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Use Of Transgenic Mice In UDP-Glucuronosyltransferase (UGT) Studies

Zhimin Ou^{1,2}, Min Huang², Lizi Zhao², and Wen Xie^{1,3,*}

¹Center for Pharmacogenetics and Department of Pharmaceutical Sciences, University of Pittsburgh, Pittsburgh, PA 15261, USA

²Institute of Clinical Pharmacology, Sun Yat-Sen University Guangzhou 510080, China

³Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, PA 15261, USA

Abstract

Transgenic mouse models are useful to understand the function and regulation of drug metabolizing enzymes *in vivo*. This article is intended to describe the general strategies and to discuss specific examples on how to use transgenic, gene knockout, and humanized mice to study the function as well as genetic and pharmacological regulation of UDP-glucuronosyltransferases (UGTs). The physiological and pharmacological implications of transcription factor-mediated UGT regulation will also be discussed. The UGT-regulating transcription factors to be discussed in this article include nuclear hormone receptors (NRs), aryl hydrocarbon receptor (AhR), and nuclear factor erythroid 2-related factor 2 (Nrf2).

1. Strategies to create transgenic and gene knockout mice

Mouse models represent useful tools to study the regulation of UDP-glucuronosyltransferases (UGTs), as well as the physiological implications of this regulation *in vivo*. Nuclear receptors (NRs) have recently been shown to play an important role in the transcriptional regulation of UGTs. In this section, we will use NR mouse models as examples to describe the general strategies to create transgenic and gene knockout mice. More specifically, using the xenobiotic receptor pregnane $\times$ receptor (PXR) as an example, we will describe strategies on the creation of “loss-of-function” gene knockout mice, “gain-of-function” transgenic mice, and the “humanized” mice as summarized in Fig. 1 (Gong et al., 2005). Similar strategies have been used to create constitutive androstane receptor (CAR) transgenic, knockout, and humanized mice. CAR is a sister xenobiotic receptor of PXR that has also been shown to regulate the expression of drug metabolizing enzymes including UGTs.

1.1. Creation of PXR knockout mice

1.1.1. Construction of PXR targeting vector—Mouse PXR genomic DNA was isolated by screening a 129/Sv library (Stratagene, La Jolla, CA) using a PXR cDNA probe. A targeting vector was generated by replacing the second and third exons of PXR with a PGK-Neo selection marker, in conjunction with a negative selection marker (PGK-TK) (Fig. 2A). The resulting mutant allele has a deletion of two exons spanning nucleotides 339-660 that include amino acids 63-170 of the PXR DNA-binding domain (DBD).

*Correspondence author: Dr. Wen Xie, Center for Pharmacogenetics, 633 Salk Hall, University of Pittsburgh, Pittsburgh, PA 15261. Telephone: 412-648-9941; Fax: 412-648-1664; wex6@pitt.edu.

1.1.2. Embryonic stem (ES) cell transfection and selection—The targeting vector was linearized by Not I digestion and transfected into J1 ES cells by electroporation. G418 (200 µg/ml) and ganciclovir (0.2 mM) resistant ES clones were screened for designated homologous recombination by Southern blot analysis (data not shown).

1.1.3. Generation of chimeric mice, germ line transmission, and confirmation of loss of PXR expression—The PXR heterozygous (PXR^{+/-}) ES cells were microinjected into C57BL6/J blastocysts, which were subsequently transplanted into the uteri of pseudopregnant ICR mice. Chimeric male progeny were crossed with C57BL6/J females. Germline transmission of the mutant allele was detected in agouti progeny by Southern blot analysis (Fig. 2B). The absence of PXR mRNA expression in the liver and small intestine of PXR knockout mice was confirmed by Northern blot analysis (Fig. 2C) (Xie et al., 2000).

