretion after oral administration of glucose. So GLP-1 plays an important role in T2D (Sonne et al., 2014). The secretion of GLP-1 is dose-dependent. The overexpression of TGR5 enhances the level of cAMP and GLP-1 secretion. It suggests that TGR5 induces GLP-1 secretion via intracellular cAMP production (Katsuma et al., 2005). This study aroused the interest of many groups in exploring potential treatment of T2D through the management of glucose homeostasis by activating TGR5. In 2007, OA isolated from olive leaves was found as a natural TGR5 agonist. It decreased plasma glucose and insulin via the activation of TGR5 (Sato et al., 2007). Recent years, it is found that TGR5 induces differential translation of the C/EBPβ isoform LIP by AKT-mTOR pathway in macrophages. And the activation of TGR5 can alter adipose tissue macrophage (ATM) function and improve insulin action. So TGR5 activation in macrophages may prevent insulin resistance and treat T2D (Perino et al., 2014, Table 1).

In 2015, a small compound WB403 was identified as a TGR5 agonist. It was tested in the different mouse models of T2D for glycemic control. As a result, TGR5 could be activated by WB403 to improve glucose tolerance, decrease fasting blood glucose and the glycosylated hemoglobin A1c (HbA1c) in T2D mice (Zheng et al., 2015). In the new reports, Kumar et al. (2016) shown that TGR5 induced GLP-1 release from pancreatic α cells via an Epac-mediated PKA-independent mechanism. Agarwal et al.
also shown the important roles of TGR5 in T2D. All of these studies indicate the important functions of TGR5 in T2D treatment.

**TGR5 and Obesity**

Obesity becomes great threat to public health in the world. The energy intake exceeds expenditure, resulting in obesity (Nalliah et al., 2016). It is now known that brown adipose tissue (BAT) dissipates energy as heat by thermogenesis (Chen et al., 2011). In human BAT, the mitochondria are powerful generators of heat. It metabolizes fat, protecting people from obesity. Because of the key role of BAT in energy burning, increasing BAT amount could be used for treatment of obesity. The administration of bile acids to mice can increase energy expenditure in BAT. This effect is dependent on activation of TGR5, but not FXR (Chen et al., 2011). TGR5 activation increases the level of cAMP-dependent thyroid hormone-activating enzyme, type 2 iodothyronine deiodinase (D2). D2 is one of major thermogenic protein. It can convert thyroxine (T4) into active tri-iodothyronine (T3) in BAT. Bile acid treatment in BAT and human skeletal muscle cells increases D2 activity, oxygen consumption and extracellular acidification rate (Watanabe et al., 2006). In the recent years, different groups also show that the new roles of TGR5 in obesity (Chen et al., 2015; Donepudi et al., 2016; Pierre et al., 2016; Wang et al., 2016). For example, Wang et al. (2016) reported TGR5 inhibited kidney disease in obesity and diabetes through inducing mitochondrial biogenesis and preventing renal oxidative stress and lipid accumulation. These reports suggest that TGR5 agonists may be the potential drugs for treating obesity.

**TGR5 and Inflammation**

Inflammation is one of the responses of the organism to harmful stimuli, such as pathogens, damaged cells, or irritants (Wang et al., 2008c; Meng et al., 2011). Chronic inflammation is increasingly recognized as an important component of tumorigenesis and metabolic diseases (Goussens and Werb, 2002). Therefore, the precise control of inflammation is essential for the prevention of chronic inflammatory disorders, as well as for inhibiting the exacerbation or progression of diseases, including many types of cancers (Shacter and Weitzman, 2002; Wang et al., 2011).

Our group found the activation of TGR5 could inhibit inflammation in liver and stomach (Wang et al., 2011; Guo et al., 2015a). In liver, TGR5 inhibits the expression of inflammatory mediators in response to NF-κB activation induced by LPS in wild-type (WT), but not TGR5−/− mice (Wang et al., 2011). Yang et al. (2016) reported that during ischemia/reperfusion injury TGR5 inhibited inflammatory response through suppression of the Toll-like receptor 4 (TLR4)-NF-κB pathway. TGR5 activation can also suppress LPS-induced production of cytokines in Kupffer cells and TGR5-overexpressed THP-1 cells (Kawamata et al., 2003; Keitel et al., 2008). But in human monocytes, co-triggering of TGR5 and TLR4 enhances the activation of NF-κB and the production of inflammatory cytokines. The two different and simultaneous events associate with the function of human monocytes, contributing to increasing inflammation (Mobraten et al., 2015). Hepatic encephalopathy (HE) can be a major neurological complication of acute and chronic liver failure. It causes neuroinflammation. The activation of TGR5 by betulinic acid decreases neuroinflammation via neuron and microglia paracrine signaling during HE (McMillin et al., 2015, Table 1). Last year, our group found that TGR5 activation also suppresses gastric inflammation (Guo et al., 2015a). Chronic inflammation is connected with various diseases such as liver, colon and gastric cancer (Guo et al., 2015b). TGR5 may be a potential target for treatment of chronic inflammation and related cancer.

