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The Roles of Xenobiotic Receptors: Beyond Chemical Disposition

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Running title: Non-canonical Effects of Xenobiotic Receptors

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Abbreviations

CAR, constitutive androstane receptor; CITCO, 6-(4-chlorophenyl)imidazo [2,1-beta][1,3]thiazole-5-carbaldehyde-*O*-(3,4-dichlorobenzyl)oxime; DBD, DNA-binding domain; DME, drug-metabolizing enzymes; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; FGF, fibroblast growth factor; FOXO1, forkhead box protein O1; G6Pase, glucose-6-phosphatase; HCC, hepatocellular carcinoma; HNF-4α, hepatic nuclear factor 4 alpha; LBD, ligand-binding domain; NR, nuclear receptor; PB, phenobarbital; PCN, pregnenolone 16 α-carbonitrile; PEPCK, phosphoenolpyruvate carboxykinase; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator-1α; PPAR, peroxisome proliferator activated receptor; PXR, pregnane X receptor; PP2Cα, protein phosphatase 2Cα; RACK1, receptor for activated C kinase 1; RXR, retinoid X receptor; TCPOBOP, 1,4-bis[2-(3,5-dichloropyridyloxy)] benzene; SGK2, serum/glucocorticoid regulated kinase 2; VRK1, vaccinia related kinase 1; XR, xenobiotic receptor

ABSTRACT

Over the past 20 years, the ability of the xenobiotic receptors to coordinate an array of drug-metabolizing enzymes and transporters in response to endogenous and exogenous stimuli has been extensively characterized and well documented. The constitutive androstane receptor (CAR) and the pregnane X receptor (PXR) are the xenobiotic receptors that have received the most attention, as they regulate the expression of numerous proteins important to drug metabolism and clearance and formulate a central defensive mechanism to protect the body against xenobiotic challenges. However, accumulating evidence has shown that these xenobiotic sensors also control many cellular processes outside of their traditional realms of xenobiotic metabolism and disposition, including physiological and/or pathophysiological responses in energy homeostasis, cell proliferation, inflammation, tissue injury and repair, immune response, and cancer development. This review will highlight recent advances in studying the non-canonical functions of xenobiotic receptors with a particular focus placed on the roles of CAR and PXR in energy homeostasis and cancer development.

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1. Introduction

Nuclear receptors (NRs) are transcription factors that are activated by both endogenous and exogenous ligands, leading to the initiation of biological responses through alteration of target gene transcription (Forman and Evans, 1995). Utilizing classical endocrinology approaches, a number of steroid hormone receptors such as estrogen receptor, androgen receptor, glucocorticoid receptor, and progesterone receptor were initially isolated (Jensen and Jacobson, 1960; Hollenberg et al., 1985; Misrahi et al., 1987; Lubahn et al., 1988). Containing relatively compact ligand-binding domains (LBDs), these receptors are responsive primarily to endogenous steroid hormones with high binding sensitivity often at nanomolar concentration ranges (Nagy and Schwabe, 2004; Sonoda et al., 2008). Different from these traditional endocrine receptors, receptors that respond to a diverse array of foreign compounds including environmental chemicals and clinically used drugs while lacking physiologically relevant endogenous ligands are termed xenobiotic receptors (XRs). These include but are not limited to the constitutive androstane receptor (CAR; NR1i3), the pregnane X receptor (PXR; NR1i2), the aryl hydrocarbon receptor (AhR; though it is not categorized in the NR family), and the peroxisome proliferator-activated receptors (PPARs) (Issemann and Green, 1990; Dreyer et al., 1992; Kliewer et al., 1998; Moore et al., 2000; Denison and Nagy, 2003; Wang and LeCluyse, 2003). Notably, XRs have bulky and less conserved LBDs which allow them to accommodate a structurally diverse library of ligands (Ekins et al., 2009). For instance, PXR, the primary regulator of CYP3A4 transcription, probably has the largest ligand-binding pocket in the entire NR superfamily, which enables the fitting of large and structurally diverse ligands (Watkins et al., 2001). Indeed, the broad spectrum of ligands that can activate PXR matches the substrate diversity of CYP3A4, the most abundant human liver cytochrome P450 enzyme that is responsible for the metabolism of 30-50% of clinically used drugs (Kumar and Surapaneni, 2001; Zanger et al., 2008). In response to xenobiotic challenges, XRs coordinate a defensive

network by regulating the transcription of genes encoding drug-metabolizing enzymes (DMEs) and transporters, which facilitate the breakdown and excretion of foreign substances from the body (Handschin and Meyer, 2003; Qatanani and Moore, 2005; Wang et al., 2012). Consistent with their metabolism/detoxification roles, the majority of XRs are highly expressed in the liver and intestines, which are the primary organs responsible for metabolism and clearance of exogenous chemicals. Typically, XRs are sequestered in the cytoplasm and translocate to the nucleus of primary hepatocytes *in vitro* and intact liver *in vivo*, upon agonistic stimulation (Ikuta et al., 1998; Kawamoto et al., 1999; Kawana et al., 2003; Li et al., 2009). Once inside the nucleus, XRs heterodimerize with their protein partners and bind to specific response elements located upstream of their target genes to trigger transcription. While this process is beneficial to rid toxic compounds from the body in general, induction of DMEs and transporters by XR activation in response to pharmaceuticals is known to cause unexpected drug-drug interactions that can lead to severe toxicity and/or loss of therapeutic efficacy (Honkakoski et al., 2003; Köhle and Bock, 2009; Tolson and Wang, 2010).

As xenobiotic sensors, CAR and PXR have been extensively studied over the past 20 years, due mostly to their broad and critical roles in governing the inductive expression of major DMEs such as phase I cytochrome P450 enzymes (i.e., CYP2B6, CYP2Cs, and CYP3A4) and phase II UDP-glucuronosyltransferases (i.e., UGT1A1 and UGT1A9) and sulfotransferases (i.e., SULT1A1, and SULT1D1), as well as drug transport proteins including organic anion-transporting polypeptides (uptake) and multidrug resistance proteins (efflux) (Xie et al., 2000; Timsit and Negishi, 2007; Köhle and Bock, 2009; Banerjee et al., 2015). Research thus far has clearly established that these receptors form the backbone of xenobiotic response, especially in the liver and intestines, by upregulating the expression of an overlapping yet distinctive array of important DMEs and transporters. Of note, although the effects of XRs as xenobiotic sensors dictating chemical metabolism and disposition have been extensively investigated, accumulating

evidence reveals that XRs can also function as signaling molecules that modulate physiological and pathophysiological functions including energy metabolism, insulin signaling, inflammation, immune response, cell proliferation, apoptosis, autophagy, and cancer development (Gao and Xie, 2012; Yan et al., 2014; Banerjee et al., 2015; De Mattia et al., 2016; Kazantseva et al., 2016; Roman et al., 2017; Gutiérrez-Vázquez and Quintana, 2018). This review aims to highlight the recent advances in our understanding of the non-traditional endobiotic roles of CAR and PXR with particular emphases on energy homeostasis and cancer development.