1.2. Creation of PXR transgenic mice

1.2.1. Construction of Albumin (Alb)-hPXR transgene—To generate transgenic mice expressing the wild-type human PXR (hPXR) in the liver, the hPXR cDNA was cloned downstream of the liver-specific mouse Alb promoter/enhancer (Pinkert et al., 1987). A SV40 intron/poly (A) sequence was placed downstream of hPXR cDNA (Fig. 3A).

1.2.2. Generation of transgenic mice and confirmation of transgene expression—Transgene was excised and purified from the plasmid vector and microinjected into the pronuclei of fertilized mouse eggs. The injected eggs were then implanted into the oviduct of pseudopregnant female mice. Transgene positive mice were screened by PCR on tail DNA (Xie et al., 2000). The transgene expression at the mRNA level was confirmed by Northern blot analysis (Fig. 3B).

1.3. Creation of hPXR “humanized” mice

1.3.1. Generation of hPXR humanized mice—As diagramed in Fig. 1, the Alb-hPXR transgene was bred into the PXR knockout background, and the resulting PXR-knockout/hPXR-transgenic mice are predicted to lack mPXR but have hPXR transgene expressed in the liver.

1.3.2. Confirmation of the humanized drug response profile in “humanized” mice—The drug induction of *CYP3A11*, a PXR target gene, was compared between the wild-type and humanized mice by Northern blot analysis (Fig. 4). In humanized mice, *CYP3A11* was no longer induced by PCN but was efficiently induced by the human-specific PXR inducer rifampicin (RIF). In contrast, no induction occurred in wild-type mice by RIF. Expressing hPXR and responding to human-specific PXR inducer exclusively, the “humanized” mice represent a major step toward generating humanized toxicological models to predict xenobiotic enzyme inducibility and drug-drug interactions.

2. Use of NR transgenic mice to study UGT regulation

2.1. Regulation of UGTs by PXR and CAR

2.1.1. Background of UGT regulation by PXR and CAR—Nuclear receptors PXR and CAR function as xenobiotic receptors that regulate the expression of genes encoding the Phase I and Phase II drug metabolizing enzymes and transporters (Baes et al., 1994; Bertilsson et al., 1998; Blumberg and Evans, 1998; Blumberg et al., 1998; Choi et al., 1997; Kliewer et al., 1998; Savas et al., 1999; Sonoda et al., 2003; Xie and Evans, 2001; Xie et al., 2004; Xie et al., 2003). This regulation is achieved through the binding of PXR and CAR to their response elements found within the target gene promoters. Emerging evidence has demonstrated that PXR and CAR are tissue-specific transcription factors that play an important role in the

regulation of UGTs, and the NR-mediated UGT regulation may have contributed to the tissue-specific expression pattern of UGTs. The regulations of UGTs by NRs can be receptor- and UGT isoform-specific. The NR transgenic mouse models have been proven to be useful tools and widely used in studying the regulation of UGTs by NRs *in vivo*.

2.1.2. Use of PXR and CAR transgenic mice to study UGT regulation

PXR transgenic mice: Using transgenic mice expressing the activated hPXR (VP-hPXR) in the liver, Xie and colleagues showed that UGT1A1 was under the positive control of PXR. VP-hPXR was created by fusing the VP16 activation domain from the herpes simplex virus to the N-terminal of hPXR. VP-hPXR has the intact DNA-binding domain (DBD) and transactivate PXR target gene expression in a constitutively manner. UGT1A1 mRNA and protein expression was up-regulated in VP-hPXR transgenic mice (Xie et al., 2003). The microsomal glucuronidation activity towards β -estradiol (a UGT1A1 substrate), thyroid hormones, corticosterone, and xenobiotics such as 4-nitrophenol and 4-OH-PhIP, was also increased in VP-hPXR transgenic mice. Consistent with the up-regulation of UGT1A1 by hPXR and the physiological function of this enzyme, VP-hPXR transgenic mice showed an increased bilirubin clearance in a hyperbilirubinemia model induced by bilirubin fusion (Xie et al., 2003). The UGT1A6 was also suggested to be positively regulated by PXR (Blumberg et al., 1998), which was supported by the activation of UGT1A6 gene expression in VP-hPXR transgenic mice (Xie et al., 2003).

