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TGR5: an emerging bile acid G-protein-coupled receptor target for the potential treatment of metabolic disorders

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Over the past decade, new roles for bile acids in paracrine and endocrine regulation of cholesterol homeostasis, lipid and carbohydrate metabolism and immunomodulatory functions have been discovered. Most of the early discoveries focused on the genomic actions of bile acids through the activation of families of nuclear receptors, such as the farnesoid X receptor and vitamin D receptors, until a new chapter in the bile acid receptor discovery unfolded in the form of TGR5; a novel G-protein-coupled receptor mediating several non-genomic functional responses induced by binding of bile acids. The key involvement of TGR5 in mediating energy homeostasis and glucose homeostasis made it an attractive target for the potential treatment of metabolic disorders.

The global spread and ever-increasing incidence of metabolic syndrome over the past few decades has not only categorized it as an 'epidemic' but has also dramatically increased the risk of cardiovascular diseases and diabetes. The increase in prevalence of diabetes in the United States alone has been estimated at 24 million and more than 180 million people world wide [1]. As a result, there is concern over the increasing incidence of microvascular and macrovascular complications. Given the relationship of lipid and carbohydrate metabolism abnormalities in type 2 diabetes, obesity and associated cardiovascular diseases, it is imperative to have a range of pharmacological interventions in addition to lifestyle modifications to achieve successful long-term management. The global pharmaceutical sales for diabetes has witnessed an average growth rate of nearly 20% from around US$4 billion in 1995 to over US$17 billion in 2005. Overall, anti-diabetic drug sales are expected to grow dramatically over the next five years to over US$ 22 billion in 2012 to address the unmet needs of ever increasing patient population given the severity of disease and associated co-morbidities [2]. The pharmacological management of type 2 diabetes, obesity, hyperlipidemia and related co-morbidities requires an aggressive and comprehensive approach with early intervention required to delay or even prevent the progression of the disease or the use of combination therapies to reach acceptable glycemic and lipid control during the late stages of the disease. There are various drugs available with different modes of actions, relating to insulin secretion (sulfonylureas, meglitinides, DPP-IV inhibitors; glucagon-like peptide-1 (GLP-1) analogues or mimetic); hepatic glucose production (metformin, thiazolidinediones); peripheral tissue insulin resistance (metformin, thiazolidinediones); inhibition of dietary carbohydrate breakdown (α-glucosidase inhibitors) and reduction of circulating lipid (statins, fibrates), yet there is a need for new pharmacological tools, not dealing solely with reducing blood glucose or lipid but tackling the problem of metabolic syndrome as a whole. In the search for such emerging tools, bile acid receptors have been identified as potential targets and are being explored to address the various aspects of metabolic disorders. Bile acids have long been considered as the products of cholesterol catabolism and viewed as components involved in solubilization of cholesterol, fatty acids and lipophilic vitamins, owing to their detergent-based amphiphatic properties [3]. The historical perspective related to the roles of bile acids in digestion, absorption and transport of dietary lipids underestimated the therapeutic applications of the bile acids. Recent discoveries have ushered a new chapter in the profile of bile acids, recognizing those paracrine and endocrine functions related to the homeostasis of cholesterol, lipid and carbohydrate metabolism

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1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2009.02.005

