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CYP1A1 Gene: A Cancer Risk Modifier

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Review Article

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วารสารไทยเภสัชศาสตร์และวิทยาการสุขภาพ 2554;6(3):240-250

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Thai Pharmaceutical and Health Science Journal 2011;6(3):240-250

บทคัดย่อ

ยีนไซโตโครม พี 450 1 เอ 1 (CYP1A1) เป็นยีนเป้าหมายสำคัญหนึ่งของมะเร็ง เนื่องจากมีหน้าที่หลักในการเปลี่ยนแปลงทางชีวภาพของสารกลุ่ม polycyclic aromatic hydrocarbon (PAHs) ให้อยู่ในรูปสารกึ่งกลางจำพวก epoxide หรือสารก่อมะเร็งที่มีฤทธิ์สูงสุดซึ่งสามารถกระตุ้นกระบวนการกลายพันธุ์ในสายดีเอ็นเอที่เกี่ยวข้องกับการเกิดมะเร็งได้ mRNA ของ CYP1A1 จะถูกเหนี่ยวนำให้เพิ่มขึ้นด้วยการกระตุ้นการจับระหว่างตัวรับ aryl hydrocarbon (AhR) กับ ligand)ได้แก่ สาร PAH ที่มีอนุพันธ์ของ 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) เป็นสารต้นแบบ จากนั้นสารประกอบของตัวรับ AhR และ ligand จะผ่านเข้าสู่นิวเคลียสอันเป็นจุดเริ่มการกระตุ้นยีน CYP1A1 ดังนั้นการรบกวนกลไกการควบคุมใด ๆ จะส่งผลต่อกระบวนการเจริญเติบโตของเซลล์มะเร็ง ยิ่งไปกว่านี้การเพิ่มการทำงานของ protein kinase C (PKC), tyrosine kinase และการกระตุ้น mitogen-activated protein kinases (MAPKs) จะส่งผลให้เพิ่มการส่งสัญญาณของตัวรับ AhR นอกจากนี้ glucocorticoid receptor (GR) และ estrogen receptor (ER) ยังแสดงผลร่วมต่อกระบวนการส่งสัญญาณของตัวรับ AhR ได้ อาทิ GR สามารถลดการแสดงออกของยีน CYP1A1 ที่แสดงผลผ่านกลไกของตัวรับ AhR โดยจับกับองค์ประกอบของตัวรับ AhR หรือองค์ประกอบภายในยีนที่ตอบสนองต่อสารแปลกปลอม (xenobiotic responsive element) ในขณะที่ ER สามารถส่งผลโดยตรงต่อส่วนโปรโมเตอร์ภายในยีน CYP1A1 โดยเป็นเสมือนตัวควบคุมร่วมในการเหนี่ยวนำผ่านตัวรับ AhR ในสภาวะปกติตัวรับ AhR มีบทบาทส่งเสริมการเจริญเติบโตของเซลล์แต่ตัวรับ AhR สามารถแสดงผลตรงกันข้ามในสภาวะที่มีลิแกนด์ เนื่องจากยีน CYP1A1 สามารถพบได้ในเนื้อเยื่อหลายชนิด เช่น ปอด รก ตับและไต แม้ว่าจะพบยีนนี้ในตับเมื่อถูกเหนี่ยวนำด้วยสารกระตุ้นต่าง ดังนั้นกระบวนการเจริญของมะเร็งที่เกี่ยวข้องกับการแสดงออกที่มากเกินไปของยีน CYP1A1 จึงมีโอกาสเกิดขึ้นในอวัยวะหลายชนิดจากการได้รับลิแกนด์ภายนอกที่ปนเปื้อนอยู่ทั่วไปในสภาวะแวดล้อม นอกจากนี้ความหลากหลายทางพันธุกรรมของยีน CYP1A1 พบว่าสัมพันธ์กับโอกาสเกิดมะเร็งหลายประเภทด้วย

คำสำคัญ: ยีนไซโตโครม พี 450 1 เอ 1, ตัวรับแอริลไฮโดรคาร์บอน, มะเร็ง, AhR

Abstract

CYP1A1 gene is an important target in cancer, due to its role in metabolic bioactivation of polycyclic aromatic hydrocarbons (PAHs), to electrophilic intermediates referred to bay region epoxides, to ultimate carcinogens and capable of causing oncogenic mutations in DNA. Expression of CYP1A1 mRNA is elevated by activation of the arylhydrocarbon receptor (AhR) through binding of exogenous ligands such as PAHs, of which the halogenated derivative 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a prototype, then translocates from cytoplasm to nucleus upon the activation. Hence, disruption of these regulatory pathways is implicated in tumor progression. In addition, up-regulation of protein kinase C (PKC) and tyrosine kinase, as well as activation of mitogen-activated protein kinases (MAPKs) result in increase of AhR signal transduction. Glucocorticoid receptor (GR) and estrogen receptor (ER) also affect AhR-mediated pathway, but undergoing via different aspects. Namely, GR decreases AhR-mediated expression by interacting with xenobiotic responsive element-binded AhR, while ER has a direct interaction with CYP1A1 promotor by acting as a co-regulator of AhR-mediated transcriptional activation. In normal condition, AhR plays a promoting role in cell cycle progression. In the existence of exogenous ligands, AhR shows inhibitory effect *vice vesa*. CYP1A1 is expressed constitutively in several extrahepatic tissues such as intestine, lung, placenta, and kidney, but not in liver. However, CYP1A1 expression has been demonstrated in the liver after inducer treatment. Therefore, cancer progression regarding overexpression of CYP1A1 possibly occurred in several organs related to exogenous ligands in every day exposures, e.g. smoking, diet, and the environment. Apart from these, genetic polymorphism of *CYP1A1* gene has recently been noted to involve in difference types of cancer.

