



Cancer Activation and Polymorphisms of Human Cytochrome P450 1B1

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(Received January 25, 2016; Revised February 29, 2016; Accepted March 1, 2016)

Human cytochrome P450 enzymes (P450s, CYPs) are major oxidative catalysts that metabolize various xenobiotic and endogenous compounds. Many carcinogens induce cancer only after metabolic activation and P450 enzymes play an important role in this phenomenon. P450 1B1 mediates bioactivation of many procarcinogenic chemicals and carcinogenic estrogen. It catalyzes the oxidation reaction of polycyclic aromatic carbons, heterocyclic and aromatic amines, and the 4-hydroxylation reaction of 17 β -estradiol. Enhanced expression of P450 1B1 promotes cancer cell proliferation and metastasis. There are at least 25 polymorphic variants of P450 1B1 and some of these have been reported to be associated with eye diseases. In addition, P450 1B1 polymorphisms can greatly affect the metabolic activation of many procarcinogenic compounds. It is necessary to understand the relationship between metabolic activation of such substances and P450 1B1 polymorphisms in order to develop rational strategies for the prevention of its toxic effect on human health.

Key words: Cytochrome P450 1B1, Cancer activation, Polymorphism

INTRODUCTION

Cytochrome P450 enzymes (P450s, CYPs) are heme-thiolate monooxygenase enzymes found in a variety of living organisms including animals, fungi, bacteria, and plants (1). These are the major catalysts of oxidative metabolism of xenobiotic chemicals, and therefore have been a subject of numerous toxicological, pharmacological, and drug metabolism studies (2,3).

In 1958, Klingenberg first discovered a pigment in the liver microsomes that binds CO and demonstrates strong absorbance at 450 nm (4,5). Four years later, Omura and Sato reported additional properties of this biochemical system and coined the name "cytochrome P450" for "Pigment 450" (5,6). Early work on P450 proteins was carried out with human tissue samples (7,8). For the several human P450 enzymes, cDNA molecules were cloned in 1980s and the recombinant P450 enzymes were heterologously expressed

and purified in mammalian, yeast, and bacterial systems in late 1980s and early 1990s (9).

The human genome includes 57 P450 genes (<http://drnelson.uthsc.edu/cytochromeP450.html>) and among them, about 15 are considered to be primary catalysts of xenobiotic metabolism (10). There are three enzymes in the human P450 1 family including P450 1A1, 1A2, and 1B1. This review will focus on metabolic importance of human P450 1B1 and the role of its polymorphisms in cancer development.

HUMAN CYTOCHROME P450 1B1

P450 1B1 was originally found in cultures of keratinocytes as a new dioxin-inducible gene (11). In humans, P450 1B1 is expressed primarily in the kidney, spleen, thymus, prostate, lung, intestine, and colon but barely detectable in liver (12,13). Moreover, high P450 1B1 expression has been observed in various hormone-mediated cancers, such as breast, ovarian, endometrial, and prostate cancers (14). P450 1B1 has never been purified from human tissues (12), but Guengerich and colleagues were able to express and characterize the recombinant P450 1B1 enzyme (15).

P450 1A1, 1A2, and 1B1 share a common transcriptional regulation through the aryl hydrocarbon (Ah) system, which is comprised of the aryl hydrocarbon receptor (AhR) and AhR nuclear translocator (ARNT) (16). The P450 1B1 gene is transcriptionally activated when a ligand binds to the

