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Review

A CYP2B6-humanized mouse model and its potential applications☆

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ABSTRACT

CYP2B6 is a human microsomal cytochrome P450 enzyme with broad substrate selectivity. *CYP2B6* is the only functional member of the human *CYP2B* gene subfamily, which differs from the situation in rodents, such as mouse, where multiple functional *Cyp2b* genes are expressed. Recent studies with *Cyp2b* knockout or knockdown mouse models have yielded insights into the in vivo roles of mouse CYP2B enzymes in drug disposition and xenobiotic toxicity. A CYP2B6-humanized mouse model (CYP2A13/2B6/2F1-transgenic/*Cyp2abfgs*-null), which expresses human CYP2B6 in the liver, and human CYP2A13 and CYP2F1 in the respiratory tract, but not any of the mouse *Cyp2b* genes, has also been established. In the CYP2B6-humanized mouse, the CYP2B6 transgene is expressed primarily in the liver, where it was found to be active toward prototype CYP2B6 substrate drugs. The regulatory elements of the *CYP2B6* transgene appear to be compatible with mouse nuclear receptors that mediate *CYP2B* induction. Therefore, the CYP2B6-humanized mouse is a valuable animal model for studying the impact of CYP2B6 expression or induction on drug metabolism, drug efficacy, drug-drug interaction, and drug/xenobiotic toxicity. In this mini-review, we provide a brief background on CYP2B6 and the *Cyp2b*-knockout and CYP2B6-humanized mice, and discuss the potential applications and limitations of the current models.

1. Introduction

CYP2B6, a human cytochrome P450 enzyme with broad substrate specificity, is considered to play a key role in the metabolism of many clinical drugs, environmental toxins, and endogenous compounds [1]. The highly inducible and polymorphic nature of the *CYP2B6* gene, in addition to the broad substrate specificity of the enzyme, has triggered significant academic and industrial interests in its pharmacogenetics, structure-function relationships, enzyme inhibition, and mechanisms of gene regulation, as well as its role in xenobiotic metabolism and toxicity [2–4]. Although the substrate selectivity of CYP2B6 overlaps with some other hepatic drug metabolism CYP enzymes (e.g., CYP3A4 and CYP2C), it shows unique preference for many drugs or other xenobiotic compounds. The clinical drugs that are believed to be metabolized preferentially by CYP2B6 include central nervous system acting drugs (propofol, bupropion, methadone, and mephenytoin), anti-tumor agents (cyclophosphamide and ifosfamide), antiretroviral drugs (nevirapine and efavirenz), and many others (recently reviewed in Ref. [5]. CYP2B6 also contributes significantly to toxic metabolites formation and procarcinogen activation. For instance, CYP2B6 is believed to be responsible for 90% of efavirenz transformation via 8-hydroxylation to its neurotoxic metabolites, leading to short- and long-term adverse effects on the nervous system [6]. CYP2B6 also appears to play a predominant role in bioactivating the widely used organophosphorus insecticides chlorpyrifos and parathion to their more toxic oxon metabolites [7], which raises health concerns due to the prevalent human exposure to these pesticides [8].

The human *CYP2B* gene subfamily consists of only one functional gene, *CYP2B6*, and a pseudogene, *CYP2B7P*, both located in a large *CYP2ABFGST* gene cluster on chromosome 19 [9,10]. In contrast, rodents have multiple functional *CYP2B* genes. For example, the orthologs of human *CYP2B6* in the mouse include *Cyp2b9*, *2b10*, *2b13*, *2b19*, and *2b23*, which are located in the *Cyp2abfgs* gene cluster on mouse chromosome 7, a region syntenic to the human *CYP2ABFGST* gene cluster [9,11]. CYP2B6, which is

Abbreviations: WT, wild-type; CYPs, cytochromes P450; CAR, constitutive androstane receptor; PXR, pregnane X receptor; GR, glucocorticoid receptor; PB, phenobarbital; and DEX, dexamethasone.

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predominantly expressed in the liver, shows a large interindividual variability (up to ~300 fold) in levels of expression and catalytic activities; this variability is partly due to genetic polymorphisms and partly due to the inducibility of the gene [3,12]. Hepatic CYP2B enzymes are highly inducible in both humans and rodents by chemicals that interact with the constitutive androstane receptor (CAR) and/or pregnane X receptor (PXR) [12,13]. Typical CYP2B inducers are ligands of either receptors, such as rifampicin, phenobarbital (PB), or PB-like compounds [12,13]. The glucocorticoid receptor (GR) has been suggested to play an indirect role in the regulation of CYP2B, acting through modulation of CAR and PXR expression [13].

CYP2B6 is also expressed in the brain [14]. Studies have shown that CYP2B enzymes and transcripts are expressed in the brains of rodents, monkeys and humans [14–16] and can be regulated by exposure to ethanol, nicotine and other compounds [17–19]. Evidence from animal studies suggests that brain CYP2B may influence drug efficacy or neurotoxicity by modulating local metabolite levels and response. Other studies on expression and regulation of CYP2B in rodent brains have revealed remarkable regional differences in expression and inducibility by classic CYP2B inducers [20–22]. For example, CYP2B was detected at high levels in the cortex and midbrain of rats, relative to other brain regions examined [21]. In mice, CYP2B was found to be expressed in the hippocampus and cerebellum in both males and females, but PB treatment led to CYP2B induction only in the hippocampus of females [22]. These differences may contribute to region-selective metabolism and neurotoxicity.