Key words: *CYP1A1* gene, aryl hydrocarbon receptor, cancer, AhR

Introduction

Among phase I biotransformation, cytochrome P450 (P450) system ranks the first in catalytic versatility and the sheer number of xenobiotics it detoxifies or activates to reactive intermediates.[1] P450 is a microsomal superfamily of isoenzymes. All P450 enzymes are heme-containing proteins. The heme iron in P450 is usually in the ferric (Fe^{3+}) state. When it is reduced to the ferrous (Fe^{2+}) state, P450 can bind ligands. P450 is located on the smooth

endoplasmic reticulum of cells throughout the body. P450 enzymes are present in almost all tissues but the highest concentration of P450 enzymes is found in the endoplasmatic reticulum of the liver (microsomes).[2] The liver microsomal P450 enzymes involve in xenobiotic biotransformation belong to three main P450 gene families, namely *CYP1*, *CYP2* and *CYP3*. Liver microsomes also contain P450 enzymes encoded by *CYP4* gene family, the substrates of which include several fatty acids and eicosanoids but relatively few xenobiotics. The level and activity of each P450 enzyme have been shown to vary from one individual to the next, due to environmental and genetic factors.[3,4] In conclusion, cytochrome P450 enzymes play a dual role in the organism. On the one hand, they inactivate the drug/xenobiotic and prepare it for excretion. On the other hand, they are also capable of activating foreign chemicals to highly reactive toxic intermediates that might act as carcinogens or mutagens.

Cancer

Cancer continues to be a worldwide killer which is a complex disease where genetics, lifestyle, and environment play important roles indicating susceptibility to the disease. One defining feature of cancer is the uncontrolled growth because it has high cell proliferating rate and spread of cells. It can affect almost all part of the body. The growth often invades surrounding tissue and can metastasize to distant sites. Cancer arises from one single cell. The transformation from a normal cell into a tumor cell is a multistage process, typically a progression from a pre-cancerous lesion to malignant tumors. These changes are the result of the interaction between a person's genetic factors and external agents.[5] Environmental factors are transmitted to the cell by different regulatory mechanisms.[6]

CYP1A1: Cancer progression. Chemical carcinogens in environment are mostly chemically inert by themselves and require metabolic activation by cytochrome P450 (CYP) enzymes to more reactive metabolites in order to exhibit carcinogenicity in experimental animals and humans.[7] CYP1 family plays major roles in metabolic activation of a variety of environmental carcinogens. CYP1A1, one of the three members of CYP1 family, principally involved in metabolizing polycyclic aromatic hydrocarbons (PAHs), to reactive electrophiles intermediates, and subsequently form carcinogenic DNA adduct.[7] CYP1A1 is constitutively expressed in extrahepatic tissues, such as lung, kidney and placenta, and inducibly demonstrated in the liver after exposure to an inducer[8], thus CYP1A1 contributes to the incidence of cancers in these organs, at least in part. Expression of the *CYP1A1* gene is inducible in a ligand-dependent fashion by the aryl hydrocarbon receptor (AhR), when PAHs and other carcinogens are ingested into an animal's body[9] leading to increasing synthesis of these enzymes.

CYP1A1: Cancer prevention. It is widely accepted that CYP1A1 enzyme metabolically activates many PAHs, including benzo[*a*]pyrene (B[*a*]P), to DNA- and protein-binding intermediates that are associated with toxicity, mutagenesis, and carcinogenesis. In contrast, study of the role of CYP1A1 in an intact animal showed that CYP1A1 inducibility was essential in detoxication of oral B[*a*]P.[10] Therefore, the important role of CYP1A1 in the detoxification of environmental carcinogen became interesting according to many dietary compounds in the metabolic activation pathway to perform the cancer preventative activity.

Conclusively, the expression of CYP1A1 as cancer progression or preventive may depend on the balance of procarcinogen activation, detoxication, and naturally dietary natural product-extrahepatic metabolism.[11] The present review aims to comprehensively summarize about the bimodal roles of CYP1A1 in carcinogen-activating and cancer preventing.