2. Constitutive Androstane Receptor

Initial characterization of CAR revealed that it was an orphan nuclear receptor that binds DNA as a heterodimer with the retinoid X receptor (RXR) without the involvement of any identified ligand (Baes et al., 1994). The high basal activity of CAR in immortalized liver cells, along with the early identification of the steroid ligands androstanol and androstenol as antagonists of CAR (though at concentrations much higher than the physiological levels) gave rise to its current established name (Baes et al., 1994; Forman et al., 1998). Orthologous mouse and rat *CAR* genes were cloned in the years following the isolation of human CAR, and the murine proteins were likewise found to heterodimerize with RXR and to display similar constitutive activity (Choi et al., 1997; Yoshinari et al., 2001). Our recognition of the importance of CAR in xenobiotic metabolism began with exploration into the enzyme-inducing effects of phenobarbital (PB), a powerful antiepileptic drug. The PB-provoked expression of *CYP2B* genes was found to involve a DNA response element accordingly denominated the phenobarbital-responsive enhancer module (PBREM) in the *CYP2B* gene promoter regions, and CAR was established as the key nuclear receptor that regulates the inductive expression of CYP2B by numerous PB-like chemicals (Trottier et al., 1995; Park et al., 1996; Honkakoski and Negishi, 1997; Honkakoski et al., 1998; Sueyoshi et al., 1999; Wei et al., 2000; Staudinger et al., 2013).

The mechanisms by which CAR is activated and deactivated along with its heterodimerization with RXR have been well elucidated. In primary hepatocytes, CAR remains cytosolic prior to activation either through direct interaction with a ligand or via indirect signaling pathways (Kawamoto et al., 1999; Kanno et al., 2005; Li and Wang, 2010). Inactive cytoplasmic CAR, phosphorylated at threonine 38 of the DNA-binding domain (DBD), gains activity through dephosphorylation of this residue via protein phosphatase 2A (PP2A), which is recruited to the CAR protein complex by dephosphorylated receptor for activated C kinase 1 (RACK1) (Mutoh et al., 2009; Mutoh et al., 2013). This activation could be antagonized by the extracellular signal-regulated kinase 1/2 (ERK1/2) following the binding of epidermal growth factor (EGF) to its membrane receptor, EGFR (Koike et al., 2007; Osabe and Negishi, 2011) or via metformin-mediated activation of the AMP-activated protein kinase (AMPK) (Yang et al., 2014). Most recently, Shizu et al. described a conversion between CAR monomer and homodimer states within the hepatocellular cytoplasm, where cytosolic CAR homodimerizes when cells are treated with EGF, and the complex dissociates when cells are treated with erlotinib, a tyrosine kinase inhibitor of EGFR (Shizu et al., 2017). This report further demonstrated that RACK1 binds CAR in the monomer state but not when CAR exists as a homodimer, as the homodimer interaction interface buries the requisite binding site. Another recent study suggested, however, that rather than taking place within the nucleus, heterodimerization of both CAR and PXR with RXR occurs within the cytoplasm. Dash and colleagues reported that nuclear entry of CAR-RXR and PXR-RXR heterodimers is dependent on the intact nuclear localization signal (NLS) of at least one of the partners and is strongly influenced by the RXR NLS (Zelko et al., 2001; Dash et al., 2017). Interestingly, these results contrast with interaction energy-based predictions from the aforementioned study that would suggest that CAR-RXR heterodimerization would be favored over (and would thus preclude) CAR homodimerization in the cytoplasm if RXR were present. Although the precise cytoplasmic conditions and mechanisms of CAR dimerization remain elusive, both direct and indirect activators translocate cytosolic CAR to the nucleus as the

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essential first step of activation. Hitherto, numerous xenobiotics have been identified as either direct or indirect activators of CAR, which are able to trigger complicated cellular responses in a CAR-dependent manner.

Initial and extensive investigations have focused on the role of CAR in regulating DMEs and transporters that protectively dispose of exogenous compounds such as toxic environmental substances and drugs (Yamamoto et al., 2003; Qatanani and Moore, 2005). To date, both the molecular mechanisms and biological consequences of CAR-mediated xenobiotic metabolism and disposition have been well documented and thus will not be the focus of this review. As insight into the homeostatic effects of CAR deepens, an expanding body of literature has emerged exploring the endogenous roles of CAR beyond xenobiotic disposition. It has long been known that PB, a prototypical CAR activator, improves insulin sensitivity and decreases blood glucose levels in patients with type 2 diabetes (Lahtela et al., 1985). Metabolic benefits were observed in wild-type (WT) but not CAR-/- mice treated with 1,4-bis[2-(3,5-dichloropyridyloxy)] benzene (TCPOBOP), a potent mouse CAR agonist (Dong et al., 2009; Gao et al., 2009). Additional studies have shown that CAR is involved in liver regeneration, inflammation, hepatocarcinogenesis, and renal ischemia-reperfusion-induced kidney injury (Huang et al., 2005; Tschuor et al., 2016; Tanner et al., 2018). Here we will concentrate on the recent findings regarding the role of CAR in energy homeostasis and cell proliferation. It is provocative that modulation of CAR activity in these domains may have therapeutic potential in managing diseases such as obesity, type 2 diabetes, and cancers.

2.1. CAR and Energy Homeostasis

The involvement of CAR in energy homeostasis was first recognized over a decade ago when CAR activation by PB in mice resulted in downregulation of genes associated with gluconeogenesis and fatty acid synthesis (Ueda et al., 2002). Subsequently, growing evidence supporting a role of CAR in energy homeostasis and metabolic disorders has promoted

investigation into the broad function of CAR beyond xenobiotic disposition. In 2004, Maglich et al. reported that under caloric restriction and fasting, CAR mediated a compensatory response to limit energy expenditure in mice by downregulation of serum levels of triiodothyronine (T3) and tetraiodothyronine (T4), two major thyroid hormones that control the basal metabolic rate (Maglich et al., 2004). Notably, fasting stimulated a CAR-dependent induction of sult1a1, sult2a1, and ugt1a1, which are important for the metabolic breakdown of T3 and T4. In CAR-/- mice, however, fasting failed to induce the expression of these enzymes and the serum concentrations of T3 and T4 remained high, which led to more weight loss under caloric restriction than in WT mice (Maglich et al., 2004). Interestingly, in another report, while the authors did not observe fasting-stimulated CAR activation, the study demonstrated that CAR is required for a PB-induced decrease of T3 and T4 levels in the serum; treatment with PB or TCPOBOP induced the expression of sulfotransferases and UGTs that are important for T3 and T4 metabolism in WT but not CAR-/- mice (Qatanani et al., 2005). Given that decreased basal energy expenditure represents a major barrier for obese individuals trying to lose weight, antagonism of human CAR may potentially benefit patients under a weight loss program, if the above findings hold true in humans. In contrast to these findings, two research groups independently showed that activation of CAR by TCPOBOP markedly ameliorated symptoms of obesity, diabetes, and fatty liver induced by high-fat diet (HFD) in WT mice, while such effects were not observed in TCPOBOP-treated CAR-/- mice (Dong et al., 2009; Gao et al., 2009). Further gene expression and biochemical analyses have revealed that the metabolic benefits of CAR activation may involve the suppression of glucose and lipid production, the inhibition of triglyceride and VLDL export, and induction of β-oxidation and energy expenditure.

