Introduction

The human CYP2ABFGST gene cluster on chromosome 19 contains several functional CYP genes, which encode five cytochrome P450 enzymes (CYP2A6, CYP2A13, CYP2B6, CYP2F1, and CYP2S1), as well as several CYP pseudogenes (Wang et al., 2003). The five CYP genes are all expressed in the respiratory tract, but their contributions to xenobiotic metabolism and target tissue bioactivation remain poorly defined. To study the in vivo function and regulation of these P450 enzymes, we have been generating transgenic mice that express the cognate human CYP genes, which encode five cytochrome P450 enzymes (CYP2A13, CYP2B6, CYP2F1, and CYP2S1), as well as several CYP pseudogenes (Wang et al., 2003). The five CYP genes are all expressed in the respiratory tract, but their contributions to xenobiotic metabolism and target tissue bioactivation remain poorly defined. To study the in vivo function and regulation of these P450 enzymes, we have been generating transgenic mice that express the cognate human CYP genes. We previously reported the generation and characterization of CYP2A6-transgenic mice (Zhang et al., 2005a). In the present study, we prepared CYP2A13/2B6/2F1-transgenic (TG) mice, mainly for study of the functions of CYP2A13. CYP2A13 is located ~70 kbp downstream of CYP2B6 and immediately upstream of CYP2F1; all three genes are arranged in the same direction (Fig. 1A). To preserve regulatory sequences potentially important for the expression of CYP2A13, we selected a human genomic DNA clone encompassing all three CYP genes for transgenic mouse production.

CYP2A13, which is expressed preferentially in the respiratory tract, is the most efficient P450 enzyme in the in vitro metabolic activation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (Su et al., 2000; Jalas et al., 2005), a tobacco-specific nitrosamine and potent lung carcinogen (Hecht, 2003). CYP2A13 is also active toward many other toxicants and carcinogens, including aflatoxin B1 (He et al., 2006), 4-aminobiphenyl (Nakajima et al., 2006), naphthalene, styrene, and toluene (Fukami et al., 2008), and 3-methylindole (D’Agostino et al., 2009). CYP2A13 was hypothesized to play an important role in NNK-induced lung tumorigenesis (Ding and Kaminsky, 2003). CYP2A13-transgenic mice would be valuable not only for directly testing the ability of this human enzyme to mediate chemical carcinogenesis but also for assessing the in vivo efficacy of chemopreventive agents that target CYP2A13.

CYP2B6 is expressed primarily in the liver, where it contributes 2 to 10% of the total P450 content (Wang and Tompkins, 2008). Human CYP2B6 has also been detected, at much lower levels, in several extrahepatic tissues, including brain, kidney, intestine, lung, trachea,
FIG. 1. Structure of the transgene and Southern blot analysis of transgenic mice. A, structure of the transgene fragment (modified from Wang et al., 2003). The ~210-kbp transgene fragment included full-length CYP2A13, CYP2B6, and CYP2F1 genes, as well as three CYP2 pseudogenes. B, strategy for Southern blot analysis. An 864-bp CYP2A13 DNA probe (2A13 probe) (open box) was used. Genomic DNA was digested with HindIII. The size of the expected fragment from the CYP2A13 transgene was 5.1 kbp. C, Southern blot analysis. Increasing amounts (0.1–5 µg) of genomic DNA from a homozygous TG mouse were analyzed; genomic DNA from a WT C57BL/6 mouse (10 ng) was used as a positive control sample, whereas human DNA (10 µg) was used as a negative control sample. The approximate sizes of the detected HindIII fragments are indicated.

CYP2A13/2B6/2F1-TRANSGENIC MICE

Materials and Methods

Generation of TG Mice. A human bacterial artificial chromosome (BAC) clone (CTD-2355H15) containing CYP2A13, CYP2B6, and CYP2F1 genes was obtained from Invitrogen (Carlsbad, CA). The three P450 genes in that BAC clone have all been confirmed, through sequence analysis, to be the *f* allele (http://www.cypalleles.ki.se). The ~210-kbp BAC DNA insert (Fig. 1A) was linearized with NotI, which removes the vector, and was isolated after pulsed-field gel electrophoresis and β-agarose digestion, according to a published method (Abe et al., 2004). Transgenic mice were produced at the Transgenic and Knockout Core Facility at the Wadsworth Center (Albany, NY), according to standard procedures (Nagy et al., 2003). Purified BAC insert was microinjected into the pronuclei of fertilized eggs from the C57BL/6J strain. The eggs either were transferred the same day or were cultured to the two-cell stage and then transferred into the oviducts of pseudopregnant B6CBAF1/J mice and were allowed to develop to term. Positive transgenic mice were identified through PCR analysis of tail DNA, with use of the following CYP2A13-specific PCR primers: 5′-cctggaacagatgcctttaactccg-3′ (forward, starting at position +3144 of CYP2A13) and 5′-tgctgcatctgccgctgagc-3′ (reverse, starting at position +3475) (Zhang et al., 2002). The 332-bp PCR product encompasses CYP2A13 exons 5.