**TGR5 and Cancer**

Gastric cancer is one of the most common cancers in the world. Gastric carcinogenesis is a complex process and easily causes death (Lin et al., 2015). There are few reports about TGR5 and cancer. Our group found that TGR5 activation could suppress gastric cancer cell proliferation and migration via inhibiting STAT3 pathway (Guo et al., 2015b). Han et al. (2014) demonstrated that the aberrant hypermethylation of TGR5 promoter in serum cfDNA might serve as a biomarker for the surveillance of HCC. Hong et al. (2010) found that TGR5 receptor is overexpressed in oesophageal adenocarcinoma tissues and indicated TGR5 may play an important role in oesophageal adenocarcinoma. The functions of TGR5 in other cancers need to be investigated.

**TGR5 and Liver Regeneration**

Normal liver regeneration is important for restoring the liver mass following liver injury. Previous reports indicate that 70% hepatectomy increases BA flux and changes expression of several nuclear receptors and enzymes involved in BA metabolism (Wang et al., 2008a). The reports showed that bile salts are important for liver regeneration following partial hepatectomy through activating FXR and TGR5 (Wang et al., 2008c; Chen et al., 2010; Fan et al., 2015). In TGR5 knockout mice, exacerbated inflammatory response, severe hepatocyte necrosis, prolonged cholestasis, and delayed regeneration was observed after partial hepatectomy (Péan et al., 2013). So TGR5 has a crucial protective role on the liver in case of BA overload after partial hepatectomy through the control of bile hydrophobicity and cytokine secretion (Zou et al., 2014; Joudainne et al., 2015).

**Other Bile Acid Membrane Receptors**

Bile acids also activate other two GPCRs sphingosine-1-phosphate receptor 2 (S1PR2) and muscarinic receptor 2 (Chrm2) (Zhou and Hylemon, 2014). Conjugated bile acids activate S1PR2 to regulate inflammation, cancer development and some liver diseases (Kwong et al., 2015). Muscarinic receptors are overexpressed in colon cancer and their activation promotes proliferation, migration and invasion of human colon cancer cells (Raufman et al., 2003, 2011).
PROSPECTS

TGR5, as an important membrane receptor, is activated by bile acids and multiple compounds. The novel roles of TGR5 in different diseases make it become a new drug target. Further investigation of TGR5 will provide novel insights into the complex mechanism of metabolic diseases and cancer.

AUTHOR CONTRIBUTIONS

CG wrote the manuscript, WC and YW revised and edited the manuscript.

REFERENCES

Agarwal, S., Patil, A., Aware, U., Deshmukh, P., Darji, B., Sasane, S., et al. (2016). Discovery of a potent and orally efficacious TGR5 receptor agonist. ACS Med. Chem. Lett. 7, 51–55. doi: 10.1021/acsmedchemlett.5b00323

Akira, S., Nishio, Y., Inoue, M., Wang, X. J., Wei, S., Matsuoka, T., et al. (1994). Molecular cloning of AIPR, a novel IFN-stimulated gene factor 3 p91-related transcription factor involved in the gp130-mediated signaling pathway. Cell 77, 63–71. doi: 10.1016/0092-8674(94)90235-6

Alejandro, E. U., Gregg, B., Blandino-Rosano, M., Cras-Meneur, C., and Bernal-Mizrachi, E. (2015). Natural history of beta-cell adaptation and failure in type 2 diabetes. Mol. Aspects Med. 42, 19–41. doi: 10.1016/j.mam.2014.12.002

Breckler, M., Berthouze, M., Laurent, A. C., Crozatier, B., Morel, E., and Lezuoalch, F. (2011). Rap-linked cAMP signaling Epac proteins: compartmentation, functioning and disease implications. Cell Signal. 23, 1257–1266. doi: 10.1016/j.celsis.2011.03.007

Broeders, E. P., Nascimento, E. B., Havekes, B., Brans, B., Roumans, K. H., Tailleux, A., et al. (2015). The bile acid chenodeoxycholic acid increases human brown adipose tissue activity. Cell Metab. 22, 418–426. doi: 10.1016/j.cmet.2015.07.002