Mechanistically, CAR-mediated energy homeostasis appears to be involved in a combined repression of an array of genes associated with gluconeogenesis, fatty acid synthesis, and energy expenditure such as phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-

phosphatase (G6Pase), fatty acid synthase (FAS), and stearoyl-CoA desaturase-1 (SCD-1) (Yu et al., 2016). This downregulation is involved in the prevention of the forkhead box protein O1 (FOXO1) transcription factor from interacting with insulin response element binding sites located upstream of genes such as PEPCK1, G6Pase, and insulin-like growth factor-binding protein 1 (Kodama et al., 2004). Through direct interaction between CAR and FOXO1, activated CAR acting as a corepressor downregulates FOXO1-mediated transcription of gluconeogenic genes. Binding of CAR to the direct repeat 1 site in the PEPCK promoter in place of hepatic nuclear factor-4α (HNF4α), a key hepatic factor crucial for the expression of bile acid synthesis and gluconeogenic genes, has also been reported as a means of metabolic suppression by CAR (Miao et al., 2006). The peroxisome proliferator-activated receptor gamma coactivator-1α (PGC1α) is another key transcriptional coactivator that governs energy metabolism by regulating the expression of PEPCK and G6Pase (Herzig et al., 2001). Gao et al. recently demonstrated that when bound by its ligand, CAR alters the subcellular localization and degradation of PGC1α through direct CAR-PGC1α interaction, by which the CAR-PGC1α complex is co-redistributed to the promyelocytic leukemia protein-nuclear bodies (PML-NBs), where activated CAR facilitates the ubiquitination and degradation of PGC1α by recruiting Cullin 1 E3 ligase (Gao et al., 2015). This finding suggests that in addition to transcriptional repression, posttranslational modification of protein stability may also contribute to CAR-mediated suppression of hepatic gluconeogenesis.

In contrast with the relatively consistent repression of gluconeogenesis by CAR activation, more conflicting experimental results have been generated regarding the role of CAR in the regulation of lipogenesis. Activation of CAR in mice has been shown to mitigate hepatic steatosis, increase glucose tolerance and insulin sensitivity, and alleviate or prevent obesity in diabetic mouse models (Dong et al., 2009; Gao et al., 2009). CAR-mediated anti-lipogenic effects were also observed in hyperlipidemic HepG2 cell cultures treated with evodia alkaloids

(Yu et al., 2016). Furthermore, the hormone irisin was recently identified as a direct target of CAR and protects HFD-induced obese mice through the CAR-irisin axis (Mo et al., 2016). Results of this study corroborated prior research on the effects of the hormone and demonstrated that hepatic expression of irisin suppresses lipogenesis (Zhang et al., 2013; Polyzos et al., 2014; Mo et al., 2016).

On the other hand, the majority of current studies were carried out under metabolic/nutritional challenges such as HFD-feed or caloric restriction. Interestingly, Marmugi et al. reported that treatment with TCPOBOP provoked the expression of lipogenic and glycolytic genes and increased lipid levels in a CAR-dependent manner in the livers of healthy mice under physiological conditions (Marmugi et al., 2016). A novel CAR target gene within the lipogenic category, Pnpla3 (Romeo et al., 2008; Smagris et al., 2015), was identified that may contribute to the observed fatty liver phenotype. A separate study demonstrated that the high serum triglyceride level of leptin-function deficient (*ob/ob*) mice was completely normalized when crossed onto a CAR-/- background (Maglich et al., 2009). Notably, when maintained on a normal diet, treatment with TCPOBOP (0.3 mg/kg ip once daily for 14 days) resulted in a 50% increase in serum triglycerides in WT but not CAR-/- mice. This is in stark contrast to Gao's observation where TCPOBOP (0.5 mg/kg ip once per week for 8 weeks) reduced serum triglyceride from 230 to 132 mg/dl in wild-type mice fed with a HFD regimen (Gao et al., 2009). It is possible that HFD-induced nutritional stress contributes significantly to the contradictory results in these studies, though factors such as the TCPOBOP treatment regimen and the genetic background of the mice used cannot be excluded. Additionally, the inherited species differences between human and mouse CAR may further complicate the dispute. In primary and immortalized human hepatocytes, activation of CAR promotes the expression of lipogenic genes such as SCD1 and Pnpla3 (Marmugi et al., 2016). Another study using human primary hepatocytes found that CAR activation, while inhibiting gluconeogenesis, did not affect the

expression of genes associated with hepatic lipogenesis (Lynch et al., 2014). Collectively, a correlation between CAR and energy homeostasis has been firmly established (Fig. 1). Numerous studies have demonstrated that activation or deactivation of CAR can disturb the balance of energy metabolism/expenditure. However, the exact role of CAR in metabolic disorders continues to be uncertain or even controversial. Information pertaining to humans in particular is limited.

2.2. CAR in Cell Proliferation and Cancer

The effect of CAR activation on mitogenesis has been the subject of intense inquiry since the discovery that CAR is responsible for PB- and TCPOBOP-induced liver hypertrophic and hyperplastic responses in mice (Wei et al., 2000). This topic is intriguing from two standpoints: whereas a hyperplastic response might lead to the development of cancer in certain circumstances, a regenerative response following severe tissue injury is often critical to survival. The essential role of CAR in PB- and TCPOBOP-mediated tumor promotion was initially established by using CAR-/- and WT mice, in that activation of CAR is associated with both induction of DNA replication and suppression of apoptosis (Yamamoto et al., 2004; Huang et al., 2005; Phillips et al., 2007). Subsequent studies have further confirmed that a class of rodent CAR activators exhibit their tumor-promoting activities in CAR-dependent manners (Maeda et al., 2015; Tamura et al., 2015; Tamura et al., 2016; Okuda et al., 2017; Wang et al., 2017). Although the underlying molecular mechanisms by which CAR stimulates tumor promotion are not fully elucidated, accumulating evidence reveals that activation of CAR alters the expression of the growth arrest and DNA damage-inducible 45 β (GADD45β), the murine double minute 2 (mdm2), the tubulin alpha 8 (TUBA8), the family with sequence similar 84, member A (FAM84A), and c-Myc which are all closely correlated with cell proliferation and oncogenic signaling (Huang et al., 2005; Blanco-Bose et al., 2008; Yamamoto et al., 2010; Kamino et al., 2011a; Kamino et al., 2011b).

