J Endocrinol Reprod 18 (2014) 2 : 59 - 74 **Review Article**

The Constitutive Androstane Receptor (CAR): a nuclear receptor in health and disease

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Summary

Constitutive Androstane Receptor (CAR, NR1I3), a member of the nuclear receptor superfamily of transcription factors, has emerged as one of the key regulators of the drug and xenobiotic metabolism. The unique feature that separates CAR from other members of the superfamily is that it remains active in the absence of ligand and is further regulated by activators. From its first isolation in 1994, a number of studies related to its distribution, characteristics, functions, and relation to other members of the superfamily have been conducted that place it centrally, governing many key events of the body. Human CAR is expressed relatively higher in liver and epithelial cells of the small intestine villi and less in heart, muscle, kidney, brain and lung. Though there are some controversies regarding its subcellular localization in different cell lines, in general, the subcellular localization of CAR is reported to be predominantly cytoplasmic, in complex with co-chaperone partners HSP90 and CCRP (cytoplasmic CAR retention protein). To execute transcription functions, nuclear translocation is a prerequisite event for a NR, including CAR. In this context, existence of two pathways is suggested, i) direct mechanism of action; and ii) indirect mechanism of action that is governed via nuclear translocation of CAR. Additionally, existence of species-specific differences in its modulation with ligands acting either as an agonist, antagonist or inverse agonist is also apparent. Like the other xenobiotic receptor PXR, CAR also functions as an alternative 'xenosensor' to defend the body against persistent chemical insults. It responds to diverse array of chemically distinct compounds, including endobiotics and xenobiotics, to regulate the clearance of noxious chemicals and toxic metabolites in liver and intestine via induction of genes involved in their metabolism. The usefulness of targeting CAR in metabolic diseases including bilirubinemia, obesity, type 2 diabetes mellitus, atherosclerosis, preeclampsia, hypertension, cholestasis and also in liver cancer is being extensively studied in animal models. However, to determine the human relevance it requires further investigation. Though a large number of natural and synthetic compounds act as modulators of CAR, designing new derivatives with defined therapeutic benefit need to be investigated. The purpose of this review is to highlight the general aspects of nuclear receptor CAR, its mechanism of action and importance in human health and disease.

***Key Words**:* Nuclear receptors, Transcription factors, Constitutive Androstane Receptor, Xenosensor, Drug metabolism, Metabolic diseases, Sub-cellular localization, Cancer.

Introduction

Nuclear Receptors (NRs) belong to a superfamily of phylogenetically-related proteins comprised of 48 members in humans. They act as transcriptional switches by responding to their cognate ligands including various hormones, vitamins, lipids, steroids, etc., and share a general modular structure (Mangelsdorf et al., 1995; Nuclear Receptors Nomenclature Committee, 1999; Burris et al., 2012). The members of this superfamily have a central DNA binding domain (DBD), also termed 'C region' which is highly conserved in sequence. There is a highly variable region on the amino-terminal to the C region called region A/B which contains the activation function 1 (AF-1) whose function (transcriptional activity) is independent of the presence of ligand. On the carboxy-terminal to the DBD, another conserved region is found, which is termed as the ligand binding domain (LBD) or E region and contains the activation function 2 (AF-2) whose action (transcriptional activity) is ligand-dependant. This region is responsible for recognition and binding of the specific ligands. There is a comparatively shorter region which connects C and E regions, called the hinge region or region D. On the extreme carboxy terminal to the LBD, some receptors may contain a region of unknown function called F region (Burris et al., 2012) (Fig. 1).

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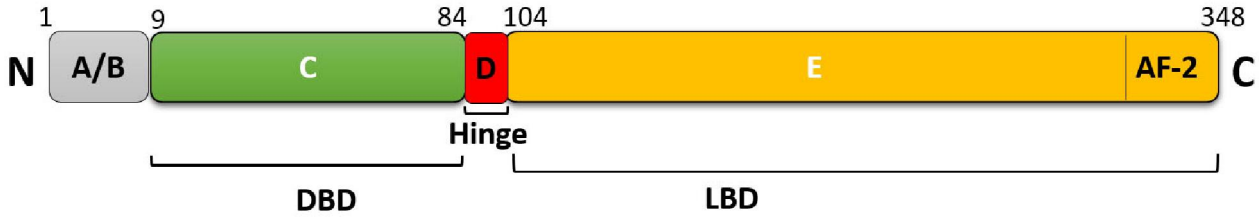

Fig.1: Schematic illustration depicting the modular structure of CAR that resembles the basic structural and functional organization of nuclear receptors. The CAR domains illustrated are marked with numbers representing the amino acid residues included for the specific regions or motifs. These residue numbers are either reported in the literature or derived from general modular structure of NRs. The presence of a functional A/B (AF-1) domain in CAR is disputed in some reports.