Heterozygous (+/-) TG mice were intercrossed to yield homozygotes (+/+) . TG mice were also crossbred with Cyp2f2-null mice (Li et al., 2001) so the resultant TG (+/-)(Cyp2f2-/-) mice were used for detection of CYP2F1 protein without interference from mouse CYP2F2. All studies with mice were approved by the Wadsworth Center Institutional Animal Care and Use Committee.

Southern Blot Analysis. Mouse genomic DNA was isolated from frozen thymus samples, whereas human genomic DNA was isolated from frozen lung tissues from an 18-year-old, black, male donor (Ling et al., 2007). HindIII-digested genomic DNA was fractionated through electrophoresis on 0.7% agarose gels, transferred to nylon membranes, and analyzed by using a 32P-labeled, 864-bp, DNA probe corresponding to CYP2A13 exon 2 (positions +593 to +1456). The transgene copy number was estimated through densitometric analysis of the 5.1-kbp CYP2A13-specific band detected. The 1-kb Plus DNA ladder (Invitrogen) was used for size determination.

Quantitative RNA-PCR Analysis. Total RNA was isolated by using a RNeasy Mini kit (QIAGEN, Valencia, CA). RNA samples were treated with DNase I (Invitrogen) before reverse transcription. Real-time RNA-PCR analysis was performed essentially as described previously (Zhang et al., 2007), with primers specific for CYP2A13 (2A13F and 2A13R) (Zhang et al., 2004), CYP2F1 (CYP2F1F1 and CYP2F1R1) (Zhang et al., 2005b), and CYP2B6 (5′-cacttccgaggtcgggtgc-3′ and 5′-cccatgtgtgcatcagatac-3′) (Rencurel et al., 2005). Serial dilutions of one reverse transcription product were used to generate a standard curve. A no-template control sample was used in each reaction. The levels of mouse GAPDH mRNA were also determined, as an internal standard (with primers 5′-ttgcaagggccggtc-3′ and 5′-tgctctggaa-3′; product size, 120 bp).

Laser-Capture Microdissection of Lung Bronchiolar Epithelial Cells. Lung tissue samples were prepared from 2-month-old male TG mice. Fresh tissues were mounted in freezing medium and rapidly chilled on dry ice. Frozen sections (10-µm thick) were prepared with Superfrost/Plus slides ( Erie Scientific Co., Portsmouth, NH). Slides were immediately placed in ice-cold 70% ethanol for 10 min, treated with a standard hematoxylin/eosin staining protocol, and then air-dried for ~10 min before they were used for laser-capture microdissection (LCM); the LCM procedure (Pixcell IIe; Arcturus, Mountain View, CA) was completed within 4 h after slide preparation. Bronchiolar epithelial cells (from distal airways) were isolated with Arcturus Capsure caps (Molecular Devices, Sunnyvale, CA). Immediately after LCM, isolated cells were transferred into 0.5-ml LoBind microcentrifuge tubes (Eppendorf North America, New York, NY), which were preloaded with 350 µl of a lysis buffer (QIAGEN). For each lung, ~10,000 cells were captured for RNA preparation.

Immunoblot Analysis. Microsomal proteins were prepared from various tissues of 2-month-old mice, as described previously (Ding and Coon, 1990). Nasal mucosa (NM), and skin (Hukkanen et al., 2002; Ding and Kaminsky, 2003; Wang and Tompkins, 2008). CYP2B6 metabolizes a large number of substrates, including clinically used therapeutic agents, recreational drugs, endogenous chemicals, pesticides, and environmental chemicals (Wang and Tompkins, 2008).