In addition to the brain, CYP2B6 expression has been reported in other extrahepatic tissues, including kidney [23], heart [23], placenta [23], intestine, and the respiratory tract [24]. However, the inducibility of CYP2B enzymes in some of these tissues are not known, and lung CYP2B enzyme appears to be refractory to induction in both rats and rabbits [23]. Tissue-specific expression of CAR or PXR in extrahepatic tissues may account for the selective inducibility of CYP2B enzymes. Besides chemical inducers, inflammation is also considered as a significant regulator in CYP2B expression, which undergoes nitric oxide-mediated degradation upon exposure to inflammatory cytokines [23].

As has been reviewed extensively [1,3,5,12,25], human CYP2B6 is highly polymorphic, with numerous coding region variants and promotor-region variants that have significant functional impact on its expression and catalytic activities. The *CYPalleles* website lists more than 30 starred allelic variations or haplotypes for the *CYP2B6* gene, 19 of which were proven via in vivo or *in vitro* experiments to cause functional changes [http://www.cypalleles.ki.se/; [26]]. Additional studies have revealed promotor variants that not only affect basal expression but also inducibility [27]. The mechanisms underlying the genetic effects on CYP2B6 function may also be complicated by the interaction between effects on expression and effects on catalytic properties, the latter may be substrate-dependent. Such complexities make it very challenging to predict phenotypes from CYP2B6 genetic variations regarding an individual's capacity to metabolize various drugs or propensity to fall victim to the adverse effects of drug-drug or drug-exposome interactions.

Most published studies on possible in vivo roles of CYP2B6 and its variants in drug disposition or toxicity relied on pharmacogenetic studies that associate genetic polymorphisms of CYP2B6 with drug disposition or toxicity in patients, CYP2B6 probe substrate disposition studies, or pharmacological inhibition studies with known CYP2B6 inhibitors [3,5]. Complementary genetic studies in animal models where the contribution of CYP2B6 can be examined under well-controlled experimental conditions have not been possible until now, as a CYP2B6 transgenic mouse model was not available until recently [28]. Notably, extrapolation from studies on the function of mouse *Cyp2b* genes to predict in vivo functions of human CYP2B6 is particularly problematic, given the presence of five different mouse *Cyp2b* genes, compared to the single functional CYP2B6 gene in humans. The presence of the mouse *Cyp2b* genes and other functionally similar *Cyp* genes also necessitate the development of "humanized" mouse models, where human CYP2B6 in expressed, in place of mouse CYP2Bs. Such an animal model, which circumvents at least some of the species difference between mouse and human CYP enzymes in expression, regulation and catalytic efficiencies, provides an effective approach to directly studying in vivo function and regulation of CYP2B6 gene and its roles in drug metabolism and chemical toxicity in humans.

In this review, we briefly describe the generation and characterization of the CYP2B6-humanized mouse model on a *Cyp2b*-knockout genetic background (CYP2A13/2B6/2F1-transgenic/*Cyp2abfgs*-null), as well as available *Cyp2b* knockout and knockdown mouse models. We further discuss their potential applications, including advantages and limitations, in studying the in vivo function, regulation, and clinical implications of human CYP2B6.

2. Properties of a CYP2B6-humanized mouse

Currently, there is only one CYP2B6-humanized mouse model available, which was originally named CYP2A13/2B6/2F1-transgenic/*Cyp2abfgs*-null [28]. This model was generated via cross-breeding from a CYP2A13/2B6/2F1-transgenic mouse model [29] and a *Cyp2abfgs*-null mouse model [30], as depicted in Fig. 1. The following sections describe the properties of these two parental mouse models, as well as other available *Cyp2b* knockout/knockdown mouse models, and the resultant CYP2B6-humanized mouse model.

2.1. CYP2A13/2B6/2F1 transgenic mouse model

The CYP2A13/2B6/2F1-transgenic mouse is currently the only available transgenic mouse model that expresses human CYP2B6 in mice. The transgene was obtained from a bacterial artificial chromosome clone of a human genomic DNA fragment that contained, sequentially, *CYP2B6*, *CYP2A13*, and *CYP2F1*, in the same orientation [29]. The three human genes are a part of the human *CYP2ABFGST* gene cluster on chromosome 19, which contained two additional functional *CYP* genes, *CYP2A6* and *CYP2S1*, located upstream and downstream, respectively, of the CYP2B6-2A13-2F1 genes [9]. The transgenic mouse contained approximately five copies of the transgene, most likely as closely associated or tandem repeats, and its sequence corresponded to the *1A allele (http://www.cypalleles.ki.se).

The insertion of the transgene did not seem to disrupt any critical cellular function, as the CYP2A13/2B6/2F1 transgenic mice were normal in gross morphology, development, and reproduction [29]. Interestingly, whereas CYP2B6 was primarily expressed in the liver, CYP2A13 and CYP2F1 were expressed mainly in the respiratory tract [29]. This differing tissue specificity of the three genes in the transgenic mouse is consistent with their expression patterns in human tissues, and suggests presence of regulatory elements (such as a locus control region) for concordant regulation of CYP2A13 and CYP2F1, and for specific regulation of CYP2B6.

The presence of proper regulatory machinery for CYP2B6 expression in the transgenic mouse was further evidenced by "human-like" hepatic CYP2B6 expression levels and inducibility by classic CYP2B6 inducers. In the CYP2A13/2B6/2F1-transgenic mice, CYP2B6 protein was detected in the liver at a level of ~0.2 pmol/mg microsomal protein, which is close to the low end of the range of levels reported in human livers, ~0.4 pmol/mg microsomal protein [31]. This basal level of CYP2B6 expression is approximately 1000

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