CAR transgenic mice: The *in vivo* regulation of UGT1A1 by CAR was reported in the VP-CAR transgenic mice in which the activated form of mCAR (VP-CAR) was targetedly expressed in the liver using the “Tet-Off” tetracycline inducible transgenic system as outlined in Fig. 5A (Saini et al., 2005). In this transgenic system, first, the TetRE-VP-CAR transgene that encodes VP-CAR under the control of a minimal cytomegalovirus promoter and the TetRE were created. The TetRE-VP-CAR mice were then bred with the Lap-tTA activator line to generate bitransgenic mice. Driven by the liver-specific Lap (CCAAT/enhancer-binding protein- β) promoter, the Lap-tTA transgene directs the expression of the tetracycline-responsive transcriptional activator (tTA) exclusively in the hepatocytes (Kistner et al., 1996). It was predicted that tTA binds to TetRE and consequently induces the expression of VP-CAR only in the absence of Dox. Addition of Dox (supplied in drinking at a concentration of 2 mg/ml) will result in the displacement of tTA from TetRE and silencing of VP-CAR expression (Tet-Off). Northern blot analysis demonstrating the expected transgene expression and Dox regulation is shown in Fig. 5B. In addition to gene regulation, VP-CAR transgenic mice were also used to evaluate the effect of CAR activation on bilirubin clearance. VP-CAR transgenic mice showed resistance to hyperbilirubinemia induced by phenylhydrazine. The resistance was VP-CAR dependent, because no protection was observed in mice pre-treated with Dox to silence the expression of VP-CAR (Saini et al., 2005).

PXR knockout mice: Consistent with the notion that PXR plays a positive role in UGT regulation, Chen and colleagues showed that PCN, a rodent-specific PXR agonist, induced UGT expression in wild-type mice and this induction was abolished in PXR knockout mice (C. Chen et al., 2003). In this study, wild-type and PXR knockout mice received control or PCN-supplemented diet for 21 days before liver harvesting and determination of UGT expression at enzymatic and mRNA levels. In wild-type mice, PCN treatment significantly increased UGT activities toward bilirubin, 1-naphthol, chloramphenicol, thyroxine, and triiodothyronine. However, UGT activities in PXR knockout mice were not increased by PCN. In agreement with the changes in enzymatic activities, the mRNA expression of mouse UGT1A1 and UGT1A9, which are involved in the glucuronidation of bilirubin and phenolic compounds, was increased about 100% in wild-type mice after PCN treatment, whereas this

induction was abolished in PXR knockout mice. The expression of UGT1A2, 1A6, and 2B5 was not affected by PCN regardless of PXR genotype (C. Chen et al., 2003).

Interestingly, when the effect of individual or combined loss of PXR and CAR was examined using PXR and CAR single or double knockout mice, it was found that the loss of PXR and/or CAR did not significantly alter the basal level of serum bilirubin. Upon bilirubin infusion, while the CAR knockout and the double knockout mice showed similar sensitivity as the wild-type mice, the PXR knockout mice exhibited a surprisingly complete resistance to hyperbilirubinemia (Saini et al., 2005). The increased bilirubin clearance in PXR knockout mice was also confirmed in another independent hyperbilirubinemia model induced by phenylhydrazine-mediated hemolysis (Saini et al., 2005). The increased bilirubin resistance in PXR knockout mice was associated with the induction of bilirubin detoxifying genes, such as UGT1A1, OATP4/SLC21A6, GSTA2, and MRP2. The expression of these genes remained largely unchanged in CAR knockout or double knockout mice, consistent with the sensitivity to hyperbilirubinemia found in both genotypes. Based on these results, it was proposed that PXR has both negative and positive roles in bilirubin clearance and that this dual role of PXR is achieved, at least in part, by cross-talking with CAR (Saini et al., 2005).

