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Mini review

Cytochrome P450-mediated metabolism of estrogens and its regulation in human

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Abstract

Estrogens are eliminated from the body by metabolic conversion to estrogenically inactive metabolites that are excreted in the urine and/or feces. The first step in the metabolism of estrogens is the hydroxylation catalyzed by cytochrome P450 (CYP) enzymes. Since most CYP isoforms are abundantly expressed in liver, the metabolism of estrogens mainly occurs in the liver. A major metabolite of estradiol, 2-hydroxyestradiol, is mainly catalyzed by CYP1A2 and CYP3A4 in liver, and by CYP1A1 in extrahepatic tissues. However, CYP1B1 which is highly expressed in estrogen target tissues including mammary, ovary, and uterus, specifically catalyzes the 4-hydroxylation of estradiol. Since 4-hydroxyestradiol generates free radicals from the reductive-oxidative cycling with the corresponding semiquinone and quinone forms, which cause cellular damage, the specific and local formation of 4-hydroxyestradiol is important for breast and endometrial carcinogenesis. Changes in the expression level of estrogen-metabolizing CYP isoforms not only alter the intensity of the action of estrogen but may also alter the profile of its physiological effect in liver and target tissues. Generally, many CYP isoforms are induced by the substrates themselves, resulting in enhanced metabolism and elimination from the body. Of particular interest is a novel finding that human CYP1B1 is regulated by estradiol via the estrogen receptor. This fact suggests that the regulation of CYP enzymes involved in estrogen metabolism by estrogen itself would be physiologically significant for the homeostasis of estrogens at local organs. In this mini-review, we discuss the CYP-mediated metabolism of estrogens and the regulation of the estrogen-metabolizing CYP enzymes in relation to the risk of cancer.

Keywords: Cytochrome P450; Estrogen; Metabolism; Regulation; Carcinogenesis

1. Introduction

Estrogens exert diverse biological effects such as female sexual differentiation and development, arterial vasodilation, the maintenance of bone density, and neuroprotective actions. Many of these effects result from direct interaction between estrogen and the estrogen receptor (ER), which activates the expression of target genes encoding proteins with important biological functions [1–3]. One of the most notable effects of estrogens is their contribution to

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the development and evolution of breast cancer and endometrial cancer [4,5]. Prolonged exposure to estrogens is considered an important etiological factor for the induction of estrogen-associated cancers [6,7]. Estrogens are eliminated from the body by metabolic transformation to estrogenically inactive metabolites that are excreted in the urine and/or feces. The metabolism of estrogens includes oxidation (mainly hydroxylation) by cytochrome P450s (CYPs), glucuronidation by UDP-glucuronosyltransferase, sulfation by sulfotransferase, and *O*-methylation by catechol *O*-methyltransferase (COMT) [8–11]. The first step in the metabolism of estrogens, hydroxylation, is mediated by CYP enzymes. CYP consists of a superfamily of heme-containing monooxygenases and is responsible for the oxidative metabolism of many drugs and environmental chemicals as well as endogenous substances including steroids [12]. Three families (CYP1, CYP2, and CYP3) mainly catalyze the oxidative metabolism of exogenous and endogenous compounds. Some isoforms in these CYP families are also responsible for the metabolism of estrogens. The expression level of the estrogen-metabolizing CYP enzymes is regulated by many factors in the liver and target tissues. Therefore, the metabolism of estrogen not only alters the intensity of its action but may also alter the profile of its physiological effects in target tissues. In this mini-review, we discuss the CYP-mediated metabolism of estrogens, the regulation of the estrogen-metabolizing CYP enzymes, and the association with cancer susceptibility.

2. Biotransformation of estrogens in human

The biosynthesis of estrogens from cholesterol involves a series of enzymatic steps (Fig. 1). CYP11A catalyzes pregnenolone formation from cholesterol. CYP17 catalyzes androgen formation from pregnenolone. CYP19 catalyzes estrogens from androgens. Estrone is converted to potent estradiol by 17β-hydroxysteroid dehydrogenase (17β-HSD) [13]. In pre-menopausal females, ovary and adrenal glands are the principal source of estradiol [14–16]. In males or post-menopausal females, the ovary ceases to produce estrogens and the circulating inactive steroids such as androstenedione, testosterone, and estrone sulfate are considered to be major precursor substrates of local estrogen production [17,18].

