Published in final edited form as:

Curr Drug Metab. 2011 December ; 12(10): 997–1006.

Humanized Transgenic Mouse Models for Drug Metabolism and Pharmacokinetic Research

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Abstract

Extrapolation of the metabolic, pharmacokinetic and toxicological data obtained from animals to humans is not always straightforward, given the remarkable species difference in drug metabolism that is due in large part to the differences in drug-metabolizing enzymes between animals and humans. Furthermore, genetic variations in drug-metabolizing enzymes may significantly alter pharmacokinetics, drug efficacy and safety. Thus, humanized transgenic mouse lines, in which the human drug-metabolizing enzymes are expressed in mouse tissues in the presence or absence of mouse orthologues, have been developed to address such challenges. These humanized transgenic mice are valuable animal models in understanding the significance of specific human drug-metabolizing enzymes in drug clearance and pharmacokinetics, as well as in predicting potential drug-drug interactions and chemical toxicity in humans. This review, therefore, aims to summarize the development and application of some humanized transgenic mouse models expressing human drug-metabolizing enzymes. The limitations of these genetically modified mouse models are also discussed.

Keywords

Humanized; transgenic; mouse model; drug metabolism; pharmacokinetics; drug interaction; toxicity; cancer

INTRODUCTION

Xenobiotic drugs entering the body may be subjected to a variety of oxidative and conjugative reactions that are catalyzed by Phase 1 [e.g., cytochrome P450 (P450 or CYP)] and/or Phase 2 [e.g., uridine 5'-diphospho-glucuronosyltransferase (UGT) and arylamine N-acetyltransferase (NAT)] enzymes in liver, small intestine or other tissues. Generally, these drug-metabolizing enzymes convert medications, as well as other foreign chemicals such as dietary compounds, environmentally derived toxicants and carcinogens, into inactive metabolites (e.g., 4-hydroxy-diclofenac from diclofenac). In some cases, biotransformation

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produces active metabolites (e.g., morphine from codeine) or toxic and reactive metabolites (e.g., *N*-acetyl-*p*-benzoquinone imine from acetaminophen) [1–3]. Most importantly, drug-metabolizing capacity may determine the rate of drug clearance that ultimately defines the exposure to drug. Therefore, understanding biotransformation and the enzymes involved in the process is critical for the assessment of pharmacokinetic properties, drug efficacy and toxicity during drug discovery and development.

Both *in vitro* and *in vivo* models are utilized for preclinical investigation of drug metabolism. Studies using recombinant enzymes, human hepatic subcellular fractions including microsomes, cytosols, and S9 fractions, freshly prepared or cryopreserved human hepatocytes and human liver slices [4] may provide mechanistic understanding of drug metabolism process. Recent efforts have been directed to quantitative extrapolation of *in vitro* data into *in vivo* pharmacokinetic properties although there are many uncertainties including scaling factors, models and drug-dependent effects [5–7]. On the other hand, animals are extremely useful models to understand biotransformation in a whole body system and to identify the significance of metabolic elimination in pharmacokinetics and drug toxicity. While the commonly-used laboratory animals share many similarities in anatomy, physiology and biochemistry with humans, there are marked species differences in the capacity to process a drug [8–11]. For example, CYP2D6 is the only functional CYP2D enzyme expressed in humans, whereas at least six Cyp2d (Cyp2d9, 2d 10, 2d11, 2d12, 2d13, 2d22) occur in mice. Rather, mouse liver microsomes exhibited no debrisoquine 4-hydroxylation activity [12] that is an index reaction for human CYP2D6, and murine Cyp2d22 showed a substrate specificity more similar to human CYP3A4 rather than CYP2D6 [13, 14]. Therefore, species difference in drug metabolism represents a major confounding factor in proper interpretation of animal data during drug discovery.

The development of humanized transgenic mouse models offers an unprecedented opportunity to study human enzyme-mediated processes at the systemic level [3, 15–19]. Because many species difference in drug metabolism is caused by the difference in expression and/or function of drug-metabolizing enzymes, the expression of human enzymes in humanized transgenic mice opens the door to study the impact of one specific enzyme in susceptibility to disease and risk of toxicity, as well as pharmacokinetics and dynamics. This review aims to introduce the development of drug-metabolizing enzyme humanized transgenic mouse models, and their applications to drug metabolism research. Some limitations of these mouse models are also discussed, e.g., the expression of related mouse drug-metabolizing enzymes is changed considerably.

HUMANIZED TRANSGENIC MOUSE MODELS IN DRUG METABOLISM RESEARCH

A common approach to produce humanized transgenic mouse models (Table 1) involves microinjection of a human transgene into fertilized zygotes and implantation of the eggs into pseudo-pregnant females. The founders that carry human transgene can be used as breeders to establish homozygous transgenic mouse line [20]. One may use the complete gene sequence, in a bacterial artificial chromosome (BAC) construct [21–27], which consists of all exons, introns and 5' and 3'-regulatory elements that can be critical for genetic and epigenetic regulation of the transgene under physiological or pharmacological conditions.

Another method is to employ human cDNA as transgene that is often driven by a heterologous promoter (e.g., mouse serum albumin promoter, ApoE promoter, villin promoter, and rat probasin promoter) [28–32] controlling tissue- specific expression of transgene. The resulting humanized transgenic mice in a wild-type mouse background (e.g., FVB/N and C57BL/6N) still express murine drug-metabolizing enzymes, whose activities may share some similarity as human enzyme. Thus, the transgenic mice may be bred with knockout mice, in which the mouse orthologue has been disrupted, to create a truly humanized mouse line [22, 23, 25, 29, 31, 33] (Table 1). The humanized mouse models have been widely employed to assess the regulation and function of transgene at the systemic level.