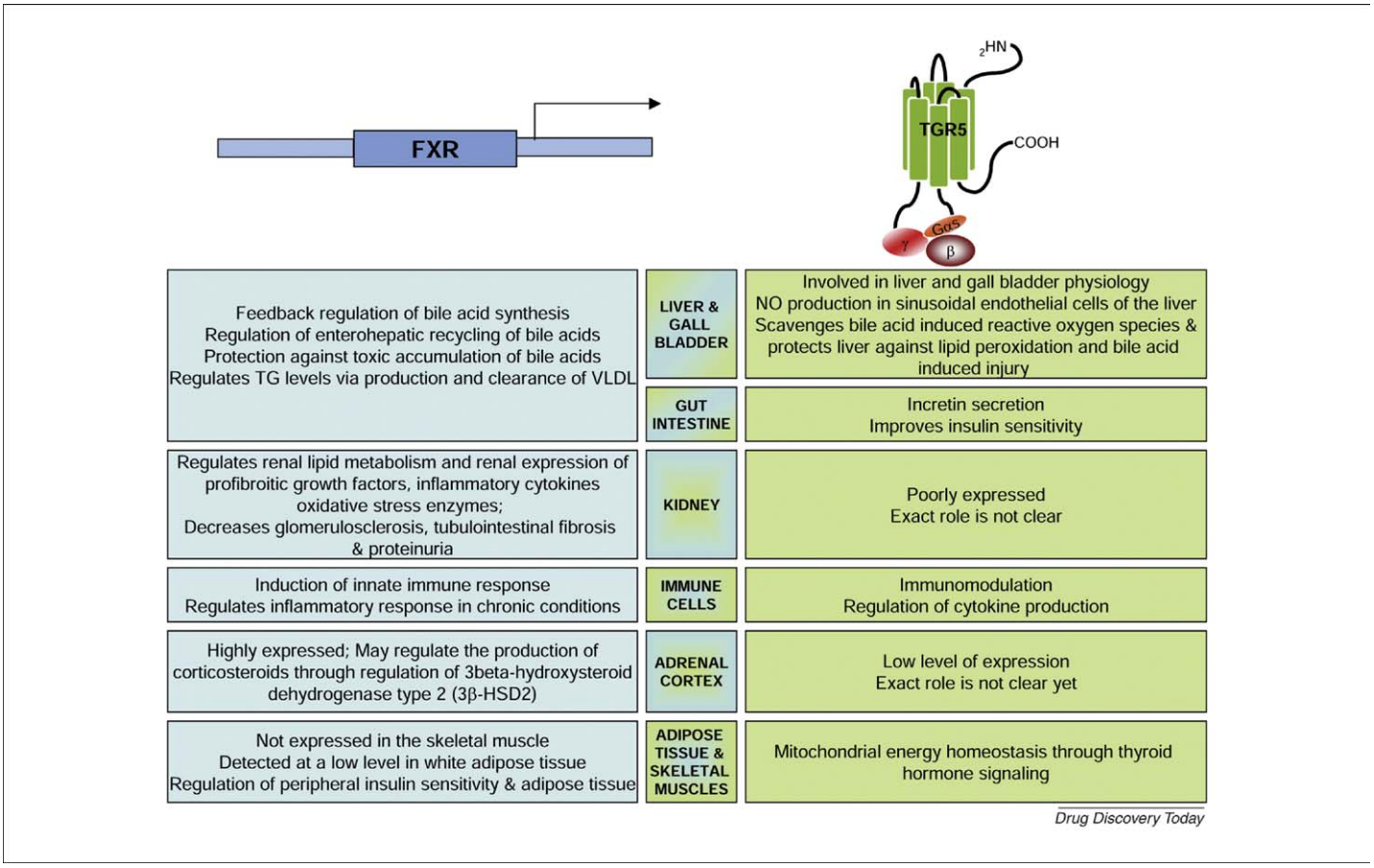

FIGURE 1

Genomic and non-genomic functions of bile acids mediated through FXR and TGR5 in key expressing tissues. Note: NO: nitric oxide; TG: triglycerides; VLDL: Very low-density lipoproteins.

and regulation of the immune system [4]. In 1999, an orphan farnesoid X receptor (FXR; also known as NR1H4) was identified as an endogenously expressing bile acid nuclear receptor [5,6]. FXR is now recognized as a master regulator of the pleiotropic actions of endogenous bile acids in the regulation of enterohepatic recycling of bile acids and in the feedback regulation of bile acid biosynthesis in the liver and intestine (Figure 1). Activation of FXR-α protects against the toxic accumulation of bile acids through increased conjugation in the liver, followed by their excretion into bile canaliculi, thereby promoting bile flow [7–9]. Apart from these roles, FXR is also implicated in regulation of lipid metabolism, renal expression of inflammatory cytokines and oxidative stress enzymes [10]; immune response under certain chronic states [11]; production of corticosteroids through regulation of 3beta-hydroxysteroid dehydrogenase type 2 in the adrenal cortex [12] and glucose metabolism through regulation of gluconeogenesis and glycogenolysis in the liver [13,14] and regulation of peripheral insulin sensitivity in the adipose tissue [15] (Figure 1). The discovery of FXR was followed by the pregnane X receptor (PXR; also known as NR1I2) and the vitamin D receptor (VDR; also known as NR1I1), which are other bile acid-activated receptors [16,17]. The genomic functions of bile acids contributed to activation of these nuclear receptors, however, the non-genomic functions of bile acids in terms of intracellular signaling and functional responses were not established until 2002, when TGR5 (also known as M-BAR or BG37 or GPBAR1)—a metabotropic receptor of the bile acid was discovered (Figure 1) [18,19]. The present review highlights the role of TGR5, therapeutic relevance, reasons for increasing therapeutic interest, current developments and the future landscape of the target.

TGR5 receptor biology

TGR5 was identified through the exploration of GPCRs in the GenBank™ database with human spleen cDNAs and finding a genomic DNA sequence (AC021016) coding for a novel GPCR, designated TGR5 [19]. The TGR5 cDNA is encoded by a single exon that maps to chromosome position 2q35 in humans and to a syntenic region on mouse chromosome 1c3. The initiation codon of human TGR5 was found on the basis of the nucleotide sequence information of AC055884 and AC021016, and the open reading frame (ORF) of human receptor was estimated to consist of 993 base pairs, with a deduced amino acid sequence of a protein consisting of 330 amino acid residues [18]. The motif analysis predicted the protein to be a seven-transmembrane receptor. The subsequent isolation of TGR5 cDNAs in various other species, including identification of homologues of TGR5 in aquatic vertebrates, highlighted the sequence conservation amongst mammals and a role in vertebrate physiology as well. Human TGR5 (GPBAR1 or BG37 or GPCR19 or GPR131 or M-BAR or MGC 40597) shares 82, 83, 86 and 90% amino acid identity with rat (Gpbar1), mouse (Gpbar1 or BG37 or GPR131 or M-BAR), bovine (GPBAR1 or MGC

