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P450 and Human Cancer

Kaname Kawajiri[1] and Yoshiaki Fujii-Kuriyama[2]

[1]*Department of Biochemistry, Saitama Cancer Center Research Institute, Ina-machi, Kitaadachi-gun, Saitama 362 and* [2]*Department of Chemistry, Faculty of Science, Tohoku University, Aoba, Sendai 980*

Most of the chemical carcinogens in our environment are activated mainly by a restricted number of cytochrome P450 species, P450 1A1, 1A2, 2E1, and 3A. This metabolic activation of procarcinogens is a crucial part of the initial host response to the environmental exposure, since most chemical carcinogens do not show any carcinogenicity by themselves. Inter-individual variability in the metabolic activity may thus be a key host factor to explain the differences in susceptibility to chemical carcinogenesis among individuals. Recent studies on P450s in cancer etiology have provided some valuable insights into this problem.

Key words: Cytochrome P450 — Metabolic activation — Cancer etiology

A large proportion of human cancers is known to be caused by synthetic or natural compounds in the environment.[1] Initiation of tumorigenesis by chemical compounds requires at least three successive reactions *in vivo*[2]: 1) most chemical carcinogens, which are not chemically reactive by themselves, must be metabolically converted into reactive forms, 2) adduct formation between these reactive metabolites and DNA somehow evades biological DNA repair processes and possibly causes base changes or DNA rearrangements, 3) these DNA alterations are fixed and lead to oncogene activation. Thus, the metabolic activation of carcinogens is an obligatory initiation step in chemical carcinogenesis, and a microsomal NADPH-dependent monooxygenase system, including some specific forms of P450, plays an essential role in oxidative activation of chemical compounds.[3]

P450 is a superfamily consisting of a large number of hemoproteins that catalyze the oxidation of a wide variety of structurally unrelated compounds ranging from endogenous substrates such as steroids, fatty acids, and prostaglandins to exogenous chemicals including drugs and lipophilic xenobiotics.[2,4–6] Some specific forms of P450 which catalyze oxidative metabolism of exogenous chemicals are also involved in the metabolic activation of chemical carcinogens.[7]

Numerous studies on the roles of specific forms of P450s in bioactivation of chemical carcinogens have been conducted by using Ames' mutation test system[8–10] and the *umu* mutation system.[11] Three methods have been applied to identification of specific forms of P450s involved in the metabolic activation of carcinogens; one is based on reconstituted systems of microsomal monooxygenase using purified P450 preparations,[12] the second is inhibition tests of the biotransforming activity in intact microsomes using specific antibodies against P450 molecules,[13] and the last uses an expression system of the isolated cDNAs driven by an appropriate promoter in cultured cells.[14]

Besides the approaches based on experimental animals, recent developments, especially in the field of molecular biology, have provided us with more direct biochemical information on human P450 proteins and genes.[4–6] At present, 10 families comprising 16 subfamilies of human P450 (Table I) are known, and have been investigated from the viewpoints of structure, function and regulation.[15] The contribution of each species of human P450 to bioactivation of various carcinogens has been evaluated extensively using cDNA- or gene-directed expression of P450 and human liver microsomes in combination with specific antibodies.[16–23] It is established that some of the human P450 species have notably different catalytic activities towards various carcinogens from those expected on the basis of the studies with experimental animals, and that the gene expression was in some cases different between human P450s and animal

Abbreviations: TCDD, 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin; XRE, xenobiotics-responsive element; BTE, basic transcription element; AHH, aryl hydrocarbon hybroxylase; 2-AA, 2-aminoanthracene; 2-AF, 2-aminofluorene; 2-AAF, 2-acetylaminofluorene; 4-ABP, 4-aminobiphenyl; 2-NA, 2-naphthylamine; Trp-P-1, 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole; Trp-P-2, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole; Glu-P-1, 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole; Glu-P-2, 2-aminodipyrido[1,2-*a*:3′,2′-*d*]imidazole; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; MeIQ, 2-amino-3,5-dimethylimidazo[4,5-*f*]quinoline; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; Dimethyl-IQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; NDMA, N-nitrosodimethylamine; NA, nitrosoamines; CAT, chloramphenicol acetyltransferase; NDEA, N-nitrosodiethylamine.

