Expression of Human CAR Splicing Variants in BAC-Transgenic Mice

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The nuclear receptor constitutive androstane receptor (CAR) is a key regulator for drug metabolism in liver. Human CAR (hCAR) transcripts are subjected to alternative splicing. Some hCAR splicing variants (SVs) have been shown to encode functional proteins by reporter assays. However, in vivo research on the activity of these hCAR SVs has been impeded by the absence of a valid model. This study engineered an hCAR-BAC-transgenic (hCAR-TG) mouse model by integrating the 8.5-kbp hCAR gene as well as 73-kbp upstream and 91-kbp downstream human genomic DNA into the genome of CAR-null mice. A series of experiments demonstrate that (1) the expression of major hCAR mRNA SVs, SV0-4, in livers of hCAR-TG mice is comparable to that in human livers; (2) the hCAR SVs are predominantly expressed in liver, which resembles the tissue distribution of CAR in humans, but diverges from that in mice; and (3) major hCAR mRNA SVs increase markedly in postnatal livers of hCAR-TG mice, which mimics the ontogeny of CAR mRNA in humans. Thus, the transgene likely contains all the functional regulatory elements controlling proper spatial and temporal expression of the hCAR gene. Moreover, hCAR-TG mice respond to the hCAR-specific agonist 6-(4-chlorophenyl)imidazo[2,1-b] [1,3]thiazole-5-carbaldehyde O-(3,4-dichlorobenzyl)oxime instead of the mouse CAR agonist 1,4-bis[2-(3,5-dichloropropylidoxyl)]benzene, as well as the common CAR activator, phenobarbital, suggesting that hCAR is fully functional in livers of transgenic mice. In summary, the hCAR-TG mice developed by this study represent a valid model for studying in vivo function and regulation of hCAR and its splicing variants.

Key Words: CAR; splicing variant; in vivo; BAC; transgenic mice.

Nuclear receptor constitutive androstane receptor (CAR; NR1I3) plays an important role in regulating the metabolism of xenobiotics and nutrients in the liver. Compared with many other nuclear receptors, CAR is unique in that it is constitutively active and can be further activated by many endogenous and exogenous chemicals (Swales and Negishi, 2004), including steroid hormones, pharmaceuticals, as well as environmental, dietary, and occupational chemicals (Hernandez et al., 2009). Upon activation, CAR translocates from the cytoplasm into the nucleus, heterodimerizes with the retinoid X receptor, binds to the phenobarbital (PB) responsive enhancer module elements, and induces the transcription of numerous phase I and II enzymes and transporters to facilitate the elimination of chemicals (Honkakoski et al., 1998). Examples of genes regulated by CAR are phase I enzymes such as cytochrome P450s (Cyps) (Huang et al., 2004; Petrick and Klaassen, 2007), phase II enzymes such as UDP-glucuronosyltransferase 1a1 (Huang et al., 2004; Sugatani et al., 2001), glutathione S-transferase al (Huang et al., 2004), and sulfotransferase 2a1 (Assem et al., 2004), as well as membrane transporters such as multidrug resistance-associated protein 4 (Assem et al., 2004; Petrick and Klaassen, 2007). Because of its constitutive activity and broad influence on xenobiotic metabolism, the hepatic expression level of CAR could potentially affect the capability of an individual in eliminating drugs from the body (Lamba et al., 2003; Vyhlidal et al., 2006).