152072) and rabbit (Gpbar1) receptor respectively [19]. Despite the fairly strong conservation amongst vertebrates, phylogenic analysis highlights that human TGR5 is convergent to monkey and rabbit receptors and is quite divergent from rodent (rat and mice) receptors [20]. Among the known GPCRs, TGR5 shared moderate amino acid sequence similarity with the endothelial cell differentiation gene (EDG) family of receptors being approximately 25, 26, 29 and 30% sequence identity with EDG7, EDG1, EDG8 and EDG6 respectively [21]. Northern hybridization using RNAs from human tissue samples, revealed the ubiquitous expression of TGR5 in various tissues, such as heart, spleen, skeletal muscle, kidney, liver, small intestine, placenta, lung and peripheral blood leukocytes [19]. Similar expression analyses in day-7, day-11, day-15 and day-17 mouse embryos and adult mouse identified the expression of TGR5 gene in heart, spleen, lung, liver, kidney, skeletal muscle, and testis. [18]. Northern hybridization studies were corroborated in human samples through reverse transcription PCR, revealing the highest level of expression in human placenta and spleen, followed by moderate expression in other tissues including lungs, liver, stomach, small intestine and adipose tissue with relatively low level of expression in kidney, skeletal muscles and pancreas. The relative expression level of human TGR5 mRNA in adipose tissues and intestine was nearly threefold to fivefold lower compared with spleen and placenta. Likewise, the relative expression level in the human skeletal muscles was nearly 25–30 fold lower compared with the expression level in the spleen and placenta [19]. TGR5 mRNA was detected in the resting $CD14^+$ monocytes in fractionated human leukocytes and in adherent alveolar macrophage cells, indicating the potential involvement of TGR5 in immune responses mediated by monocytes/macrophages [19]. The expression of TGR5 in different diseased conditions, however, is not yet known.

TGR5 is a class A GPCR, transducing signal through Gs-protein mediated cAMP accumulation, and was recently reclassified as the founder member of the bile–acid receptor subclass of GPCRs [22]. The cAMP-mediated signaling of TGR5 activation has been implicated in a range of cellular physiological activities (Figure 2) and will be discussed further in detail in the review. TGR5 is expressed on the plasma membrane and is internalized into the cytoplasm in response to its agonists [19]. Of the different bile acids serving as natural endogenous ligands for the receptor, taurine-conjugated lithocholic acid (TLCA), lithocholic acid (LCA), deoxycholic acid (DCA), chenodeoxycholic acid (CDCA) and cholic acid (CA) dose-dependently induced the accumulation of cAMP in TGR5-transfected CHO cells with the following rank order potency of—TLCA (0.33 μM) > LCA (0.53 μM) > DCA (1.01 μM) > CDCA (4.43 μM) > CA (7.72 μM) but not in mock-transfected cells, independent of nuclear receptor expression [19]. A recent study conducted with a set of bile acids and its derivatives in CHO cells transfected with TGR5 and in human intestinal