Mechanism of Procarcinogen Activation by CYP1A1

CYP1A1 is responsible for activation of most carcinogenic polycyclic aromatic hydrocarbons (PAHs) to epoxide intermediates, which are further converted to more reactive diol-epoxides, i.e., 5-methylchrysene-1,2-diol, ($\pm$)benzo(*a*)-pyrene-7,8-diol, chrysene-1,2-diol, benzo(*b*)fluor-anthrene-9,10-diol, etc., with aid of epoxide hydrolase.[9] An important and very extensively studied prototype of PAHs widely accepted to demonstrate the activation process of the carcinogen is benzo[*a*]pyrene (B[*a*]P).

Metabolic activation of the prototype carcinogen B[*a*]P was extensively studied by the research groups of Jerina and Conney in the mid-1970's.[7] It was initially thought that B[*a*]P-4,5-epoxide, the so called K-region epoxide, was the ultimate carcinogenic metabolite.[12] However, the B[*a*]P-4,5-epoxide was found to be readily hydrolyzed by epoxide hydrolase to an inactive B[*a*]P-4,5-diol metabolite and was finally concluded not to play a major role in carcinogenesis by B[*a*]P.[7] Subsequent investigation of several B[*a*]P metabolites revealed that the diastereomeric B[*a*]P-7,8-diol-9,10-epoxides (Figure 1), commonly referred to as bay region epoxides, were highly reactive towards DNA. Hence, they were concluded as the ultimate carcinogenic metabolites of B[*a*]P. The pathways leading to the formation of B[*a*]P bay region epoxides are as the following.

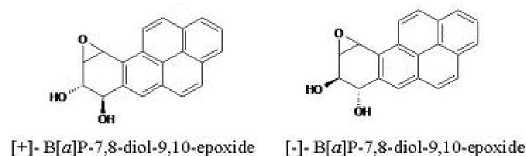

Figure 1 Chemical structure of diastereomeric B[*a*]P-7,8-diol-9,10-epoxides (Androutsopoulos et al, 2009).[11]

B[*a*]P is firstly oxidized by liver microsomes of MC-treated rats to (+)- and (-)-B[*a*]P-7,8 oxides, with the conversion rate of the (+) enantiomer being much higher than that of the (–) form. Subsequently, microsomal epoxide hydrolase rapidly hydrolyzes these oxides to produce (-)- and (+)-B[*a*]P-7,8-diol.[13] Last step is oxidative activation of each of these metabolites by P450s to produce four diol epoxides (Figure 2), namely (-)-B[*a*]P-7,8-diol-9,10-epoxide-1, (+)-B[*a*]P-7,8-diol-9,10-epoxide-2, (+)-B[*a*]P-7,8-diol-9,10-epoxide-1, and (-)-B[*a*]P-7,8-diol-9,10-epoxide-2 respectively[14], which are highly mutagenic to *Ames Salmonella* tester strains and Chinese Hamster V-79 cells. Additionally, the epoxides are denoted as bay region epoxides due to their ability to cause oncogenic mutations in specific parts of the DNA.[11]

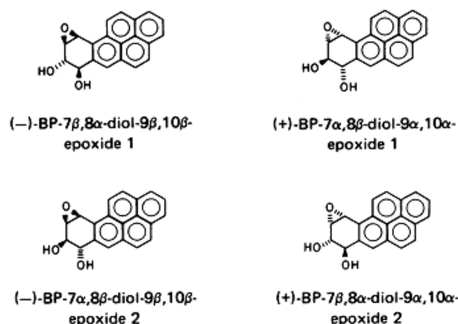

Figure 2 Absolute stereochemistry of the optical enantiomers of the diastereomeric BP-7,8-diol-9,10-epoxides derived from BP-7,8-dihydrodiol (Buening et al, 1978).[14]

The metabolite (+)-B[*a*]P-7,8-diol-9,10-epoxide-2 was identified as the most reactive of the four metabolites in producing tumors in newborn mice. This diol epoxide was considered to be the ultimate carcinogenic conversion product of B[*a*]P, because it had almost the same level of carcinogenicity as B[*a*]P itself and (-)-B[*a*]P-7,8-diol. This diol epoxide is considered to be the ultimate carcinogenic metabolite of B[*a*]P.[7,15]

The bay region theory has also been applied to other PAHs to investigate their carcinogenic activation by CYPs and epoxide hydrolase and found the result as same metabolic activation pattern as B[*a*]P.[7]

Other typical PAHs have been investigated for their carcinogenic actions and considered to be activated in accordance with the bay region theory including benz[*a*]-anthracene, benzo[*b*]fluoranthrene, benzo[*c*]phen-anthrene, chrysene, benzo[*g*]chrysene and 5,6-dimethyl-chrysene and 5-methylchrysene[16] (Figure 3). Certainly, these reactive intermediates of PAHs dihydrodiols were activated selectively in *in vitro* by human recombinant CYP1A1 and CYP1B1 to DNA-modifying products in *Salmonella typhimurium* NM2009 tester strain.[17] The carcinogenic potential of CYP1A1 in the activation of PAHs has been investigated further in *in vivo*, according to its activation of aflatoxin B1 to 8,9-epoxide in rabbit lung and liver.[18] In addition, B[a]P also induced carcinogenesis in mice which were positive for the aryl hydrocarbon receptor, *AhR (+/+).*[19] Therefore, CYP1A1 in the activation of PAHs has many studies both *in vitro* and *in vivo.*