Chen, W. D., Wang, Y. D., Zhang, L., Shah, S., Wang, M., Yang, F., et al. (2010). Farnesoid X receptor alleviates age-related proliferation defects in regenerating mouse livers by activating forkhead box m1b transcription. Hepatology 51, 953–962. doi: 10.1002/hep.23390

Chen, X., Lou, G., Meng, Z., and Huang, W. (2011). TGR5, as an important membrane receptor, is activated by bile acid receptor plays a key role in bile acid metabolism and fasting-induced hepatic steatosis in mice. Hepatology. doi: 10.1002/hep.28707. [Epub ahead of print].

Faes, S., and Dormond, O. (2015). PI3K and AKT: unfaithful partners in cancer. Int. J. Mol. Sci. 16, 21138–21152. doi: 10.3390/ijms160921138

Fan, M., Wang, X., Xu, G., Yan, Q., and Huang, W. (2015). Bile acid signaling and liver regeneration. Biochim. Biophys. Acta 1849, 196–200. doi: 10.1016/j.bbabmb.2014.03.021

Fan, C., Q. H., Yu, Y., Zhang, Q., Su, J., Yu, D., et al. (2015a). The G-protein-coupled bile acid receptor Gpbar1 (TGR5) inhibits gastric inflammation through antagonizing NF-κB signaling pathway. Front. Pharmacol. 6, 283. doi: 10.3389/fphar.2015.00287

Fan, C., Su, J., Li, Z., Xiao, R., Ren, J., Li, Y., et al. (2015b). The G-protein-coupled bile acid receptor Gpbar1 (TGR5) suppresses gastric cancer cell proliferation and migration through antagonizing STAT3 signaling pathway. Oncotarget 6, 34402–34413. doi: 10.18632/oncotarget.5353

Guo, C., Su, J., Li, Z., Xiao, R., Ren, J., Li, Y., et al. (2015b). The G-protein-coupled bile acid receptor Gpbar1 (TGR5) suppresses gastric cancer cell proliferation and migration through antagonizing STAT3 signaling pathway. Oncotarget 6, 34402–34413. doi: 10.18632/oncotarget.5353

Han, L. Y., Fan, Y. C., Mu, N. N., Gao, S., Li, F., Ji, X. F., et al. (2014). aberrant DNA methylation of G-protein-coupled bile acid receptor Gpbar1 (TGR5) is a potential biomarker for hepatitis B Virus associated hepatocellular carcinoma. Int. J. Med. Sci. 11, 164–171. doi: 10.7150/ijms.6745

Hong, J., Behar, J., Wands, J., Resnick, M., Wang, L. J., Della, M., et al. (2010). Role of a novel bile acid receptor TGR5 in the development of oesophageal adenocarcinoma. Gut 59, 170–180. doi: 10.1136/gut.2009.188375

Horiga, H., Katsukawa, M., Mita, M., and Sato, R. (2015). Dietary obacunone supplementation stimulates muscle hypertrophy, and suppresses hyperglycemia and obesity through the TGR5 and PPARgamma pathway. Biochem. Biophys. Res. Commun. 463, 846–852. doi: 10.1016/j.bbrc.2015.06.022

Johnston, C. A., Moreno, J. P., and Foreyt, J. P. (2014). Cardiovascular effects of space limitations. This work is supported by the National Natural Science Foundation of China (Grant No. 81370522 and Grant No. 81472232), Program for Science & Technology Innovation Talents in Universities of Henan Province (HASTIT, Grant No. 13HASTIT024) and Plan for Scientific Innovation Talent of Henan Province to WC.

ACKNOWLEDGMENTS

We apologize to colleagues whose work could not be cited due to space limitations. This work is supported by the National Natural Science Foundation of China (Grant No. 81370537 and No. 81672433) and the Fundamental Research Funds for the Central Universities (Grant No. YS1407 and Grant No. 2050205) to YW, the National Natural Science Foundation of China (Grant No. 81270522 and Grant No. 81472232), Program for Science & Technology Innovation Talents in Universities of Henan Province (HASTIT, Grant No. 13HASTIT024) and Plan for Scientific Innovation Talent of Henan Province to WC.
kidney disease in obesity and diabetes. *J. Am. Soc. Nephrol.* 27, 1362–1378. doi: 10.1681/ASN.2014121271

Wang, Y. D., Chen, W. D., and Huang, W. (2008a). FXR, a target for different diseases. *Histol. Histopathol.* 23, 621–627.