Alternative splicing is a major source of protein diversity in higher eukaryotes and plays critical roles in differentiation, development, and disease. In humans, 35 splicing variants (SVs) of CAR mRNA have been identified (Ensembl: ENSG00000143257). In addition to the first-cloned SV0 (CAR1), SV1 (CAR2), and SV2 (CAR3) are the most abundant human CAR (hCAR) SVs in human livers (Lamba et al., 2004). Although most of hCAR SVs do not encode functional proteins, several in vitro assays showed that some hCAR SVs encode proteins that can be activated by chemicals that do not activate the constitutively active wild-type (WT) hCAR (SV0 or CAR1) (DeKeyser et al., 2009; Dring et al., 2010). For example, SV1 responds to the common plasticizer di(2-ethylhexyl)phthalate (DeKeyser et al., 2009), whereas SV2 exhibits low basal activity, but it is strongly activated by the prototypical hCAR agonist 6-(4-chlorophenyl)imidazo[2,1-b] [1,3]thiazole-5-carbaldehyde O-(3,4-dichlorobenzyl)oxime (CITCO) (Auerbach et al., 2005). However, the in vivo function and expression profiles of these hCAR SVs remain unknown, mainly due to the lack of a valid animal model.

Human hepatoma cell lines, human primary hepatocytes, and human liver tissues are model systems that are commonly
used to study the regulation and function of human genes. Significant disadvantages of these systems include compromised regulatory properties (cell lines), lack of in vivo environment (cell lines and primary hepatocytes), and limited accessibility (primary hepatocytes and human liver tissues). Laboratory mice provide useful models of human development and drug metabolism, but hCAR is different from mouse CAR (mCAR) in regard to ligand specificity and pre-mRNA alternative splicing. mCAR has a specific ligand, 1,4-bis[2-(3,5-dichloropyridyl)oxy]benzene (TCPOBOP) (Nims et al., 1999), and does not respond to the hCAR-specific ligand CITCO. In comparison to the 35 hCAR SVs, mice only express 2 SVs of CAR mRNA (Choi et al., 1997). hCAR transgenic mice provide a promising tool to study in vivo function and regulation of hCAR. Whereas two lines of hCAR-transgenic mice have been engineered, they either express only WT (SV0) hCAR (Zhang et al., 2002) or lack the promoter and 5′-untranslated region of hCAR gene (Scheer et al., 2008). Therefore, the purpose of this study was to engineer a new line of hCAR-transgenic mice by integrating the entire hCAR gene encoding region as well as 73-kbp upstream and 91-kbp downstream of human genomic DNA into the mouse genome. Characterization of these hCAR-transgenic mice indicates that they are suitable for studying the developmental regulation and in vivo transcriptional function of this human nuclear receptor.

MATERIALS AND METHODS

Chemicals and reagents. CITCO was obtained from Enzo Life Science (Farmingsdale, NY). TCPOBOP and PB were obtained from Sigma Aldrich (St Louis, MO). All other reagents were obtained from Sigma Aldrich.

Generation of hCAR-BAC-transgenic mice. The hCAR-BAC-transgenic (hCAR-TG) mouse founders were generated by the Transgenic and Gene-Targeting Institutional Facility at the University of Kansas Medical Center. Briefly, bacterial artificial chromosome (BAC) RP11-297K8 DNA, constructed by the Sanger Center Chromosome 1 Mapping Group, was linearized with endonuclease NotI and microinjected at the concentration of 0.1 ng/µl into pronuclei of fertilized eggs of FVB × C57BL/6 mice. Two founder lines (no. 22 and no. 25) were generated, as detected by PCR analysis of genomic DNA from tail biopsies. The transgenic founders were bred with CAR-null mice (provided by Dr. Ivan Rusyn, University of North Carolina, Chapel Hill, NC) on the C57BL/6 background to generate hCAR+/mCAR− F1 hybrids. The F1 offspring were further crossed with CAR-null mice to generate the hCAR+/mCAR− F2 generation that expressed only hCAR but not mCAR as determined by PCR analysis. The F2 generation of hCAR+/mCAR− mice were bred with their littermates to generate homozygous hCAR+/mCAR−. The zygosity of hCAR genomic DNA in hCAR-TG mice was determined by SYBR Green real-time quantitative PCR (real-time qPCR) as described (Haurowne et al., 2007). PCR primers (5′-cagctcgaactttttttcttct-3′ and 5′-cctcttgcttcttctct-3′), targeting the 3′-end of the hCAR gene and resulting in a 201-bp band, were used to determine the zygosity of the hCAR gene in hCAR-TG mouse. The mouse gap junction channel protein alpha 5 (mGJA5; GenBank: NM_008121) was used as the endogenous reference. Real-time qPCR primers for mGJA5 were 5′-aactttggcattgtggaagg-3′ and 5′-aactttggcattgtggaagg-3′; and glyceraldehyde-3-phosphate dehydrogenase (Gapdh) (GenBank accession number AF128849), 5′-aaggaggaggttaacacctg-3′ and 5′-gctgacaggggctact-3′; mCAR (GenBank accession number NM_009803), 5′-ctcagaggggagtacagenctc-3′ and 5′-agttcctcggcccatattct-3′; Cyp3a11 (GenBank accession number NM_007818), 5′-aactgctgaagttggagaag-3′ and 5′-gtagaggagcagaacctc-3′; and glyceraldehyde-3-phosphate dehydrogenase (Gapdh) (GenBank accession number M32599), 5′-aactggctgaagttggagaag-3′ and 5′-gtagaggagcagaacctc-3′. The Power SYBR Green Master Mix (Applied Biosystems) was used for real-time PCR analysis. Differences in gene expression between groups were calculated using cycle threshold (Ct) values, which were normalized to Gapdh mRNA.

