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REGULATION, FUNCTION, AND TISSUE-SPECIFIC EXPRESSION OF CYTOCHROME P450 CYP1B1

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■ **Abstract** Cytochrome P450 CYP1B1 is a relatively recently identified member of the *CYP1* gene family. The purpose of this commentary is to review the regulatory mechanisms, metabolic specificity, and tissue-specific expression of this cytochrome P450 and to highlight its unique properties. The regulation of CYP1B1 involves a variety of both transcriptional and post-transcriptional mechanisms. CYP1B1 can metabolize a range of toxic and carcinogenic chemicals in vitro but in some cases with a unique stereoselectivity. Estradiol 4-hydroxylation appears to be a characteristic reaction catalyzed by human CYP1B1. However, there are considerable species differences regarding the regulation, metabolic specificity, and tissue-specific expression of this P450. In humans CYP1B1 is overexpressed in tumor cells, and this has important implications for tumor development and progression and the development of anticancer drugs specifically activated by CYP1B1.

INTRODUCTION

Until fairly recently, the cytochrome P450 *CYP1* gene family was thought to consist of a single subfamily containing two very well characterized members, *CYP1A1* and *CYP1A2*. However, in 1994 a new member of the human *CYP1* gene family was cloned from a tetrachloro-dibenzo-ρ-dioxin-treated human keratinocyte cell line (1). This formed part of a series of investigations to identify the differential expression of genes that results from exposure to dioxin (2). Nucleic acid and amino acid sequence analysis indicated that CYP1B1 showed ~40% homology with both CYP1A1 and CYP1A2. Despite this low degree of similarity with CYP1A1 and CYP1A2, this new P450 was assigned by the P450 nomenclature committee (3) to

a new CYP1 subfamily, CYP1B, which currently contains only the single member CYP1B1 (GenBank accession nos. U56438 and U03688). DNA hybridization studies suggest that there is in fact only one member of the *CYP1B* subfamily (1, 4).

Although assigned to the *CYP1* gene family, the *CYP1B1* gene has several distinct properties that clearly separate this P450 from the other well-established members of the CYP1 family. The human *CYP1B1* gene is located on chromosome 2p22-21 spanning approximately 12 kilobases (kb) of DNA and is composed of three exons and two introns (4). [It is interesting that an aryl hydrocarbon hydroxylase gene locus was assigned to human chromosome 2 as early as 1976 (5).] The mRNA is 5.2 kb and the open reading frame begins at the 5′ end of the second exon, in contrast to other P450s, which all begin in exon 1. The predicted protein sequence is 543 amino acids (Figure 1). The long 3′-untranslated region contains multiple polyadenylation sites (1). Analysis of the predicted amino acid sequence indicates that there are three potential furin cleavage sites commencing at amino acid 38, which suggests that there could be post-translational N-terminal processing. CYP1B1 is the largest known human P450, both in terms of mRNA size and number of amino acids, but it is paradoxically the simplest in gene structure.

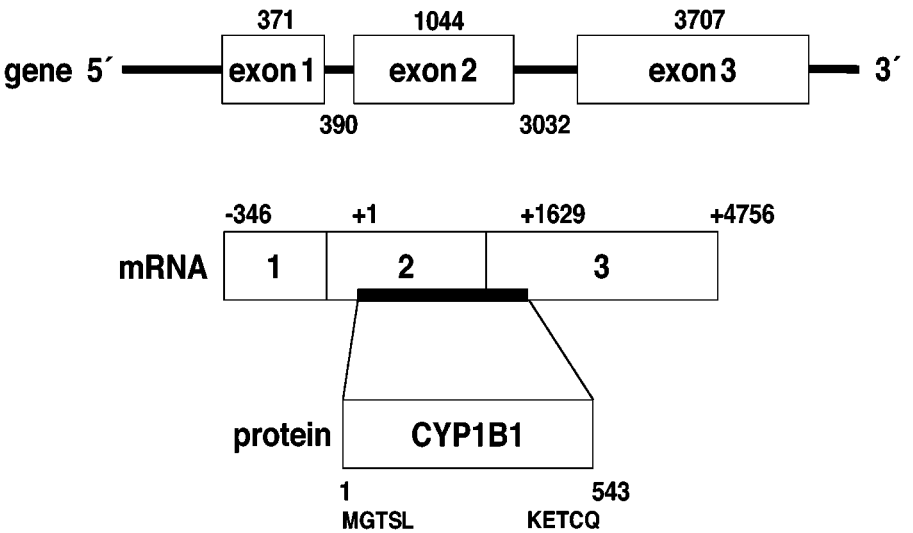

Figure 1 The structure of the human *CYP1B1* gene. The *CYP1B1* gene is localized to chromosome 2p21 and encompasses ~12 kb of genomic DNA. The gene is composed of three exons and two introns and gives rise to a 5.2-kb mRNA. Translation begins close to the 5′ end of exon 2 and continues into exon 3 to produce a predicted protein composed of 543 amino acids. There is a large 3-kb untranslated 3′ region that contains multiple polyadenylation sites. The first five N-terminal and last five C-terminal amino acids are indicated.

For comparison, both the human *CYP1A1* and *CYP1A2* genes are located on chromosome 15 (*CYP1A1* on chromosome 15q22-q24 and *CYP1A2* on chromosome 15q22-qter), and each gene is composed of seven exons and six introns with mRNAs of 2.8 and 3.2 kb, respectively, and proteins of 512 and 515 amino acids, respectively.