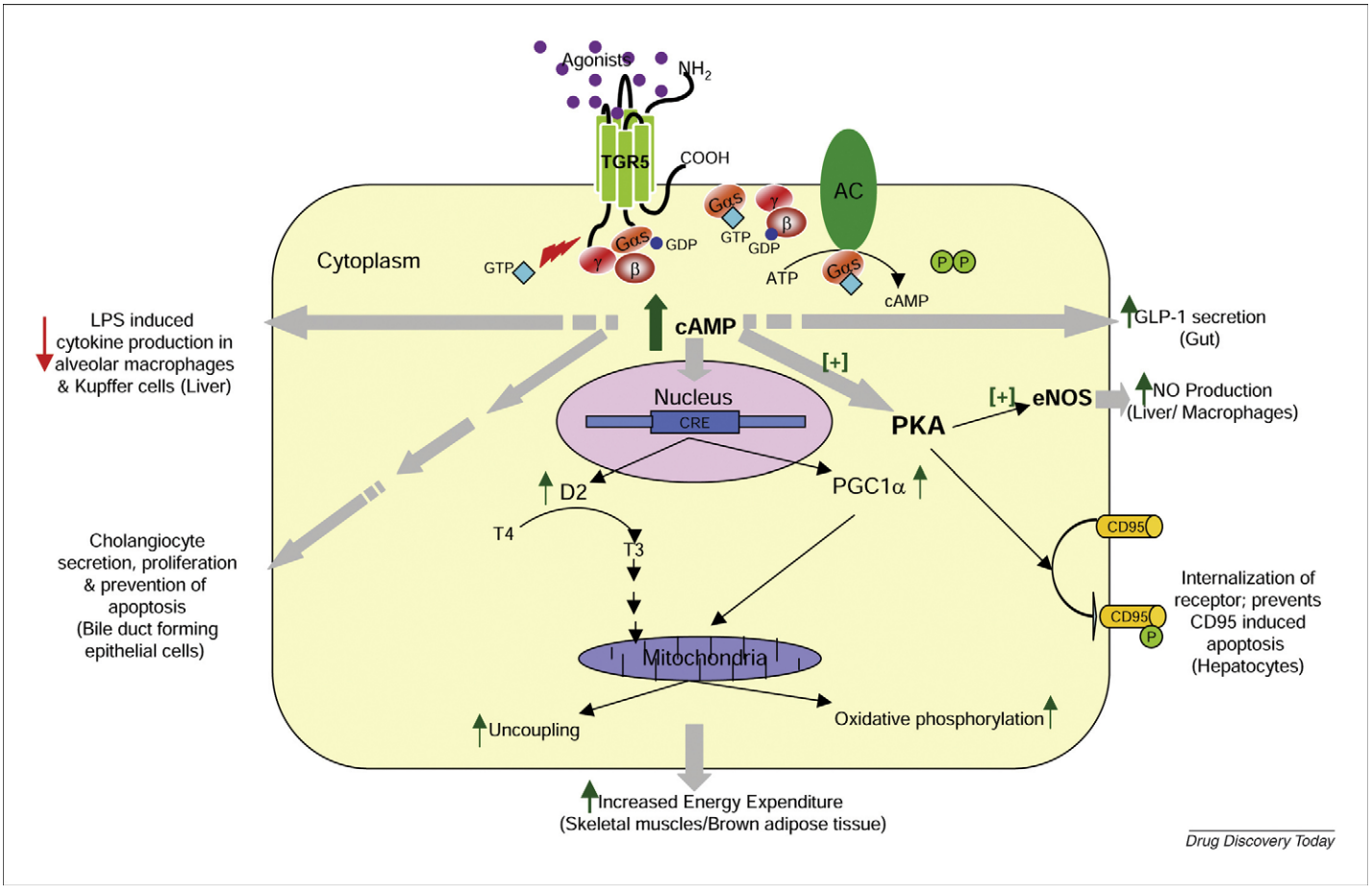

FIGURE 2

TGR5: Potential signaling mechanism and functional relevance of receptor activation. Note: AC: Adenylate cyclase; CRE: Cyclic AMP response element; eNOS: endothelial nitric oxide synthase; PGC1a: Peroxisome proliferator activated-receptor gamma coactivator-1alpha; PKA: protein kinase A.

NCI-H716 cells, has identified some potent, selective TGR5 agonists with the following rank order of potency: 7ξ-Me-LCA (0.076 μM) 7α-F-LCA (0.25 μM) and CDC-Sul (0.44 μM) with respective fold selectivity over FXR binding of 213, >400 and >200 [23]. Agonist potency appeared to be directly related to hydrophobicity. Ursodeoxycholic acid and cholesterol exhibit poor activity; however, pregnandione had a potency ~50% of that of TLCA, implying the importance of hydroxyl groups, as well as 5β-cholanic acid structures in the agonistic activity on TGR5. The absence of little or no activity with (E)-([tetrahydrotetramethyl-napthalenyl]propyl) benzoic acid (TTNPB), rifampicin, and 22(R)-hydroxysterol, which are potent agonists for FXR, pregnane X receptor and liver X receptor, respectively, and a difference in rank order potency of bile acids for TGR5 compared with bile acid nuclear receptors demonstrated an independent signaling pathway for bile acids through TGR5 for rapid response [19].

TGR5 and energy homeostasis

Bile acids have been reported to inhibit diet-induced obesity and prevent the development of insulin resistance through their effects on regulation and expression of key genes involved in hepatic fatty acid and triglyceride biosynthesis, very low density lipoprotein production and through increased energy expenditure in metabolically active tissues in the body [24–28]. The former effects are mediated by sterol-regulatory-element-binding protein 1c through activation of FXR-α [29]; however, energy homeostasis is maintained by the activation of TGR5 receptor [28]. The administration of bile acids to mice increased energy expenditure in brown adipose tissue (BAT) through induction of the cAMP-dependent thyroid hormone-activating enzyme, type 2 iodothyronine deiodinase (D2). Bile acid treatment of rodent BAT and human skeletal muscle cells increases D2 activity, oxygen consumption (a measure of aerobic mitochondrial oxidation) and extracellular acidification rate (a measure of glycolysis, lactate production and anaerobic metabolism) [28]. The effect was independent of nuclear bile acid receptors and was mediated through the activation of TGR5 as demonstrated by the use of highly selective synthetic agonist (benzyl 2-keto-6-methyl-4-(2-thienyl)-1,2,3,4-tetra-hydropyrimidine-5-carboxylate) for TGR5 [28]. The bile acid-TGR5-cAMP-D2 signaling pathway, therefore, represents a crucial mechanism for regulating energy homeostasis in order to improve metabolic control (Figure 3). The targeted disruption of the TGR5 (Gpbar1) gene reduced energy expenditure with increased body weight owing to accumulation of fat in homozygous mice compared with wild-type mice [30] also confirms the therapeutic importance of TGR5 as a metabolic switch to control energy homeostasis and consequent management in diet-induced obesity and insulin resistance.

Thyroid hormone 3,5,3′-triiodothyronine (T3) and its precursor, thyroxine (T4), are iodinated compounds, which are known to