NRs, in general, can be divided into three distinct classes. The 'type I' classical steroid receptors include Androgen Receptor (AR), Estrogen Receptor α and β (ER α/β), Glucocorticoid Receptor (GR), Mineralocorticoid Receptor (MR) and Progesterone Receptor (PR). The 'type II' NRs are mainly the heterodimeric partners of Retinoid X Receptor (RXR) which include Vitamin D Receptor (VDR), Thyroid Receptor (TR), Retinoid Acid Receptor (RAR), and the Peroxisome Proliferator-Activated Receptors (PPAR), etc. The 'type III' Orphan Nuclear Receptors comprise of Constitutive Androstane Receptor (CAR), Pregnane & Xenobiotic Receptor (PXR, NR1I2), along with a few others (Shank et al., 2005).

CAR gene (NR1I3) was initially isolated from human and was called MB67 and, subsequently, termed as CARα (human CAR) (Baes et al., 1994). A cDNA from mouse was later identified and named CARβ (mouse CAR) (Choi et al., 1997). The similarity between human CAR and mouse CAR in the DBD and LBD is found to be 88%, and 72%, respectively. Human CAR is located on the chromosome 1, locus 1q23, and extended nearly 8.5 kilobases (kbs) (Thigpen, 2004). The size of this gene is about 8,545 base pairs (bps), having 9 exons that are separated by 8 introns (Auerbach et al., 2003; Savkur et al., 2003).

Analogous to its first cousin PXR, CAR acts as a defense receptor, and can jointly be described as the defenders against endogenous and exogenous chemical insults that are inflicted upon the body (Saradhi et al., 2006). The name Constitutive Active Receptor was proposed for this receptor during its initial cloning due to its inherent transcriptional activity in the absence of ligands until androstanes were identified as its inverse agonists (Baes et al., 1994; Forman et al., 1998). Till date, though many xenobiotic compounds have been identified as its agonists, the phenobarbital-like compounds are most widely used to study CAR functions (Gao & Xie, 2012).

CAR induces Phase I, II and III drug metabolizing enzymes in the liver and intestine where its expression is relatively higher (Tolson & Wang 2010; Ihunnah et al, 2011) and may extend both beneficial and sometimes harmful effects to the body. For example, ligand-activated CAR is believed to have a protective effect on diet-induced obesity and several metabolic diseases (Gao et al., 2009). On the contrary, it may enhance not only the metabolism of therapeutic drugs but also drug-drug interactions. Also, owing to its role in cell proliferation it may potentially enhance tumor propensity (Takizawa et al., 2011). Therefore, ongoing investigations into molecular mechanism of CAR signaling are expected to provide not only new avenues of pharmacological opportunities but also novel clues to many CAR-related pathological conditions, most notably in areas of metabolic disorders and cancer.

Receptor expression, localization and ligands

Since CAR helps in protecting the living organisms from many toxic compounds, the expression of CAR is justly expected to be higher in the liver, which is the major site of metabolism. CAR is also expressed at higher levels in epithelial cells of the small intestine villi. It is expressed as a transcript of 1.4-1.7 kb, 2.1 kb, 2.9 kb and a 7 kb mRNA. The 7.0 kb transcript is expressed rather weakly. CAR is also observed as a 3.0 kb transcript but at a rather lower level in heart and muscles and even lower levels in kidney (1.6 kb transcript), brain and lung (Baes et al., 1994; Choi et al., 1997; Doherty & Charman, 2002; Swales & Negishi, 2004). Interestingly, Yoshinari et al. (2001) reported more CAR protein levels in male Wistar-Kyoto rats when compared to its female counterpart raising gender-related differences in CAR expression.

Historically, subcellular localization of NRs mostly began with controversy (Kumar et al., 2006). Receptor localization results obtained are as good as the advancement in available technologies with their limitation hampering fair conclusions (Saradhi et al., 2005; Kumar et al., 2006). In this context, CAR is no exception. However, conflicting reports suggesting it to be cytoplasmic have outweighed the reports that advocate it to be nuclear. The specificity of available antibodies, nature of tags utilized, the cell types used for receptor expression etc., have all been instrumental in reporting contradictory observations (Kawamoto et al., 1999; Zelko et al., 2001) (Fig. 2).

The major characteristic of CAR that makes it distinct from its close NR partners is its high ligand-independent transcriptional activity (known as the constitutive activity). Observations made in cell cultures and in animal models have shown that a herbal drug 'yin zhi huang' is perhaps the best activator for CAR (Huang et a.l, 2004; Chang et al., 2009). A comprehensive list of compounds affecting the biological activity of human, mouse and rat CAR is presented in tables 1 and 2. From the list of diverse ligands that are being reported to modulate the function of CAR it is apparent that the promiscuous nature of CAR is somewhat comparable to its closest relative PXR.