Nucleic acid sequences of orthologous forms of CYP1B1 have also been isolated from both mouse and rat cells. Mouse CYP1B1 (GenBank accession nos. U03283 and X78445) has been cloned from a mouse embryo fibroblast-derived cell line (MCA-10T1/2) induced with dioxin (6), and a similar cell line (C3H 10T1/2) treated with dimethylbenzanthracene (7). The orthologous rat CYP1B1 (GenBank accession nos. X83867 and U09540) has been cloned from the adrenal cortex of adult rats, which had been pretreated with either adrenocorticotrophic hormone [ACTH (8)] or dioxin (9). Each of these P450s has an mRNA of 5.2 kb and a predicted protein of 543 amino acids. Although there is a high degree ($>80\%$) of both nucleic acid and amino acid sequence similarity between human, mouse, and rat CYP1B1, several studies (see below) indicate that there are considerable species differences regarding the regulation, metabolic specificity, and tissue-specific expression of this P450 (1, 6, 8, 10).

Both the mouse and rat CYP1B1 P450s were cloned in an extensive series of experiments by Jefcoate's laboratory. These researchers initially identified products of polycyclic aromatic hydrocarbon metabolism in a mouse embryo fibroblast-derived cell line [C3H 10T1/2 (11)] and in rat adrenal gland cells (12, 13) that could not be attributed to any of the then known P450s. This led to the purification and partial immunological and metabolic characterization of P450-like proteins from both mouse embryo fibroblasts (initially designated P450EF) and adult rat adrenal gland cells (initially designated P450RAP) and finally to the identification of these P450s as the murine and rat forms of CYP1B1, respectively. Neither the mouse *Cyp1b1* gene nor the rat *CYP1B1* gene have as yet been assigned to a specific chromosome.

The identification of this new member of the *CYP1* gene family has already stimulated considerable research activity. There has been particular interest in the field of cancer research because studies have shown that CYP1B1 is capable of metabolizing a variety of putative human carcinogens and also that CYP1B1 shows increased expression in a wide range of human tumors (14). Thus, CYP1B1 appears to have potentially important roles in tumor development and progression, as a potential target for anticancer drugs and as a tumor biomarker. CYP1B1 is also important in toxicology, given its inducibility by dioxin and other Ah receptor agonists.

This commentary reviews the pharmacology and toxicology of CYP1B1 as well as its regulation, metabolic specificity, and tissue-specific expression. The potential biological roles of CYP1B1 are also reviewed. The opportunity is taken to indicate areas of controversy and also outline possible directions for future research.

MECHANISMS OF REGULATION OF CYP1B1

Regulation of Human CYP1B1

The *CYP1B1* gene is transcriptionally activated by polycyclic aromatic hydrocarbons, which act via the Ah receptor complex. The most potent of these Ah receptor agonists for activating transcription of the *CYP1B1* gene appears to be dioxin. Initial analysis of the upstream regulatory region of the human *CYP1B1* gene has identified several positive and negative regulatory elements within the promoter region (15) that are structurally distinct from the promoters of the related *CYP1A1* and *CYP1A2* genes. Both basal regulatory sequences and dioxin-responsive elements have been identified in the 5′ regulatory region of the *CYP1B1* gene (15). Maximum expression in a reporter construct required the presence of transcriptional enhancer elements in the regions from nucleotide (nt)-1022 to nt-835 and from nt-2300 to nt-1356. The region from nt-1022 to nt-835 also contains several dioxin-responsive elements (4). Four other regulatory regions have also been identified that are required for maximal activity of the CYP1B1 promoter (15). Constitutive expression of the human *CYP1B1* gene also appears to involve at least one of the dioxin or xenobiotic-response elements (16). Multiple xenobiotic responsive elements have also been identified in the upstream regulatory region of the mouse *Cyp1b1* gene (17).

Studies examining both the basal and inducible expression of CYP1B1 in human tumor-derived cell lines indicate that there is distinct cell-type-specific expression of CYP1B1. For example, in HepG2 hepatoma-derived cells, CYP1A1 (mRNA and protein) but not CYP1B1 was highly induced by dioxin, whereas in ACHN cells (a kidney tumor–derived cell line) the converse was true, with only CYP1B1 (mRNA and protein) being inducible by dioxin. In the ACHN cell line, there was also weak constitutive expression of CYP1B1 mRNA (18), but there was no detectable constitutive expression of CYP1B1 protein. In HepG2 cells, there was no constitutive expression of either CYP1A1 or CYP1B1 mRNA. Since both cell lines contained a functional Ah receptor and structurally intact *CYP1A1* and *CYP1B1* genes, it was suggested that transcriptional repression was occurring due to the presence of an as-yet-uncharacterized repressor protein (18). The inducibility of CYP1B1 and not CYP1A1 in ACHN cells has also been demonstrated by Spink et al (19), who showed that CYP1B1 induced by dioxin in ACHN cells catalyzed the 4-hydroxylation of estradiol.

Expression of both immunoreactive CYP1A1 and CYP1B1 proteins has been identified in dioxin-treated MCF-7 (a breast cancer–derived cell line) cells, whereas in noninduced MCF-7 cells, there is a very low constitutive level of CYP1B1 but not CYP1A1 (20). In different breast cancer cell lines, there is a similar variable expression of CYP1A1 and CYP1B1 mRNAs, both constitutive and induced in response to dioxin (21). However, the expression of CYP1B1 mRNA, either constitutive or induced, does not correlate with the expression of either Ah receptor mRNA or estrogen receptor mRNA, although "cross-talk" between Ah receptor and

estrogen receptor (21) signaling pathways has been shown to be important in the context of endocrine disrupters. Treatment of MCF-7 cells with tetradecanoylphorbol-13-acetate also results in differential changes in the induction of CYP1A1 mRNA and CYP1B1 mRNA by dioxin (22). There is enhanced expression of CYP1B1 mRNA as a result of treatment with tetradecanoylphorbol-13-acetate prior to induction with dioxin, perhaps related to the observed up-regulation of the Ah receptor and suppression of estrogen receptor mRNA and reduced expression of CYP1A1 mRNA. The mechanisms that enable distinct patterns of inducibility of CYP1A1 and CYP1B1 in different cell types by Ah receptor agonists remain to be fully elucidated. In addition, inducible expression of CYP1B1 may also involve non-Ah receptor-mediated mechanisms.