CYP2F1, which is the least well characterized of the three human P450s, is expressed primarily in the respiratory tract (Carlson, 2008; Zhang and Ding, 2008; Weems et al., 2010). Little CYP2F1 mRNA expression was detected in other tissues (Carr et al., 2003). Studies using mammalian cells containing low levels of heterologously expressed CYP2F1 suggested that CYP2F1 is active toward several pulmonary xenotoxicants, including naphthalene, styrene, 3-methylindole, and benzene (Nakajima et al., 1994; Lanza et al., 1999; Powley and Carlson, 2000); however, heterologous expression of CYP2F1 in nonmammalian systems yielded nonfunctional P450 proteins (e.g., Baldwin et al., 2005). Therefore, CYP2F1-transgenic mice would be useful for studying the function of CYP2F1 in chemically induced lung toxicity.

TG mice were characterized generally with respect to viability, growth, and fertility and then were examined thoroughly regarding the tissue distribution of transgene expression, both at the mRNA level and at the protein level. Our results show that CYP2A13 and CYP2F1, but not CYP2B6, are expressed in the lung and NM of the TG mice, whereas CYP2B6 is expressed in the liver of the TG mice, albeit at low levels. Furthermore, metabolic studies were conducted and demonstrated that the transgenic CYP2A13 is capable of bioactivating NNK in vitro and in vivo, in the mouse NM. The value and limitations of this unique TG mouse model for study of the in vivo functions of the three human P450s are discussed.

Lung tissue samples were prepared from 2-month-old male TG mice. Fresh tissues were mounted in freezing medium and rapidly chilled on dry ice. Frozen sections (10-µm thick) were prepared with Superfrost/Plus slides (Erie Scientific Co., Portsmouth, NH). Slides were immediately placed in ice-cold 70% ethanol for 10 min, treated with a standard hematoxylin/eosin staining protocol, and then air-dried for ~10 min before they were used for laser-capture microdissection (LCM); the LCM procedure (Pixcell IIe; Arcturus, Mountain View, CA) was completed within 4 h after slide preparation. Bronchiolar epithelial cells (from distal airways) were isolated with Arcturus Capsure caps (Molecular Devices, Sunnyvale, CA). Immediately after LCM, isolated cells were transferred into 0.5-ml LoBind microcentrifuge tubes (Eppendorf North America, New York, NY), which were preloaded with 350 µl of a lysis buffer (QIAGEN). For each lung, ~10,000 cells were captured for RNA preparation.
A monoclonal antibody against human CYP2A6 (A106; BD Gentest, Woburn, MA) was used for detection of CYP2A13 in microsomes prepared from mouse NM. For detection of CYP2A13 protein in lung microsomes, a rabbit anti-CYP2A5 polyclonal antibody (Gu et al., 1998) was used, and the CYP2A5 and CYP2A13 bands were separated with a high-resolution gel electrophoresis system, as described previously (Wong et al., 2005). Heterologously expressed CYP2A13 in a S9 cell microsomal preparation (Su et al., 2000) and purified recombinant CYP2A5 (Gu et al., 1998) were used as standards for immunoblot quantification.

A CYP2B6-specific monoclonal antibody (BD Gentest) was used for detection of CYP2B6 protein. Recombinant CYP2B6 in a S9 cell microsomal preparation (BD Gentest) was used as a standard for immunoblot quantification. A rabbit anti-peptide antibody to CYP2F1/2, which was used previously for characterization of Cyp2F2-null mice (Li et al., 2011), was used for detection of CYP2F1 protein in microsomes from TG (+/−)/Cyp2F2(−/−) mice. The sequence of the antigenic peptide [NH2-CysPheGlyPhenLeuDECOOH, corresponding to amino acids 404−415 of CYP2F1; the first cysteine was added for conjugation] is shared by CYP2F1 and CYP2F2. The antibody did not cross-react with recombinant CYP1A1, CYP2A6, CYP2C9, CYP2D6, CYP2E1, CYP2S1, or CYP3A4 on immunoblots (data not shown). Heterologously expressed human CYP2F1 protein contained in a S9 cell microsomal preparation was used as a positive control sample for immunoblot preparation. Baculoviral expression of the CYP2F1 protein in S9 cells was achieved by using the Bac-to-Bac baculovirus expression system (Invitrogen) and a full-length CYP2F1 cDNA entry clone purchased from GeneCopoeia (Rockville, MD). The amount of CYP2F1 protein was not determined; the recombinant CYP2F1 protein did not produce the typical reduced CO-difference P450 length CYP2F1 cDNA entry clone purchased from GeneCopoeia (Rockville, MD). For immunoblot analysis of CYP2B6 and CYP2F1, NuPAGE Bis-Tris mini-gels (10%; Invitrogen) were used. Calnexin, a marker protein for the endoplasmic reticulum, was detected by using a mouse monoclonal antibody against human CYP2A6 (A106; BD Gentest, Woburn, MA) was used for detection of CYP2A13 in microsomes prepared from mouse NM. The sequence of the antigenic peptide [NH2-CysPheGlyPhenLeuDECOOH, corresponding to amino acids 404−415 of CYP2F1; the first cysteine was added for conjugation] is shared by CYP2F1 and CYP2F2. The antibody did not cross-react with recombinant CYP1A1, CYP2A6, CYP2C9, CYP2D6, CYP2E1, CYP2S1, or CYP3A4 on immunoblots (data not shown). Heterologously expressed human CYP2F1 protein contained in a S9 cell microsomal preparation was used as a positive control sample for immunoblot preparation. Baculoviral expression of the CYP2F1 protein in S9 cells was achieved by using the Bac-to-Bac baculovirus expression system (Invitrogen) and a full-length CYP2F1 cDNA entry clone purchased from GeneCopoeia (Rockville, MD). The amount of CYP2F1 protein was not determined; the recombinant CYP2F1 protein did not produce the typical reduced CO-difference P450 length CYP2F1 cDNA entry clone purchased from GeneCopoeia (Rockville, MD).