CYP1A1/CYP1A2-Humanized Mice

CYP1A2 accounts for about 10% of the total amount of hepatic P450s in human liver tissues, and CYP1A1 is mainly expressed in the lung, placenta, and lymphocytes [34–37]. Xenobiotic agents metabolized by CYP1A include medications (e.g., caffeine, theophylline and acetaminophen) and environmental and dietary arylamines [e.g., 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)]. The oxidation of poly cyclic aromatic hydrocarbon and heterocyclic aromatic amines/amides produces electrophilic reactive metabolites that may bind to DNA and protein and lead to carcinogenesis or toxicity. Though *CYP1A* genes are highly conserved among different species [10], human and rodent CYP1A2 enzymes are quite different in the metabolism of some drugs [38]. Thus, a number of CYP1A1/1A2-humanized transgenic mouse lines were developed using BAC constructs carrying human *CYP1A1* and *CYP1A2* genes and bred into *Cyp1a1*^{-/-}, *Cyp1a2*^{-/-}, and *Cyp1a1/1a2*^{-/-} knockout mouse lines, respectively (Table 1). The resulting *CYP1A1/1A2*-humanized mouse models have been employed to delineate the role of CYP1A1/1A2 in drug metabolism and assess the risk of xenobiotic drugs (Table 2) [22, 23, 39–46].

The *CYP1A1/1A2*-humanized transgenic mice lacking mouse *Cyp1a2* (*CYP1A1/1A2_Cyp1a2*^{-/-}) were used to evaluate theophylline pharmacokinetics [39]. After a single i.p dose of 8 mg/kg of theophylline, *CYP1A1/1A2_Cyp1a2*^{-/-} mice and wild-type mice showed a 1.7- and 3.8-fold higher systemic clearance than *Cyp1a2*^{-/-} mice, respectively. The replacement of mouse *Cyp1a2* with human *CYP1A2* not only restored the ability to metabolize theophylline, but also revealed a humanized metabolic profile. For example, the human-specific metabolite, 3-methylxanthine, was found only in *CYP1A1/1A2_Cyp1a2*^{-/-} mice. In addition, pretreatment of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a CYP1A1/1A2 inducer, markedly elevated the expression of human CYP1A2 and consequently enhanced the systemic clearance of theophylline in *CYP1A1/1A2_Cyp1a2*^{-/-} mice.

Another study utilized the *CYP1A1/1A2_Cyp1a1*^{-/-} and *CYP1A1/1A2_Cyp1a2*^{-/-} mice as well as *Cyp1a1*^{-/-}, *Cyp1a2*^{-/-} and wild-type mice to investigate the metabolism of PhIP, a food derived procarcinogen [23]. Compared to wild-type mice, both *CYP1A1/1A2_Cyp1a1*^{-/-} and *CYP1A1/1A2_Cyp1a2*^{-/-} mice produced more N²-hydroxylated metabolite and less 4'-hydroxy lated metabolite. The results were consistent with a previous finding that N²-hydroxylation is the predominant metabolic pathway for

PhIP in humans. When *CYP1A1/1A2_Cyp1a1^(-/-)/1a2^(-/-)* and wild-type mice were treated with 200 mg/kg of PhIP orally combined with dextran sodium sulfate for 7 days to induce colon carcinogenesis, tumors were only found in *CYP1A1/1A2_Cyp1a1^(-/-)/1a2^(-/-)* mice [41]. These results indicate that *CYP1A1/1A2*-humanized mouse models are very useful for studying drug metabolism and risk of carcinogenesis.

CYP2A6-humanized Transgenic Mice

CYP2A6 is mainly expressed in human liver. Although it is involved in the biotransformation of a limited number of medications, CYP2A6 metabolizes many toxicants and carcinogens [47, 48]. CYP2A6 is also known as the primary enzyme responsible for nicotine metabolism. To evaluate the role of CYP2A6 in drug metabolism in a whole body system, a CYP2A6-humanized transgenic mouse model was developed using the CYP2A6 cDNA driven by a mouse liver-specific transthyretin promoter/enhancer [28] (Table 1). Expression of CYP2A6 in transgenic mouse liver was confirmed by Western blots, which led to a significant higher coumarin 7- hydroxylation activity in transgenic mouse liver microsomes than wild-type controls. Further pharmacokinetic studies revealed that CYP2A6-transgenic mice had a lower exposure to coumarin and a higher exposure to the 7- hydroxylated metabolite, as compared to wild-type mice. The CYP2A6-transgenic mice, especially when they are bred into the *Cyp2aJ*-null mouse background [49], should be valuable models for studying the *in vivo* function of CYP2A6 in drug metabolism and chemical toxicity. In addition, a more ideal model in which CYP2A is expressed in extrahepatic tissues such as lung and olfactory mucosa would help to determine the regulation and function of this CYP2A in metabolism or carcinogenesis [50].

CYP2C18/2C19-Humanized Transgenic Mice

The human CYP2C subfamily consists of four CYPs, CYP2C8, CYP2C9, CYP2C18 and CYP2C19, which contribute to the metabolism of many drugs. CYP2C19 is known for its genetic variations in different populations and its ability to metabolize a number of antidepressants and proton pump inhibitors [51, 52]. To examine the regulation and function of CYP2C18/CYP2C19 *in vivo*, a CYP2C18/CYP2C19-humanized transgenic mouse model was developed using a BAC clone containing the whole *CYP2C18/CYP2C19* genes [26] (Table 1). The expression of CYP2C19 protein in CYP2C18/CYP2C19-transgenic mouse livers led to a higher *R*-omeprazole and *S*-mephenytoin hydroxylase activities than those in wild-type mice. Interestingly, CYP2C18 and CYP2C19 mRNA expression exhibited sexual dimorphism in transgenic mouse liver and kidney [26], and both were subjected to hormonal regulation [53]. These findings indicate that the *CYP2C18/CYP2C19-trans-*genic mouse line may be of value for assessing *CYP2C* gene regulation *in vivo* and the impact of CYP2C19 in drug metabolism and pharmacokinetics.