In human breast cancer samples we have shown that there is also differential expression of CYP1B1 mRNA and CYP1A1 mRNA, with consistent constitutive expression of CYP1B1 mRNA, but expression of CYP1A1 mRNA has been identified in only 25% of samples (23). We have found no expression of CYP1A2 mRNA in any of the samples examined. More recently we have also found similar differential expression of CYP1B1 mRNA and CYP1A1 mRNA in kidney tumors (24). CYP1B1 mRNA is expressed in all tumor samples, whereas CYP1A1 mRNA is present in 81% of kidney tumors. Increased constitutive expression of CYP1B1 protein has been shown in a variety of human tumors (14). The mechanisms controlling the expression of CYP1B1 in actual human tumor cells (as opposed to cultured cancer-derived cell lines) have not been elucidated, although transcriptional processing and translational stability could potentially contribute. The possibility of transcriptional up-regulation via the Ah receptor by an endogenous ligand is suggested by a report of activation of the Ah receptor complex in the absence of exogenous ligand; this mechanism could conceivably contribute to the expression of CYP1B1 (25, 26). The potential for interaction with other receptor pathways exists, particularly in breast cancer with the estrogen receptor as indicated above.

Although inducible expression of CYP1B1 by Ah receptor agonists, especially dioxin, has been well documented, other mechanisms possibly contribute to the regulation of CYP1B1, including non-Ah receptor-mediated pathways of transcriptional regulation and also post-transcriptional mechanisms, but these mechanisms have received much less study. The human CYP1B1 mRNA contains multiple polyadenylation sites, and it has been suggested that there is cell-type-specific alternative processing of the CYP1B1 mRNA, which may regulate the amount and/or the ability of the final transcript to be translated (16). The use of alternative processing may also influence the stability of the mRNA (16).

Regulation of Rodent CYP1B1

Both mouse and rat CYP1B1s are inducible by dioxin and are also regulated by cyclic AMP (cAMP)-mediated pathways (17, 27). Steroidogenic transcription factor-1 motifs associated with cAMP-dependent transcriptional activation

of genes have recently been identified in the 5′ upstream regulatory sequences of the mouse *Cyp1b1* gene (17). However, it is not known whether cAMP-dependent transcriptional regulation is involved in regulating human CYP1B1. Constitutive expression of CYP1B1 was absent in Ah-receptor-deficient mouse embryo fibroblasts (17), suggesting a key role for the Ah receptor in both constitutive and inducible expression of mouse CYP1B1. Recently, however, it has been suggested that aryl hydrocarbon nuclear translocator (ARNT) may function as a repressor of basal CYP1B1 expression independently of its interaction with the Ah receptor (28). In a mouse hepatoma-derived cell line (hepa-1), cells with a defective ARNT gene and no detectable immunoreactive ARNT protein had constitutive expression of CYP1B1 at a much higher level compared with wild-type cells with a normal ARNT gene and detectable immunoreactive ARNT protein. Moreover, those cells with a defective ARNT gene were also unresponsive to dioxin (28). A recent study has shown that overexpression of the β1 isoform of protein kinase C in C3H10T1/2 cells resulted in decreased expression of both CYP1B1 mRNA and protein (29), thus providing further evidence for the involvement of multiple cellular pathways in regulation of this form of P450.

Inducible expression of CYP1B1 may also involve non-Ah receptor-mediated mechanisms. For example, in Ah receptor knockout mice, CYP1B1 mRNA (but not CYP1A1 mRNA) is weakly inducible in liver by piperonyl butoxide (30). This finding appears to contrast, but not necessarily conflict, with the results of Zhang et al (17), who found that no induction by dioxin of a *Cyp1B1* promoter construct occurred when the construct was transiently transfected into Ah receptor-deficient mouse embryo fibroblasts. These cells had been derived from Ah receptor knockout mice. In cultured cells derived from mouse embryo fibroblasts, it has been shown that CYP1B1 is a labile protein in the absence of substrate (benzanthracene) and that protein stabilization by substrate may contribute to the regulation of CYP1B1 (31). Once again it is not known whether a similar mechanism regulates human CYP1B1. If stabilization of human CYP1B1 protein does occur, then this could in part explain the apparent discordant results regarding CYP1B1 mRNA and protein levels in human tissues and tumors (see below).

The transcriptional regulation of rat CYP1B1 has received less attention and, in contrast to both human and murine CYP1B1, there have been no studies to map the regulatory regions of the rat *CYP1B1* gene. Rat CYP1B1 is inducible by Ah receptor agonists and is also inducible by ACTH (27). ACTH treatment of cultured cells derived from either the zona fasciculata or zona glomerulosa of rat adrenal cortex resulted in an approximately fourfold increase in CYP1B1 expression as assessed by the increases in CYP1B1 mRNA, immunoreactive CYP1B1 protein, and stereoselective metabolism of 7,12-dimethlybenz[a]anthracene. However, the response towards ACTH was greater than the corresponding response elicited by treatment with dioxin, which resulted in a twofold increase in CYP1B1 protein but a sixfold increase in CYP1B1 mRNA. In contrast, angiotensin had no effect on

CYP1B1 expression (27). Inhibition of CYP1B1 activity using 1-ethynylpyrene, a suicide inhibitor of CYP1B1, had no effect on corticosteroid hormone synthesis. It is interesting that, in contrast to P450s in other types of cultured cells, especially hepatocytes, high basal expression of CYP1B1 protein appears to be maintained in cultured rat adrenocortical cells, thus further highlighting the unique aspects of this P450.