Mode of action

Nuclear Receptors either reside in nuclear compartment or are transported from cytoplasm to nucleus when bound to their ligand(s) with the involvement of nuclear localization signal (NLS). Typically, a basic amino acid-rich sequence (RRARQARRR) serves as NLS and is normally found in DBD region. CAR is reported to be translocated into the nucleus by importins that recognize and bind to its NLS (Chiang, 2009). Intriguingly, a functional NLS is absent in human CAR but two NLS were reported in rat CAR (Kanno et al., 2005). According to Zelko et al. (2001), within the 30 amino acid residues in C-terminal region of CAR of both human and mouse CAR a leucine-rich sequence (L/MXXLXXL) is present. This sequence functions as a signal for nuclear translocation in presence of xenobiotics and, therefore, is termed as the 'xenobiochemical response signal' (XRS). Kawana et al. (2003) reported that mouse CAR NLS has rather a weak role to play due to the possibility of mutations in its NLS. Thus, the nuclear translocation of CAR is both ligand-dependent and independent (Kawamoto et al., 1999; Zelko et al., 2001). When bound to its ligand CAR shifts predominantly from cytoplasmic to nuclear compartment that is reported to be governed by a receptor de-phosphorylation event (Squires et al., 2004; Pondugula et al., 2009). Subsequently, CAR associates with its obligate heterodimeric partner RXR and docks onto the specific DR4 motifs to execute its transcription functions (Kawamoto et al., 1999; Sueyoshi & Negishi, 2001). Microarray studies performed using CAR null and wild type mice have revealed that 98 genes are either up-regulated or down-regulated in response to CAR activation (Hernandez et al., 2009). Presently, two pathways for CAR activation are known to be operational i.e., i) direct mechanism of activation; and ii) indirect mechanism of activation.

Table 1 : Compounds affecting biological activity of human CAR

Chemicals	Source	Biological activity	References
Androstanol	Synthetic	Inverse agonist	Forman et al., 1998
5β-pregnane-3,20-dione	Synthetic	Agonist	Moore et al., 2000
Clotrimazole	Synthetic	Antagonist, Inverse agonist, agonist	Moore et al., 2000; Jyrkkarinne et al., 2008; Lynch et al., 2012
5 β -Pregnanedione	Steroids	Agonist	Maglich et al., 2003
6-(4-Chlorophenyl)imidazo [2,1-b][1,3]thiazole-5-carbaldehyde O-(3,4-dichlorobenzyl)oxime (CITCO)	Synthetic	Agonist	Maglich, et al., 2003
Meclizine	Synthetic	Inverse agonist	Huang et al., 2004
6,7-Dimethylesculetin	Natural	Agonist from Yin Chin	Huang et al., 2004
Phenytoin	Synthetic	Activator, Agonist	Wang et al, 2004; Kublbeck et al., 2011a
Phenobarbital	Synthetic	Agonist	Wagner et al., 2005
Efavirenz	Synthetic	Agonist	Faucette et al., 2007
Nevirapine	Synthetic	Agonist	Faucette et al., 2007
Valproic acid	Synthetic	Agonist	Cerveny 2007
Carbamazepine	Drug	Agonist	Faucette et al., 2007
Triaryl phosphates	Plasticizers	Agonist	Jyrkkarinne et al., 2008
Thiazolidin-4-ones	Synthetic	Agonist	Kublbeck et al., 2008
Sulfonamides	Synthetic	Agonist	Kublbeck et al., 2008
A series of chemotypes	Synthetic	Agonist	Li et al., 2008
1-(2-Chlorophenylmethylpropyl)-3-isoquinoline-carboxamide (PK11195)	Synthetic	Antagonist; inverse agonist	Li et al., 2008; Kublbeck et al., 2011b; Lynch et al., 2012
Di(2-ethylhexyl) phthalate	Synthetic	Agonist for hCAR2	DeKeyser et al, 2009
Flexible diaryl compounds (FL81)	Synthetic	Agonist	Kublbeck et al., 2011b
Organochlorines (e.g., methoxychlor, PCB153, o,p ?-DDT)	Pesticides	Agonist	Kublbeck et al., 2011a
Pyrethroids (e.g., permethrin, cypermethrin)	Pesticides	Agonist	Kublbeck et al., 2011a
1-[(2-Methylbenzofuran-3-yl)methyl]-3-(thiophen-2-ylmethyl) urea (S07662)	Synthetic	Inverse agonist	Kublbeck, et al., 2011b
Carbamates (e.g., benfuracarb)	Pesticides	Agonist	Abass et al., 2012
Artemisinin	Drug	Agonist	Burk et al., 2012
Food-derived flavonoids (e.g., chrysin)	Natural polyphenols	Agonist	Yao et al., 2011
Alcohol-derived flavonoids (e.g., ellagic acid)	Natural polyphenols	Agonist	Yao et al., 2011
Androstan-3 α -ol and androsten-3 α -ol	Steroids	Inverse agonist	Dau et al., 2013
3,17 β -Estradiol and 17 α -ethinylestradiol	Steroids	Inverse agonist	Dau et al., 2013
T0901317	Synthetic	Inverse agonist	Kanno et al., 2013
Nigramide J	Naturally occurring cyclohexane-type amide alkaloid from *Piper nigrum*	Inverse agonist	Y. Kanno et al., 2014
Allyl isothiocyanate (AITC)	Natural	Antagonist	Lim et al., 2015