CYP2D6-humanized Transgenic Mice

CYP2D6 is one of the most important P450 enzymes in humans. CYP2D6 contributes to the metabolism of over 20% of medications and many CYP2D6 substrate drugs act on the central nervous system or cardiovascular system [54–56]. CYP2D6 is also known for its genetic polymorphism in humans that may cause large variability in drug metabolism, pharmacokinetics and drug response during clinical pharmacotherapy. There is notable

species difference in CYP2D6-mediated drug metabolism between humans and rodents. Debrisoquine is 4-hydroxylated in humans and Sprague-Dawley rats, whereas a decreased debrisoquine 4-hydroxylation activity is found in Dark Agouti rats and no debrisoquine 4-hydroxylation activity is shown in liver microsomes prepared from three strains of mice or recombinant mouse Cyp2d enzymes [12–14]. To investigate CYP2D6 function at the physiological condition, a CYP2D6-humanized transgenic mouse model was developed using λ *X* phage genomic clone containing the whole *CYP2D6* gene [21] (Table 1). Human CYP2D6 is expressed in transgenic mouse liver, small intestine and kidney, a similar distribution pattern as that in humans [21, 57].

The *CYP2D6*-transgenic mice were first employed to study debrisoquine pharmacokinetics [21]. Expression of functional CYP2D6 significantly enhanced debrisoquine metabolic elimination in mice, as manifested by a 6.2-fold higher systemic clearance of debrisoquine in CYP2D6-transgenic mice (94.1 L/h/kg) than wild-type mice (15.2 L/h/kg). Consistently, the systemic exposure (AUC) to 4-hydroxy-debrisoquine was 8.6-fold higher in *CYP2D6*-transgenic mice than that in wild type mice. In addition, the urinary metabolic ratios (debrisoquine/4-hydroxy-debrisoquine) were revealed to be around 12.5 in wild-type mice and 0.5 in *CYP2D6*-transgenic mice, which are close to the values noted in human CYP2D6 poor metabolizers and extensive metabolizers, respectively. These findings suggest that *CYP2D6*-transgenic mice could be a model for studying CYP2D6 pharmacogenetics.

The *CYP2D6*-transgenic mouse model was successfully used to delineate the impact of CYP2D6 status in indolealkylamine metabolism, pharmacokinetics and dynamics, as well as the involvement of CYP2D6 in a serotonin-melatonin cycle (Fig. 1) [58–64]. Indolealkylamines are a major class of natural psychoactive drugs that have seen widespread abuse while some may be potentially used as psychopharmacotherapeutic agents [65]. Overdosing or concomitant use of serotonergic indolealkylamines can cause severe or even fatal “serotonin toxicity”. 5-Methoxy-*N,N*-dimethyltryptamine (5-MeO-DMT) and 3,4-dihydro-7-methoxy-1-methyl-beta-carboline (harmaline) represent two indolealkylamine drugs that are commonly used together. 5-MeO-DMT is known as a nonselective 5-HT receptor agonist, and harmaline is a selective MAO-A inhibitor (MAOI) [66]. CYP2D6-mediated *O*-demethylation is revealed as an important pathway for harmaline metabolism, and MAO-A-catalyzed deamination is the predominant route for 5-MeO-DMT metabolism. Studies in *CYP2D6*-transgenic versus wild-type mice showed that CYP2D6 genotype/phenotype had significant impact on harmaline pharmacokinetics and pharmacodynamics [62]. Co-administration of harmaline led to an increased and prolonged exposure to 5-MeO-DMT [64], as well as a potentiation of 5-MeO-DMT-induced hyperthermia and toxicity (unpublished data). The results obtained from *CYP2D6*-transgenic mouse models suggest that CYP2D6 genetic polymorphism may cause considerable variability in indolealkylamine metabolism, pharmacokinetics and dynamics.

CYP2E1 -humanized Transgenic Mice

CYP2E1 is mainly expressed in liver tissues, accounting for about 7% of total hepatic P450s in humans. CYP2E1 metabolizes many toxicologically important low-molecular-weight chemicals (e.g., ethanol, acetone, chloromethane, and benzene), as well as medications [e.g.,

acetaminophen (APAP)] and physiologically important fatty acid metabolites [67–70]. CYP2E1 is readily induced by ethanol and plays an important role in the pathogenesis of alcohol-induced liver injury and other associated symptoms like oxidative stress, mitochondrial damage and glutathione depletion. Like other P450s, species difference in CYP2E1 regulation, catalytic activity and toxicological consequence have been documented between mice and humans [11, 70].