The effect of other steroid hormones, including estradiol and progesterone, on CYP1B1 expression has also been studied in cultured stromal fibroblasts isolated from rat mammary gland cells (32). A low level of immunoreactive CYP1B1 protein was detected in isolated rat mammary stromal fibroblasts cultured for 1–2 days without any pretreatment, whereas there was no significant CYP1B1 identified in isolated mammary epithelial cells prepared at the same time as the stromal fibroblasts (32). The expression of CYP1B1 was increased by exposure to benzanthracene and estradiol but not to cortisol, which suppressed the induction of CYP1B1 (32). The suppression of CYP1B1 expression by cortisol in rat mammary fibroblasts appears to be caused by glucocorticoid receptor suppression of the Ah receptor complex (27, 33).

METABOLIC SPECIFICITY OF CYP1B1

Metabolic Reactions Catalyzed by Human CYP1B1

Most of the studies examining the metabolic specificity of human CYP1B1 have been performed using recombinant CYP1B1 expressed in either yeast (*Saccharomyces cerevisiae*) (34, 35) or human (36) lymphoblastoid cells, because native human CYP1B1 protein has as yet not been purified. Recently, human CYP1B1 has also been expressed at a high level in *Escherichia coli* (37) and insect cells (Gentest Corp., Woburn, Mass.). Initial experiments (34) to express CYP1B1 in *S. cerevisiae* resulted in a much higher expression of CYP1B1 when, instead of using the full coding sequence, the construct contained a deletion corresponding to an absence of the N-terminal amino acid residues 2–4 (34). Subsequent studies have been performed with this 5′-modified CYP1B1 cDNA to achieve satisfactory expression of CYP1B1 protein. Highly purified, functionally active expressed human CYP1B1 has been obtained from *E. coli* using diethylaminoethyl cellulose and carboxymethyl-sepharose chromatography (37). Once again the highest level of expression was obtained from a construct that had a deletion of N-terminal amino acids 2–4 (37). Sufficient purified, expressed protein was obtained to determine carbon monoxide-binding spectra (37).

CYP1B1 is capable of activating a variety of putative human carcinogens as assessed by mutagenicity assays (35, 36). The Ames test was used to measure the ability of CYP1B1 expressed in *S. cerevisiae* to activate a wide range of polycyclic aromatic hydrocarbons and both aromatic and heterocyclic amines (35). CYP1B1 had higher activity than either CYP1A1 or CYP1A2 for the activation of various

promutagens and was particularly efficient in the activation of dibenzo [11, 12][a,l] pyrene 11,12 diol (35). The preferential metabolism of this latter compound (which is one of the most potent known procarcinogens) by CYP1B1 has been studied more extensively using V79 Chinese hamster cells containing expressed human CYP1B1 (38). This P450 showed distinct stereoselectivity towards this substrate, preferentially forming dibenzo[a,l]pyrene 11,12-dihydrodiol-13,14 epoxides with a significantly higher turnover number than CYP1A1 (38). CYP1B1 is one of the most effective P450s in activating aflatoxin B1 to a mutagenic metabolite (36). The metabolism by human CYP1B1 expressed in *S. cerevisiae* of a dietary heterocyclic amine, 2-amino-1 methyl-6-phenylimidazo[4,5-b] pyridine, which has been implicated in the aetiology of breast and colon cancer, has been studied in detail (39). CYP1B1 metabolized this compound to several products including its mutagenic N2-hydroxylated derivative (39).

CYP1B1 activity has also been characterized by using several xenobiotics that are considered model substrates for individual P450s (36, 40). The activity of human CYP1B1 in a number of standard probe reactions for clinically important P450 forms has been tested using CYP1B1 expressed in either human lymphoblastoid cells (36) or *S. cerevisiae* (40). CYP1B1 was active in the hydroxylation of benzpyrene and bufuralol, the oxidation of caffeine and theophylline, and the *O*-dealkylation of ethoxycoumarin, ethoxy-trifluoromethyl-coumarin, and ethoxyresorufin. It was less active in the metabolism of ethoxyresorufin than CYP1A1. However, CYP1B1 was inactive in the hydroxylation of chlorzoxazone, coumarin, diclofenac, lauric acid, nifedipine, *p*-nitrophenol, *S*-mephenytoin, and tolbutamide (40), which are all metabolic probes for other P450s. Interestingly, CYP1B1 hydroxylated testosterone when expressed either in lymphoblastoid cells (36) or *E. coli* (41) but not when expressed in *S. cerevisiae* (40), although only a low level of testosterone hydroxylation was observed.

Dioxin has previously been observed to induce a novel pathway of estradiol metabolism in MCF-7 cells (42) involving the induction of the 4-hydroxylation of 17β-estradiol. This activity was not attributable to CYP1A1, which catalyzes the 2-hydroxylation of estradiol and was inhibited by an antibody raised to mouse CYP1B1. Subsequent studies with human CYP1B1 expressed in *S. cerevisiae* (34) or *E. coli* (37) have confirmed CYP1B1 as a highly selective estradiol 4-hydroxylase that is inefficient in catalyzing the 2-hydroxylation of estradiol. Indeed, 4-hydroxylation of estradiol currently represents the most specific assay available to investigate the activity of human CYP1B1.