orthologues, although some of the structural and functional characteristics were shared.[4, 5]

Individual difference in susceptibility to chemical carcinogens is one of the most important subjects in the risk assessment of human cancers.[24] Although many different types of host factors may be involved in the differences in susceptibility, genetic differences in metabolism of carcinogens have been suggested to be associated with different predispositions to cancers. Thus, one of the most important targets in human P450 research is to examine whether inter-individual variability in phenotypic levels of specific P450s is associated with susceptibility to chemically induced cancers.[4, 7] Although phenotypic variability of some drug oxidation activities among individuals was clearly shown to be associated with genetic polymorphisms of P450 genes,[25–29] etiological association between cancer incidences and genetic variations of P450 is controversial and is being reinvestigated using newly developed technology.[30–37]

With this background, the current state of knowledge on the relationship between human P450s and cancer is briefly reviewed here.

Overview of Human P450 Enzymes

Table I lists structurally characterized human P450 species[15] together with some of their features, including chromosomal localization, numbers of amino acid residues, main organs of P450 expression, and typical carcinogens activated. The nomenclature used in this article is that described by Nebert *et al.*[15] An evolutionary tree of human P450s is displayed in Fig. 1, in which major branch points of P450 divergence were taken from Gotoh.[38] From this figure and table, the following conclusions may be reached. 1) All P450 molecules in humans have evolved from a common ancestor gene. 2) After the first divergence at about 1.5×10^9 years ago between microsomal and mitochondrial species, the number of microsomal P450 species has greatly increased, accompanied with changes in substrate specificities, from rigid specificities toward steroids to loose ones toward xenobiotics. 3) P450s responsible for activation of chemical carcinogens mainly cluster in three families, P450 1, 2 and 3, which are known as drug-metabolizing P450s. It has been suggested that the

Table I. Update of Human P450s Structurally Characterized[15] Together with Some of Their Features, Including Chromosomal Localization, Numbers of Amino Acid Residues, Main Organs of P450 Expression, and Typical Carcinogens Activated

P450 gene symbol	Trivial name	Chromosomal location	Amino acid residues	Organs	Carcinogens activated
CYP1A1	P_1, c, form6	15q22-qter	512	Extrahepatic	B(a)P, 2-AAF etc.
1A2	P_3, d, form4	15	516	Liver	2-AAF, 4-ABP, Glu-P-1, IQ, AFB_1, Trp-P-2, 2-AF etc.
CYP2A6	IIA3, P450(I)	19q13.1-13.2	494	Liver	AFB_1, NDEA etc.
2B7	IIB1	19q12-q13.2	491	Liver	AFB_1
2C8	form 1, IIC2		490	Liver	
2C9	IIC1, mp-4	10q24.1-24.3	490	Liver	
2C10	mp, mp-8		490	Liver	
2D6	db1	22q11.2-qter	497	Liver	
2E1	j	10	493	Liver	NDMA, NDEA etc.
2F1	IIF1	19q12-13.2	491	Lung	
CYP3A3	HLp		504	Liver	AFB_1
3A4	NF		503	Liver	AFB_1, B(a)P-7, 8-diol, 6-AC etc.
3A5	hPCN3, HLp2	7q21.3-q22	502	Liver	
3A7	HFL33		503	Liver	AFB_1, IQ
CYP4B1	IVB1, P450HP	1p12-p34	511	Placenta, Lung	
CYP7	7α		504	Liver	
CYP11A1	SCC	15	521	Steroidogenic	
11B1	11β	8q21-22	503	Adrenal	
CYP17	17α	10	508	Steroidogenic	
CYP19	arom	15q21.1-21.3	503	Steroidogenic	
CYP21A2	C21B	6p21.3	494	Adrenal	
CYP27		2q33-qter	499	Liver	