CAR knockout mice: Huang and colleagues first reported that bilirubin clearance in mice was markedly increased by pretreatment with the CAR agonist phenobarbital (PB) or 1,4-bis[2-(3,5 dichloropyridyloxy)] benzene (TCPOBOP), resulting in 3-5 fold lower level of residual total serum bilirubin. However, the CAR agonist effect was abolished in CAR knockout mice. At the gene regulation level, the PB and TCPOBOP responsive induction of UGT1A1 seen in wild-type mice was abolished in CAR knockout mice (Huang et al., 2004).

Humanized PXR mice: To generate humanized PXR mice, the Alb-hPXR transgene was bred into a PXR knockout background, and the resulting PXR knockout/hPXR-transgenic mice lacked mPXR but had hPXR transgene expressed in the liver. As expected, treatment of PXR humanized mice with the prescription antibiotic RIF, a potent hPXR-specific activator, elevated UGT1As mRNA expression within 24 hrs of a single oral dose (Xie et al., 2003).

Humanized CAR mice: Humanized CAR mice were created by breeding the hCAR transgenic mice with the CAR knockout mice to produce a humanized line expressing only hCAR in the liver. Treatment of CAR-humanized mice with PB increased clearance of bilirubin. At the gene expression level, PB treatment in CAR humanized mice resulted in the induction of UGT1A1, MRP2, SLC21A6, and GSTA1 (Huang et al., 2003).

2.2. UGT regulation by peroxisome proliferator-activated receptors (PPARs)

PPARs have also been implicated in UGT regulation. PPARs, including PPAR α , PPAR δ/β , and PPAR γ , belong to the orphan nuclear receptor superfamily. Upon ligand activation, PPARs can regulate target gene transcription through heterodimerization with RXR and binding of PPAR-RXR heterodimers to PPAR response elements (PPREs), which usually consist of a direct repeat of hexanucleotide AGGTCA separated by one or two nucleotides (DR1 or DR2).

Among PPARs, PPAR α is highly expressed in liver, muscle, kidney, and heart where it stimulates fatty acid β -oxidation (Kota et al., 2005). Incubation of primary human hepatocytes, HepG2, or Huh7 cells with the PPAR α agonists fenofibric acid or Wy14643 resulted in an increase of UGT2B4 mRNA level and induced glucuronidation of hyodeoxycholic acid, a specific bile acid substrate for UGT2B4. Further study revealed that PPAR α activation could induce UGT2B4 promoter activity through a DR1 type PPRE (nt -1199 to nt -1175) in the promoter region of UGT2B4. EMSA and mutagenesis studies confirmed that this UGT2B4/

DR1 is both necessary and sufficient to confer the transactivation of UGT2B4 by PPAR α (Barbier et al., 2003).

PPAR α knockout mice were used to investigate the PPAR α -dependent induction of UGT2B expression *in vivo*. In wild-type mice, fenofibrate treatment led to a significant increase of UGT2B mRNA levels compared to the vehicle-treated mice. In contrast, UGT2B transcripts were undetectable in either fenofibrate or vehicle treated PPAR α knockout mice. These results suggest that PPAR α is a key regulator of basal and fibrate-activated murine UGT2B gene expression (Barbier et al., 2003).

3. Use of aryl hydrocarbon receptor (AhR) knockout mice to study UGT regulation

AhR is a member of the basic helix-loop-helix Per-Arnt-Sim (PAS) domain transcription factor family. PAS proteins have been described as biological sensors and at least part of the AhR function can be viewed as adaptive response to cellular environment and as a sensor for endo- and xenobiotic signals (Kewley et al., 2004). Activation of AhR has been reported to simultaneously induce both Phase I and Phase II enzymes (Ma, 2001; Rushmore and Pickett, 1990).