To evaluate the role of CYP2E1 at the physiological condition, a *CYP2E1*-transgenic mouse model was developed using a CYP2E1 cDNA driven by a mouse albumin enhance/promoter [71] (Table 1). Chronic treatment with ethanol led to a significantly higher hepatic CYP2E1 protein expression and enzymatic activity in the *CYP2E1*-transgenic mice than wild-type control mice. A notably higher serum alanine aminotransferase levels indicated that *CYP2E1*-transgenic mice had a more severe liver damage, which was also supported by histologic examination. Further study on hepatic gene expression [72] revealed the association of an increased expression of stress related genes, cytokeratin 8 and 18 genes, with the progression of alcoholic liver damage. Interestingly, overexpression of CYP2E1 in the *CYP2E1*-transgenic mouse liver also led to a reduction of hepatic insulin signaling and glycogen storage, as well as an increase of glucose synthesis, suggesting an association of hepatic CYP2E1 with insulin signaling pathway [73]. In addition, this *CYP2E1*-transgenic mouse model was used along with the *Cyp2e1*-null and wild-type mice to investigate the preventive role of CYP2E1 in mallory body formation, a complex phenomenon formed in human chronic alcohol liver disease [74].

Another *CYP2E1*-transgenic mouse model was developed using a BAC clone consisting of the whole *CYP2E1* gene, and then bred with *Cyp2e1*-null mice [25] (Table 1). The resultant *CYP2E1*-humanized (*CYP2E1*_Cyp2e1^(-/-)) mouse line expresses functional human CYP2E1 while lacking mouse Cyp2e1 background. Several CYP2E1 substrates were tested in humanized *CYP2E1*_Cyp2e1^(-/-) versus wild-type *Cyp2e1*^(+/+) mice [25]. No significant difference was found in chlorzoxazone 6-hydroxylation but higher level of O- hydroxylated metabolite of *p*-nitrophenol was observed in humanized *CYP2E1*_Cyp2e1^(-/-) mice than wild-type *Cyp2e1*^(+/+) mice. Another interesting finding obtained from this humanized mouse model is that there are some difference in APAP-induced hepato- toxicity associated with human CYP2E1 and mouse Cyp2e1 [25]. After treated with the same dose of APAP (400 mg/kg, p.o.), *Cyp2e1*-null mice had no centrilobular hepatic necrosis lesions, wild-type mice showed mild to moderate degrees of centrilobular hepatic necrosis lesions and *CYP2E1*-humanized mice exhibited moderate to severe centrilobular hepatic necrosis lesions. This *CYP2E1*_Cyp2e1^(-/-) mouse line was also employed to study alcohol-induced liver damage [75]. Following chronic treatment with alcohol, the *Cyp2e1*-null mice had a decreased liver injury and oxidative stress whereas all the liver-injury symptoms were restored in the *CYP2E1*-humanized mice. These studies have clearly demonstrated that *CYP2E1*-humanized transgenic mice are unique animal models for in assessing the risk of xenobiotic drugs in humans.

CFP3A4-humanized Transgenic Mice

CYP3A4 is the most abundant P450 enzyme expressed in human liver and small intestine, and it is responsible for the metabolism of over 50% of xenobiotic drugs (e.g., benzodiazepines, antivirals and steroids) [16, 76]. There are also other CYP3A isozymes in humans; CYP3A5 is expressed in specific populations while CYP3A7 is mainly present in fetal liver and CYP3A43 is expressed in prostate. In rodents, six mouse *Cyp3a* genes (*Cyp3a11*, *3a13*, *3a16*, *3a25*, *3a41* and *3a44*) and six rat *CYP3A* genes (*CYP3A1*, *3A2*, *3A9*, *3A18*, *3A23* and *3A62*) are found. Although mouse and male rat liver microsomes exhibit similar testosterone 6 β -hydroxylase activity as human liver microsomes, intestinal midazolam and nisoldipine oxidation rates are much lower in rats than in humans and the impact of ketoconazole on midazolam pharmacokinetics is much greater in humans than in rodents [11, 77].

To study CYP3A4 function at the systemic level, a *CYP3A4* humanized transgenic mouse model was developed using a BAC clone consisting of the whole *CYP3A4* gene [24] (Table 1). CYP3A4 was initially found to be expressed only in small intestine of adult male *CYP3A4*-transgenic mice. Therefore, midazolam pharmacokinetic profiles after intravenous administration were comparable between wild-type and *CYP3A4*-transgenic mice. In contrast, midazolam pharmacokinetics was significantly different between *CYP3A4*-transgenic and wild-type mice after oral administration. A 3.4-fold lower C_{max} , 1.6-fold lower AUC and 2.1-fold lower bioavailability in *CYP3A4*-transgenic mice demonstrated the importance of intestinal CYP3A4 in the first-pass metabolism of midazolam. In addition, pretreatment with ketoconazole resulted in a significant increase of the exposure to midazolam, a much higher degree in *CYP3A4*-transgenic mice (7.7-fold) than wild-type mice (3.4-fold) [24]. This mouse model is valuable in studying first-pass metabolism in gut mucosa and *CYP3A4*-mediated drug-drug interactions, while no or low expression of hepatic CYP3A4 may limit its other applications.

Another *CYP3A4*-transgenic mouse model was generated using the CYP3A4 cDNA driven by an ApoE promoter-HCR1 cassette [30] (Table 1). CYP3A4 was selectively expressed in transgenic mouse livers, which reduced the systemic exposure to intravenously-administered midazolam and cyclosporin A. Consistently, formation of metabolite 1'-hydroxy-midazolam was 2-fold higher in *CYP3A4*-transgenic mice than wild-type mice.