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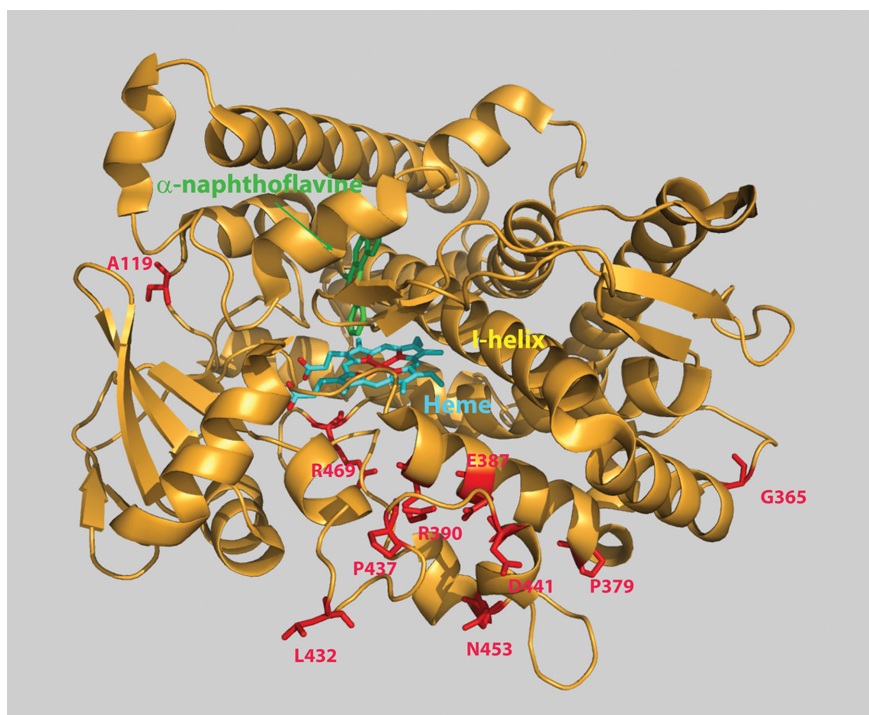


Fig. 1. The X-ray crystal structure of P450 1B1 in the complex with α -naphthoflavone (18). Polymorphic residues are indicated in red color.

cytoplasmic AhR complex (17). The ligand bound AhR translocates to the nucleus, where it forms a heterodimer complex with ARNT (17). This tertiary complex of the ligand (e.g., dioxin), AhR and ARNT subsequently binds to enhancer sites of the P450 1B1 gene and opens up the chromatin structure, so that transcription factors can bind to the promoter and cause more rapid transcription (17).

The X-ray crystal structure of P450 1B1 in the complex with α -naphthoflavone was determined by Eric Johnson's group (Fig. 1) (18). The narrow substrate binding cavity of P450 1B1 contains a slot-like active site, which is well adapted to bind to its characteristic substrates that possess hydrophobic and planar aromatic ring structures (18). The Phe231 residue of P450 1B1 induces a distortion of the F-helix accommodating for π - π stacking with α -naphthoflavone, a feature also present in the structure of P450 1A2 (Fig. 1) (18).

O-deethylation of 7-ethoxyresorufin (EROD) is used as a model reaction catalyzed by P450 1B1. The catalytic activity P450 1B1 is similar to that of P450 1A1 and 1A2 (12). The roles of P450 1B1 in the bioactivation of a very broad spectrum of chemical carcinogens including polycyclic hydrocarbons, heterocyclic amines, aromatic amines, and nitropolycyclic hydrocarbons have been well documented (12,19) (Table 1). An *E. coli* strain overexpressing P450 and *N*-acetyltransferase has been used to characterize the chemical genotoxicity of P450 enzymes (20,21). A P450 1B1-based genotoxicity system has been utilized to characterize potent inhibition of the enzyme by tetramethylstilbene (22).

ASSOCIATIONS OF CANCER AND DISEASES

P450 1B1 activity has been under a close experimental scrutiny because this enzyme mediates metabolic bioactiva-

Table 1. Chemical carcinogens metabolically activated by human P450 1B1^a

Polycyclic aromatic carbons	Heterocyclic amines	Aromatic amines	Nitropolycyclic hydrocarbons	Estrogens
Benzo[<i>a</i>]pyrene	MeIQ	2-Aminoanthracene	1-Nitropyrene	17 β -Estradiol
Dibenzo[<i>a,l</i>]pyrene	MeIQx	2-Aminofluorene	2-Nitropyrene	Estrone
Benzo[<i>a</i>]anthracene	IQ	4-Aminobiphenyl	6-Nitrochryrene	
Dimethylbenz[<i>a</i>]anthracene	Trp-P1	<i>O</i> -Aminoazotoluene	2-Nitrofluoranthene	
Benzo[<i>c</i>]phenanthrene-3,4-diol	Trp-P2	6-Aminochrysene	1,8-Dinitropyrene	
5-Methylchrysene	PhIP		1-Aminopyrene	