Immunoblot analysis. Nuclear and cytoplasmic fractions were prepared from three individual mouse livers in each treatment group using NE-PER nuclear protein extraction kit (ThermoFisher Scientific Inc., Rockford, IL). Thirty micrograms of nuclear protein and 100 µg of the cytosol protein were separated by SDS-polyacrylamide gel electrophoresis and electrophoretically transferred to a polyvinylidene fluoride membrane. Nuclear proteins were probed with an anti-CAR1/2 (M-150) polyclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and anti-TATA-binding protein (TBP) monoclonal antibody (ThermoFisher Scientific Inc.). Cytosolic proteins were probed with rabbit anti-rabbit CYP2B1/2 polyclonal antibody (Xenotech) and rabbit β-actin polyclonal antibody (Abcam, Cambridge, MA). Secondary anti-rabbit or mouse antibodies were diluted 10,000-fold. Immunoreactions were detected by Pierce ECL Western blotting substrate (ThermoFisher Scientific Inc.). The density of blots was quantified by Quantity One 1-D Analysis Software (Bio-Rad Laboratories, Hercules, CA). The individual blot densities of CAR and Cyp2b10 were normalized to the nuclear and cytosol loading control, TBP, and β-actin, respectively.

Statistical analysis. Statistical significance was assessed by two-way ANOVA when two variables were examined or Student’s t-test when one variable was tested. The criterion for statistical significance was p < 0.05.
RESULTS

Generation and Characterization of hCAR-TG Mice

BAC clone RP11-297K8 containing a 173-kbp DNA fragment from human chromosome 1 (Fig. 1A) was used to generate hCAR-TG mice. In addition to the hCAR gene, RP11-297K8 encompasses other human gene loci such as ubiquitin specific protease 21 (USP21), protoporphyrinogen oxidase (PPOX), and apolipoprotein A2 (APOA2). Further details about RP11-297K8 can be found on NCBI website http://www.ncbi.nlm.nih.gov/clone/270914/. The transgenic founders were generated and bred with CAR-null mice on the C57BL/6 background for two generations to obtain the hCAR+/+/mCAR−/− F2 generation that expresses only hCAR but not mCAR (Fig. 1B). Homozygous hCAR-TG mice were identified by real-time qPCR as described (Haurogne et al., 2007). The primer designed for the hCAR gene was able to detect one Ct difference for two-fold DNA quantities between heterozygous and homozygous, whereas the reference gene mGJA5 showed similar Ct values in all examined samples (Fig. 1C). The homozygous hCAR-TG mice from line no. 22 were used in this study and their WT littermates were used as controls.