After successful disruption of murine *Cyp3a* gene cluster, a *Cyp3a*-null (*Cyp3a*^(-/-)) mouse model was developed and bred with the ApoE-*CYP3A4*-transgenic mice to generate a liver *CYP3A4*-humanized mouse line (*CYP3A4*_*Cyp3a*^(-/-) A) [29] (Table 1). In addition, a villin-*CYP3A4*-transgenic mouse model was developed with selective expression of CYP3A4 in small intestine, and bred with *Cyp3a*-null mice to produce an intestine *CYP3A4*-humanized mouse line (*CYP3A4*_*Cyp3a*^(-/-) V) [29]. Comparative studies in the *Cyp3a*-null, wild-type, liver and intestine *CYP3A4*-humanized mice demonstrated that *Cyp3a*/CYP3A4 largely determined docetaxel pharmacokinetics and toxicity. In addition, it was revealed that intestinal CYP3A4 rather than hepatic CYP3A4 controlled the systemic exposure to orally administered triazolam, as well as the drug-drug interaction between ketoconazole and triazolam [78]. These results indicate that *CYP3A4*-humanized mice are

useful animal models to investigate the role of CYP3A4 in first-pass metabolism, as well as systemic clearance of drugs.

Interestingly, CYP3A4 expression was subjected to developmental and hormonal regulation in the *CYP3A4*-transgenic mice developed with BAC clones [79]. CYP3A4 protein was indeed expressed at high levels in the livers of male mice before puberty but disappeared completely after puberty. CYP3A4 protein was constitutively expressed in female mouse livers, albeit a lower level after puberty. This finding demonstrates the influence of age and sex on hepatic CYP3A4 expression in a whole animal body system (Fig. 2), which was further confirmed by studies with *CYP3A4/3A7*-transgenic [80] and *CYP3A4/2D6-transgenic* mice [81]. Comparatively, sexual dimorphism was not found for hepatic CYP2D6 expression in the *CYP3A4/2D6*-transgenic mice [81]. Moreover, continuous infusion of recombinant growth hormone [74] or treatment with pregnenolone 16 α -carbonitrile (PCN) or 1,4-bis-2-(3, 5- dichloropyridyloxy)-benzene (TCPOBOP) [81] induced hepatic CYP3A4 expression in adult male *CYP3A4*-transgenic mice, indicating the presence of nuclear receptor-controlled regulatory mechanisms in transgenic mouse models. While the exact mechanisms under developmental expression of hepatic CYP3A4 are still unclear, emerging studies on epigenetic and posttranslational regulatory factors [82–86] likely open a new door to understand CYP3A4 regulation for which these transgenic mouse models may serve as useful tools.

NAT2-humanized Transgenic Mice

Two NAT isoforms, NAT1 and NAT2, are found in humans and recognized for their functions in acetylation of therapeutic drugs and environmental aromatic or heterocyclic amines such as 4- aminobiphenyl [87, 88]. Ten *NAT1*- or NAT2-transgenic mouse lines were developed using NAT1 and NAT2 cDNA driven by multiple promoters [89, 90]. The NAT1-transgenic mice were unable to reach homozygosity [89] or only had a lower level of NAT1 expression in kidney [90], and the CMV promoted *NAT2*-transgenic mice just showed a minor increase in hepatic isoniazid *N*- acetyltransferase activity [89]. Another NAT2-transgenic mouse model developed with a prostate-specific rat probasin promoter showed a prostate-specific expression of NAT2 [32], and the *N*- acetyltransferase activity of sulfamethazine was 15-fold higher in prostate cytosol from *NAT2*-transgenic mice than that from control mice. Rather, the *N*-acetylation or *O*-acetylation of other compounds such as 2-aminofluorene, *N*-hydroxy-2-aminofluorene, *N*- hydroxy-PhIP, and *N*-hydroxy-2-acetylaminofluorene, as well as the formation of PhIP-DNA adduct in the prostate were comparable between transgenic or control mice. Recently a new *NAT2*- transgenic mouse model was developed using NAT2 cDNA driven by mouse liver-specific albumin enhance/ promoter, and bred to *Nat1/2-null* mice to generate a NAT2-humanized transgenic mouse line [31]. The *NAT2*⁽⁺⁾*Nat1/2*^(-/-) mice showed high NAT2 expression in humanized mouse livers, exhibited 40- to 80-fold higher *in vitro* NAT2 activity, and had 35-fold lower exposure to sulfamethazine, compared to the wild-type C57BL/6 mice. These mouse models could be valuable in determining the risk of arylamine chemicals.

UGT1A-humanized Transgenic Mice

UGT drug-metabolizing enzymes consist of UGT1 and UGT2 isozymes that mediate the glucuronidation of many xenobiotic drugs and endogenous compounds in livers as well as extrahepatic tissues [91]. Among nine functional UGT1A isozymes, UGT1A1 is of specific interest due to its genetic polymorphism and its clinical importance in the metabolism of bilirubin and some medications/ metabolites (e.g., SN-38). Therefore, a *UGT1A*-humanized transgenic mouse line was developed using a BAC clone consisting of the entire UGT1 locus to study the function and regulation of UGT1A *in vivo* [27] (Table 1). Studies on the *UGT1A*-transgenic mice revealed a tissue-specific, xenobiotics-inducible and hormonal controlled UGT1A expression.

The *UGT1A*-transgenic mice were further crossed with *UGT1*- null mice [92] to produce *UGT1A*-humanized mouse lines (*UGT1A*_JgtI^(-/-)). Specifically, UGT1A locus containing wild-type *UGT1A1**I allele gave a UGT1^{A1*1}-humanized mouse line (*JGT1A1**1_UgtI^(-/-)), and UGT1A locus containing the Gilbert's *JGT1A1**28 allele produced a UGT1^{A1*28}-humanized mouse line (*UGT1A1**28_UgtI^(-/-)). Comparative studies with *UGT1A1**I_UgtI^(-/-) and *UGT1A1**28_UgtI^(-/-) mice confirmed that the *UGT1A1**28 allele contributes to hyperbilirubinemia. In addition, the *UGT1A1**I and *UGT1A1**28-humanized mice were employed to evaluate the importance of UGT1A1-mediated glucuronidation in drug clearance [93]. Compared to wild-type mice, the *JGT1A1**28-humanized mice showed about 3-fold higher exposure to SN-38 (7-ethyl-10-hydroxyl-camptothecin) the active metabolite of the chemotherapeutic drug irinotecan, an analog of camptothecin following intravenous administration. These studies demonstrate that *UGT1A*-humanized mouse models are valuable in studying the physiological role of UGT1A, as well as the impact of UGT1A1 on drug metabolism and pharmacokinetics.