A large number of hydroxylated (2-, 4-, 6α-, 6β-, 7α-, 12β-, 15α-, 15β-, 16α-, and 16β- hydroxylated) metabolites of estrogens via CYP isoforms have been detected in vivo and in vitro [19,20]. These metabolic pathways of estrogens are identified in liver and extrahepatic tissues. Since most P450 enzymes are abundantly expressed in liver, the metabolism of estrogens mainly occurs in liver. In liver approximately 80% of estradiol is biotransformed to 2-hydroxyestradiol and 20% to 4-hydroxyestradiol [21]. CYP1A1, CYP1A2, and CYP3A4 exhibit catalytic activity dominantly for the 2-hydroxylation rather than the 4-hydroxylation of estradiol [22]. In contrast, CYP1B1 exhibits the catalytic activity specifically for the 4-hydroxylation of estradiol [22]. CYP3A5 also exhibits distinct activity for the 4-hydroxylation of estrogens (in particular estrone). Since CYP1A1 is not expressed in liver, the 2-hydroxylation in liver is mainly catalyzed by CYP1A2 and CYP3A [23–25]. In extrahepatic tissues, 2-hydroxyestradiol formed by CYP1A1 and CYP3A4 has also been detected in breast, uterus, placenta, brain and pituitary [26–28]. In human breast and uterus, since the expression level of CYP1B1 is high, 4-hydroxylation is the dominant pathway of estradiol [26,29]. In addition to the quantitatively major metabolic pathways of estrogens (2- and 4-hydroxylations), 6α-, 6β-, 7α-, 12β-, 15α-, 15β-, 16α-, and 16β-hydroxyestrogens are also formed as minor metabolites by several CYP isoforms such as CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2C8, CYP3A4, CYP3A5, and CYP3A7 [22].

3. Role of estradiol and its metabolites in carcinogenesis

In animals and humans, elevated circulating estrogen levels increase the risk of breast or endometrial cancer [30]. One postulated mechanism for the carcinogenesis is that estrogens themselves act as a hormone stimulating cell proliferation. The other postulated mechanism is that estrogens act as a procarcinogen that induces genotoxicity. A catechol metabolite, 4-hydroxyestradiol, formed by CYPs generates free radicals from reductive-oxidative cycling with the corresponding semiquinone