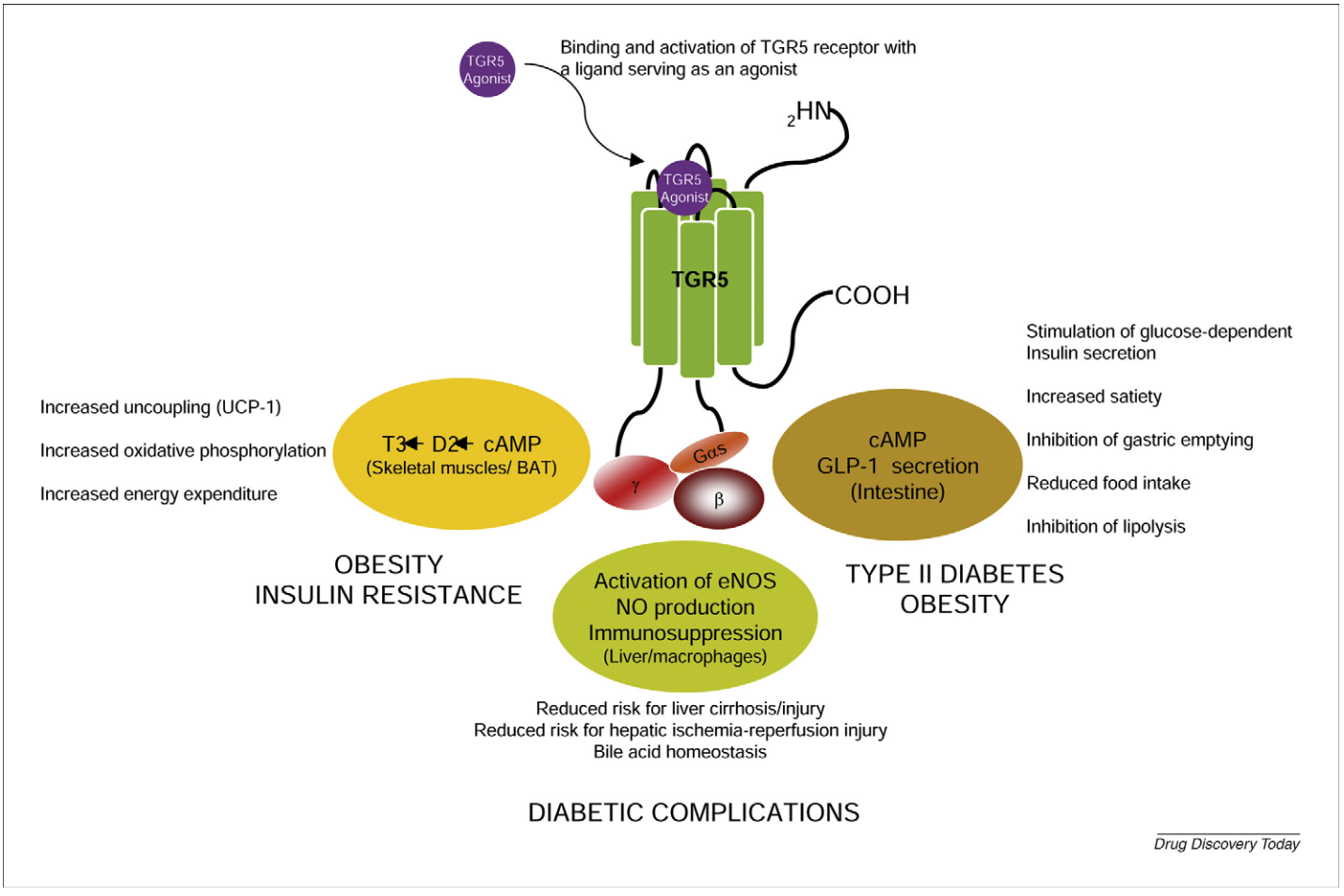

FIGURE 3

TGR5: Potential mechanism and therapeutic relevance of receptor activation by TGR5 agonists.

regulate the expression of genes involved in various biological processes, such as development, growth and metabolic control. The iodothyronine deiodinase types I, II and III (D1, D2 and D3 respectively) regulate the activity of thyroid hormone via removal of specific iodine moieties from the precursor T4 molecule. Type II enzyme (D2) is mainly responsible for deiodination of T4, thereby generating the active form of thyroid hormone T3, which is implicated in controlling the thermogenesis in mammals [31]. BAT is the major site of adaptive thermogenesis in rodents, with heat being generated as a result of the actions of uncoupling protein 1(UCP-1) [32]. D2 and TGR5 are co-expressed in mouse BAT, which has the highest relative expression level of both genes. The increased expression and activity of D2 in BAT in mice fed on a high fat diet with bile acids confers the resistance offered by bile acids to diet-induced obesity [28]. In contrast to rodents, adult humans do not have significant amount of BAT and utilize skeletal muscle as the component of crucial importance to energy homeostasis. Human skeletal muscle expresses both D2 and TGR5 and bile acids have been reported to increase D2 expression, D2 activity and energy expenditure by means of TGR5 cAMP-mediated pathway (Figure 2) [28]. This would also be more important in maintaining energy homeostasis by bile acids or TGR5 agonists, particularly in adult human subjects carrying BAT in the neck and paravertebral regions [33] and in subjects with increased BAT under high catecholamine states [34] than was once thought. Taken together, all information, in addition to the recent information that adult human skeletal muscles expressing physiologically relevant UCP-1 serve as progenitors of BAT cells [35], indicate that TGR5 agonists should induce thermogenesis, regulating energy expenditure in obese human subjects. It would be however interesting to observe the effects of TGR5 agonists in terms of thermogenesis and energy homeostasis in both healthy and obese populations over a period of time.

The bile acid-TGR5-cAMP-D2-T3 pathway could be therapeutically significant in the management of obesity, through the control of thermogenesis and food intake (Figure 3). Human obesity is associated with altered cholesterol homeostasis including increased production and turnover [36,37] and secretion of excess cholesterol from the liver into bile [38] thereby making a highly saturated bile and, though not proven, could be a possible reason for low energy expenditure in such individuals [39]. Interestingly, the low bile acid synthesis in familial hypercholesterolemia patients was previously regarded as a risk factor for coronary heart disease [40]. Bile acid levels increase in the liver following a meal and a significant amount of bile acid is secreted into the systemic circulation. The postprandial serum levels of bile acids increase up to 15 μM, consistent with the pharmacological concentration required to stimulate TGR5 and D2 [41]. Serum bile acid levels could serve as a hormonal signal for food intake and also as a key factor in diet-induced thermogenesis. The induction of thermogenesis by bile acids under insulin resistant state, however, is not known. With the highest association of insulin resistance with obesity, it would be really interesting and worthwhile to study the effects of bile acids and TGR5 agonists in increasing thermogenesis in such subjects.