We could not detect CYP2A13 protein in lung microsomes from TG mice when we used the monoclonal anti-CYP2A6 antibody (data not shown). However, CYP2A13 protein was detected in the lung (Fig. 2C) when we used a polyconal anti-CYP2A5 antibody (Gu et al., 1998), which had a much higher titer than the monoclonal antibody did. Notably, the relative band intensities for CYP2A13 and CYP2A5 in Fig. 2C, left, could not be used directly for determination of the relative levels of the two CYP2A proteins because, as shown in Fig. 2C, right, the antibody apparently had greater reactivity toward CYP2A5 than toward CYP2A13. A quantitative analysis indicated...
lung and NM microsomes from male TG mice were gous TG mice (data not shown). In experiments not CYP2A5 protein level determined in lung microsomes from homozygous samples (Zhang et al., 2007) but still levels detected previously in microsomes from human lung biopsy 200 fmol/mg protein, which was that the content of CYP2A13 protein in mouse lung microsomes was 25 times lower than the highest levels detected previously in microsomes from human lung biopsy samples (Zhang et al., 2007) but still 25 times lower than the CYP2A5 protein level determined in lung microsomes from homozygous TG mice (~5 pmol/mg) (data not shown). In experiments not shown, we also observed that the levels of CYP2A13 protein in the lung and NM microsomes from male TG mice were ~30% lower than the levels in corresponding tissues from female TG mice.

We also determined the cellular distribution of CYP2A13 mRNA in the lungs of TG mice, through RNA-PCR analysis of LCM-isolated lung bronchiolar epithelial cells. We estimated that CYP2A13 mRNA levels (normalized to GAPDH levels) were ~12-fold higher in bronchiolar epithelial cells than in the intact lung (n = 4) (data not shown). This result agreed well with the findings of a previous immunohistochemical study of CYP2A13 expression in human lung, where the strongest CYP2A13 immunoreactivity was detected in bronchiolar epithelial cells (Zhu et al., 2006).

CYP2A13 protein was not detected in liver microsomes from either male or female TG mice, with a detection limit of ~100 fmol/mg microsomal protein (data not shown). The very low (if any) hepatic expression of CYP2A13 protein in TG mice is concordant with the essential lack of CYP2A13 mRNA expression in human liver (Su et al., 2000).

**CYP2F1 Expression.** CYP2F1 mRNA was detected in transgenic mice at the highest levels in the NM and lung, was barely detectable in the testis, and was not detected in the other tissues examined (Fig. 3A). Efforts to resolve human CYP2F1 and mouse CYP2F2 proteins by using the same high-resolution gel electrophoresis system that was effective for separation of CYP2A13 and CYP2A5 were unsuccessful, and none of the available anti-CYP2F antibodies could distinguish between the two CYP2F proteins (data not shown). There-
In experiments not shown, we observed that there was no noticeable difference in the levels of CYP2F1 protein in lung or NM microsomes from male and female TG/Cyp2f2-null mice. CYP2F1 protein was not detected in liver microsomes from male or female TG/Cyp2f2-null mice (Fig. 3B and data not shown).