Metabolism by Murine CYP1B1

In contrast to human CYP1B1, recombinant mouse CYP1B1 expressed in *E. coli* does not act as an estradiol hydroxylase (10). The recombinant mouse CYP1B1 was produced by creating an expression construct in which the 20 N-terminal amino acids of mouse CYP1B1 were replaced with 8 amino acids of human CYP17, because this facilitated expression of P450 in *E. coli*. The recombinant mouse

CYP1B1 also had a modified C terminus with an additional 12 amino acids, including 6 histidine residues to aid purification. Compared with the expression of human CYP1B1 in *E. coli* (37), only a relatively low level of expression of mouse CYP1B1 was achieved (10). Mouse CYP1B1 expressed in *E. coli* shows a stereoselective metabolism of 7,12-dimethylbenz[a]anthracene, which is similar to this same compound's metabolism by CYP1B1 constitutively or inducibly present in murine C3H10T1/2 cells. The major metabolites produced by both native and expressed mouse CYP1B1 are the 3,4- and 10,11-dihydrodiols of 7,12-dimethylbenz[a]anthracene. Expressed murine CYP1B1 also binds polycyclic aromatic hydrocarbons in a manner similar to that observed for native mouse CYP1B1 induced in C3H10T1/2 cells (10).

Rat CYP1B1 Metabolism

Rat CYP1B1 present in adrenal cortex, testis, or ovary cells shows a stereoselectivity towards the metabolism of 7,12-dimethylbenz[a]anthracene, which is identical to that of mouse CYP1B1 isolated from embryo fibroblasts (12, 13). Rat CYP1B1 expressed in *S. cerevisiae* shows a stereoselectivity in the metabolism of benzo[a]pyrene and benzo[a]pyrene-7,8-diol (43) that is similar to that shown by human CYP1B1 expressed either in yeast or baculovirus (43) cells, although rat CYP1B1 is more active than human CYP1B1 (43).

Metabolic Probes for CYP1B1 Activity

The investigation of the metabolic specificity of CYP1B1 and the identification of functionally active CYP1B1 in biological tissues would be considerably enhanced by the identification of further specific metabolic probes with straightforward assay methods, especially single-step or direct fluorescent methods. The alkoxyresorufin group of substrates, especially ethoxyresorufin, has been used extensively to study and characterize other P450-associated monooxygenase activity. Recently in our laboratories, using a series of homologous alkoxyresorufin substrates, we compared the *O*-dealkylation activities of recombinant human CYP1B1, CYP1A1, and CYP1A2 expressed in lymphoblastoid cells. We found that CYP1B1 was less active overall than CYP1A1 and CYP1A2 but had a different substrate selectivity (Table 1). In particular, CYP1B1 was relatively inefficient in catalyzing the de-ethylation of ethoxyresorufin in comparison with CYP1A1. CYP1B1 expressed in *E. coli* had slightly higher activity towards ethoxyresorufin compared with expressed CYP1A2, but in both cases activity was significantly less than that of expressed CYP1A1 (37). The low overall rate of metabolism by CYP1B1 towards all of the alkoxyresorufin substrates tested means that none of them can be recommended as specific probes for CYP1B1. The study of the metabolic specificity of CYP1B1 will also be made much easier by the further development of selective chemical inhibitors (44, 45) and the production of specific inhibitory antibodies.

TABLE 1 Alkoxyresorufin *O*-dealkylase activities of recombinant human CYP1A1, CYP1A2, or CYP1B1 expressed in human lymphoblastoid cells towards a series of alkoxyresorufin substrate

P450	Methoxyresorufin	Ethoxyresorufin	Propoxyresorufin	Butoxyresorufin	Benzyloxyresorufin
CYP1A1	5.7[a]	9.0	5.8	3.6	0.2
CYP1A2	5.56	1.10	0.15	0.11	0.014
CYP1B1	0.01	0.23	0.18	0.04	0.14

[a]Values are nanomoles of resorufin formation per minute per nanomole of P450. The P450s were supplied by Gentest Corporation, Woburn, Mass.

CYP1B1 EXPRESSION IN NORMAL TISSUES AND TUMORS

In reviewing the literature regarding the expression of CYP1B1 in individual tissues from different species, it became clear that a number of potentially misleading conclusions regarding the presence of CYP1B1 have been published. This is especially true for those studies that have used the reverse transcriptase polymerase chain reaction (RT-PCR) to detect CYP1B1 mRNA in human tissues (46–49). In all of the studies documenting the presence of CYP1B1 in cells or tissues, it is therefore important to distinguish between the identification of mRNAs, especially in RT-PCR studies, which may detect low amounts of CYP1B1 mRNA of uncertain biological significance and the presence of either immunoreactive or catalytically active protein. It is also important to distinguish between constitutive expression of CYP1B1 and the presence of CYP1B1 induced by different agents. Furthermore, inferences about the presence of CYP1B1 in human cells and tissues based on data from corresponding isolated cells or cell lines should not be made. Ideally the presence of CYP1B1 should be confirmed by demonstrating functionally active protein. However, this is difficult given the current paucity of specific substrates for CYP1B1 as described above.

CYP1B1 Expression in Normal Adult Human Tissues

A low level of CYP1B1 mRNA has been detected by Northern blotting in several normal human tissues including kidney, liver, intestine, eye tissue, and brain (1, 35, 50, 51). However, the results obtained from the studies by Sutter et al (1), Shimada et al (35), and Rieder et al (51) cannot be considered definitive because, in each case, only a single set of commercially obtained tissue samples was investigated. No information was provided for these tissues regarding potential prior exposure in vivo to chemicals, drugs, or dietary factors capable of inducing the expression of CYP1B1 nor whether RNA was obtained from single individuals or was pooled from several. Furthermore, no clinicopathological information was provided regarding the samples.