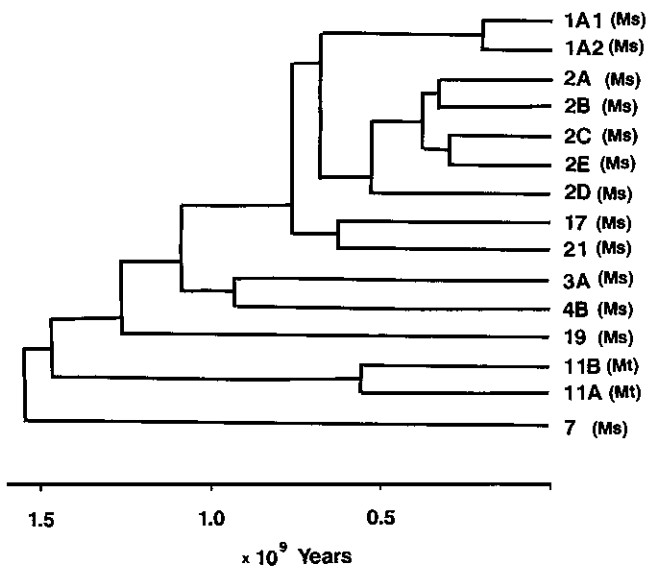

Fig. 1. The phylogenetic tree of human P450 proteins. This is a portion of an overview of all P450 proteins.[38] Ms, microsomal P450; Mt, mitochondrial P450.

increasing numbers of drug-metabolizing P450 genes may have coincided with the adaptation to terrestrial life of the vertebrates, one aspect of which would have been the need to detoxify toxic lipophilic compounds present in ingested land vegetation by oxidation. However, oxidized chemicals are sometimes more toxic than their precursors, even being mutagenic or carcinogenic. Thus, drug-metabolizing P450s seem to work as a double-edged sword for either detoxication or activation of chemical compounds.

P450s Responsible for Bioactivation of Carcinogens

a) P450 1A1

Although P450 1A1 has not so far been isolated from human tissues, considerable information on both its structure and function was obtained by the use of molecular cloning technology. The overall sequence similarity of human 1A1[39-41] to the rat[42,43] and mouse[44] orthologues amounts to 78 and 80% in amino acids, and 80 and 83% in coding nucleotide sequence, respectively. Human 1A1 (512 amino acid residues) is smaller by 12 amino acids than the rodent equivalent (524 residues). In experimental animals, 1A1 is induced in both the liver and extrahepatic tissues by exogenous polycyclic hydrocarbons such as benzo[*a*]pyrene, TCDD, and 3-methylcholanthrene.[45,46] In contrast, 1A1 in human is considered to be primarily an extrahepatic enzyme, because considerable amounts of both mRNA and protein could be detected in lung,[47-49] lymphocytes[40,50] and placenta,[50] in contrast with an undetectable level of the mRNA and protein in most human livers examined.[19]

The contribution of 1A1 to bioactivation of benzo[*a*]-pyrene in human liver was estimated by determining the catalytic activity after cDNA- or gene-directed expression of the enzyme molecules.[19] Human 1A1 can metabolize benzo[*a*]pyrene to mutagenic active forms, but its contribution to the activation process of benzo[*a*]pyrene in human liver appears to be minor because of its marginal content. Instead, recent reports by McManus *et al.*[19] and by Shimada *et al.*[20,21] showed a large contribution of P450 3A4, not 1A1, to the bioactivation of benzo-[*a*]pyrene in human liver. However, since one of the main target organs of benzo[*a*]pyrene-induced human cancer is the lung, a major role of 1A1 in the extrahepatic metabolism of benzo[*a*]pyrene cannot be ruled out. In fact, studies with normal lung tissues indicated that significant levels of mRNA for human 1A1 were expressed among active cigarette smokers but not among non-smokers.[47]