Comparison of Hepatic Expression of Major SVs of hCAR mRNA in hCAR-TG Mice and Humans

Major SVs of hCAR mRNA reported in a previous study (Jinno et al., 2004) were determined in livers of male adult hCAR-TG mice and compared with those in human livers. As shown in Figure 2, the WT hCAR SV0 was the predominant isoform (~60%), followed by SV2 (~30%), and SV1 (~8%) in both human and hCAR-TG mouse livers. Marked interindividual differences in hCAR SV0 were observed in human liver samples. The expressions of SV0, SV1, SV2, and SV3 were comparable in human livers and hCAR-TG mouse livers, whereas SV4 was expressed lower in hCAR-TG mice than in humans.

Comparison of Prototypical CAR-Target Genes Induced by Species-Specific CAR Activators in the Liver

Both WT and hCAR-TG mice (male) were subjected to the following four treatments: corn oil (vehicle control), TCPOBOP (mCAR-specific ligand), CITCO (hCAR-specific ligand), and PB (nonligand activator for both mCAR and hCAR). As shown in Figure 3A, hCAR and mCAR mRNAs were only detected in livers of hCAR-TG and WT mice, respectively. The prototypical CAR-target gene Cyp2b10 was induced by TCPOBOP and

![FIG. 1. Generation of hCAR-TG mice. (A) The BAC clone RP11-297K8, containing about 73-kbp human DNA upstream of CAR gene and 91-kbp downstream of CAR gene, was integrated into mouse genome to generate the hCAR-TG mice. Abbreviations of genes surrounding hCAR gene locus were shown. For detailed information about RP11-297K8, please refer to NCBI http://www.ncbi.nlm.nih.gov/clone/270914/. (B) PCR analysis of transgenic mice that contain hCAR but not mCAR using hCAR– and mCAR-specific primers. L, DNA ladder; P, positive plasmid control; WT, wild-type mice; −/−, CAR-null mice; 1 and 2 are two hCAR-TG/mCAR-null mice. (C) Real-time quantitative PCR analysis to identify homozygous hCAR-TG mice. The mouse gap junction channel protein alpha 5 (mGJA5; GenBank: NM_008121) was used as the endogenous reference. A, B, C, and D represent four hCAR-TG mice that are either homozygous (+/+) or heterozygous (+/−) of hCAR gene.]}
PB in WT mice, but not by CITCO. On the contrary, Cyp2b10 mRNA was increased by CITCO and PB in livers of hCAR-TG mice, but not by TCPOBOP. Cyp3a11 is a shared target gene by CAR and pregnane X receptor. Cyp3a11 mRNA was predominantly induced by TCPOBOP and PB in WT mice, and by CITCO and PB in livers of hCAR-TG mice. However, TCPOBOP also slightly increased Cyp3a11 mRNA in hCAR-TG mice, suggesting off-target induction of Cyp3a11 by this mCAR-specific ligand in mouse liver. Interestingly, the induction of CAR target genes by PB appears to be more potent in livers of hCAR-TG mice than that in WT mice. Moreover, TCPOBOP in WT mice and CITCO in hCAR-TG mice both increased nuclear accumulation of mCAR and hCAR proteins, respectively, as well as markedly induced Cyp2b10 protein (Fig. 3B). In summary, the above data demonstrate that the hCAR gene is functional in livers of the transgenic mice.

**Ontogenetic Expression of Major hCAR SVs, Cyp2b10, and Other Human Genes in Livers of hCAR-TG Mice**

The expression of hCAR SVs was determined in livers of male hCAR-TG mice at ages of 2 days before birth (~2 days), 5, 20, and 60 days. hCAR mRNA SV0, 1, 2, 3, and 4 were detectable in fetal livers of hCAR-TG mice. The expression of these hCAR SVs was two- to seven-fold higher in neonates (5-day-old hCAR-TG mice) livers than in fetal livers (Fig. 4A). Whereas the expression of SV1, SV2, SV3, and SV4 remained stable from 5 to 60 days of age, the WT SV0 further increased in adult mice (60 days old) compared with adolescents (20 days old).