Wang, Y. D., Chen, W. D., Moore, D. D., and Huang, W. (2008b). FXR: a metabolic regulator and cell protector. *Cell Res.* 18, 1087–1095. doi: 10.1038/cr.2008.289

Wang, Y. D., Chen, W. D., Wang, M., Yu, D., Forman, R. M., and Huang, W. (2008c). Farnesoid X receptor antagonizes nuclear factor κB in hepatic inflammatory response. *Hepatology* 48, 1632–1643. doi: 10.1002/hep.22519

Wang, Y. D., Chen, W. D., Yu, D., Forman, B. M., and Huang, W. (2011). The G-protein-coupled bile acid receptor, Gpbar1 (TGR5), negatively regulates hepatic inflammatory response through antagonizing nuclear factor κ light-chain enhancer of activated B cells (NF-κB) in mice. *Hepatology* 54, 1421–1432. doi: 10.1002/hep.24525

Watanabe, M., Houten, S. M., Mataki, C., Christoffolete, M. A., Kim, B. W., Sato, H., et al. (2006). Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. *Nature* 439, 484–489. doi: 10.1038/nature04330

Yamanaka, Y., Nakajima, K., Fukada, T., Hibi, M., and Hirano, T. (1996). Differentiation and growth arrest signals are generated through the cytoplasmic region of gp130 that is essential for Stat3 activation. *EMBO J.* 15, 1557–1565.

Yang, H., Zhou, H., Zhuang, L., Wang, X., and Lv, L. (2016). GPBAR1/TGR5 Attenuates Liver Ischemia/Reperfusion Injury via the Inhibition of TLR4 Signaling in Mice. *Liver Transplant.* doi: 10.1002/lit.24628. [Epub ahead of print].

Yin, W., Cheepala, S., Roberts, J. N., Syson-Chan, K., DiGiovanni, J., and Clifford, J. L. (2006). Active Stat3 is required for survival of human squamous cell carcinoma cells in serum-free conditions. *Mol. Cancer* 5:15. doi: 10.1186/1476-4598-5-15

Yoneno, K., Hismatsu, T., Shimamura, K., Kamada, N., Ichikawa, R., Kitazume, M. T., et al. (2013). TGR5 signalling inhibits the production of pro-inflammatory cytokines by in vitro differentiated inflammatory and intestinal macrophages in Crohn's disease. *Immunology* 139, 19–29. doi: 10.1111/imm.12045

Yoshimura, A., Naka, T., and Kubo, M. (2007). SOCS proteins, cytokine signalling and immune regulation. *Nat. Rev. Immunol.* 7, 454–465. doi: 10.1038/nri2093

Zambad, S. P., Tuli, D., Mathur, A., Ghalsasi, S. A., Chaudhary, A. R., Deshpande, S., et al. (2013). TRC210258, a novel TGR5 agonist, reduces glycemic and dyslipidemic cardiovascular risk in animal models of diabetes. *Diabetes Metab. Syndr. Obs.* 7, 1–14. doi: 10.2147/DMSO.S50299

Zarrinpaz, A., and Loomba, R. (2012). Review article: the emerging interplay among the gastrointestinal tract, bile acids and incretins in the pathogenesis of diabetes and non-alcoholic fatty liver disease. *Aliment. Pharmacol. Ther.* 36, 909–921. doi: 10.1111/apt.12084

Zheng, C., Zhou, W., Wang, T., You, P., Zhao, Y., Yang, Y., et al. (2015). A novel TGR5 activator WB403 promotes GLP-1 secretion and preserves pancreatic β-cells in type 2 diabetic mice. *PLoS ONE* 10:e0134051. doi: 10.1371/journal.pone.0134051

Zhou, H., and Hylemon, P. B. (2014). Bile acids are nutrient signaling hormones. *Steroids* 86, 62–68. doi: 10.1016/j.steroids.2014.04.016

Zhu, J., Ning, M., Guo, C., Zhang, L., Pan, G., Leng, Y., et al. (2013). Design, synthesis and biological evaluation of a novel class of potent TGR5 agonists based on a 4-phenyl pyridine scaffold. *Eur. J. Med. Chem.* 69, 55–68. doi: 10.1016/j.ejmech.2013.07.050

Zou, Q., Duan, H., Ning, M., Liu, J., Feng, Y., Zhang, L., et al. (2014). 4-Benzofuranyloxy nicotinamide derivatives are novel potent and orally available TGR5 agonists. *Eur. J. Med. Chem.* 82, 1–15. doi: 10.1016/j.ejmech.2014.05.031

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2016 Guo, Chen and Wang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.