Regulatory gene expression of 1A1 has been investigated by the use of rodent equivalents. It was clearly demonstrated that at least two different types of *cis*-acting DNA elements were required for a high level of inducible expression of the gene in response to xenobiotics such as TCDD and 3-methylcholanthrene.[51-54] One was an inducible enhancer designated XRE (xenobiotics-responsive element), which was localized far upstream of the gene and exhibited an inducible enhancer activity in response to added inducers.[51-53] The other was located in the region proximal to the TATA sequence of the gene and acted as a promoter element to regulate the basic transcription activity; it was designated BTE (basic transcription element).[54] Several lines of evidence suggest that the TCDD-receptor interacts with the XRE sequence, and that Sp1 or BTEB factor interacts with BTE. Since homologous sequences were also found in human gene,[40,41] the mode of human 1A1 gene regulation[55,56] seems to be fundamentally the same as that of the rodent genes. The mechanism of extrahepatic expression of human 1A1 gene remains an important topic for future study.

About two decades ago,[30,57] it was suggested that high AHH inducibility was an important risk factor for human lung cancer. High AHH inducibility of peripheral blood lymphocytes was more frequently observed among lung cancer patients than it was among patients with benign diseases. A rationale for such an association was that individuals with high inducibility of AHH could readily and efficiently activate the carcinogens in cigarette smoke. Although the results were very controversial, the association between AHH and human lung cancer has recently been confirmed by using an improved

assay system of lymphocyte AHH.[31, 32] The observed trimodal inducibility of AHH[57, 58] was suggested to be genetically determined. If this is the case, it may be reasonable to conjecture that levels of inducible expression of 1A1 in human lung may correspond to lymphocyte AHH inducibility. Apart from the genetic difference among individuals, induction of 1A1 mRNA in lung by cigarette smoking[47] and the formation of benzo[*a*]-pyrene-DNA adducts within pulmonary tissues from active cigarette smokers[59] also suggest that the level of inducible expression of 1A1 in human lung is important in lung cancer etiology.

Human P450 1A1 gene shows a genetic polymorphism in the 3′-flanking region with restriction nuclease *Msp* I.[60] Using this *Msp* I polymorphism, investigations on the inter-individual differences in susceptibility to lung cancer were carried out by two different approaches: i.e., comparison of the genotype frequencies between patients and the healthy population,[35, 36] and relative risk estimation of people with a susceptible genotype in relation to cigarette smoking dose.[36, 61] The results can be summarized as follows: 1) significant differences in frequencies of the genotypes were observed between patients with lung cancer and healthy controls, and the observed high-risk elevation of genotype C (homozygous rare allele having an *Msp* I site) was specific to Kreyberg I type, but not to Kreyberg II (adenocarcinoma), 2) an intra-patient study on cumulative cigarette doses in relation to squamous cell carcinoma revealed that patients with genotype C contracted the carcinoma after a smaller number of cigarettes smoked as compared with other genotypes; a case-control study showed that the individuals with this susceptible genotype were at a remarkably high risk (odds ratio 7.31) at a low dose level of cigarette smoking and that the difference in susceptibility between genotypes reduced at high dose levels.

