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Mini-review

CYP1B1 and hormone-induced cancer

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ABSTRACT

Cancers in hormone-responsive tissues (*e.g.,* breast, ovary, endometrium, prostate) occur at high incidence rates worldwide. However, their genetic basis remains poorly understood. Studies to date suggest that endogenous/exogenous oestrogen and environmental carcinogens may play a role in development and/or progression of hormone-induced cancers *via* oxidative oestrogen metabolism. Cytochrome P450 1B1 is a key enzyme in its oestrogen metabolism pathway, giving rise to hydroxylation and conjugation. Although *CYP1B1* is expressed in many cancers, particularly high levels of expression are observed in oestrogen-mediated disease. CYP1B1 is more readily found in tumour tissue compared to normal. Given the role of CYP1B1 in pro-carcinogen and oestrogen metabolism, polymorphisms in *CYP1B1* could result in modifications in its enzyme activity and subsequently lead to hormone-mediated carcinogenesis. CYP1B1 may also be involved in progression of the disease by altering the tissue response to hormones and clinical response to chemotherapy. The exact mechanism behind these events is complex and unclear. Only a few functional single nucleotide polymorphisms of *CYP1B1* are known to result in amino acid substitutions and have been extensively investigated. Studies examining the contribution of different *CYP1B1* alleles to hormone-mediated cancer risks are inconsistent. The main focus of this review is to appraise the available studies linking the pathogenesis of the hormone-induced cancers to various *CYP1B1* polymorphisms. Additionally, we explore the role of a neuronal protein, γ-synuclein, in CYP1B1-mediated pathogenesis.

1. Introduction

Originally discovered in rat liver microsomes [1,2], cytochrome P450 enzymes are so named on the basis of the presence of a heme group (P stands for pigment) and a strong spectral absorption band at 450 nm, first observed in 1958 [3–5]. The nomenclature system for symbolizing P450 enzymes and their genes is *CYP*, followed by a number for the family, a letter for the subfamily, and a number for the polypeptide. Currently, the members of the human CYP1 family identified have been CYP1A1, CYP1A2 and CYP1B1. The enzyme CYP1B1 (cytochrome P450, family 1, subfamily B, polypeptide 1) is a heme-thiolate monooxygenase capable of metabolizing xenobiotics, such as polycyclic aromatic hydrocarbons (PAHs) [6] and endogenous compounds, *e.g.,* oestrogen, testosterone [4,7].

CYP1B1 is transcriptionally induced by compounds such as TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) or dioxin, and regulated by several key transcriptional factors including oestrogen receptor (ER) and aryl hydrocarbon receptor (AhR) [6]. Apart from its role in xenobiotic metabolism, CYP1B1 is implicated in the bioactivation of pro-carcinogens [8–10]. The enzyme also appears to play a role in the metabolism of certain anticancer agents used in the treatment of hormone-induced cancers [11]. Over-expression of CYP1B1 has been linked with reversible resistance to the anticancer agent docetaxel in both clinical [11,12] and pre-clinical studies [13]. Resveratrol, a naturally-occurring phyto-oestrogen found in red wine, undergoes CYP1B1-mediated hydroxylation to generate the anti-leukaemic agent, piceatannol [14]. Piceatannol possesses anticancer properties [15] and inhibits a variety of tyrosine kinases involved in cell proliferation [16]. Thus, CYP1B1 may act as a tumour-suppressor or rescue enzyme *via* bioactivation of non-toxic dietary constituents into growth inhibitory substances [14].

Human *CYP1B1* is located on chromosome 2 at the 2p21–22 region [6,17]. The length of DNA is 12 kilobases (kbs) and, the length

Abbreviations: AhR, aryl hydrocarbon receptor; ARNT, aryl hydrocarbon receptor nuclear translocator; bp, base pair; CaP, prostate adenocarcinoma; CE, catechol oestrogen; COMT, catechol-*O*-methyltransferase; CYP1B1, cytochrome P450 1B1; DRE, dioxin-responsive element; E_1, oestrone; E_2, 17β-oestradiol; E_2-3,4-Q, oestradiol-3,4-quinone; E_2-3,4-SQ, oestradiol-3,4-semiquinone; EPT, oestrogen-progestin therapy; ER, oestrogen receptor; ERE, oestrogen responsive element; ET, oestrogen therapy; Hsp, heat shock protein; kb, kilobase; 4-OH E_2, 4-hydroxyoestradiol; PAH, polycyclic aromatic hydrocarbon; PCR, polymerase chain reaction; PR, progesterone receptor; PZ, peripheral zone; SERM, selective oestrogen receptor modulator; SNCG, γ-synuclein; SNP, single nucleotide polymorphism; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TZ, transition zone; XAP2, X-associated protein 2; XRE, xenobiotic-responsive element.