Table 2: **Compounds affecting biological activity of mouse / rat CAR**

Chemicals	Source	Biological activity	References
Androstanol	Synthetic	Inverse CAR agonist	Forman et al., 1998
1,4-bis[2-(3,5-Dichloropyridyloxy)]benzene (TCPOBOP)	Synthetic	Agonist for mouse CAR; suppressing gluconeogenic and lipogenic genes, anti-obesity	Tzameli et al., 2000
5 β -Pregnanedione	Steroids	Inverse mouse CAR agonist	Maglich et al., 2003
chlorpromazine	Synthetic	Agonist for mouse CAR	Makinen et al., 2003
Meclizine	Synthetic	Agonist for mouse CAR	Huang et al., 2004
6,7-Dimethylesculetin	Natural	Agonist for mouse CAR from Yin Chin	Huang et al., 2004
Phenobarbital	Synthetic	Agonist for mouse CAR	Wagner et al., 2005
Diallyl sulfide	Natural. From garlic oil	Agonist for mouse CAR	Fisher et al., 2007
Wy-14,643	Synthetic	Inverse agonist for mouse CAR	Guo et al., 2007
Ciprofibrate	Synthetic	Inverse agonist for mouse CAR	Guo et al., 2007
cis- and trans-guggulsterone	Natural	Inverse agonist for mouse CAR	Chang., 2009
Artemisinin	Natural	Agonist for mouse CAR	Burk et al., 2012
cis-2,4,6-Triphenyldioxane-1,3	Synthetic	Agonist for rat CAR; suppressing gluconeogenic and lipogenic genes	Kachaylo et al., 2012
3,17 β -Estradiol and 17 α -ethinylestradiol	Steroids	Agonist for mouse CAR	Dau et al., 2013
Paclitaxel	Natural	Agonist for mouse CAR	Fukumasu et al., 2014

i) Direct mechanism of activation of CAR

In general, the subcellular localization of unliganded CAR is reported to be predominantly cytoplasmic where it is retained in complex with co-chaperone partners like cytoplasmic CAR retention protein (CCRP) and HSP90. Upon binding to an agonist CAR dissociates from CCRP and HSP90 resulting in receptor translocation from cytosol to the nuclear compartment. This multi-step event, for initiation of nuclear translocation, is suggested to depend on a protein phosphatase PP2A recruitment for dephosphorylation of CAR resulting in dissociation of CAR-CCRP-HSP90 complex (Fig. 3). Subsequently, CAR heterodimerises with RXR when inside the nucleus, and recruits co-regulators to modulate gene transcription function (Timsit & Negishi, 2007; Chang et al., 2009). Some of the major CAR coactivators reported to participate in this function include SRC-1, transcription factor Sp1, signal cointegrator-2, etc. (Timsit & Negishi, 2007), and corepressor NCoR (Lempiainen et al., 2005).

ii) Indirect mechanism of activation of CAR

Phenobarbital, an anticonvulsant drug, activates CAR by indirect pathway (Yoshinari et al., 2003). According to Mutoh et al. (2013), PB activates CAR by inducing the dephosphorylation of CAR through PP2A. The PP2A is suggested to be activated through another pathway including the EGFR (epidermal growth factor receptor) and the RACK1 (receptor for activated C kinase 1). In the absence of PB, the epidermal growth factor (EGF) binds to EGFR, thereby activating the Src kinase, which in turn phosphorylates RACK1. Upon PB-exposure, PB binds competitively to EGFR and leads to inactivation of Src kinase. As a consequence the unphosphorylated RACK1 interacts with PP2A to activate CAR. The recruitment of PP2A has been shown to be mediated by the multi-protein complex 8. As PB is involved in the activation of AMPK (AMP-activated protein kinase), it has been suggested that AMPK activates PP2A (Rencurel et al., 2005). The proposed mechanism is depicted in figure 3.