UGTs are among the Phase II enzymes regulated by AhR. *In vivo* studies showed that AhR agonists 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and β -naphthoflavone (BNF) were able to induce the mRNA and protein expression of UGT1A1 as well as the 17 β -ethynyl estradiol glucuronidation. UGT1A1 promoter driven reporter activity was induced by TCDD and BNF treatment in HepG2 cells in an AhR-dependent manner. A xenobiotic response element (XRE) (CACGCA) was found in the human UGT1A1 gene promoter region and proved to be responsible for the induction of UGT1A1 by AhR agonists in HepG2 cells. Deletion or mutation of this XRE abolished the promoter response to TCDD and BNF. The specific binding of AhR to the XRE on UGT1A1 promoter was confirmed by electrophoretic mobility shift assay (EMSA) and supershift assay using antibodies against AhR and its dimerization partner Arnt (Yueh et al., 2003). In addition to UGT1A1, UGT1A6 was also subjected to the regulation by AhR. TCDD was shown to induce the expression of human UGT1A6 in Caco-2 cells (Munzel et al., 1998). In rodents, both UGT1A1 and UGT1A6 promoter regions were mapped and "TGCGTG" type of XREs were identified, which have been shown to be necessary and sufficient to mediate the transactivations of both UGT genes by AhR (Emi et al., 1996; Shelby et al., 2003).

The *in vivo* evidence to support the regulation of UGT1A6 and 1A1 by AhR was obtained by using AhR knockout mice. Nishimura and colleagues treated pregnant AhR heterozygous (AhR^{+/-}) and AhR knockout (AhR^{-/-}) mice with a single oral dose of 10 μ g/kg TCDD at gestation day 12.5. The pups born from TCDD-treated mice were sacrificed and analyzed 21 days after birth. It was reported in this study that the expression of UGT1A6 in the liver was induced markedly by TCDD in AhR^{+/-} but not in AhR^{-/-} mice. UGT1A6 is known to glucuronidate thyroid hormones. The results of Nishimura and colleagues suggest that the induction of UGT1A6 may be at least partly responsible for the reduced serum thyroid hormone level in TCDD-treated mice (Nishimura et al., 2005). In an independent study, exposure of mice to 2,4,6-triphenyl-1-hexene (ST-1), an isomer of STs (styrene trimers), influenced thyroxine (T₄) level in the same manner as TCDD. ST-1 blocked the formation of AhR-XRE complex in the nucleus, and decreased the mRNA expression and activity of UGT1A1 and UGT1A6 in wild-type mice. The inhibitory effect of ST-1 is believed to be mediated by the suppression of the AhR, because the inhibition was abolished in AhR^{-/-} mice (Yanagiba et al., 2008).

4. Use of nuclear factor erythroid 2-related factor 2 (Nrf2) knockout mice to study UGT regulation

Nrf2 belongs to a family of basic leucine zipper transcription factors. Nrf2 binds to an AP1-NF-E2 tandem repeat sequence regulating globin gene expression in hematopoietic cells (Moi et al., 1994). The actin-associated kelch-domain protein, Keap1, mediates Nrf2 transactivation in response to oxidative stress signals (Itoh et al., 1999). Under basal conditions, Nrf2 is sequestered in the cytoplasm by Keap1, which negatively regulates Nrf2 by facilitating its proteasomal degradation (McMahon et al., 2003). Upon oxidative or xenobiotic stimulation, the dissociation of Nrf2 from Keap1 allows Nrf2 to migrate to the nucleus where it functions as a transcriptional activator of its target genes (Dinkova-Kostova et al., 2002). *In vitro* studies indicated that Nrf-2 was another important regulator of UGTs. Both UGT1A1 mRNA level and enzyme activity in HepG2 cells were induced by the Nrf2 activators sulforaphane, *tert*-butylhydroquinone (tBHQ), BNF, and H₂O₂, whereas overexpression of Keap-1 or infection with siNrf2-lentivirus abolished the induction. An Nrf-2 binding site ARE has been found in the UGT1A1 gene promoter. The regulation of UGT1A1 by Nrf2 was further suggested with the use of UGT1A transgenic (Tg-*UGT1*) mice. Tg-*UGT1* mice were created by transgene the bacterial artificial chromosome (BAC) clone containing the human UGT1A gene locus (see Section 5 for details). Consistent with the *in vitro* results, treatment of Tg-*UGT1* mice with tBHQ induced the UGT1A1 mRNA level and enzyme activity. Interestingly, the tBHQ-responsive induction of UGT1A1 was only significant in the liver and intestine, suggesting the tissue specificity of tBHQ-activated UGT1A1 gene expression (Yueh and Tukey, 2007).