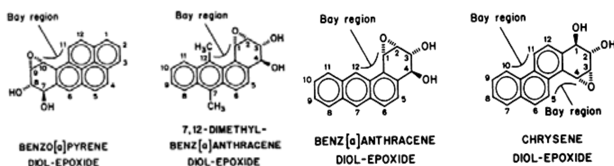

Figure 3 Structures of bay-region diol-epoxides of several polycyclic aromatic hydrocarbons (Conney AH, 1982).[7]

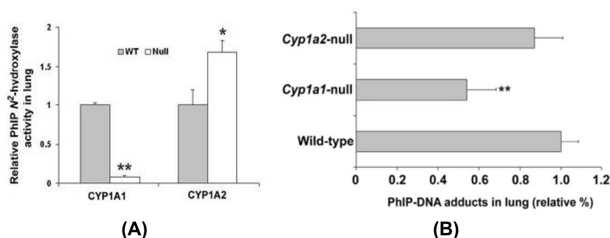

Figure 4 Relative quantification of (A) PhIP N2-hydroxylase activity and (B) PhIP-DNA adducts in lung homogenates of *Cyp1a1*-null mice, *Cyp1a2*-null mice, and wild type (WT) mouse (Ma & Whitlock, 1996)[20]

AhR-Mediated CYP1A1 Induction

The aryl hydrocarbon receptor (AhR) or dioxin receptor mediates the carcinogenic and other toxic effects of a variety of environmental pollutants, including an industrial byproduct 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and some polycyclic aromatic hydrocarbons (PAHs)[22]. As a consequence of AhR activation, induction of CYP1A1 expression is also mediated through AhR.[23] Unliganded AhR exists as a part of a cytosolic protein complex. There are two chaperone molecules of Hsp-90 heat-shock proteins, a co-chaperone protein called p23 and the immunophilin-like protein XAP2 (hepatitis B virus X-associated protein 2).[24] Entry of an exogenous ligand such as B[*a*]P or TCDD, through the cell membrane leads to binding to the receptor followed by translocation of the cytosolic heat-shock chaperone complex to the nucleus, various MAP kinases are involved in this step. The AhR undergoes dissociation from the chaperone proteins and heterodimerization with ARNT (aryl hydrocarbon nuclear translocator) that recruits p300[23] (Figure 5).

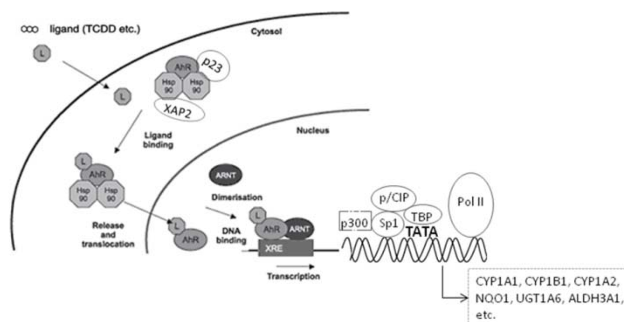

Figure 5 AhR ligand-mediated activation of phase I and II metabolizing enzyme genes (Conney, 1982[7]; Westwell 2004[25]).

The AhR-ARNT heterodimer binds to consensus regulatory sequences termed AhREs (aryl hydrocarbon response elements), XREs (xenobiotic response elements), or DREs (dioxin response elements), located in the promoter region of the CYP1A1 gene. In addition, AhR/ARNT heterodimer directly interacts with Sp1 and the two transcription factors (p300 and p/CIP) to synergistically enhance the expression of CYP1A1 gene which eventually leads to binding with TBP (TATA binding protein) and subsequent recruitment of RNA polymerase II.[26] Regarding induction of the CYP1A1 gene, TCDD causes a broad spectrum of biochemical and toxicological effects, and tumor promotion. This proposed mechanism of CYP1A1 induction also applies to certain Phase II xenobiotic metabolizing enzymes, such as NQO1 (NAD(P)H-dependent quinone oxydoreductase-1), UGT1A6 (UDP-glucuronosyl transferase), ALDH3A1 (aldehyde dehydrogenase) and several glutathione S-transferases.[27]