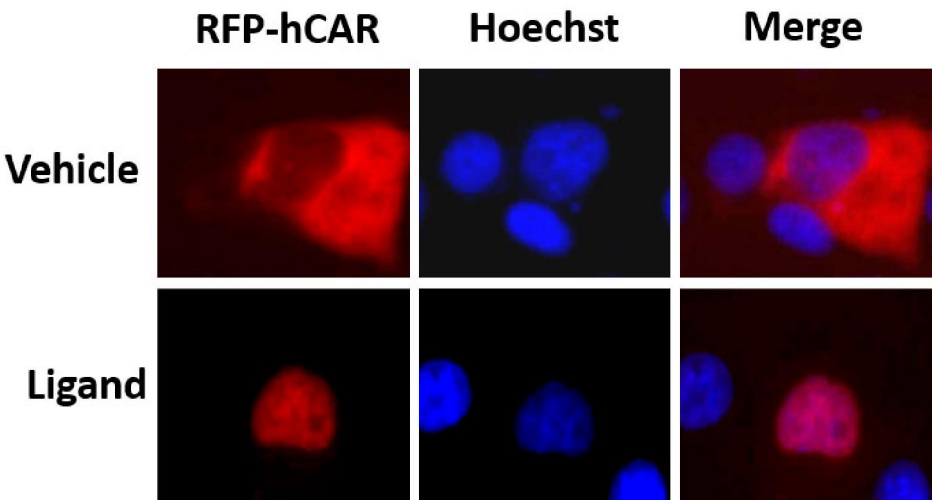

Fig. 2: A representative subcellular localization of RFP-tagged human CAR expressed in COS-1 cell line. CAR distribution in the interphase of living cells in the absence and presence of a ligand are shown. The cells were transiently transfected with plasmid construct RFP-CAR and cultured for 24 hours in steroid-stripped medium for protein expression. Hoechst was added to the cultures for visualization of the nucleus. In the absence of ligand the subcellular localization of human CAR was predominantly towards cytoplasm (upper panel). However, ligand binding shifted the receptor towards the nuclear compartment (lower panel).