Nrf2 knockout mice were also used to study UGT regulation. Nrf-2 knockout mice, first reported by Chan and colleagues in 1996, were created by replacing a 4.2-kb segment of DNA containing part of exon 4 and all of exon 5 of Nrf2 with a 5.5-kb fragment of DNA coding for the bacterial gene LacZ followed by the positive selection neomycin resistance cassette (Chan et al., 1996). Microarray analysis revealed that the basal level of UGT expression in wild-type mice were significant higher than the Nrf2 knockout mice, and Northern blot analysis provided further evidence that sulforaphane could induce UGT1A6 mRNA expression in wild-type mice, but not in Nrf2 knockout mice (Thimmulappa et al., 2002). Another research group also found that the AhR-Nrf2 pathway played a major role in UGT regulation (Yeager et al., 2009). It was reported in this study that the AhR agonist TCDD activated Nrf2 in livers of wild-type mice by increasing the translocation of Nrf2 to the nucleus and subsequent binding of Nrf2 to AREs. The same study reported that Nrf2 knockout mice had lower basal mRNA expression of NQO1, UGT1A6, UGT1A9, UGT2B34, UGT2B35, and UGT2B36 than wild-type mice. TCDD treatment in wild-type mice could significantly induce the expression of UGT1A5, UGT1A6, UGT1A9, UGT2B34, UGT2B35, and UGT2B36, but this induction was markedly reduced or abolished in Nrf2 knockout mice (Buckley and Klaassen, 2009).

5. Use of UGT transgenic mice to study UGT function and regulation

The *UGT1A* genes in humans have been shown to be differentially regulated in a tissue-specific manner. Moreover, the glucuronidation patterns between rodent and human were proved to be different. In rodents, maternal liver glucuronidation activity is lower during pregnancy (Inoue et al., 2005; Luquita et al., 2001), whereas selective glucuronidation activities during pregnancy are induced in humans, as evidenced by increased oral clearance of paracetamol (Miners et al., 1986), a phenolic compound that can be glucuronidated by UGT1A1 and UGT1A6 (Court and Greenblatt, 2000; Krishnaswamy et al., 2005), and lamotrigine, which undergoes *N*-glucuronidation (Rommel and Sinz, 1991) by UGT1A4 (Green and Tephly, 1998).

In order to study the homeostasis controlled by the human *UGT1* gene locus in adults as well as during fetal development and lactation, Chen and colleagues developed a UGT transgenic

model (Tg-*UGT1*) that expresses a bacterial artificial chromosome (BAC) encoding the entire human *UGT1* locus. The Tg-*UGT1* mice express each of the nine *UGT1A* genes in selective tissues and the expression was regulated by the nuclear receptor PXR. The Tg-*UGT1* mice have provided not only a useful tool to examine the regulatory properties that control the tissue-specific and xenobiotic receptor-elicited expression pattern of individual *UGT1A* genes, but also a better opportunity to understand how the *UGT1* locus is regulated at times where changes are apparent in the physiological levels of circulating hormones (S. Chen et al., 2005).