Recently, Petersen *et al.*[62] reported a possible correlation between the AHH inducibility phenotype and the *Msp* I polymorphism. In one family of 3 generations consisting of 15 individuals, they found that individuals with genotype B (heterozygote) segregated with higher AHH inducibility than those with genotype A (predominant homozygote), although ones with genotype C were not included. It remains to be seen how the presence of this *Msp* I site may influence AHH inducibility. Further work should be done with larger numbers of individuals, including ones with genotype C, before a definite conclusion can be drawn concerning possible correlation between AHH inducibility phenotypes and the *Msp* I polymorphism. In this context, it is interesting to note that a novel point mutation in the coding region of the gene resulted in an amino acid substitution of Ile for Val at residue 462 in the heme-binding region, which is strongly linked to the *Msp* I polymorphism.[63]

b) P450 1A2

Human P450 1A2 has been successfully purified from liver microsomes as an enzyme catalyzing phenacetin O-deethylation.[25, 64] This protein was considered to be the orthologue of rat P450d or mouse P_3-450, a species of P450 with a high-spin state, from their N-terminal amino acid sequences determined by protein chemistry and complete primary structures deduced from cDNA sequences. The predicted amino acid sequence of human 1A2[65] shares 70 and 69% identical amino acids with its rat[66] and mouse[44] counterparts, respectively. Similarity of the two molecular species of the 1A subfamily in humans, 1A1 and 1A2, is 68% in amino acid sequence. The human 1A2 carries 4 more amino acid residues than the 1A1 does. This is opposite to the situation in the rat and mouse, where the number of constituent amino acids of 1A2 (513 residues) is smaller by 11 amino acids than that of 1A1 (524 residues).

An extensive investigation has been performed on the roles of 1A2 in metabolic activation of various carcinogens[16–20] and it was concluded that the enzyme activated a series of aromatic amines (2-AA, 2-AF, 2-AAF, 4-ABP, 2-NA), heterocyclic amines (Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2, IQ, MeIQ, MeIQx, Dimethyl-IQx), and aflatoxin B_1. Some of these carcinogens have been identified as causative agents in occupational carcinogenesis, and they also exist in cigarette smoke, agricultural chemicals, and cooked foods. Since a good correlation was found between the mutagenic activity and 1A2 content in microsomes of individual livers,[19, 20] the levels fo hepatic 1A2 may be an important determinant of the susceptibility of different individuals to arylamine-induced cancers.

Large inter-individual differences in the levels of hepatic 1A2 were suggested from the results of several earlier studies including the N-oxidation activity toward 2-acetylaminofluorene,[67] 2-naphthylamine[68] and 4-aminobiphenyl,[69] and the O-deethylation activity toward phenacetin.[69] In fact, content of human 1A2 was found to vary up to 10-fold among individuals.[19, 64] It was found that human hepatic microsomal caffeine 3-demethylation was selectively catalyzed by 1A2.[70] As caffeine is a commonly ingested and relatively innocuous compound, determination of the 3-demethylated product in human urine may be useful in the estimation of the P450 1A2 activity of individuals. This could provide us with a useful tool to investigate how P450 1A2 activity in human individuals is correlated to the susceptibility to cancers. Unfortunately, genetic polymorphisms responsible for inter-individual variability of the P450 1A2 activity have not yet been determined.

P450 1A2 is expressed predominantly in liver cells and is induced by isosafrole[45, 46] in addition to polycyclic

hydrocarbons such as benzo[*a*]pyrene, TCDD, and 3-methylcholanthrene. In humans, 1A2 is induced by cigarette smoking. The increase in hepatic 1A2 mRNA is due to both transcriptional activation and mRNA stabilization.[71] Transcriptional regulation of human 1A2 gene was studied through functional analysis of the 5′-flanking sequence.[72] Transfection of the 5′-flanking sequences of the gene fused to bacterial CAT gene resulted in tissue-specific CAT expression that was regulated by 3-methylcholanthrene. Since consensus sequences of both the binding regions of liver-specific transcription factor HNF-1 and XRE are localized in the 5′-flanking region of the gene, a further study on synergistic effects of the two elements on gene expression would be desirable.