^a(12)

tion of many chemical carcinogens (Table 1). This functional feature of P450 1B1 helps to understand its role in cancer initiation and progression. As a recognized driver of cancer development, P450 1B1 has been considered a promising cancer biomarker and a potential target for anticancer therapy. In humans, P450 1A1 and 1A2 are believed to be the major enzymes that catalyze the activation of procarcinogenic polycyclic aromatic hydrocarbons (PAHs). However, several studies have established that P450 1B1 also plays a very important role in metabolic activation of PAHs (13,23,24). There have been several reports about the effects of the P450 1B1 gene knockout *in vivo*. In mice with a knockout of the P450 1B1 gene, lower rates of tumor growth and elevated protection against DNA adduct formation were observed when carcinogenic agents such as 7, 12-dimethylbenz[*a*]anthracene and dibenzo[*a,h*]pyrene were administered (23,25,26). In addition, P450 1B1 gene-knockout mice displayed attenuated tumor tissue metastasis induced by benzo[*a*]pyrene (27). These reports strongly imply that the mechanisms of P450 1B1-induced cell proliferation, migration, and invasion may have a considerable preclinical and clinical significance.

P450 1B1 is an efficient catalyst for estrogen hydroxylation. It catalyzes the 4-hydroxylation reaction of 17 β -estradiol (E_2) that produces the less active metabolite, 4-hydroxyestradiol (12,17,28,29). P450 1A2 and 3A4 can hydroxyl-

ate 17 β -estradiol but the major hydroxylated product is 2-hydroxyestradiol (30). 4-Hydroxyestradiol is believed to cause estrogen-dependent tumors (12). It can be converted to chemically more reactive species, quinones and semiquinones, which covalently bind DNA (31). Ortiz de Montellano's group reported that the mutation of Val395 to Leu in human P450 1B1 changed the specificity of the 17 β -estradiol reaction from 4-hydroxylation to 2-hydroxylation (32), which suggested that estradiol carcinogenicity of P450 1B1 depends on that single amino acid residue (32).

Chun and colleagues previously described a selective and potent inhibitor of P450 1B1 and its utility in preventing cancer development (22). Presence of 2,4,3',5'-tetramethoxystilbene (TMS) inhibited EROD activity of P450 1B1 with an IC_{50} value of 6 nM, demonstrating a 500-fold selectivity over P450 1A2. TMS also strongly and selectively inhibited 4-hydroxylation of 17 β -estradiol by P450 1B1-expressing membranes (IC_{50} 90 nM) or purified P450 1B1 (IC_{50} 390 nM) (22,33). These studies showed that TMS is a selective and potent competitive inhibitor of P450 1B1 and can be considered as a prospective preventive agent for estrogen-dependent tumors. The molecular mechanism of cancer cell proliferation by P450 1B1 has been revealed recently. The transcription factor Sp1, involved in cell growth and metastasis, was found to be positively regulated by P450 1B1 (unpublished data). It is likely that P450 1B1 promotes cell

Table 2. Allelic variants of CYP1B1 with polymorphisms located in the coding region

Allelic variants	Nucleotide changes	Amino acid changes	References
<i>CYP1B1</i> *2	142C>G; 255G>T	R48G; A119S	(35,37)
<i>CYP1B1</i> *3	4326C>G	L432V	(35)
<i>CYP1B1</i> *4	4390A>G	N453S	(35)
<i>CYP1B1</i> *5	142C>G; 4326C>G	R48G; L432V	(38)
<i>CYP1B1</i> *6	142C>G; 355G>T; 4326C>G	R48G; A119S; L432V	(38)
<i>CYP1B1</i> *7	142C>G; 355G>T; 4326C>G; 4360C>G	R48G; A119S; L432V; A443G	(38)
<i>CYP1B1</i> *8	4326C>G; 4353G>C; 4379C>T	L432V; D441H	Rahman <i>et al.</i> , unpublished
<i>CYP1B1</i> *9			
<i>CYP1B1</i> *10			
<i>CYP1B1</i> *11	171G>C	W57C	(35)
<i>CYP1B1</i> *12	182G>A	G61E	(35)
<i>CYP1B1</i> *13	501_502insT	167Frameshift	(35)
<i>CYP1B1</i> *14	841G>T	E281X	(35)
<i>CYP1B1</i> *15	863_864insC	288Frameshift	(34)
<i>CYP1B1</i> *16	Large deletion	Splicing defect	(34)
<i>CYP1B1</i> *17	4096_4108del	355Frameshift	(34)
<i>CYP1B1</i> *18	4125G>T	G365W	(35)
<i>CYP1B1</i> *19	4168C>T	P379L	(35)
<i>CYP1B1</i> *20	4191G>A	E387K	(35)
<i>CYP1B1</i> *21	4201G>A	R390H	(35)
<i>CYP1B1</i> *22	4232_4241dup	404Frameshift	(35)
<i>CYP1B1</i> *23	4342C>T	P437L	(35)
<i>CYP1B1</i> *24	4377delG	449Frameshift	(35)
<i>CYP1B1</i> *25	4437C>T	R469W	(35)
<i>CYP1B1</i> *26	4435_4461dup	477Frameshift	(35)