UGT2B7-humanized Transgenic Mice

UGT2B7 is an important enzyme in UGT2 subfamily and it is involved in the metabolism of many therapeutic agents such as nonsteroidal anti-inflammatory drugs, anticarcinogens and analgesics [94]. UGT2B7 is the principal enzyme catalyzing glucuronidation of morphine to the inactive metabolite morphine-3-glucuronide and the active metabolite morphine-6-glucuronide [95]. UGT2B7 also plays an important role in controlling the homeostasis of fatty acids and steroid hormones [96]. To examine the regulation and function of UGT2B7 *in vivo*, a UGT2B7-transgenic mouse line was developed using a BAC clone consisting of the entire *UGT2B7* gene [97]. Expression of human UGT2B7 in transgenic mouse liver significantly enhanced the glucuronidation of hyodeoxycholic acid. In addition, UGT2B7 mRNA was also detected in extrahepatic tissues including brain, intestine, kidney, ovary and uterus, exhibiting a similar expression profile as that in humans. Treatment with TCPOBOP, a constitutive androstane receptor (CAR) ligand, reduced UGT2B7 expression in the *UGT2B7*-transgenic mouse liver. The role of CAR in regulation of UGT2B7 is also supported by studies with *UGT2B7*⁽⁺⁾_Car^(-/-) mice, in which the treatment with TCPOBOP had no effects on *UGT2B7* expression [97]. These studies demonstrate the utilization of *UGT2B7*-humanized transgenic mice in understanding the regulation of UGT2B7, as well as its function in the metabolism of therapeutic drugs and endogenous substrates.

LIMITATIONS OF HUMANIZED TRANSGENIC MOUSE MODELS

The humanized transgenic mouse models discussed above exemplify their values in mechanistic understanding of drug metabolism, pharmacokinetics, dynamics and drug toxicity. However, these models also have some limitations. One is the potential influence of mouse drug-metabolizing enzymes when they exhibit overlapped substrate specificity with human isozyme. For instance, following the administration of desipramine, a well-known CYP2D6 substrate, urinary metabolic ratios of desipramine were not different in *CYP2D6-transgenic* and wild-type mice despite desipramine metabolic profile in CYP2D6-transgenic mouse liver microsomes was closer to that in human CYP2D6 extensive metabolizer liver microsomes [98]. Further studies revealed that mouse CYP enzymes had considerable contribution to desipramine 2-hydroxylation (unpublished data), which is predominantly mediated by CYP2D6 in humans. Another example is that similar inhibitory effects of BAS 100, a mechanism-based CYP3A4 inhibitor isolated from grapefruit juice, on erlotinib pharmacokinetics were observed in CYP3A4-transgenic and wild-type mice [99]. While CYP3A4 plays a major role in the metabolism of erlotinib in humans, it is not clear how much the murine P450 enzymes contribute to erlotinib clearance and if erlotinib is metabolized differently in mouse models. Therefore, one needs to consider the potentially significant influence of mouse drug-metabolizing enzymes or elimination pathways on drug clearance when using these transgenic mouse models.

Another confounding factor is that the expression of endogenous murine genes related to drug metabolism might be altered after the introduction of a human gene or disruption of a target mouse gene in the humanized transgenic mice. For example, expression of murine Cyp3a and Cyp2b enzymes was different in the CYP3A4-transgenic mouse liver tissues, resulting in a distinct developmental gene expression pattern (Fig. 2) [79]. It was also reported that the *Cyp3a-null* mice had a remarkably increased expression of Cyp2c enzymes [29, 100, 101]. Although the functional consequences of altered gene expression have not been studied extensively, it is necessary to be cautious when interpreting data obtained from those humanized transgenic mouse models because those mouse P450 enzymes are also able to metabolize CYP3A4 substrate drugs.

Depending on the construct of transgene and mouse genetic background, genetic and epigenetic regulation of transgene might be different in different humanized transgenic mice. Transcriptional regulation is solely controlled by mouse nuclear receptors unless a mouse model also expresses human xenobiotic receptor [16, 19]. Regulation of transgene at the 3'-untranslated region (3'UTR) [102, 103] cannot be studied completely in a mouse model developed with a transgene construct lacking the 3'UTR sequence. It is also noteworthy that humanized mice are still animal models with distinct physiological conditions. Furthermore, it is hard to define what level of transgene expression in mouse models is relevant to that in humans. Therefore, caution is advised for any intention of quantitative extrapolation of humanized mouse data to humans.

CONCLUSION

Drug-metabolizing enzymes determine metabolic elimination of xenobiotic drugs from the body. Studying the function and regulation of drug-metabolizing enzymes is essential for the assessment of drug efficacy and safety in drug discovery and development. These humanized transgenic mouse models expressing human drug- metabolizing enzymes with or without mouse orthologues are helpful tools for mechanistic understanding of the significance of a specific enzyme in drug metabolism, drug interactions, pharmacokinetics, dynamics and chemical toxicity at the systemic level. Nevertheless, one should be aware of the physiological difference between mouse models and humans, as well as the limitations of humanized transgenic mice, and care should be taken when interpreting data obtained from these humanized transgenic mouse models.