Recently, Dong and colleagues studied the relationship between mouse CAR activation and the Wnt/β-catenin pathway in the development of liver tumors (Dong et al., 2015). Although no evidence was found of direct interaction between CAR and β-catenin at the transcriptional level, results of the study showed that CAR activation prevented the senescence that would otherwise be triggered by Wnt/β-catenin activation over time and that the two act synergistically to promote liver cell proliferation and hepatocellular carcinoma (HCC) development. Another study by Braeuning et al., however, found that in Apc-/- mice (APC forms part of the protein complex that is essential to normal degradation of β-catenin), treatment with PB did not result in a persistent proliferative advantage (Braeuning et al., 2016). PB was shown to promote adenoma but inhibit carcinoma in liver cells of Apc-/- mice. Although mechanisms other than those directly involving CAR in the inhibition of HCC were not ruled out, the study points to the paradoxical properties of PB in tumor promotion and the need for additional investigation. Most recently, Tschuor and colleagues studied the regenerative effects of CAR in mouse liver following extreme (91% of liver volume resected), extended (86% resected), and standard (70% resected) hepatectomy (Tschuor et al., 2016). Marked impairment in mouse CAR activation following extended hepatectomy was observed, and liver dysfunction and lack of regeneration corresponded with similar phenomena in Car-/- mice that had undergone standard hepatectomy. Following administration of the mouse CAR activator TCPOBOP, survival was significantly improved in WT but not CAR-/- mice. As a regenerative response is essential to avoid potential liver failure after significant resection in the setting of tumor invasion or following transplantation with reduced-size liver grafts, therapeutic human CAR intervention may play a role in recovery from compromising liver surgery in the future (Clavien et al., 2010; Tschuor et al., 2016).

MicroRNAs (miRNAs) are short noncoding RNAs that play important roles in the post-transcriptional regulation of genes associated with various diseases, including HCC. miR-122,

the most abundant hepatic miRNA, has been established as a tumor suppressive miRNA in the liver (Coulouarn et al., 2009). Of note, the expression of miR-122 was markedly downregulated in C3H/HeN mice *in vivo* and HepG2 cells *in vitro* treated with PB (Shizu et al., 2012). Kazantseva et al. further demonstrated that activated CAR represses the expression of miR-122 through direct competition with HNF4a for binding to the DR1 response element located upstream of the pri-miR-122 promoter, a mechanism by which CAR suppresses a number of other HNF4a target genes (Kazantseva et al., 2015). Using deep sequencing approaches, Hao et al. profiled the global miRNA expression patterns in livers from C57BL/6J mice treated with TCPOBOP or DMSO as vehicle control (Hao et al., 2016). Among the 51 miRNAs significantly altered by TCPOBOP treatment in this study, known oncogenic miRNAs, such as miR-148a, miR-let-7f, and miR-671, are upregulated, supporting the idea that CAR may modulate a network of miRNAs in facilitating mouse hepatocyte proliferation. In addition to CAR-mediated regulation of miRNA expression, the expression of CAR itself can also be repressed by miRNA such as miR-137, which was observed in cellular models of hepatocellular and colon cancers (Takwi et al., 2014). More in-depth analysis of the rather comprehensive roles of miRNA in CAR-dependent hepatocarcinogenesis is warranted.

Another mechanism by which CAR may influence cancer development is through its involvement in circadian rhythm homeostasis. CAR expression has been shown to elevate during the night in mice, corresponding to their regular feeding patterns (Gachon et al., 2006). More recent studies have expanded on the link between CAR activity and circadian rhythms. For example, the period circadian regulator 2 protein (PER2) has been found to directly interact with CAR, with implications that have yet to be explored (Martini et al., 2017). Additionally, a shifted feeding schedule in rats (i.e., daytime rather than nighttime feeding) likewise caused a shift in CAR expression (de Vries et al., 2017). Significant disruption in circadian rhythms over time, in turn, has recently been demonstrated by Kettner and colleagues to provoke non-

alcoholic fatty liver disease, fibrosis, and hepatocellular carcinoma in correlation with elevated bile acid and CAR levels in mice. Increased levels of CAR were found to be related to disruption in sympathetic nervous system signaling and peripheral tissue clock activity (Kettner et al., 2016).

Compared to what we have learned from rodent animals with regard to the role of CAR in cancer development, the function of CAR in human hepatocarcinogenesis continues to be controversial, and in-depth studies are limited. Indeed, although PB represents a prototypical CAR activator and known nongenotoxic carcinogen that promotes liver cancer in rodents, PB-induced replicative DNA synthesis and hepatocellular proliferation in rodents were not observed in either cultured human hepatocytes *in vitro* or in chimeric mice with humanized liver *in vivo* (Elcombe et al., 2014; Yamada et al., 2014; Soldatow et al., 2016; Haines et al., 2018). Moreover, epidemiological studies have shown that PB and a number of PB-like nongenotoxic rodent carcinogens do not increase the incidence of liver tumors in humans, even after long therapeutic applications at doses producing plasma concentrations challenging those that are carcinogenic in rodents (Braeuning, 2014; La Vecchia and Negri, 2014). When the human CAR transcriptome was recently analyzed using WT and CAR-/- HepaRG cells, many cell proliferation-associated genes were upregulated in CAR-/- but not WT cells (Li et al., 2015a). Additionally, in human brain tumor stem cells, activation of CAR by CITCO (6-(4-chlorophenyl)imidazo [2,1-beta][1,3]thiazole-5-carbaldehyde-*O*-(3,4-dichlorobenzyl)oxime) was associated with cell cycle arrest and enhanced apoptosis both *in vitro* and in an *in vivo* xenograft model (Chakraborty et al., 2011). Collectively, these studies raise significant concerns regarding direct extrapolation of findings from rodents to humans, particularly with regard to the role of CAR in cancer development.

2.3. Additional Endobiotic Functions of CAR

In addition to the roles discussed above, CAR has important endobiotic metabolism

functions, including its regulation of bilirubin and bile acid processing genes (Huang et al., 2003; Wagner et al., 2005). A 2017 study addressing a potential role of CAR in prevention of cholesterol gallstone disease found that CAR activation by TCPOBOP in lithogenic diet-fed mice prevented the development of cholesterol gallstones (Cheng et al., 2017). Furthermore, although primarily studied in the liver, CAR has also been investigated in other organs, such as brain and intestine. Boussadia et al. recently explored the role of CAR in pathophysiological brain processes and found that CAR-/- mice displayed inferior memory function and greater levels of anxiety, as indicated by behavioral tests, when compared with WT mice (Boussadia et al., 2016). Electroencephalographic changes in CAR-/- mice were found to correlate with memory impairment, and microvessels exhibited morphological changes that were suggestive of inflammatory processes. Additionally, when a seizure-inducing neurotoxin was peripherally administered, CAR-/- mice experienced quicker-onset and more prolonged seizure episodes than did WT mice, reinforcing the increased vascular permeability suggested by other experimental results (Boussadia et al., 2016). The effects of CAR in intestinal tissue were also studied by Hudson and colleagues recently. The expression of CAR in inflamed, non-ulcerated intestinal mucosal tissue from patients with ulcerative colitis and Crohn's disease was found to be markedly reduced when compared with corresponding tissue from healthy donors, results that were duplicated in intestinal mucosal samples from mice with chemically-induced inflammation (Hudson et al., 2017). When intestinal tissue was collected from CAR-/- mice after a week's recovery time following chemically-induced mucosal damage, mucosal tissue had failed to recover to the extent observed in WT mice in terms of both damage and inflammation. CAR activation by CITCO in Caco-2 intestinal epithelial cells was found to increase the migratory distance of these cells, an effect that was correlated with increased p38 MAP kinase activation, and to aid wound closure while having no effect on cell proliferation. Most recently, Choi et al. demonstrated an interesting kidney-liver cross-talk in response to acute kidney injury, where TCPOBOP alleviates serum IL-6 elevation induced by renal ischemia-reperfusion in a

CAR-dependent manner (Choi et al., 2018).