**CYP2B6 Expression.** CYP2B6 mRNA was detected in multiple tissues. The levels were highest in NM, liver, and kidney, intermediate in small intestine and testis, and barely detectable in lung and heart (Fig. 4A). CYP2B6 protein was detected only in liver, at an estimated level of 200 fmol/mg protein (Fig. 4B); it was not detected in NM, lung, small intestine, kidney, brain, or testis, with a detection limit of ~40 fmol/mg protein. There was no sex difference in CYP2B6 protein expression in the liver of TG mice (data not shown).

**NNK Bioactivation in Lung and NM of TG Mice.** Microsomes prepared from lung and NM of WT and TG(+/+) mice were analyzed for their in vitro metabolic activities toward NNK, a CYP2A13 substrate. Rates of formation of two stable metabolites of NNK were analyzed. As shown in Table 1, the rates of formation of the ketoaldehyde (OPB), which represents the α-hydroxylation pathway that leads to O6-mG DNA adduct formation (Peterson et al., 1993; Hecht, 1998), were significantly increased in both lung and NM microsomes from TG mice, compared with WT mice. Rates of formation of the ketoalcohol (HPB) were also increased but to smaller extents. These data indicated that the transgenic CYP2A13 and/or CYP2F1 is active in the bioactivation of NNK in vitro. The greater increase in the rates of formation of OPB than in the rates of formation of HPB is consistent with the knowledge that CYP2A13 primarily produces OPB, whereas CYP2A5 preferentially produces HPB (Jalas et al., 2005).

In vivo formation of O6-mG also was examined in NNK-treated WT and TG mice. At a NNK dose commonly used for lung tumor bioassays (100 mg/kg) (e.g., Weng et al., 2007), NNK-induced O6-mG formation was significantly increased (by 40%) in the NM of the TG mice, compared with WT mice (Table 1). An apparent increase (10%) was also seen in the lung, but the difference was not statistically significant. These in vivo results confirm that transgenic CYP2A13 and/or CYP2F1 is active in the bioactivation of NNK. Given a previous report that CYP2F1 heterologously expressed in a mammalian cell system was a poor catalyst in NNK metabolism (OPB, whereas CYP2A5 preferentially produces HPB (Jalas et al., 2005).

**TABLE 1**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Strain</th>
<th>Product Formation In Vitro</th>
<th>DNA Adduct Formation In Vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ketoaldehyde</td>
<td>Ketonealcohol</td>
</tr>
<tr>
<td>Nasal Mucosa</td>
<td>WT</td>
<td>275 ± 53</td>
<td>388 ± 80</td>
</tr>
<tr>
<td></td>
<td>TG</td>
<td>597 ± 12* (220%)</td>
<td>575 ± 46* (150%)</td>
</tr>
<tr>
<td>Lung</td>
<td>WT</td>
<td>2.9 ± 0.4</td>
<td>16.2 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>TG</td>
<td>5.7 ± 0.4* (200%)</td>
<td>19.8 ± 4.0 (120%)</td>
</tr>
</tbody>
</table>

*P < 0.01, compared with WT mice (Student’s t test).

**FIG. 4.** Expression of the CYP2B6 transgene. A, relative CYP2B6 mRNA expression levels in various tissues of TG mice. Total RNA was isolated from tissues of 2-month-old male mice. The values presented (mean ± S.D., n = 4, in arbitrary units, relative to the highest level detected) were normalized with respect to the levels of mouse GAPDH transcript determined for the same samples. SI, small intestine. B, immunoblot detection of CYP2B6 protein. Microsomal proteins prepared from tissues pooled from three 2-month-old, male, homozygous TG mice, as well as a recombinant CYP2B6 standard (in SF9 cell microsomes), were analyzed by using an anti-CYP2B6 antibody. Typical results are shown.
Discussion

To accomplish our main goal of achieving adequate, human-like, CYP2A13 expression in a mouse model, we considered it necessary to include CYP2B6 and CYP2F1 (and the neighboring CYP pseudo-genes) in the transgene construct, given their close proximity to CYP2A13 and the potential for the presence of long-distance and/or shared regulatory elements (such as a locus control region) in a CYP gene cluster. For the same reason, the human CYP1A1 and CYP1A2 genes had to be coexpressed in a transgenic mouse model (Jiang et al., 2005).