Cyp2b10 is the prototypical target gene of CAR in mouse livers. The mRNA of Cyp2b10 was determined along with hCAR SV0 in male hCAR-TG mice at ages of 2 days before birth, 5, 10, 15, 20, and 60 days. As shown in Figure 4B (left panels), two major increases of hCAR SV0 were observed at 5 days (newborn) and 60 days (adult). In comparison, Cyp2b10 mRNA constantly increased along with liver development in the hCAR-TG mice. Specifically, hCAR SV0 was 2.9-fold and Cyp2b10 was 7.2-fold higher in livers of 5-day than those in ~2-day-old mice. At 60 days of age, hepatic hCAR SV0 and Cyp2b10 mRNA were both ~1.9-fold higher than those at 20 days of age.

In addition to hCAR, other human genes on the BAC construct, such as apolipoprotein A2 (APOA2) and protoporphyrinogen oxidase (PPOX), were also expressed in livers of the hCAR-TG mice (Fig. 4B, right panels). APOA2 encodes an abundant apolipoprotein, which stabilize the high-density lipoprotein particles. Human APOA2 (hAPOA2) mRNA gradually increased in hCAR-TG mice during liver development and maturation. In contrast, the mRNA of human PPOX (hPPOX), the enzyme that catalyzes the seventh step in heme production, was expressed in fetal livers and markedly reduced in neonates and progressively decreased to a minimal level in adults.

**Tissue Distribution of hCAR SVs in hCAR-TG Mice and Species-Specific Activation of CAR in Jejunum**

The expression of hCAR SVs in major organs in male hCAR-TG mice was shown in Figure 5A. The SVs of hCAR mRNA were predominantly expressed in liver. The expression of hCAR mRNA SVs in kidney and colon were ~2% of that in liver, even lower in small intestine, and almost undetectable in adipose tissues and muscle. In organs, where hCAR SVs were detected, SV0 and SV2 were more abundant than other SVs. In order to determine the function of hCAR in organs that express low levels of hCAR in the transgenic mice, mRNAs of hCAR, mCAR, and Cyp2b10 were determined in jejunums of WT and hCAR-TG mice that were treated with species-specific ligands of CAR or the common CAR activator, PB. As shown in Figure 5B, hCAR mRNA was expressed at a very low level in jejunums of the hCAR-TG mice, and neither CITCO nor PB induced the CAR-target gene, Cyp2b10. In comparison, WT mice expressed much more mCAR in jejunum. The mCAR-specific ligand, TCPOBOP, efficiently induced Cyp2b10 mRNA in jejunums of WT mice. Interestingly, PB was not able to increase Cyp2b10 expression in jejunums of WT mice. Moreover, the basal expression of the CAR-target gene Cyp2b10 was also higher in jejunums of WT mice than those in hCAR-TG mice, which is consistent with the higher expression of CAR in WT mice than in hCAR-TG mice. These data indicate that, in contrast to the liver, in jejunums of the hCAR-TG mice, hCAR is expressed at very low levels and the CAR-target genes are not inducible by hCAR activators.

**Gender Divergence of hCAR mRNA Expression and Transcriptional Function**

Male and female hCAR-TG mice expressed similar levels of each of the five SVs of hCAR mRNA in their livers (Fig. 6, upper panel). Female hCAR-TG mice expressed about onefold more Cyp2b10 mRNA than male hCAR-TG mice, although the difference was not statistically significant. The induction
of Cyp2b10 by TCPOBOP in livers of WT mice and its induction by CITCO in hCAR-TG mice were both more profound in females than in males (Fig. 6, lower panel). The hCAR-TG mice appear to retain the female-predominant pattern of CAR activation in liver.

**DISCUSSION**

CAR together with its closest mammalian relative, the pregnane X receptor, are nuclear receptors that regulate hepatic genes that are responsible for the biotransformation and distribution of drugs. Recently, numerous SVs of hCAR mRNA have been identified (Lamba et al., 2004) and some of them have been shown to have transcriptional activity by in vitro assays (DeKeyser et al., 2009; Dring et al., 2010; Omiecinski et al., 2011). To obtain insights into the in vivo functions and expression profiles of hCAR SVs, as well as to overcome the marked species differences between human and mouse, this study engineered a new line of hCAR transgenic mice utilizing the BAC-transgenic technology.