c) P450 2E1

P450 2E1 was first purified and characterized from rabbits as an ethanol-inducible form of P450 named P450 LM3a[73] and later P450j[74] from rats and P450HLj[75] or P450ALC[76] from humans. The purified 2E1 enzyme metabolizes substrates such as N-nitrosodimethylamine (NDMA)[77] as well as aniline, ethanol and acetone. The rabbit,[78] rat,[79] and human[79] cDNAs for 2E1 have been characterized, and their deduced amino acid sequences share about 80% similarity. The number of amino acid residues of P450 2E1 is 493 in all these species. Only a single gene for 2E1 was observed in rats[80] and in humans,[81] whereas two highly homologous genes were found in rabbits.[82]

Nitrosoamines (NA) are potent carcinogens for many animals and are believed to be important in human carcinogenesis.[83] One nitroso compound, NDMA, is widely distributed in the environment, and has been detected in food, alcoholic beverages, cigarettes and polluted air, in addition to its endogenous formation in the gut from amines and nitrite.[84] The contribution of human 2E1 to bioactivation of NDMA has been estimated by several approaches. 1) NDMA demethylase activity in human liver microsomes[85] and in a reconstituted system of purified enzymes[75] was inhibited by antibody against rat 2E1. 2) A good correlation between 2E1 content in human liver microsomes and NDMA demethylase activity was observed.[86] 3) A full-length cDNA for human 2E1 was expressed in COS 1 cells and the expressed protein was found to show demethylase activity toward NDMA.[81] 4) cDNA-expressed human 2E1 metabolized NDMA to mutagenically active forms.[87] However, it still remains to be established whether levels of 2E1 expression are correlated with nitrosoamine-associated cancers or not.

Levels of 2E1 expression are regulated at both transcriptional and post-transcriptional steps in rats. The 2E1 gene is transcriptionally activated immediately after birth, and this activation of the gene occurs in parallel with demethylation of certain cytosine residues at the 5′ end of the gene.[80] Administration of inducers such as acetone, pyrazole, and ethanol to rats causes an increase of 2E1, mainly due to protein stabilization.[88] On the other hand, 2E1 also increased under some physiological conditions such as starvation[89] and diabetes,[90] where stabilization of mRNA seems to be involved.

Variable levels of expression of either 2E1 mRNA or protein were reported in the livers of human individuals.[85] Since ethanol consumption increases the incidence of human esophageal or liver cancer, possibly as a result of changed metabolism of nitrosoamines,[91] the enhanced level of 2E1 expression in hepatic and extrahepatic tissues induced by ethanol or other factors may play an essential role in nitrosoamine-induced cancers. Recently, it was found that a genetic polymorphism in the 5′-flanking region with *Rsa* I[92] resulted in different transcriptional regulation of human 2E1 gene.[93] A DNA segment of the 5′-flanking region from a homozygous rare allelic gene enhanced the expression of the fused CAT gene 10 times more than that of the predominant homozygote. From DNase I footprint analysis and gel retardation assay, it was found that this polymorphic region interacted with a transcription factor, possibly HNF-1, as suggested by the existence of the consensus sequence of the HNF-1 binding site. Although the HNF-1 binding site consensus sequence was also found in the promoter region of the human gene as well as the rat equivalent,[94] the same binding site sequence in the promoter was observed in both genotypes. This may indicate that this genetic polymorphism in the distal regulatory region participates in inter-individual differences of microsomal NDMA demethylase activity through an effect on transcriptional activation.

d) P450 3A

The human P450 3A gene subfamily codes for proteins that appear to be involved in metabolism of testosterone 6β-hydroxylation in liver.[4] They also metabolize many clinically important drugs such as nifedipine (calcium channel blocker), cyclosporin (immunosuppressant), 17α-ethynylestradiol (contraceptive agent), and erythromycin (antibiotic). At present, four P450s have been identified in this subfamily. These were designated as HLp (3A3),[95] NF (3A4),[96] hPCN3 (3A5),[97] HLp2 (3A5),[98] and HFL33 (3A7).[99] The amino acid sequences of these P450s deduced from their cDNA sequences contained 504, 503, 502, and 503 residues, respectively. The deduced N-terminal amino acid sequence of the HFL33 protein was identical to that of P450HFLa, a major P450 expressed in human fetal liver.[100]