CYP1B1 mRNA has also been identified by RT-PCR in several normal tissues and cell types, including liver (46), lymphocytes (46), endometrium (46, 47),

normal breast tissue (48), and lung epithelial cells obtained by bronchoalveolar lavage from both current smokers and nonsmokers (49). Quantitative RT-PCR showed that CYP1B1 mRNA was present at a very low level in lung epithelial cells obtained from nonsmokers, whereas there was a higher level of CYP1B1 mRNA in cells obtained from current smokers (49). However, in a similar study, CYP1B1 mRNA was not detected in cells (a mixture of lung epithelial cells and macrophages) obtained by bronchoalveolar lavage from nonsmokers, whereas CYP1B1 mRNA was detected in current smokers (52). CYP1B1 mRNA has also been detected by semiquantitative RT-PCR in nontumorigenic breast epithelial cell lines (22).

However, the level of expression of CYP1B1 protein (as opposed to mRNA) in normal human tissues is another matter. It has been proposed, based particularly on the Northern blot results described above, that CYP1B1 has widespread expression in extra-hepatic tissues and hence is potentially important in tumor development in these tissues. Native CYP1B1 protein has not, however, been isolated and purified from any normal human tissue, suggesting that constitutive expression of CYP1B1 protein in normal human tissues is at best at a very low level. (The failure to detect human CYP1B1 at all until 1994 also suggests that CYP1B1 protein is not significantly present in normal human tissues; otherwise, it would seem reasonable, given the extensive research into P450s over the previous 15–20 years, to expect that CYP1B1 would have been identified previously.) In contrast, nonhuman CYP1B1 has been partially purified in a form that is metabolically active from both mouse embryo fibroblasts (11) and rat adrenal gland (12). In both cases protein purification, which required an extensive amount of cells or tissue, preceded cloning of the gene in the respective species.

The first direct demonstration of CYP1B1 protein in human tissues was by our group, using polyclonal antibodies to CYP1B1 to identify it in breast cancer using immunoblotting (24) and in a variety of cancers using immunohistochemistry (14). However, we were unable to detect CYP1B1 protein in normal liver by either immunohistochemistry or immunoblotting of microsomal fractions, even though CYP1B1 mRNA is detectable in normal liver by Northern blotting (1, 35) and RT-PCR (46; GI Murray, MCE McFadyen, WT Melvin, unpublished observation). The absence of CYP1B1 protein in normal adult human liver microsomes has subsequently been confirmed by Edwards et al (53), who used immunoblotting with a polyclonal antibody raised against a peptide corresponding to the five C-terminal amino acid residues of the CYP1B1 protein. We have also failed to detect CYP1B1 in human liver by immunoblotting with a monoclonal antibody specific for CYP1B1 (54) and a highly sensitive chemiluminescence detection system. This antibody was produced using, as the immunogen, a synthetic peptide corresponding to amino acids 437 to 451 of the human CYP1B1 amino acid sequence (54).

A very low and variable level of CYP1B1 protein has also recently been detected by immunoblotting of the microsomal fraction of cultured breast epithelial cells (55) and cultured breast stromal cells (56), which were prepared from nonneoplastic breast tissue. However, it is not clear whether the presence of CYP1B1 in either cultured epithelial or stromal cells was representative of the cells in vivo or whether expression of CYP1B1 was induced either as a result of the isolation

of the cells or their culture over several days. No information regarding exposure of donors to potential inducing agents was presented. During the isolation and primary culturing of cells, there is a phase of proliferation, and it has been shown that CYP1B1 may be up-regulated during cellular proliferation in mouse embryo-derived fibroblasts (57).

Our results showing the highly selective expression of CYP1B1 protein in tumors do not exclude the possibility of a low level of CYP1B1 in normal tissues under certain physiological conditions, although to date the normal tissues we have examined by using both monoclonal and polyclonal antibodies to CYP1B1 have not shown CYP1B1 expression. A low level of CYP1B1 in normal tissues would not preclude the utility of the overexpression of CYP1B1 in tumors for cancer cell growth, detection, and treatment.

The Presence of CYP1B1 in Human Fetal Tissues

CYP1B1 mRNA has been identified in several human fetal tissues by Northern blotting, using a commercially available Northern blot (35). CYP1B1 mRNA was identified in all the fetal tissue samples studied with the strongest signal present in fetal kidney, which suggests that CYP1B1 is a developmentally regulated gene. For the reasons stated above for the results of Northern blotting of adult tissues, the findings obtained from this fetal study must be regarded as nondefinitive because only a single set of tissue samples was used, and the gestational ages of the fetuses were not provided. Consistent expression of CYP1B1 mRNA has also been identified by RT-PCR in several human fetal tissues (kidney, lung, adrenal gland, and brain) obtained from normal first and mid-trimester fetuses (46). In the same study, CYP1B1 mRNA was also detected in fetal liver but only in half of the samples investigated, and it was not stated whether the expression of CYP1B1 mRNA in liver varied with gestational age. CYP1B1 mRNA was also consistently present in both first-trimester and full-term placental tissues (46).

The presence of CYP1B1 mRNA in a variety of fetal tissues has led to the suggestion that CYP1B1 may have an important role in normal fetal development. This hypothesis has received recent support as a result of mutations in the CYP1B1 gene being linked with the development of a form of primary congenital glaucoma, which has an autosomal recessive pattern of inheritance (50, 58). Several mutations of the CYP1B1 gene were identified, all of which are present in the coding region of the gene (50, 59). The mutations included missense mutations, nonsense mutations, frame-shift mutations, and deletions, and some of the mutations were predicted to lead to the formation of a truncated protein. The mechanism by which abnormal CYP1B1 plays a role in the development of this form of glaucoma has not been established.