Taken together, these findings demonstrate that the role of CAR has extended well beyond its traditional function in xenobiotic metabolism and transport. Endogenous roles involving energy homeostasis, cancer development and prevention, and tissue integrity and regeneration continue to be elucidated. New insight into the mechanisms by which CAR exerts its effects and the precise conditions in which it does so will likely lead to therapeutic advances in many pathological conditions.

3. Pregnane X receptor

PXR, also known as the steroid and xenobiotic receptor (SXR) and pregnane activated receptor (PAR), has been firmly established as the master regulator of the expression of numerous phase I and II DMEs and drug transporters, with CYP3A4 as its most investigated and prototypical target gene in humans (Kliewer et al., 1998; Sueyoshi and Negishi, 2001). Owing to its broad ligand specificity, perturbation of PXR activity can alter the bioavailability, absorption, excretion, and overall disposition of xenobiotics, leading to potentially significant drug-drug, drug-herbal, and drug-environment interactions that impact vital medical treatments (Lehmann et al., 1998; Kliewer and Willson, 2002; di Masi et al., 2009).

Originally cloned in 1998 from a mouse fragment in the EST database (Kliewer et al., 1998), or by screening a human gene library to identify homologs of the Xenopus benzoate X receptor (Blumberg et al., 1998), PXR is classified in the NR1i nuclear receptor family as a ligand-dependent transcription factor. PXR is predominantly expressed in the liver and intestine, which are routinely exposed to numerous xenobiotics, where it functions as a signaling molecule for the generation of metabolic byproducts of exogenous and endogenous compounds (Kliewer et al., 1998; Lehmann et al., 1998; Jones et al., 2000). Many NRs are known for their 3-stranded β-sheet ligand-binding pocket; however, PXR exhibits a 5-stranded β-sheet ligand-binding

pocket that is malleable and largely hydrophilic, permitting the binding of a broad array of structurally diverse chemicals including drugs, endogenous metabolites, and exogenous compounds (Watkins et al., 2001; Ekins and Schuetz, 2002; Ekins et al., 2009). Similar to CAR, inactivated PXR resides in the cytoplasm of hepatocytes of untreated mice, where it translocates to the nucleus when bound to an agonistic ligand, pregnenolone 16α-carbonitrile (PCN) (Kawana et al., 2003; Squires et al., 2004). Mechanistically, nuclear translocation of PXR requires an intact NLS, which resides within the DBD of PXR (Squires et al., 2004). Although ectopically expressed CAR and PXR spontaneously accumulate in the nucleus of immortalized cell lines such as HepG2 cells, without agonist stimulation, nuclear-localized PXR remains inactive, whereas nuclear translocation alone is sufficient to activate CAR (Kawamoto et al., 1999; Kawana et al., 2003). Upon agonist binding, the PXR/RXR heterodimer recruits coactivators such as steroid receptor coactivator 1 and triggers the expression of its target genes (Kliewer et al., 1998). While CAR and PXR both regulate numerous DMEs and transporters, they exhibit different preferential regulation over these genes. This is partly due to the differential binding affinities of CAR and PXR to AG(G/T)TCA repeats in the promoters of these genes (Xie et al., 2000; Faucette et al., 2006). Together, PXR and CAR form a defensive mechanism against xenobiotic exposures by coordinately regulating a pleiotropic array of hepatic genes encoding various DMEs and transporters.

In addition to its well-characterized roles in xenobiotic metabolism and detoxification, evidence has shown that PXR also plays important roles in energy metabolism, inflammation, and cell proliferation. Notably, while PXR and CAR exhibit similar roles in xenobiotic disposition by coordinating the inductive expression of DMEs and transporters, PXR appears to differ significantly from CAR in its non-classical regulatory roles, including energy metabolism and cancer development.

3.1. PXR and Energy Homeostasis

Previous studies reveal that activation of PXR by PCN results in decreased blood glucose levels in mice, an effect attributable to PXR-mediated repression of genes such as PEPCK1 and G6Pase that are pivotal to hepatic gluconeogenesis (Bhalla et al., 2004; Kodama et al., 2004). In human hepatocarcinoma Huh7 cells overexpressing transfected human PXR, addition of cAMP induced the expression of G6Pase and PEPCK mRNAs 13- and 20-fold, respectively, while the induction of these genes was markedly repressed by rifampicin, the prototypical activator of human PXR (Kodama et al., 2007). Mechanistically, PXR acts as a corepressor of FOXO1 and FOXA2 and downregulates FOXO1-mediated insulin response sequence (IRS) activation and transcription of gluconeogenic genes (Kodama et al., 2004). GST pull-down and co-immunoprecipitation assays demonstrated that PXR directly binds CREB, a cAMP-response element-binding protein, and represses cAMP-mediated expression of G6Pase thereafter (Kodama et al., 2007). Further, this study showed that the binding affinity between PXR and CREB was strengthened by PCN treatment, which led to a decreased binding of CREB to the G6Pase promoter in mice. Additional studies investigating the effect of PXR on bile acid synthesis and gluconeogenesis in HepG2 cells found that human PXR interacts with the coactivator PGC1α in the presence of rifampicin (Bhalla et al., 2004). This ligand-dependent PGC1-PXR interaction prevents PGC1α from binding to HNF4α and forms a functionally inhibitory cross-talk between PXR and HNF4α, leading to the repression of PEPCK1.