Interaction of AhR with Protein Kinase C, Tyrosine Kinases, and MAPK Kinases

Phosphorylation regulates AhR activity in the physiological signaling pathway regulating cell cycle progression as well as the xenobiotic signal transduction pathway. Recently, it has been found that modulation of the protein kinase C (PKC) pathway also affects AhR signaling. In addition, phosphorylation at the tyrosine residues of the carboxy terminal half of AhR is required for the formation of the functional AhR/ARNT heterodimer.[23] Therefore PKC and tyrosine kinase are also involved in AhR signal transduction, as inhibitors of these kinases block the induction of target genes.[23,28] The inhibition of PKC blocks ligand-activated DNA

binding of AhR/ARNT heterodimers and leading to a decreased *Cyp1* gene expression.[28]

Activation of MAPKs (mitogen-activated protein kinases) activities are becoming increasingly accepted in the AhR pathway because they are the members of the signal transduction system involved in the control of gene expression and various events in eukaryotic cells.[29] MAPKs family, comprises of the ERK and JNKs, along with the p38s, are seine and threonine protein kinase which can phosphorylate a large panel of substrates on these residues directly to transcriptional factors or via down-stream MAPK-activated protein kinases.[23]

The interplay between MAPKs and AhR is modulated by various exogenous stimuli; an example of them is UV radiation which activates p38, JNK, and ERK kinases. The activation of MAPKs and parallel formation of tryptophan photoreactive products leads to increased expression of *CYP1* genes.[29] In addition, the up-regulation of ERK and JNK by three AhR ligands, TCDD, B[*a*]P, and benzo[*a*]-pyrene-diolepoxide (BPDE) performed in human lung carcinoma A549 cells and mouse hepatoma Hepa-1 cells critically link to the AhR activity and receptor-dependent gene expression. Similarly, inhibition of the ERK and JNK pathway using specific inhibitors completely blocked TCDD-induced ERK, or JNK activation, respectively, and partially suppressed AhR activity and CYP1A1 induction. Most significantly, dual inhibition of ERK and JNK pathways caused nearly complete suppression of CYP1A1 induction.[30] Conclusively MAPK activation is a novel and generalized cellular response to xenobiotics. Many studies indicated that the MAPK pathways are important signaling mechanism involved in the activation of AhR, in TCDD toxicity, and possibly in B[*a*]P carcinogenicity.[30]

AhR interaction is also present with other pathways. Glucocorticoid receptor (GR) both *in vitro* and *in vivo*, GR decreases AhR-mediated expressions by interacting with XRE-binded AhR. Decreased expression of P450s means prolonged half-life of some anti-cancer drugs or slower activation of procarcinogens.[31] Cross talk of AhR and ARNT with the estrogen receptor α (ERα) has also been established in a number of different systems[32,33] and could be important in estrogen-related cancers. TCDD does not bind to ERα, but it inhibits ERα signaling. More importantly, ERα plays a significant role in modulating AhR activity, as it has been reported by in *vitro* and *in vivo* studies that co-treatment of TCDD and E2 resulting in an increased induction of CYP1A1, compared to TCDD treatment alone. ERα has a direct interaction with the CYP1A1 promoter, suggesting that it acts as a co-regulator of AhR-mediated transcriptional activation[34]. The interaction of ERβ with AhR and ARNT has also been suggested[35]. It has been implied that both ERα and ERβ can regulate expression of carcinogen-metabolizing genes such as CYP1A1.

Role of AhR in Cell Cycle Progression

Several published accounts point to a role for AhR in cell cycle control. Ma and Whitlock (1996)[20] revealed that an AhR-defective cell exhibited a prolonged doubling time compared with its wild-type counterpart. This effect was attributed to delayed progress through the G1 phase.[23] These findings revealed that decrease of AhR content was also associated with decreased proliferation of mouse hepatoma (Hepa1c1c7) cells in culture.

Another AhR promotes progression of the cell cycle as shown in mouse embryo fibroblasts (MEF) from AhR null mice were found to grow more slowly. The DNA synthesis rate was also markedly different by 24 hours, the wild-type MEF cultures had incorporated 3-fold more [^{3}H]thymidine than the AhR-null MEFs. By 72 hours, the AhR-null MEFs incorporated only 35% of [^{3}H]thymidine compared with the control cultures.[36] These results indicated that in the absence of an exogenous ligand, the AhR promoted progression through the cell cycle.[23]

There was extensive evidence in several different cell lines supporting the conclusion that exogenous AhR ligands, especially TCDD, actually inhibited cell proliferation and induced cell cycle arrest in normally cycling cell populations.[37] TCDD also inhibited 17β-estradiol-induced growth of MCF-7 human breast cancer cells[38], and inhibited proliferation of the fish hepatocellular carcinoma PLHC-1 cell line[39] and the androgen-induced proliferation of G0/G1-synchronized human prostate cancer LNCaP cells.[40]

The inhibition of cell cycle progression by AhR ligands was a direct interaction between the AhR and hypophospho-rylated retinoblastoma (RB).[23] The AhR formed complexes with the RB protein, then synergisting to repress E2F-dependent gene expression (Figure 6A), which related to proteins such as cyclinE, DNA polymerase α, and DHFR, leading to slow down the progression of cells from G1 into

S-phase[41]. In addition, AhR functioned as a co-repressor of RB, mediating repression of RB target genes, such as CDK2 and cyclin A, to cause cell cycle arrest[42] (Figure 6B).