CYP1B1-null mice have recently been developed and, from initial reports, are apparently normal with no pathological defect (60). However, it is not known whether the eyes were specifically examined to exclude glaucoma or whether mice are even susceptible to this type of abnormality.

Several polymorphisms of the CYP1B1 gene have also been identified (59, 61, 62), and these show ethnic variation in their allelic frequency (62). The CYP1B1 polymorphisms result in amino acid substitutions at codons 48 (Arg→Gly), 119 (Ala→Ser), 432 (Val→Leu), and 453 (Asn→Ser), respectively (61). The polymorphism at codon 432 has been correlated with both estrogen and progesterone receptor status in breast cancer, with a stronger association with progesterone receptor positivity (61). Polymorphic variants of CYP1B1 with amino acid substitutions at codons 432 and 453 have been expressed in *E. coli*, along with NADPH cytochrome P450 reductase, and the metabolic activity of the different variants have been assessed using a panel of procarcinogens and several steroid hormones, including estradiol, testosterone, and progesterone (41, 63). The CYP1B1 variant with the valine-to-leucine substitution at amino acid 432 resulted in a fourfold increase in the K_m of the 4-hydroxylation of estradiol, suggesting that amino acid substitution at codon 432 causes alteration in CYP1B1 binding of estradiol (63). The other CYP1B1 variants did not show any altered catalytic properties towards the 4-hydroxylation of estradiol (63). Interestingly, none of the variants including the codon 432 (Val→Leu) variant displayed altered activity towards ethoxyresorufin (63).

CYP1B1 Expression and Activity in Tumors

Possibly the most significant finding regarding CYP1B1 expression is the high frequency of expression of CYP1B1 protein in various types of malignant tumors (14; Figure 2) without expression in various corresponding types of normal tissues. The majority of these observations were made using immunohistochemistry, which showed that CYP1B1 was specifically localized to tumor cells. The presence of CYP1B1 protein in breast cancer was confirmed by immunoblotting (14). Significant expression of CYP1B1 in breast cancer is also consistent with the findings of Liehr & Ricci (64), who demonstrated increased estradiol 4-hydroxylase activity in microsomes prepared from breast cancer. This activity was not detected in normal breast tissue, where 2-hydroxylation of estradiol occurred. They also found increased 4-estradiol hydroxylation in fibroadenoma, a common type of benign breast tumor. The same group has also found estradiol 4-hydroxylation that was inhibited by an antibody to mouse CYP1B1 in uterine leiomyomas (65), a benign tumor derived from smooth muscle of the uterine wall. The studies by Liehr's group of estradiol 4-hydroxylation in individual types of tumor currently represent the only demonstration of CYP1B1 activity in human tissues or tumors (as opposed to tumor cell lines). These results of increased CYP1B1 activity in tumors are consistent with the concept of overexpression of CYP1B1 in tumors. Further studies are required to examine the expression of CYP1B1 in different types of benign tumors, because it will be important to investigate the stage during tumor development and progression at which alterations of CYP1B1 expression occurs. These investigations are also important in determining the utility of CYP1B1 as an early-stage tumor marker.

CYP1B1 in Murine Cells and Tissues

CYP1B1 has been purified from a C3H10T1/2 mouse embryo fibroblast cell line after benzanthracene induction, with only a low level of constitutive CYP1B1 protein in these cells (11). Expression of CYP1B1 in C3H10T1/2 cells has been suggested to be associated with enhanced transformation of these cells (66). Cultured mouse bone marrow stromal cells also contain a detectable constitutive level of CYP1B1 protein, which is active in the metabolism of 7,12-dimethylbenz[a]anthracene (67). The level of constitutive expression of CYP1B1 is approximately fourfold higher in cultured bone marrow stromal cells than in C3H10T1/2 cells.

A very low amount of immunoreactive CYP1B1 has also been detected in several normal adult murine tissues including kidney and uterus, whereas no immunoreactive CYP1B1 was detected in lung, liver, and brain. However, CYP1B1 was induced in vivo by β-naphthoflavone in all of those tissues, with the highest level of induction observed in lung tissue (6). CYP1B1 mRNA is inducible in mouse liver by dioxin, whereas there is a very low level of constitutive expression of CYP1B1 mRNA in murine liver (68, 69).

CYP1B1 mRNA has a higher level of induction than CYP1A1 mRNA in two different strains of mice (C57BL/6J and DBA/2J), which have different allelic forms of the Ah receptor (69). Both CYP1A1 and CYP1B1 showed a higher level of inducible expression in the mouse strain (C57BL/6J) with the higher affinity form of the Ah receptor, indicating that inducible expression of CYP1B1 is linked to the Ah receptor complex.

In mice, a lower frequency of lymphomas induced by high-dose 7,12-dimethlybenz[a]anthracene has been found in CYP1B1 knockout mice compared with wild-type mice (60). This finding emphasizes the importance of CYP1B1 in tumor development and progression.

CYP1B1 in Rat Tissues

CYP1B1 is present constitutively in the cortex of rat adrenal gland, ovary, and testis (8, 12, 13). It is present in testis and ovary at 10-fold- and 25-fold-lower concentrations, respectively, than in adrenal gland. Within the adrenal cortex, CYP1B1 appears to be present in cells of both the zona fasciculata and the zona glomerulosa (27). However, it has not been established if there is preferential localization of CYP1B1 to a particular zone of the adrenal cortex. Similarly, the cell type or types expressing CYP1B1 in ovary and testis have not been identified although it is likely that CYP1B1 is present in cells involved in steroid hormone metabolism, that is, Leydig cells in the testis and luteal cells in the ovary.