ACKNOWLEDGEMENT

The authors would like to thank financial support (award number R01DA021172) from the National Institute On Drug Abuse, National Institutes of Health (NIH).

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Fig. (1).

Involvement of CYP2D6 in a serotonin-melatonin cycle. Arylalkylamine *N*-acetyl transferase (AANAT) catalyzes the formation of *N*-acetylserotonin, which is processed by hydroxyindole *O*-methyltransferase (HIOMT) to melatonin. HIOMT and CYP2D6 mediate the reversible reactions between serotonin (5-HT) and 5-methoxytryptamine. AANAT and arylacylamidase (AAA) catalyze the transformations between 5-methoxytryptamine and melatonin. Note that serotonin and 5-methoxytryptamine are mainly inactivated through monoamine oxidase-A (MAO-A)-mediated deamination metabolism to produce 5-hydroxyindoleacetic acid (5-HIAA) and 5-methoxyindoleacetic acid (5-MIAA), respectively.

Fig. (2). Expression of human CYP3A4 transgene and murine Cyp3a and Cyp2b in mouse livers exhibits sexual dimorphism and/or developmental pattern. Male or female, 2–16 week (W) mouse liver microsomes were subject to immunoblot analyses with selective antibodies [79]. HLM, human liver microsomes

Table 1.

Transgenic Mouse Models that Express Human Drug-metabolizing Enzymes

Mouse Model	Transgene	Generation	Expression of Transgene	Reference
CYP1A1/1A2_ <i>Cyp1a1</i> ^(-/-) and <i>CYP1A1/1A2_ Cyp1a2</i> ^(-/-) transgenic mice	BAC consisting of complete human <i>CYP1A1</i> and <i>CYP1A2</i> genes	Founders were generated by zygotic pronuclear microinjection of BAC. Transgenic mice were crossed into <i>Cyp1a1</i> ^(-/-) or <i>Cyp1a2</i> ^(-/-) mouse lines to produce humanized mouse lines.	Mouse liver	[22]
<i>CYP1A1/1A2_ Cyp1a1</i> ^(-/-) and <i>CYP1A1/1A2_ Cyp1a2</i> ^(-/-) transgenic mice	BAC consisting of complete human <i>CYP1A1</i> and <i>CYP1A2</i> genes	Transgenic founders were produced by microinjection of BAC into fertilized mouse eggs. Offsprings were crossed into <i>Cyp1a1</i> ^(-/-) or <i>Cyp1a2</i> ^(-/-) mouse lines.	Mouse liver, kidney, spleen, lung, heart, colon, testis, ovary	[23]
<i>CYP1A1/1A2_ Cyp1a1</i> ^{(-/-)/1a2} ^(-/-) humanized mice	BAC consisting of complete <i>CYP1A1</i> and <i>CYP1A2</i> genes	Generated by breeding <i>CYP1A1/1A2</i> transgenic founders with <i>Cyp1a1/1a2</i> ^(-/-) mice	Mouse liver	[40]
<i>CYP2A6</i> -transgenic mice	<i>CYP2A6</i> cDNA driven by TTR promoter and enhancer	<i>CYP2A6</i> transgene was electroporated into ES cells. Transgenic founders were bred with B6 mice and hemizygotes were backcrossed into B6 mice.	Mouse liver	[28]
<i>CYP2C18/2C19</i> -transgenic mice	BAC consisting of <i>CYP2C18</i> and <i>CYP2C19</i> genes	Generated by microinjection of BAC into C57BL/6 eggs and then backcrossing.	mRNA expression in mouse liver and small intestine	[26]
<i>CYP2D6</i> -transgenic mice	BAC consisting of complete human <i>CYP2D6</i> gene	Generated by microinjection of BAC into fertilized FVB/N eggs and then backcrossing	Mouse liver, small intestine, kidney	[21]
<i>CYP2E1</i> -transgenic mice	<i>CYP2E1</i> cDNA driven by mouse albumin promoter; mouse MP1 intron was used to enhance transgene expression	Transgene was microinjected into fertilized B6D2F2 eggs and transgenic founders were mated with Balb/c mice	Mouse liver	[71]
<i>CYP2E1_ Cyp2e1</i> ^(-/-) humanized mice	BAC consisting of complete human <i>CYP2E1</i> gene	Transgenic founders were produced by microinjection of BAC into FVB/N eggs and then mated with <i>Cyp2e1</i> ^(-/-) mice	mRNA expressed in mouse liver, kidney, small intestine, colon, heart, lung, spleen, brain	[25]
<i>CYP3A4</i> -transgenic mice	BAC consisting of complete human <i>CYP3A4</i> gene	Generated by microinjection of BAC into fertilized FVB/N eggs	Mouse small intestine; in livers before puberty	[24, 79]
<i>CYP3A4</i> -transgenic mice	<i>CYP3A4</i> cDNA driven by ApoE promoter-HCR1	Generated by microinjection of transgene into pronuclei of FVB/N mice	Mouse liver and kidney	[30]
<i>CYP3A4_ Cyp3a</i> ^(-/-) humanized mice	<i>CYP3A4</i> cDNA driven by ApoE promoter-HCR1	Generated by mating <i>Cyp3a</i> ^(-/-) mice with <i>CYP3A4</i> -humanized mice constructed with <i>CYP3A4</i> cDNA driven by ApoE promoter	Mouse liver, kidney	[29]