TGR5 and glucose homeostasis

The induction of GLP-1 secretion by bile acids through TGR5 in an enteroendocrine cell line (STC-1) [42] aroused the interest of the pharmaceutical industry in exploring this particular target for the potential treatment of type 2 diabetes through the management of glucose homeostasis. The hypothesis was strengthened by a recent finding with oleanolic acid, a natural TGR5 agonist isolated from olive leaves (*O. europaea*) that decreased plasma glucose and insulin levels in C57BL/6J mice maintained on a high fat diet for 10 weeks before the start of the 7 days treatment with oleanolic acid [43]. Oleanolic acid improved metabolic homeostasis in high fat-fed mice and partially corrected glucose tolerance in an intraperitoneal glucose tolerance test (IPGTT). The anti-hyperglycemic activity of oleanolic acid, which is a highly specific and potent TGR5 agonist, supports the potential value of targeting TGR5 in type 2 diabetes. Similarly, the expression of TGR5 in the small intestine and other areas of gut [19], coupled with the secretion of GLP-1 in enteroendocrine cells, indicates the therapeutic relevance of TGR5 agonists in the potential treatment of type 2 diabetes. Endocrine L-cells are present throughout the small and large intestine, with the majority localized to the distal ileum and colon [44], where the primary bile acids, such as cholic acid, are converted to the secondary bile acids such as deoxycholic acid, by gut microorganisms [45]. Since secondary bile acids, are more potent at TGR5 than primary acids [18,19], it possibly implicates TGR5 in the release of GLP-1 in the gut. Given the importance of various activities exhibited by GLP-1 in terms of regulating glucose homeostasis through stimulation of glucose dependent insulin secretion and inhibiting glucagon secretion in the pancreas; inhibition of gastric emptying; increased satiety through neuroendocrine centers thereby reducing food intake, the TGR5 receptor may represent a promising emerging target in the therapeutic management of type 2 diabetes and obesity (Figure 3). To investigate the correlation between TGR5 activation and glucose homeostasis, and to evaluate the therapeutic potential of TGR5 as a target to stimulate GLP-1 secretion, knockout studies (siRNA/shRNA at cellular level or TGR5$^{-/-}$ homozygous mice at *in vivo* level) may prove to be useful tools.

TGR5 agonists: current developments and future landscape

The amphipathic nature of bile acids is crucial in aiding the digestion and absorption of vitamins and fats. With the emerging roles of bile acids in the regulation of energy and glucose homeostasis through TGR5, the pharmaceutical industry has focused their efforts towards the discovery and development of potent and selective TGR5 small molecule agonists belonging to bile acid or non-bile acid classes (Figure 4). TGR5 has a binding pocket, which is selective for different bile acid derivatives allowing the pharmacological discrimination over the genomic effects mediated by FXR. The key amino acids involved in the binding of bile acids in the binding pocket of the two receptors are not entirely conserved, which allows the development of different bile acid derivatives with varying degrees of selectivity. Structural features contributing to selectivity include the head piece carboxylic acid binding site. [46]. Screening of various naturally occurring bile acids, semi-synthetic bile acid derivatives and steroid hormones allowed the development of a structure–activity relationship (SAR) that indicated that (i) chain length of the carboxylic acid head piece, (ii) substitution at the alpha position of the carboxylic acid and (iii) rigidity of the cyclic system play crucial roles with respect to