CYP1B1 is not constitutively present in rat liver (8), but it is inducible in liver by dioxin (70, 71) or β-naphthoflavone (8). Induction of hepatic CYP1B1 occurs only after high doses of tetrachloro-dibenzo-ρ-dioxin (71). After chronic exposure to dioxin, there is a marked zonal distribution of CYP1B1, with this enzyme present mainly in hepatocytes of zone 3 (perivenular) of the liver acinus

as determined by immunohistochemistry (71). The intra-acinar distribution of CYP1B1 parallels the localization of CYP1A2 within the liver acinus. In rat liver cells in culture CYP1B1 mRNA is expressed constitutively in hepatic stellate cells and myofibroblasts but not in hepatocytes or Kupffer cells (72). There is no constitutive expression of CYP1B1 protein in several tissues including kidney, lung, and uterus (8).

CONCLUSIONS

This review outlines current knowledge regarding the regulation, metabolic specificity and tissue-specific expression of CYP1B1, which appears in many respects to be a unique form of P450. Despite relatively intensive investigation, there remain many important areas to be studied. For example, it is important particularly in the context of toxicology and drug metabolism to establish the precise metabolic specificity of CYP1B1 towards substrates and to identify its capability for activating and detoxifying carcinogens and other toxic chemicals. Also important is its regulation by individual Ah receptor agonists and factors regulating the cell-type-specific expression of this P450. However, this classic toxicological approach to P450s runs the risk of underestimating the potentially important biological significance of CYP1B1. Possibly one of the most important findings to date is our own observation of enhanced expression of CYP1B1 protein in a variety of human cancers (14) and the suggestion that CYP1B1 may be a marker of tumorigenesis. Given the frequency of its occurrence in tumors, there may also be an important endogenous function for this enzyme. Future research into the role of CYP1B1 will need to elucidate its regulation in tumors and its metabolism of anticancer drugs. If CYP1B1 can be shown to have a distinct metabolic specificity, this could lead to the development of cytotoxic anticancer prodrugs designed to be selectively activated by CYP1B1 in tumors.

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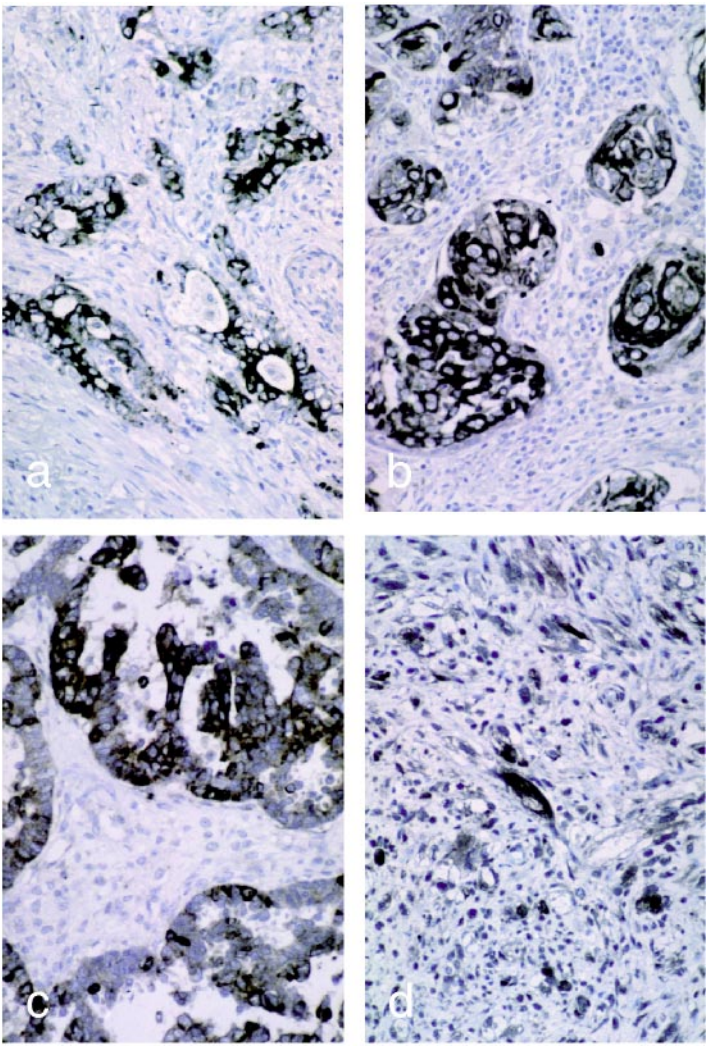

Figure 2 The immunohistochemical localization of CYP1B1 in individual types of human malignant tumors. The tumors have been immunostained with a monoclonal antibody to human CYP1B1. Sites of antibody binding were detected using a tyramide amplification system with peroxidase as the reporter molecule and were visualized using diaminobenzidine as the peroxidase substrate to give a brown reaction product (see 54 for details of CYP1B1 antibody and immunohistochemical techniques). Each tissue section has been counterstained with hematoxylin, which stains nuclei blue and allows visualization of morphological detail. In each type of tumor, there is strong CYP1B1 immunoreactivity in tumor cells. Panels: *a*, stomach cancer; *b*, esophageal cancer; *c*, ovarian cancer, and *d*, rhabdomyosarcoma (malignant tumor of muscle). There is no immunoreactivity for CYP1B1 in nontumor cells.

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