The preferential expression of CYP2A13 and CYP2F1 transgene mRNAs in the respiratory tract is comparable to the respiratory tract-predominant expression reported previously for CYP2A13 (Su et al., 2000; Wong et al., 2005) and CYP2F1 (Carr et al., 2003) transcripts in humans. It was reported that CYP2A13 is expressed at appreciable levels in human bladders (Nakajima et al., 2006). However, CYP2A13 mRNA levels in the bladders of TG mice were barely detectable. This discrepancy could be attributable to species differences in the expression of relevant transcription factors in the bladder.

The expression level of transgenic CYP2A13 appeared to be higher than the CYP2A13 levels found in human NM and lung microsomes. This observation may be explained by the fact that the CYP2A13 transgene existed in multiple copies and the quality of mouse tissues was undoubtedly better than that of human biopsy or autopsy samples. Expression of CYP2A13 may be suppressed in lung tissues from patients, because of disease-related inflammation (H. Wu and X. Ding, unpublished observations).

It is interesting to note that, although mouse CYP2A5 and CYP2F2 are both expressed in the liver as well as in the respiratory tract, human CYP2A13 and CYP2F1 are essentially not expressed in either human liver or the liver of TG mice. This observation indicated that the regulatory sequences responsible for suppressing the expression of CYP2A13 and CYP2F1 in human liver and/or for activating the expression of CYP2A13 and CYP2F1 in the respiratory tract are contained within the transgene fragment.

With the exception of CYP2A13 expression in the NM, the levels of expression of the transgenic human P450s were much lower than the levels of their orthologous mouse P450s. This fact limits the usefulness of the TG mice for direct analysis of the functions of the human P450s. Even for transgenic human P450s, because the activities of the mouse P450s were both expressed in the liver as well as in the respiratory tract, where human CYP2A13 and CYP2F1 are preferentially expressed in human liver or the lung (Hukkanen et al., 2002). Efforts are underway to produce a CYP2B6-humanized mouse model, through crossbreeding between the TG mice described here and mice of a novel Cyp2 gene cluster-null mouse model (Y. Wei and X. Ding, unpublished observations) in which all five mouse Cyp2b genes are removed.

It should be noted that there were apparent inconsistencies in the relative tissue levels of P450 proteins and P450 mRNAs for CYP2F1 and CYP2B6. Whereas CYP2F1 mRNA levels in NM and lung were not significantly different, the protein level was ~4 times greater in the NM than in the lung. Likewise, the mRNA expression of CYP2B6 was comparable between NM and liver; however, CYP2B6 protein was detected only in the liver and not in the NM. These observations may be explained, at least in part, on the basis of tissue- and genespecific differences in the efficiency of post-transcriptional and/or post-translational processes that govern the levels of P450 protein expression.

In summary, we have generated a novel transgenic mouse model in which human CYP2A13 and CYP2F1 are expressed preferentially in the NM and lung, whereas CYP2B6 is expressed in the liver. We provide evidence indicating that the transgenic CYP2A13 is active toward the lung carcinogen NNK, both in vitro and in vivo. This mouse model should be valuable for a number of applications in molecular toxicology, including studies on 1) in vivo functions of CYP2A13 and CYP2F1 in xenobiotic metabolism and toxicity in the respiratory tract, 2) in vivo function and regulation of CYP2B6 in the liver, 3) mechanisms of regulation of tissue-specific expression of CYP2A13 and CYP2F1, 4) identification of potential CYP2A13 and CYP2F1 inducers, and 5) determination of in vivo efficacy of CYP2A13 and CYP2F1 inhibitors.

Acknowledgments

We gratefully acknowledge the use of the services of the Biochemistry, Molecular Genetics, and Transgenic and Knockout Mouse Core Facilities of the Wadsworth Center. We thank Dr. Jun Gu for helpful discussion and assistance with LCM, Ying Liu for assistance with DNA sequence determination, and Weizhu Yang for assistance with mouse breeding.

Authorship Contributions

Participated in research design: Wei, Wu, Q. Zhang, Kluetzman, and Ding.
Performed data analysis: Wei, Wu, Li, Liu, Zhou, Q. Zhang, X. Zhang, and Ding.
Wrote or contributed to the writing of the manuscript: Wei, Wu, Li, Q. Zhang, and Ding.
References


Address correspondence to: Dr. Xinxin Ding, Wadsworth Center, New York State Department of Health, Empire State Plaza, Box 509, Albany, NY 12201-0509. E-mail: xding@wadsworth.org