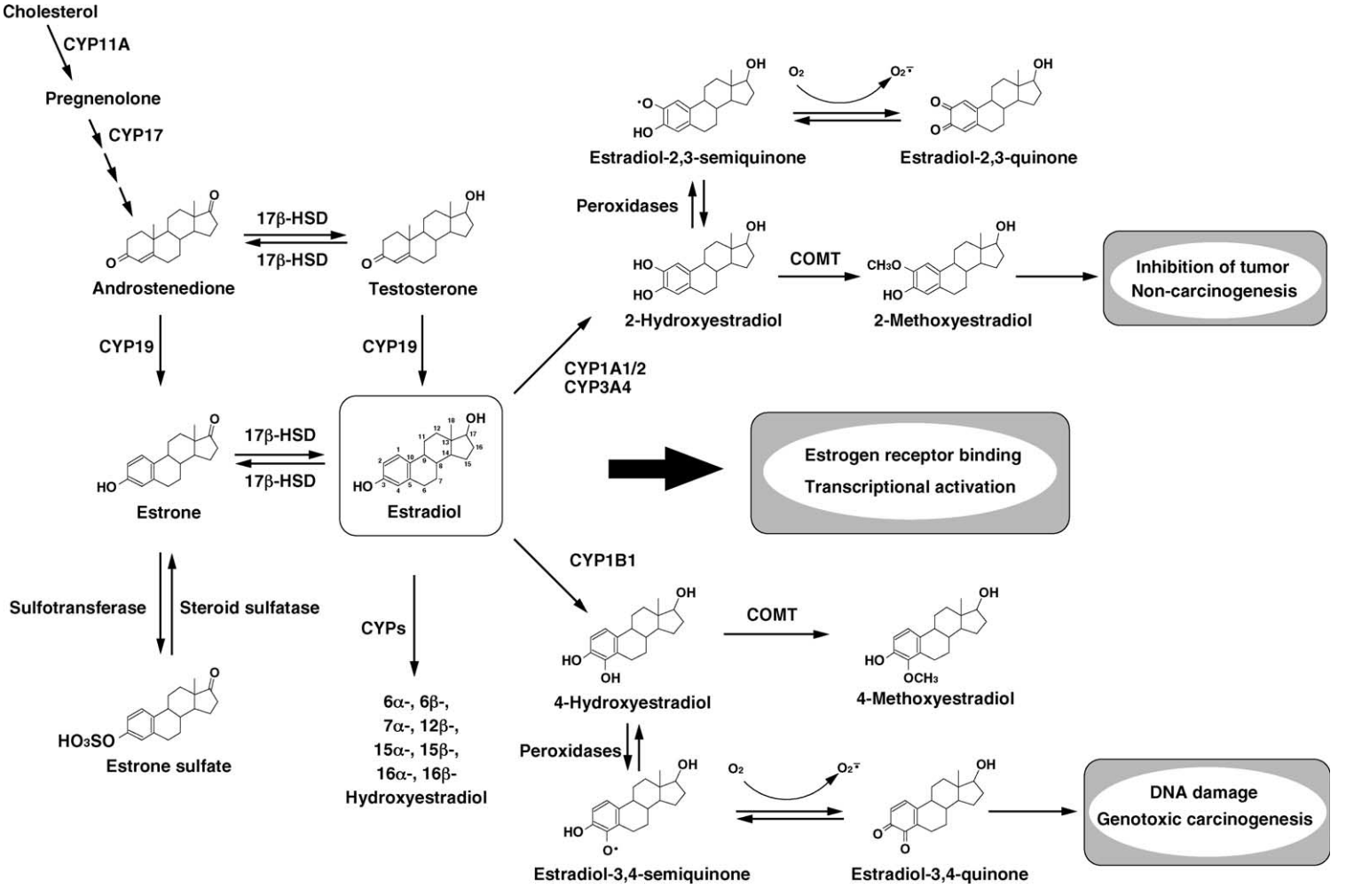

Fig. 1. Cytochrome P450-mediated metabolic pathways of estradiol. Estradiol is biosynthesized by both CYP19 and 17β-hydroxysteroid dehydrogenase (17β-HSD) from androstenedione via testosterone or estrone. Estradiol is metabolized to 2- and 4-hydroxyestradiol. These catechol metabolites can be subsequently *O*-methylated to monomethoxy estradiol metabolites by catechol *O*-methyltransferase (COMT). 2-Methoxyestradiol appears to be non-carcinogenic and inhibits the proliferation of cancer cells. 4-Hydroxyestradiol undergoes metabolic redox cycling to generate free radicals such as superoxide and the reactive semiquinone/quinone intermediates, which cause DNA damage.

and quinone forms, which cause DNA damage [31,32]. It has been shown that treatment with 4-hydroxyestradiol induces endometrial adenocarcinoma in mice [33]. In human uterine myometrium and benign uterine leiomyoma [26], and in benign and malignant mammary tumors [27,34], high levels of 4-hydroxyestradiol are detected. As mentioned above, the 4-hydroxylation of estrogens is mainly catalyzed by CYP1B1. CYP1B1 is expressed in estrogen target tissues, including mammary, ovary, and uterus [35–37]. Furthermore, the expression level of CYP1B1 is abundant in tumor tissues [38]. These reports strongly suggest that the specific and local formation of 4-hydroxylation of estradiol is important for breast and endometrial carcinogenesis, and implicate the CYP1B1 as a key player in this process.

In contrast to 4-hydroxyestradiol, 2-hydroxyestradiol is not carcinogenic, although it might also undergo metabolic redox cycling to generate free radicals and the reactive semiquinone/quinone intermediates [39,40]. Since 2-hydroxyestradiol is methylated by COMT at a faster rate than 4-hydroxyestradiol, free radicals are not easily generated [41]. Furthermore, 2-methoxyestradiol has an inhibitory effect on cell proliferation [42–44]. These may be the reasons for the non-carcinogenicity of 2-hydroxyestradiol.

It has been reported that the serum concentration of 16α-hydroxyestradiol was more pronounced in women with breast cancer [45]. In contrast, another study found no elevation of 16α-hydroxyestradiol in the urine of patients with breast cancer as compared to healthy controls [46]. 16α-Hydroxyestradiol and 16α-hydroxyestrone are in abundance during normal pregnancy, and the risk of breast cancer is low in pregnant women [47]. Thus, the role of 16α-hydroxyestradiol in carcinogenesis is controversial.