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of mRNA is ≈5.2 kb. The open reading frame starts in the second exon, which is 1629 base pairs (bps) in length, and encodes a protein with 543 amino acids [17,18]. The gene contains three exons (371, 1044 and 3707 bp) and two introns (390 and 3032 bp) [6,10,19]. *CYP1B1* also plays a role in ocular development and differentiation, and mutated forms are associated with primary congenital glaucoma [18,20].

In addition to their role in the regulation of growth and development, steroid hormones are implicated in the genesis of benign conditions including fibroids, endometriosis, and pituitary adenomas. A large and compelling body of epidemiological and experimental data implicate hormones including oestrogen, progesterone and testosterone in the aetiology of cancers affecting hormone-responsive tissues such as the breast, uterus, ovary and prostate [21–24]. Although the exact mechanisms for oestrogen-induced hormonal carcinogenesis are not fully elucidated, it is known that cytochrome P450 (CYP)-mediated metabolism of oestrogen plays a role by generating genotoxic metabolites of oestrogen. The role of hormones other than oestrogen is less clear.

During oxidative metabolism, endogenous oestrogens like 17β-oestradiol (E_2) and oestrone (E_1) are oxidized to 2-OH and 4-OH catechol oestrogens (CEs) by phase I enzymes, CYP1A1 and CYP1B1 [21,25,26]. CYP1B1 preferentially exhibits catalytic activity for the 4-hydroxylation of E_2 to yield 4-hydroxyoestradiol (4-OH E_2) [7]. This preferential action of CYP1B1 is attributed to the presence of a hydrogen bond between E_2 and CYPs [27]. If the catechol metabolites (2-OH E_2 and 4-OH E_2) are not eliminated by the process of conjugation (*i.e.*, methylation, glucuronidation or sulphonation), they go on to form semiquinones and quinones [28]. The catechol metabolite 4-OH E_2 is mainly oxidized to produce oestradiol-3,4-semiquinone (E_2-3,4-SQ) and oestradiol-3,4-quinone (E_2-3,4-Q). The E_2-3,4-Q reacts with purines in DNA to form depurinating adducts. These adducts generate apurinic sites, which potentially give rise in oncogenic mutations [29,30]. In contrast to 4-OH E_2, 2-OH E_2 catechol metabolites are weakly carcinogenic and predominantly oxidized to form stable adducts [28,30]. Additionally, 4-OH E_2 and oestrogen quinones/semiquinones undergo redox cycling, which result in production of reactive oxygen species capable of causing oxidative damage [31,32]. Furthermore, E_2-3,4-Q impairs anti-tumour activity of docetaxel by direct structural alteration and interference with this drug's microtubule stabilizing action [33].

Taking into consideration that CYP1B1 catalyzes the formation of genotoxic 4-OH E_2, it is reasonable to suppose that inter-individual variations (polymorphisms) in *CYP1B1* have the potential to influence oestrogen-mediated carcinogenic activity. Single nucleotide polymorphisms (SNPs) of *CYP1B1* have been linked with modified risk of hormone-responsive cancers; however, such observations have been inconsistent. In this review, we evaluate the existing literature to determine the role of common *CYP1B1* polymorphisms implicated in breast, uterine, prostate and ovarian cancers. Additionally, we hypothesize a role for γ-synuclein (SNCG), a neuronal protein required for efficient transcriptional activation of ERα [34], in *CYP1B1*-mediated hormonal carcinogenesis.