Mouse Model	Transgene	Generation	Expression of Transgene	Reference
<i>CYP3A4</i> _ <i>Cyp3a</i> ^(-/-) humanized mice	<i>CYP3A4</i> cDNA driven by villin promoter- SV40	Generated by mating <i>Cyp3a</i> ^(-/-) mice with <i>CYP3A4</i> -humanized mice constructed with <i>CYP3A4</i> cDNA driven by villin promoter.	Mouse small intestine, colon, kidney	[29]
<i>CYP3A4/2D6</i> -transgenic mice	BAC consisting of <i>CYP2D6</i> and <i>CYP3A4</i>	Generated by mating <i>CYP3A4</i> -transgenic mice with <i>CYP2D6</i> -transgenic mice developed with <i>CYP3A4</i> and <i>CYP2D6</i> BAC, respectively	Mouse liver, small intestine	[81]
<i>CYP3A7</i> -transgenic mice	<i>CYP3A7</i> cDNA driven by mouse MT-1 promoter	Microinjection of transgene construct into C57BL/6N DBA/2 oocytes and then back-crossed into C57BL/6N mouse line	Mouse liver and testes	[104]
<i>CYP3A4/3A7</i> -transgenic mice	BAC consisting of <i>CYP3A4</i> and <i>CYP3A7</i>	Microinjection of BAC into FVB/N eggs and crossing into C57BL/6 mouse line	<i>CYP3A4</i> in mouse liver and small intestine <i>CYP3A7</i> in fetal liver	[80]
<i>NAT2</i> -transgenic mice	<i>NAT2</i> *4 cDNA driven by rat probasin promoter	Microinjection of transgene construct into pro-nuclei of FVB mice	Mouse prostate	[32]
<i>NAT2</i> _ <i>Nat1/2</i> ^(-/-) humanized mice	<i>NAT2</i> *4 cDNA driven by mouse albumin promoter	Generated by mating <i>Nat1/2</i> ^(-/-) mice with <i>NAT2</i> -transgenic mice constructed with <i>NAT2</i> cDNA driven by mouse albumin promoter	Mouse liver and colon	[31]
<i>UGT1A</i> -transgenic mice	BAC consisting of entire human <i>UGT1</i> locus	Generated by microinjection of BAC into Balb/c and C57BL/6N eggs and backcrossing into C57BL/6N mouse line	Mouse liver, small intestine,	[27]
<i>UGT1A1</i> *1_ <i>Ugt1a</i> ^(-/-) and <i>UGT1A1</i> *28_ <i>Ugt1a</i> ^(-/-) humanized mice	BAC consisting of <i>UGT1A1</i> *1 or <i>UGT1A1</i> *28 allele	Generated by mating <i>Ugt1a</i> ^(-/-) mice with <i>UGT1A1</i> *1 or <i>UGT1A1</i> *28 transgenic founders created with BAC	Mouse liver, small intestine	[33]
<i>UGT2B7</i> -transgenic mice	BAC consisting of the whole <i>UGT2B7</i> gene	Generated by microinjection of BAC into CB6F1 mouse eggs	mRNA expressed in mouse liver, kidney, intestine, stomach, heart, lung, spleen, brain, adipose, muscle, ovary/testis	[97]

Table 2.

Applications of Transgenic Mouse Models Expressing Human Drug-Metabolizing Enzymes.

Mouse Model	Application	References
<i>CYP1A1/1A2</i> -humanized mice	1.Metabolism and pharmacokinetics of medications, e.g., theophylline and melatonin	[39, 42]
	2.Metabolism and toxicity of procarcinogens, e.g., PhIP	[23, 41, 44]
<i>CYP2A6</i> -transgenic mice	Function of CYP2A6 in drug metabolism, e.g., coumarin	[28]
<i>CYP2C18/2C19</i> -transgenic mice	Regulation and function	[26, 53]
<i>CYP2D6</i> -transgenic mice	Metabolism, pharmacokinetics and dynamics of xenobiotic drugs, e.g., debrisoquine, desipramine, 5-MeO-DMT, harmaline and pinoline	[21, 59, 62, 64]
<i>CYP2E1</i> -transgenic or humanized mice	Role of CYP2E1 in acetaminophen or alcohol-induced hepatotoxicity and related symptoms in chronic liver disease	[25, 71–75]
<i>CYP3A4</i> -transgenic or humanized mice	1.Metabolism and pharmacokinetics of xenobiotic drugs, e.g., midazolam, docetaxel, cyclosporine A, lopinavir, estradiol and triazolam 2.Liver- or intestinal-specific first-pass metabolism, e.g., midazolam, docetaxel and triazolam 3.Drug-drug interaction, e.g., midazolam and ketoconazole, erlotinib and BAS100, triazolam and tyrosine kinase inhibitors	[24, 29, 30, 78, 99]
	4.Age and gender-dependent gene regulation	[79, 80]
<i>CYP3A7</i> -transgenic mice	Expression and induction of CYP3A7	[104, 105]
<i>NAT2</i> -transgenic or humanized mice	Metabolism, pharmacokinetics, and toxicity of procarcinogens, e.g., sulfamethazine, <i>p</i> -aminosalicylic acid, 4-aminobiphenyl, 2-aminofluorene, and PhIP.	[31, 32]
<i>UGT1</i> -transgenic mice	Gene regulation	[27]
<i>UGT1A1</i> *1- or <i>UGT1A1</i> *28-humanized mice	Pharmacokinetics of UGT substrate drugs, e.g., SN-38	[93]
<i>UGT2B7</i> -transgenic mice	Xenobiotic receptor-dependent regulation of UGT2B7	[97]