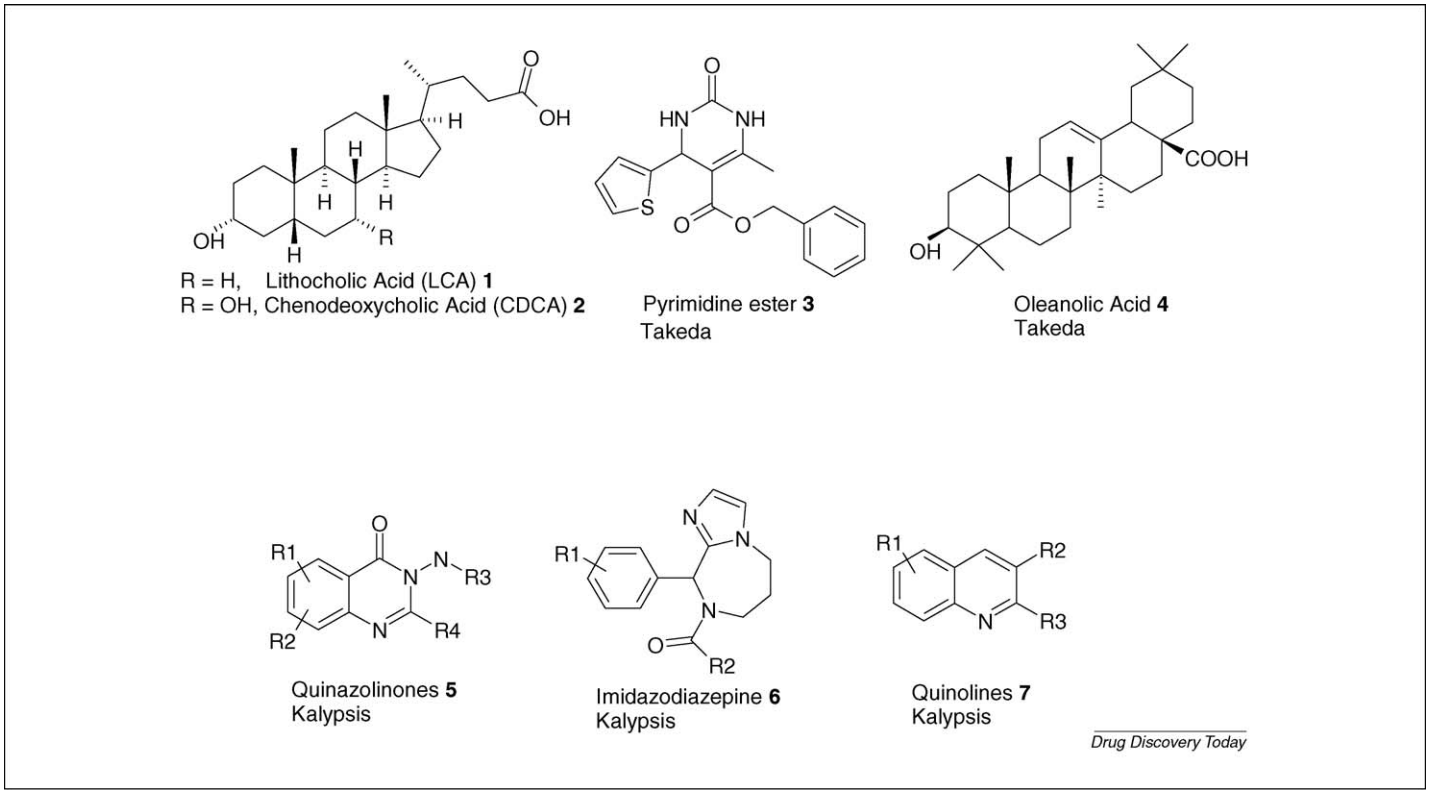

FIGURE 4

Bile acid and Non-bile acid TGR5 receptor agonists.

potency and selectivity of bile acids at the TGR5 receptor [23]. Shortening the chain length of bile acid derivatives reduces their potency on TGR5, whereas introduction of S methyl group at the α-position of carboxylic acid increases potency and provides highest selectivity over FXR. On the contrary, introduction of a double bond to the cyclic systems of bile acids provides rigidity to the molecular system and, therefore reduces binding to TGR5, suggesting a flexible and spacious binding pocket. Such SAR observations provided important clues for the development of compounds binding selectively at the TGR5 receptor over steroid hormone receptors. The SAR indicated that the TGR5 receptor exhibits highly conserved binding pocket with three hydrogen bond acceptors to anchor three hydroxyl groups of bile acids and a charged surface to bind with carboxylic acid groups.

The unique structural features of bile acids, which provide potency and selectivity towards TGR5 can be divided into four important parts (Figure 5). Region A: a large pocket, which consists of negatively charged residues to accommodate oppositely charged functional groups of the bile acids. Neutral groups like alcohols diminish the potency of bile acids binding to the TGR5 receptor. Region B: an accessory-binding pocket, which spans over C-22 and C-23. This pocket is quite large in size in TGR5 receptor compared with FXR and may be a reason why it is possible to achieve good selectivity. Pellicciari *et al*. have shown that the introduction of a small alkyl group at the C-23 position can be tolerated by TGR5 and provides selectivity against FXR [46]. The pocket is enantiomerically specific for the S-isomer over the R-isomer. Region C: This region is a large hydrophobic pocket, spanning C-6 and C-7 of the bile acids and hence tolerates alkyl (e.g. methyl, isopropyl) and fluoro substitution at C-7. Region D: is a narrow pocket with hydrogen bond acceptor groups, spanning the C-3 region. A hydroxyl (or ketone) group at C-3 anchors bile acids to the receptor through hydrogen bonds; therefore, removing these groups decreases potency at TGR5. In addition, a recent QSAR model shows two more favorable interaction sites of bile acids with TGR5: (i) hydrogen bonding at C-12 and (ii) an electrostatic interaction at the terminal of the carboxylic acid group of bile acids [47].

Takeda performed a high throughput screen in the search for a non-bile acid small molecule agonist for TGR5 [48]. Although the SAR was not entirely clear, a bicyclic system consisting of a charged

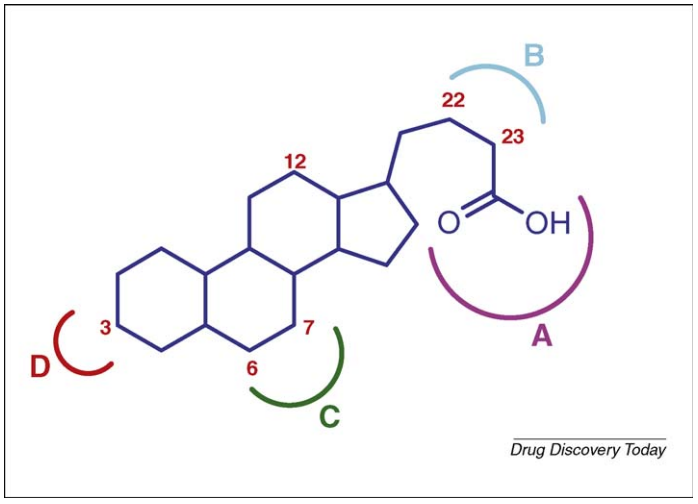

FIGURE 5

TGR5 receptor interaction sites for binding of bile acids.

seven-membered ring coupled with a five or six member aromatic ring appeared to be a unique functional motif, conferring activity at this receptor. Two non-bile acids reported from the screen with activity against the receptor were pyrimidine carboxylic ester 3 and oleanolic acid 4 (Figure 4).