In contrast with aforementioned findings suggesting a potential glucose-lowering benefit of PXR activation in mice, clinical studies have indicated that treatment with rifampicin increases blood glucose levels in both tuberculosis patients and healthy volunteers (Takasu et al., 1982; Rysa et al., 2013). Such clinical observations correlate with a recent *in vitro* study using human primary hepatocytes and HepG2 cells stably expressing human PXR, where activation of PXR by rifampicin and a statin significantly induced the expression of PEPCK1 and G6Pase in both hepatic cell systems (Gotoh and Negishi, 2014; Gotoh and Negishi, 2015). The

serum/glucocorticoid regulated kinase 2 (SGK2) that was also upregulated by human PXR activators appears to be essential for this PXR-mediated induction of gluconeogenesis, and the drug-PXR-SGK2 signaling requires the recruitment of the protein phosphatase 2Cα (PP2Cα) by ligand-activated PXR to dephosphorylate SGK2 at Thr^{193}, which in turn facilitates PXR-mediated transactivation of genes encoding gluconeogenesis, including PEPCK and G6Pase. Interestingly, this drug-PXR-SGK2 signaling is not present in mice, which may explain some of the discrepancies observed between murine and human studies (Gotoh and Negishi, 2014; Gotoh and Negishi, 2015). Most recently, Gotoh et al. further demonstrated that rather than drug challenges, a low level of glucose induced the phosphorylation of PXR at Ser^{350} and enhanced gluconeogenesis in cultured HepG2 cells (Gotoh et al., 2017). Immunoprecipitation and *in vitro* kinase assays revealed that the vaccinia related kinase 1 (VRK1), a serine/threonine kinase, is responsible for the phosphorylation of PXR at Ser^{350} under low glucose conditions, which enabled the phosphorylated PXR to scaffold PP2Cα for subsequent dephosphorylation of SGK2 at Thr^{193}. Knockdown of VRK1, on the other hand, markedly repressed the phosphorylation of PXR-Ser^{350}, increased SGK2-Thr^{193}, and nearly abolished the expression of PEPCK in HepG2 cells cultured under low glucose. Importantly, this low glucose-stimulated VRK1-PXR-PP2C-SGK2 signaling was also observed in mice under fasting conditions, suggesting that this signaling pathway may represent a novel feedback mechanism in response to low glucose that is conserved in both humans and mice. In another study, Oladimeji et al. observed that high glucose increased the expression and activity of PXR in HepG2 cells and that this induction was partially reversed by the activation of AMPK, suggesting that PXR activity can be modulated by the energy status of the cells (Oladimeji et al., 2017).

Adding yet another layer of complexity to our understanding of the role of PXR in glucose homeostasis, recent studies revealed that PXR also alters the uptake and utilization of glucose. Studies in mice and rats found that activation of PXR with PCN downregulates the expression of

glucose transporter 2 (GLUT2), the transporter responsible for glucose uptake into hepatocytes during the fed state, and glucokinase (GCK), which deactivates G6Pase by phosphorylation (Ling et al., 2016; Hassani-Nezhad-Gashti et al., 2018). Collectively, activation of PXR in various *in vivo* and *in vitro* models exhibiting different types of metabolic function has led to mixed outcomes, with PXR activation improving glucose tolerance in some models while worsening glucose homeostasis in others (Hakkola et al., 2016). Multiple confounding factors including genetic variations and experimental conditions may contribute to the observed discrepancies. Clearly, the effects of PXR activation on glucose tolerance in humans require further evaluation.

Unlike the beneficial effects of CAR activation on lipid homeostasis that have been reported by several groups, activation of PXR has been shown to enhance lipogenesis while decreasing lipid oxidation, promoting a fatty liver phenotype (Zhou et al., 2006; Nakamura et al., 2007; Bitter et al., 2015). Using PXR-/- and WT-mice, Nakamura et al. reported that treatment with PCN resulted in downregulation of CPT1A (β-oxidation) and mitochondrial 3-hydroxy-3-methylglutarate-CoA synthase 2 (Hmgcs2; ketogenesis) but upregulation of the stearoyl-CoA desaturase 1 (lipogenesis) in a PXR-dependent manner (Nakamura et al., 2007). At the molecular level, PXR affects the expression of these genes at least partly through cross-talk with the insulin response forkhead factor FoxA2. Unexpectedly, this study also found that untreated PXR-/- mice developed severe hepatic steatosis accompanied with induction of lipogenesis and repression of fatty acid β-oxidation reminiscent of those associated with the pharmacological activation of PXR (Nakamura et al., 2007). Whether unidentified endogenous ligands may contribute to this contradictory observation is largely unknown.

Studies using a combination of human PXR transgenic, PXR-/-, and WT mice found that both genetic and pharmacological activation of PXR in the liver resulted in elevated hepatic lipid accumulation, which is associated with induction of the fatty acid translocase protein CD36

without activation of the lipogenic transcriptional factor sterol regulatory element-binding protein-1c (Zhou et al., 2006). On the other hand, genetic PXR ablation protected mice from HFD- and genetically induced obesity, hepatic steatosis, and insulin resistance (He et al., 2013). In addition, Ma et al. reported that activation of PXR by PCN prevents HFD-induced obesity in AKR/J mice (Ma and Liu, 2012). Potential factors contributing to this discrepancy may include different genetic backgrounds of mice used, C57BL/6J vs AKR/J, and PCN treatment dosage and schedules. Indeed, PXR-mediated alteration of lipid homeostasis may exhibit tissue, cell type, and species specificities. Activation of PXR in human primary hepatocytes with rifampicin did not induce CD36 expression, and lipid accumulation in the hepatocytes was due to increased fatty acid synthesis and reduced fatty acid β-oxidation instead of increased free fatty acid uptake as observed in the mouse models (Moreau et al., 2009). Another possible mechanism proposed for PXR-dependent increases in hepatic lipid accumulation is the induction of the novel PXR target gene SLC13A5, an uptake transporter that imports citrate from the circulation into the hepatocyte, where it facilitates *de novo* synthesis of lipids and cholesterol (Li et al., 2015b). Collectively, activation of PXR quite consistently leads to increased hepatic lipid accumulation, while its effects on glucose balance are rather controversy (Fig. 2). The differences in mechanisms between preclinical species and humans require that caution be taken when attempting to define the physiological relevance of findings in animal models.

3.2. PXR in Cancer and Cell Proliferation

PXR-mediated alterations in drug disposition have been known to play a significant role in chemotherapy resistance, as many anticancer agents are substrates of DMEs and efflux transporters that can be upregulated by PXR activation (Zhuo et al., 2014; Oladimeji and Chen, 2018). Although PXR in the liver and intestine accelerates drug clearance in general, tumor-specific expression of PXR becomes an additional barrier to the therapeutic efficacy of anticancer agents (Mani et al., 2005; Chen et al., 2007; Chen et al., 2012). This is exemplified

by a recent study investigating the therapeutic efficacy of sorafenib in HCC treatment, where sorafenib was found to enhance its own clearance via CYP3A4 and P-glycoprotein induction in HCC by the activation of PXR (Feng et al., 2018). Outside of its traditional role of xenobiotic detoxification, accumulating evidence reveals that PXR can also regulate the expression of multiple genes associated with cell apoptosis and proliferation, which play pivotal roles in cancer progression (Masuyama et al., 2007; Gupta et al., 2008; Chen et al., 2009; Pondugula et al., 2016).