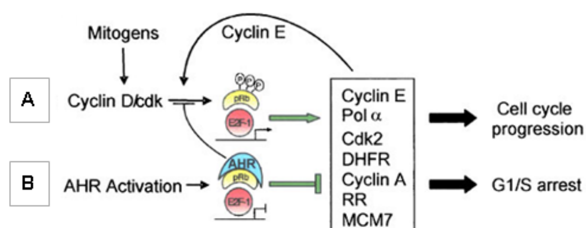

Figure 6 Role of the AhR in cell cycle regulation. (A) Under normal conditions and (B) under activation of AhR (Modified from www.interscience.wiley.com)

These results suggested that the interaction between AhR and RB acted as a negative regulator of cell cycle progression by inhibiting E2F-dependent transcriptional activity. However, there has been found a significant increase in the expression of the CDK inhibitor $p27^{kip1}$, indicating a positive regulatory mechanism involving up-regulation of cyclin kinase inhibitors.[23] In conclusion, the AhR, a potent transcriptional activator, may act as a repressor of transcription through the formation of specific protein–protein interactions[36] (Figure 6B).

Additional data showed other mechanisms to investigate the AhR role in cell cycle regulation in the absence of exogenous ligands. Use of integrated AhR variants in fibroblasts from AhR null mice showed that *AhR*+/+ fibroblasts significantly proliferated faster than *AhR-/-* fibroblasts, and exposure to TCDD did not change their proliferation rates.[43] These findings indicated that AhR function in the cell cycle was ligand-independent.

A recent study determined if constitutively active AhR affected the same transcriptional outcomes as environmental chemical-activated AhR.[44] The results shown that the constitutively active AhR maintained high baseline levels of CYP1B1 in immortalized or malignant human breast cell lines, but little or no CYP1A1 was detected (Figure 7), whereas the AhR hyper-activation by TCDD activates both genes, which implies a contribution of AhR and CYP1B1 prior to tumor formation[44] (Figure 8). These two contradictory bodies of evidence implies the wider function of AhR in the presence and absence of a ligand.

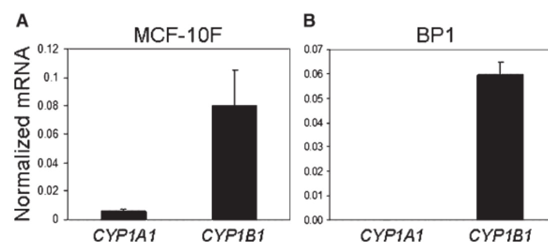

Figure 7 Preferential expression of CYP1B1 (A) in immortalized (MCF-10F) and (B) DMBA transformed, malignant (BP1) human breast epithelial cell lines(Yang et al, 2008).[44]

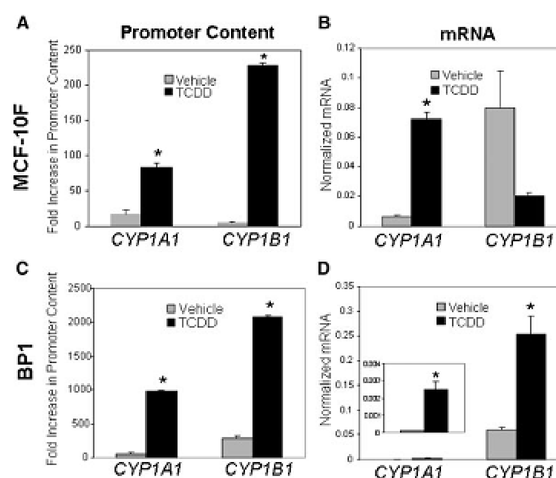

Figure 8 Hyper-activation of AhR with TCDD increased AhR binding to both CYP1A1 and CYP1B1 promoters, but not necessarily increase these gene expression (Yang et al, 2008).[44]

CYP1A1 Expression

CYP1A1 plays a major role as a carcinogen activating enzyme. Unlike most P450 enzymes, CYP1A1 is mainly expressed in extrahepatic tissues, especially lung, where it metabolizes and is markedly induced by PAHs.[8,45] CYP1A1 expression has been reported among 107 human lung adenocarcinoma, by which 48 and 29 of these were AhR high expressers, and expressed CYP1A1 respectively. After stratification by smoking status, the association between AhR and CYP1A1 was found only in smokers. These findings concluded that AhR and CYP1A1 expression was associated in smoking adenocarcinoma patients.[46]

Another study showed the occurrence of methylation in the promoter of CYP1A1 associated with tobacco smoking, as lung samples from active smokers which lacked methylation of the CYP1A1 promoter exhibited slightly higher

pulmonary EROD activity, in the regression models for age and daily consumption of tobacco.[47] In addition, the expression of CYP1A1 and AhR in small-cell lung carcinoma has been proposed as a putative diagnostic marker and has also been correlated with a history of cigarette smoking. CYP1A1 expression in lung tissues have largely influenced by the paradoxical induction of the enzyme from continuous exposure to the environmental chemicals.[11]