Alternative splicing of mRNA is a common phenomenon in mammals. The proper transcription and alternative splicing of genes require the presence of not only the coding region of the gene, but also the promoter, untranslated regions, and even distant regulatory elements (Luco et al., 2010, 2011). The first hCAR-transgenic mouse was engineered by integrating hCAR cDNA (SV0) into the mouse genome (Zhang et al., 2002). Later, another line of hCAR-transgenic mice was developed by knockin of the
coding region (exons 2–9) of hCAR gene into the mCAR gene allele, where hCAR replaced the endogenous mCAR gene and was driven by the endogenous mCAR promoter (Scheer et al., 2008). Obviously, neither of these lines of hCAR-transgenic mice contains the necessary human regulatory elements for the hCAR gene. In comparison, the hCAR-TG (hCAR+/+/mCAR−/−) mice described in this study contain the full-length hCAR gene, including the noncoding exon 1, as well as flanking fragments (73-kbp upstream and 91-kbp downstream) from the human genome (Fig. 1). Therefore, the hCAR-TG mice engineered by this study are more likely to recapitulate the in vivo transcriptional regulation of the CAR gene in humans.

The hepatic expression profiles of major SVs of hCAR in the hCAR-TG mice, namely SV0, SV1, SV2, and SV3, were comparable to those in humans, suggesting that the pre-mRNA processing of hCAR in hCAR-TG mice resembles that in humans (Fig. 2). SV4, a less-investigated hCAR SV, is expressed at a higher level in human livers than in hCAR-TG mice livers. This might be due to the small sample size we used (n = 5), considering the significant interindividual differences in hCAR expression observed in both humans and hCAR-TG mice. The hCAR knockin mice, in which the hCAR gene is regulated by the mCAR promoter, were also indicated to express hCAR SVs 2, 3, 4, and 9, although their expression levels were not specified (Scheer et al., 2008). Later work demonstrated that the hCAR-knockin mice expressed hCAR SV0 (CAR1), SV1 (CAR2), and SV2 (CAR3) at similar levels to those in human livers (Ross et al., 2010). The data from the hCAR knockin mice suggest that hCAR gene-coding region contains necessary elements for the pre-mRNA editing. However, to what extent these elements within the hCAR gene and those outside of the coding region contribute to hCAR alternative splicing is unknown. Comparing the expression profiles of a full spectrum of hCAR SVs in the hCAR-TG mice developed by this study and the hCAR knockin mice will shed light on this topic.

Species-specific activation of CAR by certain chemicals is a well-known phenomenon. In agreement with previous studies (Scheer et al., 2008), hCAR-TG mice expressed increased amounts of Cyp2b10 mRNA and protein in liver in response to the hCAR-specific ligand, CITCO, and the common CAR activator, PB, but not to the mCAR-specific ligand, TCPOBOP (Fig. 3). In WT mice, the opposite is the case; CAR is activated by TCPOBOP and PB, but not by CITCO. Therefore, the hCAR-TG mice appear to mimic the responses of humans to various CAR activators.

Differences in the potency of induction by CAR activators were observed between WT and hCAR-TG mice (Fig. 3A). The induction of Cyp2b10 and Cyp3a11 by CITCO (20 mg/kg/day for 3 continuous days) in livers of hCAR-TG mice was ~40% lower than that by TCPOBOP (3 mg/kg for a single dose) in WT mice. In contrast, PB (100 mg/kg/day for 3 continuous days) induced both Cyp2b10 and Cyp3a11 to higher levels in hCAR-TG mice than in WT mice. At dosages used in this study, TCPOBOP is a more effective activator for mCAR in WT mice, whereas PB, instead of CITCO, is the stronger activator for hCAR in hCAR-TG mice. Higher doses of CITCO could be tested in the hCAR-TG mice in future studies. However, 50 mg/kg CITCO was shown to nonspecifically induce Cyp2b10 in WT mice in a previous report (Scheer et al., 2008).