Mounting cell-based evidence thus far supports that PXR plays a pleiotropic role in cell proliferation and cancer development in a cell-type specific manner. Treatment of hepatocytes with dexamethasone, a PXR activator, inhibited spontaneous apoptosis by upregulating B-cell leukemia 2 (Bcl-2), an antiapoptotic protein that inhibits p53-mediated apoptosis signaling, and this phenomenon was also confirmed using other PXR agonists in both rat and human hepatocytes (Bailly-Maitre et al., 2001; Zucchini et al., 2005). Additionally, PXR inhibited apoptosis in LS180 colorectal adenocarcinoma cells by inducing Bcl-2 and MCL-1, another antiapoptotic protein, while downregulating proapoptotic proteins such as Bcl-2 antagonist/killer 1 and p53 (Zhou et al., 2008). Further studies probing the mechanistic interactions between PXR and p53 found that WT-p53 can directly bind to PXR, and heterodimerization of PXR and p53 appears to form a mutually repressive cross-talk through which each inhibits the other's transcriptional activity in HCT116 and LS180 colon cancer cells. This mutual inhibition protects them against chemotherapeutic-induced cell death by decreasing apoptosis and increasing malignant transformation (Elias et al., 2013; Robbins et al., 2016). In addition to its role in liver and colon cancers, PXR is also expressed in prostate cancer, breast cancer, and a number of other tumor tissues, with differential biological function and tissue and cell type/context-specific consequences (Miki et al., 2006).

In the case of colorectal cancers, Wang et al. reported that activation of PXR is sufficient to

enhance neoplastic characteristics of LS174T cells and human primary colon tumor cells both *in vitro* and in xenografted mice *in vivo*, and pointed out that mechanistically this may involve a PXR-dependent induction of FGF19 expression in cancer (Wang et al., 2011). Using similar approaches, Ouyang et al., however, observed a PXR-mediated anticancer activity in HT29 cells, another colorectal cancer cell line with relatively low expression of PXR. Stable transfection of PXR in HT29 cells led to repressed cell proliferation, migration, and xenograft growth, which is accompanied by cell-cycle arrest, elevated p21 expression, and inhibition of E2F1 (Ouyang et al., 2010). Interestingly, this report also indicated that expression of PXR is reduced in human colon cancer tissues, albeit using a relatively small sample size (Ouyang et al., 2010). A tumor-suppressive role of PXR was further supported by another report where intestine-specific activation of PXR by rifaximin significantly reduced azoxymethane/dextran sulfate sodium-induced colon cancer in human PXR transgenic but not WT or PXR-/- mice, possibly through the PXR-NF-kB axis (Cheng et al., 2014).

In addition to cancer development, PXR has been shown to be important in liver regeneration by augmenting the proliferation of hepatocytes (Dai et al., 2008; Elcombe et al., 2012). In fact, PXR was necessary for full liver regeneration in mice after a partial hepatectomy, with the PXR-/- mice exhibiting severe inhibition of hepatocyte proliferation 3 days after hepatectomy surgery (Dai et al., 2008). PXR-/- mice showed inactivation of signal transducer and activator of transcription protein 3 (STAT3) 5 days post-surgery, which was the most likely cause of hepatocyte quiescence (Dai et al., 2008). While activation of PXR in WT mice did not enhance hepatocyte proliferation, co-treatment of PCN with activators of either CAR or PPARα led to a synergistic enhancement of hepatocyte proliferation (Shizu et al., 2013). Collectively, activation of PXR perturbs the balance of cell proliferation and apoptosis in cell-, tissue-, and species-specific manners without an overarching phenotype, making the study of PXR in different cancer types complex.

3.3. Additional Non-Traditional Functions of PXR

Studies have shown that PXR is also expressed in immune cells, such as T and B lymphocytes, and in the skin of mice and humans, where perturbation of PXR expression and activity alters the immune response (Dubrac et al., 2010; Haslam et al., 2013; Elentner et al., 2015). Many patients with atopic dermatitis have compromised immune barrier function, which leads to an increase in the penetration of lipophilic pollutants (Oetjen et al., 2018). This penetration has been shown to trigger PXR activation in keratinocytes and a subsequent hyper-responsive immune response, further impairing the barrier function (Oetjen et al., 2018). Specifically, Elenter et al. reported that transgenic mice expressing constitutively activated human PXR display increased transepidermal water loss, abnormal stratum corneum lipids, focal epidermal hyperplasia, and increased expression of local T cells (Elentner et al., 2018).

The same compromise in barrier function exhibited in atopic dermatitis is also observed in the GI tract in diseases such as inflammatory bowel disease (IBD) and Crohn's disease, and PXR plays a role in both of these diseases by increasing epithelial permeability (Terc et al., 2014). Additionally, PXR has been shown to regulate the intestinal epithelial wound healing response, allowing mutations to reduce the healing response, leading to an increase in IBD risk factors. This has been shown by using PXR agonists as protective agents that prevent intestinal inflammation from occurring (Terc et al., 2014). It is suggested that PXR plays a role in the healing response by modulating gene transcription, thereby upregulating genes that are related to metabolic functioning, while hindering inflammatory genes (Mencarelli et al., 2010). Beyond the role of PXR in the disease state of the gastrointestinal tract, PXR activation plays a major role in the maintenance of homeostasis of bile acids, which can affect the potential progression of many cholesterol-related diseases.

As described throughout this review, many xenobiotics and endobiotics activate PXR, leading to the regulation of key enzymes that have been implicated in a wide array of

physiological activities. There is an abundance of knowledge on the role of PXR in xenobiotic metabolism, and recently the evidence has shifted to the more critical nontraditional role that PXR plays in the regulation of endogenous functions which has led to interest in uncovering the magnitude of PXR's influence. However, more information is needed to determine whether PXR may eventually be used as a target to prevent and treat diseases.

3. Conclusion

Over the past twenty years or so, significant advances have been achieved in our understanding of the roles of XRs in the transcriptional regulation of genes involved in xenobiotic absorption, distribution, metabolism, excretion, and toxicology. Accumulating evidence shows that these traditional xenobiotic sensors also play pivotal roles in modulating energy homeostasis, cell proliferation, cell migration, apoptosis, inflammation, and immune response, which may eventually alter the clinical consequences of metabolic disorders, obesity, and diabetes, as well as various cancers. It is evident now that although XRs such as CAR and PXR continue to be appreciated as master regulators that control xenobiotic disposition and detoxification, newly heightened researches are focusing on 1) the identification of previously unknown physiological/pathophysiological functions of XRs, 2) understanding the molecular mechanisms underlying the non-canonical roles of XRs, and 3) exploring XRs as potentially novel therapeutic targets for disease conditions such as metabolic disorders and cancers. We have witnessed rapid progression in our understanding of the endobiotic roles of CAR and PXR and in our ability to decipher the mechanisms of their activation. Unlike typical ligand-dependent nuclear receptors, CAR activity can be altered by numerous cellular signaling pathways, which themselves are often associated with important physiological and pathophysiological conditions. Notably, in animal models, while activation of both CAR and PXR benefits diabetic conditions by repressing hepatic gluconeogenesis, the two XRs display contrasting effects on lipogenesis and

fatty acid β-oxidation. Given that many drugs are dual activators of both CAR and PXR, the potential clinical application of these findings is rather complicated and requires further elucidation.