P450 3A4 apperas to be a major enzyme involved in metabolic activation of aflatoxin B_1, aflatoxin G_1, sterigmatocystin, *trans*-7,8-dihydroxy-7,8-dihydrobenzo-

[*a*]pyrene, 6-aminochrysene, and tris-(2,3-dibromoprophyl)phosphate.[20] This was indicated by positive correlations observed among mutagenic activity, nifedipine oxidation activity, and 3A4 levels in adult human liver microsomes. Recently, it was found that P450s 1A2, 2A3, 2B7, 3A3, and 3A4 activated aflatoxin B_1 to mutagenically active forms, using P450 preparations obtained by cDNA-directed expression in the Ames test system.[17] P450 3A7 also participates in the activation of aflatoxin B_1 and IQ in fetal liver.[23] Accordingly, human liver microsomes have a large capacity to activate aflatoxin B_1, but the particular form of P450 that mainly contributes to the activation of this carcinogen is different among individuals depending on the levels of the P450 molecules in liver. In fact, the level of each of the 3A proteins differs among individual human livers by 5-fold to more than 10-fold.[19, 20] Some of these forms can be induced in humans by glucocorticoids.[95] Individual variability in hepatic 3A enzymes could, therefore, have serious consequences in drug therapy as well as in the activation of chemical carcinogens.

e) Others (P450 2A, 2B, 2D)

It was reported that several species of human P450 in P450 2 family metabolized some carcinogens to mutagenically active forms. P450 2A3, coumarin 7-hydroxylase in human liver,[101, 102] was capable of metabolizing aflatoxin B_1, benzo[*a*]pyrene, N-nitrosodiethylamine (NDEA), and NDMA to cytotoxic and mutagenic forms.[103] P450 2A3 was more effective in activating NDEA than P450 2E1, while NDMA was more effectively activated by 2E1 than by 2A3. P450 2B7 also activated aflatoxin B_1 to mutagenic forms.[17] The participation of these P450s in chemical carcinogenesis cannot be assessed at present because of insufficient data.

Debrisoquine is an adrenergic blocking agent used to reduce blood pressure in hypertensive patients. The 4-hydroxylation of debrisoquine is catalyzed by P450 2D6 in human liver microsomes.[26, 104] In population studies, two distinct phenotypes have been described: extensive metabolizers (EM) excreted 10–200 times more of the urinary metabolite 4-hydroxydebrisoquine than poor metabolizers (PM).[105] The molecular basis of this phenotypic polymorphism was ascribed to abnormal mRNA splicing, resulting in an extremely low level of translatable mRNA.[27] Ayesh *et al.*[33] indicated that rapid metabolism phenotype (EM) could be associated with increased incidence of lung bronchogenic carcinomas. Caporaso *et al.*[37] confirmed by a more refined epidemiological method that this association is independent of smoking or asbestos exposure. An association between debrisoquine hydroxylation phenotypes and bladder cancer incidence was also shown.[34] Despite the broad metabolic activity of P450 2D6 towards various drugs, its role in bioactivation of specific carcinogens remains unknown. It seems likely that the 2D6 gene is linked with another gene directly involved in chemical carcinogenesis.[27]

In summary, since human carcinogenesis is a result of multiple biological reaction steps, it may be difficult to demonstrate the direct involvement of P450s in cancers. In spite of the essential role of metabolic activation of procarcinogens in chemical carcinogenesis, it remains to be established whether this step is rate-limiting. Rigorous investigations employing more direct approaches with human P450 species, cDNAs or genes are in progress to examine whether the variations in the genotype or the level of expression of P450 govern individual susceptibility and resistance to cancer incidence. Answers to these questions should lead to major advances in our understanding of the relationship between P450 and chemical carcinogenesis in humans.

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