It has been reported that CAR mRNA was expressed at low levels in fetal livers but increased after birth in humans (Huang et al., 2003; Vyhlidal et al., 2006). Consistent with the ontogenetic expression pattern of CAR in human livers, major SVs of hCAR mRNA were expressed at low levels in livers of hCAR-TG fetus and markedly increased in postnatal livers of hCAR-TG mice (Fig. 4A). The WT hCAR mRNA SV0 but not the other hCAR SVs further increased when the hCAR-TG mice matured at 60 days of age. The hepatic expression of Cyp2b10 mRNA, the prototypical CAR target gene, constantly increased during the maturation of hCAR-TG mice, including the two time points when hCAR SV0 increased. These data suggest that
the expression of CAR-target genes during liver maturation could be affected not only by the amount of CAR, but also by factors that could potentially activate CAR, as well as by other transcription factors.

In addition to hCAR, other human genes on the BAC clone were also expressed in livers of the hCAR-TG mice (Fig. 4B). The hPPOX gene, encoding an enzyme involved in heme production, was expressed higher in livers of hCAR-TG mice before birth. The mRNA of the lipid metabolism-related gene, APOA2, in contrast, increased after birth. It is known that the hematopoietic cell transcriptional signature is dominant, whereas most metabolic pathways are underexpressed in fetal livers (Lee et al., 2012). The developmental expression profiles of hPPOX, hAPOA2, hCAR, and Cyp2b10 in the hCAR-TG mice reflected the transition of fetal liver from a hematopoietic organ to a metabolic one around birth.

In mice, CAR mRNA is detected in several tissues with the highest expression in liver and small intestine (Petrick and Klaassen, 2007). In comparison, CAR mRNA is expressed at a much higher level in liver than other tissues in humans (Baes et al., 1994). In the hCAR-TG mice, the major SVs of hCAR mRNA were highly expressed in liver, but only at low levels in small intestine and other tissues (Fig. 5). Thus, the tissue distribution of hCAR in the hCAR-TG mice resembles the human pattern instead of the mouse pattern. Moreover, the low expression of hCAR in jejunum was not sufficient to respond to the hCAR-specific agonist, CITCO, to induce Cyp2b10 expression in the hCAR-TG mice. In summary, the temporal and spatial

**FIG. 5.** Tissue distribution of hCAR SVs in adult male hCAR-TG mice and species-specific activation of CAR in jejunum. (A) Tissue distribution of various hCAR SVs. Total hCAR was the sum of all five SVs in each tissue. Values are presented as mean ± SEM; n = 4–5. (B) The expression of hCAR and mCAR as well as CAR-target gene, Cyp2b10, in jejunums of wild-type (WT) mice treated with TCPOBOP (TCP) and PB, as well as hCAR-TG (TG) mice treated with CITCO (CIT) and PB. Values were presented as mean ± SEM; n = 3–5. Student’s t-test (two sided) was performed. *Statistical differences between inducer-treated and corn oil–treated control groups within the same genotype, p < 0.05.
mRNA tended to be higher in females (Fig. 6). After CITCO treatment, Cyp2b10 was more profoundly induced in livers of female than in male hCAR-TG mice, which resembles the female-predominant induction of Cyp2b10 by TCPOBOP in WT mice. Therefore, the activation of CAR is female-predominant in the hCAR-TG mice.

In summary, this study established a novel hCAR-transgenic mouse model that resembles the hepatic expression profiles of major hCAR SVs in humans in aspects including expression level, ontogeny, tissue distribution, and ligand specificity. This model is extremely useful for in vivo research on the function and regulation of this important human xenobiotic sensor, especially, for studies on in vivo roles of hCAR SVs in metabolizing environmental toxicants and pharmaceutical drugs.

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