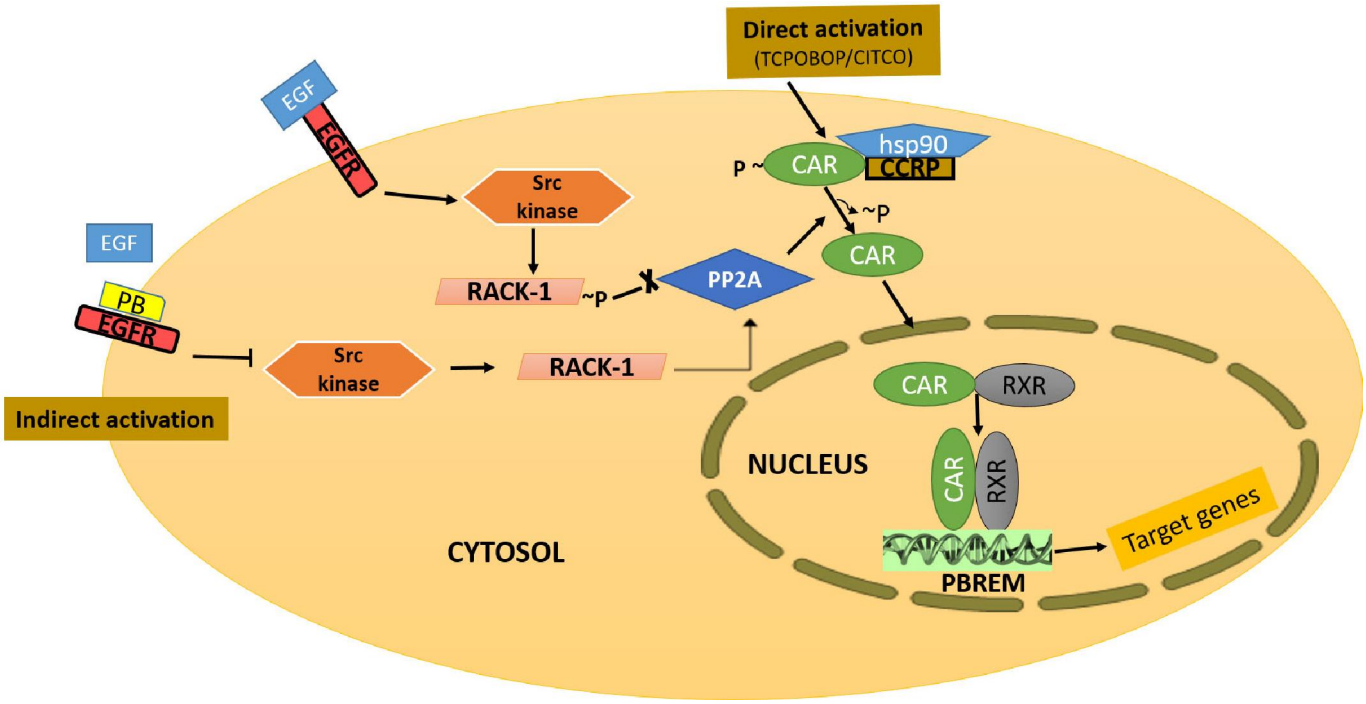

Fig. 3: Direct and indirect mechanisms of activation of nuclear receptor CAR

CAR in drug metabolism and elimination

The function of CAR first became evident with the findings that it modulates Cytochrome P450 2B (CYP2B) set of genes (Honkakoski et al., 1998). CAR prototypically binds to the NR1 and NR2 motifs in the phenobarbital responsive enhancer module (PBREM) found in the promoter region of various CYP2B genes (Makinen et al., 2002). These CYP2B genes code for monooxygenases that are involved in drug metabolism. Human CAR predominantly activates CYP2B6 whereas mouse CAR activates CYP2B10. Besides CYP2B, CAR has been reported to induce other CYP enzymes

as well. Goodwin et al (2002) showed that both human and mice CAR could induce the expression of CYP3A4 by binding to sites in the distal XREM (Goodwin et al., 2002). Moreover, CYP1A1 and CYP1A2, the prime target genes of aryl hydrocarbon receptor (AhR), were shown to be activated by CAR via an AhR-independent pathway (Yoshinari et al., 2010). Studies by Ferguson et al. (2005) suggested that CYP2C8, which is involved in the metabolism of endobiotics as well as xenobiotics, exhibits a CAR-regulated expression (Ferguson et al., 2005). In 2001, Sugatani et al demonstrated UDP-glucuronosyltransferase 1A1 (UGT1A1), a critical phase II drug metabolizing enzyme, to be regulated by CAR in response to phenobarbital. Subsequently, Osabe et al. (2008) obtained similar results while working on UGT1A6, an isoform of the UGT family of enzymes. Furthermore, various isoforms of sulfotransferases (SULTs) which are members of the phase II drug metabolizing enzymes and cause biotransformation of xenobiotics, are also upregulated by CAR. This could be inferred from the fact that administration of TCPOBOP, an agonist of mice CAR, into female mice leads to a marked increase in the hepatic levels of Sult1c2, Sult1d1, Sult1e1, Sult2a2, Sult2b1, Sult3a1, and Sult4a1 (Alnouti & Klaassen, 2008). Moreover, drug transporters that carry out efflux of the biotransformed xenobiotics, have been found to be regulated by CAR. Kast et al. (2002) showed that multidrug resistance-associated protein-2 (MRP2) was independently regulated by three nuclear receptors, viz., CAR, PXR and FXR, through a common response element at the 5'UTR of the gene (Kast et al., 2002). Similarly, Burk et al. (2005) found that cells stably expressing CAR showed elevated expression of MDR1 gene (Burk et al., 2005). Organic anion transporting polypeptide 2 (OATP2), which carries out transport of bilirubin, has also been reported to be regulated by CAR (Ding et al., 2006).

CAR and energy homeostasis

The first report on the role of CAR in regulation of metabolism and metabolic diseases came with the studies on High Fat Diet (HFD)-induced obese mice, wherein CAR was shown to prevent obesity (Gao et al., 2009). Moreover, the study also indicated a role for CAR in extenuating type II diabetes by increasing insulin sensitivity in model mice. In addition, Dong et al. (2009) obtained similar results working on leptin-deficient obese mice. Serum glucose levels were found to be significantly lower with increased glucose tolerance and insulin sensitivity. The study also pointed out the effectiveness of CAR in impeding hepatic lipogenesis and inducing β-oxidation. Zhai et al. (2010) indicated a cross talk between two NRs, CAR and LXR, which resulted in under-expression of lipogenic genes such as Srebp1, Scd1, Fas and Acc1. Roth et al. (2008) have reported a CAR-activated insulin-inducible gene-1 (Insig-1)-mediated pathway for the repression of lipogenic genes in mouse liver. Another mechanism by which CAR could play a role in the maintenance of energy homeostasis is through its inhibition of phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G6Pase) (Kachaylo et al, 2012) or competing with hepatic nuclear factor-4 (HNF-4) (a factor essential for gluconeogenesis) for binding sites on concerned genes (Miao et al., 2006). All these reports point to exploiting CAR as a potential target for therapeutics towards this receptor-related metabolic disorders.