2. Regulation and expression of cytochrome P450 1B1

2.1. Transcriptional activation of CYP1B1

CYP1B1 activity is regulated *via* several factors including aryl hydrocarbon receptor (AhR), the AhR/AhR nuclear translocator (ARNT) complex, Sp1 transcription factors, epigenetic regulation (*e.g., CYP1B1* promoter methylation), endogenous oestrogen levels, ERs and *BRCA-1* activity [35–37]. In non-cancerous tissues, *CYP1B1* is transcriptionally activated when compounds including PAHs or dioxins bind to the AhR complex [consisting of AhR, Hsp90, X-associated protein 2 (XAP2) and p23 proteins] in the cytosol [24,38]. Upon binding, the AhR complex translocates to the nucleus, where it binds with its dimerization partner known as the ARNT. This activated AhR/ARNT heterodimer complex then binds to specific DNA enhancer sequences known as dioxin-responsive elements (DREs) to induce dioxin-responsive genes such as *CYP1A1* and *CYP1B1*. DREs have been identified on the 5'-regulatory region of *CYP1B1* [39–41].

ER is a ligand-activated transcription factor which can modulate oestrogenic responses [38]. Over-expression of ERα in Ishikawa cells in the presence of E_2 induces transcriptional activation of *CYP1B1* [38]. This E_2-induced *CYP1B1* activation and expression in ER-positive cells was initially thought to be mediated *via* AhR [42]. However, it was subsequently shown that this effect may be mediated by direct interaction of ERα with oestrogen responsive elements (EREs) on *CYP1B1* [38,42]. Thus, it is possible that the ER-mediated pathway, rather than the AhR-pathway, plays an essential role in E_2-induced *CYP1B1* expression. Carcinogenic effects of E_2 could also be due to a cross-talk between *SNCG* and *CYP1B1*. This latter theory, linking SNCG to CYP1B1, remains relatively unexplored, with only one study to date examining the possible relationship between SNCG and CYPs in endometriosis [24]. Fig. 1 shows a hypothetical schematic suggesting a potential role for SNCG in CYP1B1-mediated carcinogenesis.

Tamoxifen, a selective oestrogen receptor modulator (SERM), is associated with an elevated risk of endometrial cancer [43–46]. Tamoxifen metabolites upregulate the expression of *CYP1B1*, and may play a role in inducing uterine cancers [38,43,45,46]. Endometrial cell lines treated with 4-hydroxyl tamoxifen in the absence of oestrogen, demonstrate an increase in *CYP1B1* expression *via* upregulation of the gene promoter [38,43]. Similarly, tamoxifen-treated human endometrial glandular cell and cancer cell lines exhibit elevated *CYP1B1* expression [43]. Tamoxifen is still widely used in breast cancer treatment, and for chemoprevention in at-risk women [44]. However, usage is associated with an elevated endometrial cancer risk [45], for which the underlying mechanism remains unclear [46,47].

2.2. Expression of CYP1B1 in hormone-induced cancers

CYP1B1 is differentially expressed between tissues, with the highest constitutive mRNA levels detected in extra-hepatic tissues including the breast, heart, brain, placenta, lung, liver, skeletal muscle, kidney, prostate, uterus and ovary [8,9,13,17,22,24,33,48]. CYP1B1 is present at high levels in a wide variety of tumours including those arising from hormone-responsive tissues, in contrast to typically very low or even undetectable amounts in normal extra-hepatic tissues [48–52]. CYP1B1 protein is mainly localized in nuclei, although they can also be present in the cytoplasm [48]. Regarding CYP1B1 expression in normal or cancer tissues, no definite correlation has been observed between mRNA expression and protein levels [52–54]; this could be due to altered post-transcriptional control. SNPs in the coding region of *CYP1B1* may lead to increases in consequent protein levels, thus increasing the risk of cancer by elevating the generation of bioactivated carcinogenic metabolites.

2.2.1. CYP1B1 expression in prostate cancer (CaP)

CYP1B1 mRNA and proteins have been found in both normal prostate tissue and prostate tumours (including tumour-adjacent tissues), with markedly higher levels in CaP compared with benign tissues [35]. Interestingly, within cancer-free tissue there are 2- to 6-fold higher levels of *CYP1B1* mRNA transcripts in the peripheral zone (PZ) compared to the transition zone (TZ) [22]. Additionally,