Adopted from "http://www.cypalleles.ki.se/".

proliferation and metastasis by inducing the epithelial-mesenchymal transition (EMT) and Wnt/ β -catenin signaling via Sp1 induction (unpublished data). This study suggests that Sp1 acts as a key mediator in the promotion of cancer cell proliferation and metastasis stimulated by P450 1B1.

P450 1B1 POLYMORPHISMS

Genetic polymorphisms can dramatically alter specific actions of affected proteins. In particular, polymorphisms in genes encoding P450 enzymes have a considerable impact on the fate of xenobiotics, since these subtle DNA changes represent the most frequent cause of variations in oxidative metabolism of drugs or bioactivation of toxicants (5).

To date, at least 25 allelic variants of P450 1B1 have been identified, all of which are located in the coding region (Table 2) (<http://www.cypalleles.ki.se/>). Stoilov *et al.* first identified 19 allelic variants of P450 1B1 during the search for genetic variations associated with primary congenital glaucoma (34,35). P450 1B1-deficient mice exhibit abnormalities in their ocular drainage structure and trabecular meshwork that are similar to those reported in human primary congenital glaucoma patients (36). The mechanism of these impairments is still unclear but P450 1B1 is possibly involved in the metabolism of steroids, retinol and retinal, arachidonate, and melatonin (12,36).

Ingelman-Sundberg and colleagues characterized two common P450 1B1 mutations, R48G and A119S (37). The steady-state kinetic analysis showed no differences in 17 β -estradiol hydroxylation activities in this P450 1B1*2 mutant protein and only a minor increase in the apparent K_m for EROD was observed (37). In another study, three novel allelic variants, P450 1B1*5 (R48G/L432V), *6 (R48G/A119S/L432V), and *7 (R48G/A119S/L432V/A443G) have been identified in Ethiopian population and functional consequences of these mutations have been analyzed (38). The frequencies of P450 1B1*5, *6, and *7 were 0.7, 6, and 7%, respectively (38). Recombinant P450 1B1*6 and *7 exhibited altered kinetics with a significantly high apparent K_m and low k_{cat} values for the hydroxylation of 17 β -estradiol (38).

Recently, five non-synonymous SNP allelic variants (W57X, 290Frameshift, Y81N, E229K, and R368H) were detected in coloboma/microphthalmia patients (<http://www.cypalleles.ki.se/>) (39). It is known that P450 1B1 can contribute to retinoic acid synthesis during embryonic development and, at the same time, retinoic acid receptor signaling regulates choroid fissure closure. Functional consequences of expression of these novel P450 1B1 variants observed in that study suggest that P450 1B1 may regulate proper optic fissure closure by affecting retinoic acid signaling (39).

CONCLUSIONS

In this review, we considered mechanisms of metabolic

activation of chemical carcinogens by P450 1B1. Development of selective P450 1B1 inhibitors with increased therapeutic effectiveness is a promising avenue to control cancer growth and metastasis. Pharmacological roles of P450 1B1 in metabolism of clinical drugs have not been intensively studied.

The risks of metabolic activation of chemical carcinogens in different individuals vary because of polymorphisms in genes encoding metabolic enzymes. P450 1B1 polymorphisms have been implicated as risk factors in various diseases, which may arise from impaired P450 1B1 enzymatic activity. It is therefore extremely important to carefully consider the functional significance of genetic variability of P450 enzymes in the initiation and progression of cancer.

ACKNOWLEDGEMENT

This paper was supported by Konkuk University in 2013.

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