According to Roth et al. (2008), due to activation of CAR there is a reduction in levels of SREBP-1 (master regulator of lipid metabolism) which in turn might result in supression of lipid metabolism and decrease in serum triglyceride levels. This supression of lipid metabolism may also be accredited to induction of a protein with anti-lipogenic properties (Insig-1). Some studies have shown involvement of CAR with non-alcoholic steatohepatitis, serum triglycerides level during metabolic stress and regulation of HDL (Yamazaki et al., 2007; Masson et al., 2008; Maglich et al., 2009). Induction of CAR during severe fasting suggests involement of CAR in nutrient stress (Maglich et al., 2004; Ding et al., 2006). Also, there is evidence for existence of a functional crosstalk of CAR with PPAR α, a Nuclear receptor which has an involvement in energy mobilization at the time of fasting (Wieneke et al., 2007; Guo et al., 2007).

CAR in health and disease

Although CAR has primarily been regarded as a 'xenosensor', it also plays vital roles in maintenance of energy balance, regulation of cell proliferation and development of several metabolic disorders (Table 3).

Table 3: Role of CAR in metabolic diseases

Disease	Disease Regulation	Reference
Bilirubinemia/jaundice	Down-regulation	Huang et al., 2003
Cholestasis	Down-regulation	Stedman et al., 2005
Hepatocellular carcinoma	Up-regulation	Huang et al., 2005
Hepatomegaly	Up-regulation	Huang et al., 2005
Diabetes mellitus type 2	Down-regulation	Dong et al., 2009, Gao et al., 2009
Obesity	Down-regulation	Gao et al., 2009
Atherosclerosis	Down-regulation	Sberna et al., 2011ab
Preeclampsia	Down-regulation	Masuyama et al., 2012ab
Hypertension	Down-regulation	Masuyama et al., 2012ab

Some of the wide ranging roles of CAR being reported in different metabolic disorders in human and mice are discussed here. These imply its involvement and importance in diverse physiological controls.

Bilirubinemia/jaundice

Hyper-bilirubinemia is an aberrant physiological condition due to excess bilirubin in the blood owing to the rupture of RBCs and inadequate liver function. Bilirubin, a yellow pigment, causes yellowing of the skin, eyes and other tissues, which gives rise to a condition called 'jaundice'. It is mostly common in new-borns. Phenobarbital, an indirect activator of CAR, is clinically proven to control the levels of elevated bilirubin in humans (Catz et al., 1962; Yaffe et al., 1966; Wallin et al., 1984). Huang et al. (2003) studied the effect of CAR on bilirubin clearance by using animal models and concluded that CAR has a protective action on bilirubinemia and its functional insufficiency can lead to neonatal jaundice. Additionally, it was also observed that CAR primarily targets UGT1A1, MRP2, and GSTA1 demonstrating broader regulatory effects on the pathway of bilirubin clearance.

Hepatomegaly

Hepatomegaly is a condition that results in the enlargement of the liver, the master organ of xenobiotic metabolism. CAR, which is a dominant regulator of xenobiotics detoxification and functions by inducing the hepatic expression of different enzymes and transporters involved, also increases liver size. Huang et al. (2005) reported that hepatomegaly caused due to CAR is a temporary, adaptive response to acute stress caused by different xenobiotics, and chronic CAR activation may result in hepatocarcinogenesis. It was also reported that both acute and chronic cases of xenobiotic stress may cause increase in hepatocyte DNA replication and decrease in apoptosis which otherwise were reported absent in CAR-null mice (Huang et al., 2005).

Hepatocellular carcinoma

The indirect activator of CAR, phenobarbital as well as its direct activator TCPOBOP, are strong tumor promoters in liver (Yamamoto et al., 2004) suggesting the role of CAR in tumor promotion in liver. A study conducted by Huang et al. (2005) reported that CAR knockout mice survived DEN-induced hepatocellular carcinoma (HCC), followed by phenobarbital treatment in contrast to the CAR wild-type mice having the same regimen of treatment. It was also interesting to observe that only mice expressing wild type CAR exhibited development of HCC as compared to CAR knockout mice. Though the two drugs (phenobarbitol and TCPOBOP) are mouse-specific, similar involvement of CAR in tumor promotion in human liver cannot be ruled out. There are some evidences of involvement of CAR in HCC but a thorough study is needed for understanding in detail the mode of its action in humans (Huang et al., 2005; Chakraborty et al., 2011).