A differential overexpression of CYP1A1 between tumor and normal cells, which can potentially lead to the various applications of the enzyme in cancer pathology and treatment, was explored. The most significant finding demonstrated that CYP1B1 as a tumor marker was expressed at a high frequency in a wide range of human cancers of different histogenetic types, and not detectable in normal tissues[48] (Table 1). The same finding has drawn similar associations in CYP1A1, but in a smaller range of tumors, compared to CYP1B1.[49]

Table 1 Expression of CYP1B1 in different types of malignant tumors and normal tissue[48]

Tissue	Normal#	Tumor*
Bladder	0/8	8/8 (transitional cell carcinoma)
Brain	0/12	11/12 (astrocytoma)
Breast	0/10	12/12 (invasive ductal carcinoma)
Colon	0/10	11/12 (adenocarcinoma)
Connective tissue	0/9	8/9 (sarcoma)
Esophagus	0/8	8/8 (squamous carcinoma)
Kidney	0/11	11/11 (clear cell carcinoma, n=10; transitional cell carcinoma, n=1)
Liver	0/8	Not tested
Lung	0/8	7/8 (squamous carcinoma)
Lymph node	0/5	9/9 (non-Hodgin's lymphoma)
Ovary	0/5	7/7 (adenocarcinoma)
Skin	0/5	6/6 (squamous carcinoma)
Small intestine	0/5	Not tested
Stomach	0/10	9/10 (adenocarcinoma)
Testis	0/8	8/8 (malignant germ cell tumor)
Uterus	0/7	7/7 (adenocarcinoma, n=5; malignant mixed Müllerian tumor, n=2)
Total	0/130	122/127

Note: # number positive/number tested; * number positive/number tested (histopathological diagnosis)

CYP1A1 was presented to a greater extent in malignant than in normal breast.[49,50] Oestradiol C-4 hydroxylase (4-OH-E_2) activity which is a marker of CYP1A1 activity was observed, since neoplastic mammary tissue involved in the metabolism of oestrogen[51] (Figure 9). CYP1A enzyme was present in a small percentage of non-neoplastic samples of oesophageal tissue, whereas in oesophageal carcinomas the enzyme was expressed at least 60% of the samples.[52]

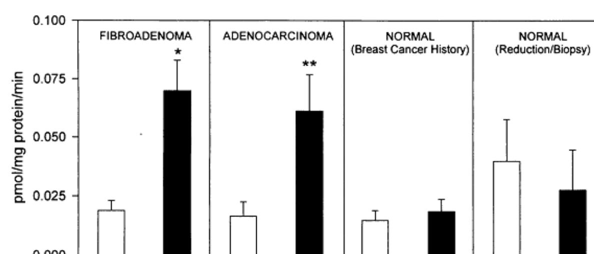

Figure 9 Microsome-mediated formation of 2-OH-E2 (open bars) and 4-OH-E2 (solid bars) obtained fresh from human mammary fibroadenomas, adenocarcinomas, and normal tissue (Liehr & Ricci, 1996).[51]

CYP1A was further detected in 68% of the urinary bladder tumors and its expression correlated with bladder tumor grade.[53] These findings revealed that carcinogen-activating role of CYP1A1 regard to the metabolism of PAHs and others exogenous substances from the continuous exposure lead to increased expression of the enzyme that well established carcinogen activity. CYP1A1 is the only placental xenobiotic metabolizing enzyme. It is well recognised in the bioactivation of many compounds critically in the first trimester, and then declines in the second and third trimestered. In addition, CYP1A1 expression in placenta involved with the substrate specificity towards oestradiol and the potential carcinogenic that can cause harmful effects during fetus developed[54]. Importantly, placental CYP1A1 is also involved in the bioactivation of many constituents in cigarettes, such as PAHs (B[*a*]P), lead to DNA-reactive species, and forming DNA adducts both in the placenta and fetal tissues. Therefore, significant induction of placental CYP1A1 activity in women exposed to cigarette smoke was extensively reported[55]. Choriocarcinoma cell line, Bewo, is a representative of human placenta to study the EROD activity assay in the presence of typical inducers (3-methylcholanthrene, β-naphthoflavone, and 1,2-benzanthracene). The results shown that CYP1A1 was induced via an increase of EROD activity compared to the levels observed in the absence of any inducer[56] (Figure 10). The same results were reported by Hakkola and colleagues.[57]