One key point discussed in this article is that both CAR and PXR present significant cell-type, tissue, and species specificities with regard to their non-canonical functions. For instance, while activation of PXR enhanced the neoplastic characteristics of LS174T cells, it repressed the proliferation of HT29 cells both *in vitro* and in xenografted mice *in vivo*. Pharmacological activation of PXR resulted in conflicting effects on HFD-induced fatty liver in mice with C57BL/6J versus AKR/J genetic background. To date, the majority these new findings have come from experiments conducted in rodent animal models, and direct extrapolation of these data to humans can be misleading and risky. It is rather convincing now that activation of CAR and PXR in mice enhances cell proliferation and tumor progression, and many of their agonists are well-known tumor promoters in rodents. Nevertheless, the role of these XRs in human cancer development is inconclusive in general and sometimes contradictory to the findings in rodent animals. In the case of CAR, both PB and TCPOBOP exhibit potent tumor-promoting effects in mice in a CAR-dependent manner. However, clinical use of PB over an extended period of time has never been associated with an increased incidence of cancer in humans. It is worth noting that, in addition to the known species differences, a lack of in-depth investigation into human XR function in appropriate models adds to the uncertainty and contradictory outcomes obtained thus far. It is anticipated that the use of novel 3D physiologically relevant human pre-clinical models, such as hepatocyte spheroid cultures, organ-on-chip platforms, and 3D bioprinted human tissues, will provide alternative approaches to overcome these challenges. Collectively, exciting new discoveries of XR-mediated endobiotic effects have been made through flourishing new studies. Extrapolation of findings from animal studies is hindered by the rather paradoxical effects observed between human and rodent CAR and PXR on energy homeostasis and cell

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proliferation. In order to fully appreciate the clinical impact of these XRs in diseases such as metabolic disorders and cancers, more intensive human studies are warranted in the future.

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Footnotes

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Figure Legends

Figure 1. Effects of CAR Activation on Energy Homeostasis. Schematic illustration of how CAR activation affects energy metabolism and balance. Activation of CAR by agonists or caloric restriction leads to the up- and down-regulation of a cluster of genes associated with gluconeogenesis, lipogenesis, β-oxidation, and energy expenditure by altering the activities of specific transcription factors such as PPARα, HNF4α, FOXO1, and PGC-1α.

Figure 2. Effects of PXR Activation on Energy Homeostasis. Schematic illustration of how PXR activation affects energy metabolism and balance. Activation of PXR by agonists or low glucose results in up- and down-regulation of a cluster of genes associated with gluconeogenesis, lipogenesis, and β-oxidation by altering the activities of specific transcription factors such as HNF4α, FOXO1, FOXA2, PGC-1α, and CREB, or protein phosphatases/kinases such as PP2Cα, and SGK2.

Figure 1

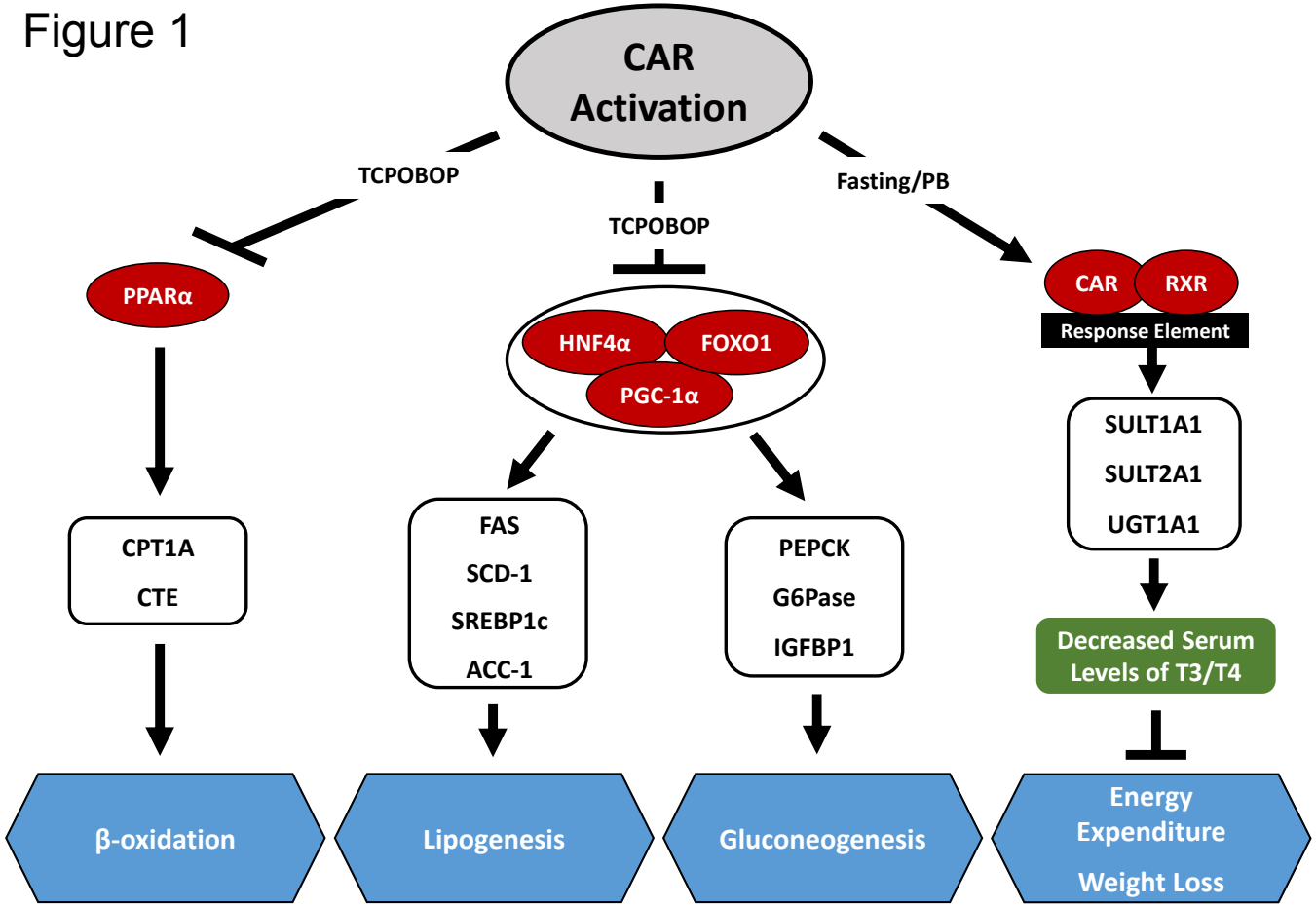

Figure 2