Obesity

Obesity can be considered as a health condition in which there is accumulation of excess body fat which imparts a detrimental effect on health and leads to many secondary health problems. A study conducted by Gao et al. (2009) on mouse model suggested that CAR has a role in preventing obesity. It was also proposed that prevention of obesity by CAR may be the result of combined effect of inhibition of gluconeogenesis, very low density cholesterol secretion, lipogenesis, export of

triglycerides and increase in energy expenditure in brown adipose tissue and peripheral fat mobilization.

Diabetes mellitus type 2

Diabetes mellitus type 2, a metabolic disorder, previously known as adult-onset diabetes or noninsulin-dependent diabetes mellitus, is characterized by hyperglycemia and is a predisposing factor to many other diseases including obesity. Dong et al. (2009) proposed that CAR activation has a protective effect on diabetes mellitus type 2 by reducing serum glucose levels, increasing glucose tolerance and insulin sensitivity. This study on mouse also showed that induction of CAR decreases glucose production, and increases glucose uptake and usage in liver.

Atherosclerosis

Atherosclerosis is the thickening of the arteries resulting from high cholesterol levels. Oxidation of LDL accumulated on the endothelial cells of arteries leads to an inflammatory response which further leads to deposition of WBCs and ultimately to the thickening of arteries. Sberna et al. (2011a), working on mouse model, observed that TCPOBOP-activated CAR could induce clearance of HDL-derived cholesterol through its conversion to bile acids. Later, with the use of LDLr-/- KO mice it was shown that CAR activation could decrease the levels of LDL in such mice (Sberna et al., 2011b). After two months of treatment with TCPOBOP, plasma triglycerides and LDL levels were reduced by 30%. Moreover, the atherosclerotic plaques were reduced by 60% suggesting that CAR may play a role in lipid metabolism that ameliorates atherosclerosis in mice models.

Preeclampsia and hypertension

Preeclampsia is a pregnancy-related complication marked by hypertension and proteinuria. It occurs in 3-5% of all pregnancies resulting in considerable maternal and neonatal morbidity and mortality (Masuyama et al., 2012a). Insulin resistance, increased body mass index and polycystic ovarian syndrome are some of the predisposing factors to preeclampsia (Seely & Solomon, 2003, Innes et al., 2001, Duckitt and Harrington, 2005). Masuyama et al. (2012a), treated high fat diet (HFD)-induced obese pregnant mice with TCPOBOP to specifically activate CAR signaling pathways in these animals. It was observed that TCPOBOP treatment reduced maternal symptoms of preeclampsia as indicated by improved systolic blood pressure, near-normal urinary protein levels and decreased maternal weight of the HFD-fed pregnant mice. Furthermore, fetal overgrowth was also ameliorated along with increased maternal glucose tolerance and decreased insulin resistance thereby reducing the possibilities of offspring developing metabolic disorders (Masuyama et al., 2012a, Masuyama et al., 2012b).

Cholestasis

Cholestasis is characterized by decreased flow of bile and its accumulation in the liver resulting in hepatic damage. A study showed that both CAR and PXR regulate cholesterol metabolism (Stedman et al., 2005). It was reported that CAR and PXR knockout mice had a high level of bile acids which led to increased hepatic damage in these mice as compared to the control mice. Also, the two nuclear receptors appeared to regulate drug transporters such as Oatp-C (organic anion transporting polypeptide C) and Oatp2 (Na^+-dependent organic anion transporter 2) further reflecting their established role in detoxification pathways.

CAR in cancer and cell proliferation

A study in 2004 showed that chronic treatment with phenobarbital, an agoinst of CAR, resulted in development of hepatocellular carcinoma and/or adenoma in mice (Yamamoto et al., 2004). Further, it was suggested that PB-activated CAR results in altered DNA methylation which ultimately leads to the hepatic tumorigenesis (Phillips et al., 2007). CAR also induced the expression of growth arrest and DNA damage-inducible 45β (Gadd45β), which is an inhibitor of apoptosis (Columbano et al., 2005). This led to cell proliferation and tumor growth in tested mice. Furthermore, in mice, TCPOBOP treatment led to increased levels of Cyclin D1 which is a major event involved in rapid hepatic cell proliferation (Ledda-Columbano et al., 2000). In another report, CAR induction appeared to upregulate Mdm2 (double minute oncogene), which further promoted DNA replication and inhibited p53 (Huang et al., 2005). However, in context

to human CAR, the available data is fairly contrasting to that of murine CAR. CITCO, a synthetic agonist of human CAR, appeared to affect cell cycle arrest and apoptosis in brain tumor stem cells without harming the normal astrocytes (Chakraborty et al., 2011). To this point, in the studies performed on mice CAR conclusively indicated its affirmative role in regulating cell proliferation. However, before considering human CAR as a therapeutic target for cancer or even metabolic disorders for that matter, extensive investigation into the functioning of the receptor in humans needs to be performed.

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