The Tg-*UGT1* mice were used to study for the regulation of UGT1A by liver X receptor (LXR), PPAR, AhR and Nrf2. Tg-*UGT1* mice treated with T0901317, a LXR agonist, for 6 days showed increased plasma levels of CDCA and LCA acyl glucuronides, indicating a higher glucuronide conjugation catalyzed by UGT1A3. Indeed, treatment of Tg-*UGT1* mice with T0901317 resulted in a significant increase in UGT1A3 mRNA levels in Tg-*UGT1* mouse liver, small intestine, large intestine, and kidney (Verreault et al., 2006). However, because T0901317 is also known to activate PXR (Mitro et al., 2007), it remains to be determined whether the T0901317 effect on UGT expression was mediated by the activation of PXR by the same drug. Treatment of Tg-*UGT1* mice with WY-14643, PPAR α activating fibrate, induced the expression of UGT1A1, UGT1A3, UGT1A4, and UGT1A6 in the gastrointestinal tract and liver, suggesting that the *UGT1* locus is subjected to PPAR α regulation *in vivo* (Senekeo-Effenberger et al., 2007).

As discussed earlier, *UGT1A* gene transcripts have been suggested to be regulated by AhR. Bonzo and colleagues reported that chrysin, an AhR activator whose activation mode is distinct from the classical AhR agonist TCDD, was able to induce UGT1A1 gene expression through a XRE *in vitro*. However, when the inductive effect of chrysin was evaluated in Tg-*UGT1* mice, it was found that oral administration of chrysin could not induce UGT1 expression as a result of rapid metabolism of chrysin by glucuronidation, which prevented adequate plasma chrysin level needed for the induction of the UGT1 locus (Bonzo et al., 2007). The use of Tg-*UGT1* mice in studying the regulation of UGT1A1 by Nrf2 has been described in Section 4.

6. Conclusions and perspectives

The creation and utilization of transgenic mouse models offer a unique and valuable tool to dissect the complexity UGT regulation. Indeed, the use of gene knockout and transgenic mice has helped to establish the regulation of UGTs by NRs and other transcription factors. The gene knockout mice and transgenic mice represent loss-of-function and gain-of-function models, respectively. The creation and use of transgenic mice humanized for NRs or UGTs represent a major step forward in generating humanized pharmacological and toxicological models. It is hoped that results from using the humanized mice will have a favorable human relevance.

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Fig. 1. Strategies to create the loss-of-function knockout, gain-of-function transgenic, and the combined “humanized” function models. Adopted from Gong *et al.* (2005), with the permission of the publisher.

Fig. 2. Creation of PXR knockout mice. (A) Restriction map of the PXR gene and strategy to generate PXR mutant allele. The PXR probes used for Southern blot and expected fragment sizes after *EcoR* I digestion are indicated. E, exon; Neo, neomycin resistance gene; TK, thymidine kinase promoter. (B) Southern blot of *EcoR* I-digested genomic DNA. WT, wild type; MT, Mutant. (C) Loss of PXR mRNA expression in the liver and small intestine of PXR knockout (PXR^{-/-}) mice. Adapted from Xie *et al.*, 2000, with the permission of the publisher.

Fig. 3. Creation of PXR transgenic mice. (A) Schematic representation of the albumin (Alb)-hPXR transgene construct. (B) Northern blot analysis of mouse and human PXR gene expression in the liver of wild type (-) and transgenic (+) mice. Adapted from Xie *et al.*, 2000, with the permission of the publisher.

Fig. 4.

Drug response profile in the humanized mice. Mice with indicated genotypes were treated with a single dose of RIF (5 mg/kg) or PCN (40 mg/kg) 24 hrs prior to liver harvesting. Liver RNA was isolated and subjected to Northern blot analysis with indicated probes. Adopted from Xie *et al.*, 2000, with the permission of the publisher.

Fig. 5. Creation of transgenic mice that harbor conditional expression of the activated CAR (VP-CAR) in the liver. (A) Outline of the TRE-VP-CAR/Lap-tTA Tet-Off transgenic system. (B) Conditional expression of VP-CAR as revealed by Northern blot analysis. Adopted from Saini et al., 2005, with the permission of the publisher.