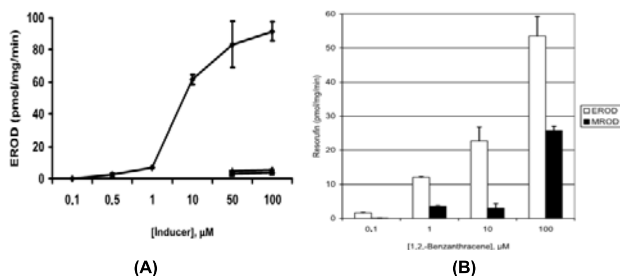

Figure 10 Induction of CYP1A1 (A) in either BeWo cells or primary cultures of human cytotrophoblasts microsomes after pretreatment with 3-MC or β-NF (B) in BeWo cell microsomes after pretreatment of the cells with 1,2-benzanthracene (Avery et al, 2003).[56]

CYP1A1 Polymorphisms

Polymorphism is another important factor that determines expression of P450s in different tissues. For CYP1A1, each of the polymorphisms is given a number in order of publication, which followed the symbol*. Thus, CYP1A1*1 is the wild-type. Two major changes causing CYP1A1 polymorphism are reported: the T→C transition in the 3' non-coding region, which has been associated with elevated enzyme activity (Crofts et al, 1994), which introduces a new *Msp*I restriction site or m1 polymorphism (CYP1A1*2A), and is found in 5% of Caucasians.[58] The CYP1A1 *Msp*1 polymorphism was found to be a significant factor in determining the risk of breast cancer among post-menopausal Chinese women in Taiwan. The same results were found in African-American patients; however, this was not apparent in Caucasians.[59] The second most commonly encountered CYP1A1 polymorphism involves an A→G transition in exon 7, which introduces Ile462Val or m2 polymorphism (CYP1A1*2C)[59] resulting in the replacement of an isoleucine (Ile) by valine (Val), which leads to an amino acid substitution in the heme-binding region and results in an increase in microsomal enzyme activity.[60]

Both CYP1A1 *m1* and *m2* polymorphisms are strongly associated with lung cancer especially in relation to tobacco smokers and in lung squamous cell carcinoma (SCC) in Japanese but not in Caucasian.[61] These findings were not comfirmed in studied conducted in Caucasian population, where the prevalence of the *CYP1A1 m1* and *m2* alleles was very low[62].However, larger studies in mixed American population point to an elevated risk of lung cancer in *m1* allele. In Brazillian populations increased in lung cancer risk was significantly associated with the presence of the *m2* allele.[63]

The variant *CYP1A1 m3* has a mutation in intron 7 and appear to be Afican-American specific. An elevated risk for lung adenocarcinoma among two populations was observed.[64] Another polymorphism (*m4*) located two bases upstream of the *m2* site, also causes an amino acid substitution of the T→A in the heme binding region of the enzyme, but effect of this polymorphism on enzyme activity is not clear yet.[63] Until now, the evidence referred to the association of genetic polymorphisms of CYP1A1 with cancer occurrence is still conflicting. Some studies have concluded that there is increase susceptibility to cancer in the presence of polymorphic variants, whereas others have reported no relationship between the two.[65,66]

Although a large body of experimental results points towards a positive association of CYP1A1 genetic polymorphisms and cancer occurrence, the relative contribution of CYP1A1 polymorphisms to the entire process of chemical carcinogenesis still remains to be evaluated. Cancer susceptibility due to carcinogen exposure is probably determined by an individual phenotype for a number of carcinogen metabolizing enzymes and the genetic susceptibility factor only becomes relevant if sufficient exposure occurs. Further investigation is required for such findings to be extrapolated successfully to human populations.

Conclusion

CYP1A1 is an enzyme involved in activation of a number of carcinogenic PAHs to reactive electrophiles that initiate cell transformation. CYP1A1 expressed mainly in extra-hepatic organs and are highly inducible by carcinogenic PAHs and TCDD through AhR which is responsible for the inducible expression of CYP1A1. Normally, AhR exists in inactive form within the cytoplasm in association with a complex of Hsp90, XAP2, and Hsp90 Co-chaperone p23. Upon ligand binding, AhR in the complex is activated, and then Hsp90 is released from the complex and the receptor translocates to the nucleus, where it forms a heterodimer with ARNT. The heterodimer binds to the XRE and alters expression of genes, leads to RNA Polymerase-II recruitment and gene transcription. In addition, AhR can affect cellular signaling through interactions with various regulatory and signaling proteins, including PKC and tyrosine

Kinase, MAPK pathways, in which inhibitors of these kinases block the induction of CYP1A1. AhR also interacts with other signaling pathways that are mediated by estrogen receptor and glucocorticoid receptor, and involves in cell-cycle regulation through growth factor signaling, if AhR activation refers to cell-cycle arrest. AhR have been implicated in the initiation and progression of cancers in multiple organs. In addition to environmental factors, genetic factor can modify CYP1A1 expression. Genetic polymorphisms in CYP1A1 determine the different susceptibilities of individual humans to carcinogenesis caused by the action of PAHs. Further study is needed to define the impact of variant forms of CYP1A1 on the incidence of cancers in humans.

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Editorial note
Manuscript received in original form on March 1, 2011;
accepted in final form on July 1, 2011