As part of their ongoing effort in the field, Kalypsis recently disclosed that quinazolinones 5 [49], imidazo[1,2-a] [1,2]diazepin 6 [50] and quinolines 7 [51] all had activity as small molecule modulators of TGR5 (Figure 4). Intercept, however, disclosed different bile acid derivatives as TGR5 modulators in recent patent information [52].

Although TGR5 is the major receptor for bile acids implicated in different disease pathways, other known bile acid receptors, such as farnesoid X receptor (FXR) and pregnane X receptor (PXR), also regulate several metabolic pathways of bile acids [13–16,26,27]. The selectivity of different bile acid derivatives for TGR5 over FXR and PXR is not significantly great, though recent evidence suggests that it could be improved through various medicinal chemistry strategies. Producing a potent TGR5 small molecule agonist with high selectivity over a range of other receptors, however, remains the aim of the medicinal chemist.

Conclusions

With an ever-increasing incidence rate of type 2 diabetes and obesity, it is imperative to develop new therapeutic regimes that may provide a benefit both in terms of efficacy and tolerability. The existing portfolio of drugs for the treatment of metabolic disorders, primarily type 2 diabetes and obesity, suffers from a range of limitations in terms of efficacy, safety and tolerability. The mutual progression of type 2 diabetes and obesity, with insulin resistance as an adjunct, highlights the need for a single therapeutic modality for addressing the issues associated with both of these disorders, without compromising safety and tolerability. The existing classes of drugs either address the extensive glycemic control through different mechanisms or are meant for weight control. With the discovery of the bile acid receptor TGR5 and its involvement in different facets of glucose and energy homeostasis through different mechanisms of actions-, D2 iodinase and GLP-1 respectively, it seems likely that TGR5 agonists may be able to address the issues associated with both type 2 diabetes and obesity (Figure 3). Further, with an expression profile of TGR5 in immune cells-(monocytes and macrophages), TGR5 has been implicated in the regulation of immunomodulation by bile acids [19]. The potential involvement of TGR5 in the regulation of pathophysiology of metabolic disorders such as obesity, insulin resistance and diabetes is, however, strengthened by the above fact, since chronic inflammation associated with visceral obesity contributes to the development of insulin resistance, leading to peripheral insulin resistance in many tissues [53]. The exact role of TGR5 in immunoregulation and its involvement in inflammatory response is not, however, clear. Amongst its other activities, the activation of TGR5 by bile acids in liver macrophages (Kupffer cells) and sinusoidal endothelial cells appears to exert protective effects over liver through a cAMP-dependent anti-apoptotic mechanism and the activation of endothelial nitric oxide synthase (eNOS) [54,55]. This mechanism may scavenge bile acid induced reactive oxygen species and protect the liver against lipid peroxidation and bile acid induced injury (Figures 2 and 3). The development of diabetes in patients with cirrhosis is well-recognized, but evidence is emerging that the development of chronic liver disease and progression to cirrhosis may occur after the diagnosis of diabetes and that diabetes plays a role in the initiation and progression of liver injury [56]. Endothelial nitric oxide synthase (eNOS) function is impaired in diabetes as a result of increased vascular generation of reactive oxygen species [57–59] and, henceforth, the augmentation of nitric oxide through eNOS activation mediated by TGR5 agonists may protect against hepatic ischemia-reperfusion (I-R) injury in type 2 diabetes mellitus (Figures 2 and 3). It is too early, however, to draw any conclusion regarding the clinical efficacy and tolerability of TGR5 agonists, until synthetic agonists provide the proof of principle for such preclinical and clinical end points. The only evidence, so far, for the efficacy of small molecule TGR5 agonists has emerged from the preliminary screening data from Takeda, [60]. The correlation of *in vitro* to *in vivo* data for small molecule TGR5 agonists is still not known. Further, the evidence of lack of cholesterol gallstone formation in mice lacking the TGR5 receptor, while being fed a lithogenic diet [61] may highlight the crucial role played by TGR5 in the formation of gallstones and, hence, raises the potential issue with the use of TGR5 agonists in inducing the gallstone formation in obese and overweight subjects. The direct involvement of TGR5 in the regulation of expression of key genes governing the negative feedback loop of bile acid synthesis remains, however, obscure. The presence of an excess of phospholipids in the gall bladders of TGR5 depleted mice, resulting in a low bile salt/phospholipid ratio makes it highly unfavorable for gallstone formation and is contradictory to the above mentioned finding [61]. Therefore, more research is warranted to address such issues.

Conflict of interest

The authors hereby declare no conflicts of interest.

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