4. Regulation of CYP enzymes catalyzing estrogen metabolism

CYP1A1, which catalyzes the 2-hydroxylation of estradiol, is induced by numerous polycyclic aromatic hydrocarbons (PAHs) and aryl amines [23] as well as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) [48]. This induction is mediated by the aryl hydrocarbon receptor (AhR) and AhR nuclear translocator (ARNT) [48–50]. The liganded-AhR/ARNT heterodimer interacts with the xenobiotic responsive element (XRE) located in the enhancer region of the target genes, and subsequently transcription of the target gene is enhanced. At least seven XREs have been identified in the 5′-flanking region (up to −1300 bp) of the human *CYP1A1* gene [48,51,52]. Recently, in a study using DNA microarray analysis it was reported that the expression level of CYP1A1 mRNA in human breast cancer MCF-7 cells is stimulated by treatment with estradiol [53]. However, the induction mechanism has not been clarified yet.

CYP1A2 is constitutively expressed preferentially in liver [54]. The basal expression of the human *CYP1A2* gene is regulated by some transcriptional factors such as liver specific transcription factor 1 and upstream stimulatory factor [55,56]. CYP1A2 is also induced by PAHs as well as TCDD via AhR/ARNT complex [49]. It has been reported that administration of estradiol increases the expression of CYP1A2 protein in hamster [57]. Therefore, estrogens may affect the expression level of CYP1A2. However, information about the regulation of *CYP1A1* and *CYP1A2* by estrogen is limited.

Human CYP1B1, which catalyzes the 4-hydroxylation of estradiol, metabolically activates numerous procarcinogens and promutagens including PAHs and aryl amines [35]. The human CYP1B1 mRNA and protein are constitutively expressed in extrahepatic tissues such as lung, kidney and endocrine-regulated tissues including breast, uterus, and ovary [35–37]. The expression of CYP1B1 is higher in various types of malignant tumors compared with normal tissues [38]. CYP1B1 is also induced by PAHs as well as TCDD. In our previous study, two XREs to which liganded AhR/ARNT binds were identified at −834 and −853 in the 5′-flanking region of the human *CYP1B1* gene, and it has been shown that the mutual interaction of these XREs is important for the regulation (Fig. 2A; [58]). In addition, we found that nuclear transcriptional factor Sp1 plays a role in the constitutive and inducible transcriptional regulations of the human *CYP1B1* gene [58].

There is extensive evidence that supports the role of AhR in the biochemical and toxic responses, such as antiestrogenic activity, elicited by TCDD and related compounds [59,60]. Several possible mechanisms for the antiestrogenic activity of TCDD have been proposed as follows: (1) the induction of CYP1

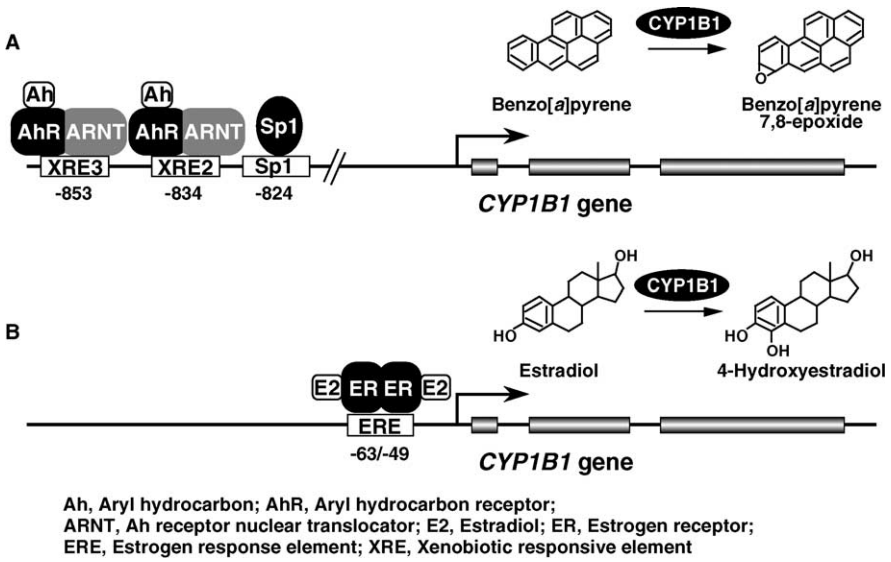

Fig. 2. Proposed induction mechanism of human CYP1B1. (A) CYP1B1, that metabolically activates various aryl hydrocarbon(Ah)s such as benzo[*a*]pyrene, is induced by Ah through the binding of AhR/ARNT to two XREs, located at −853 and −834 in the 5′-flanking region of the *CYP1B1* gene. In addition, the binding of Sp1 near the XRE cooperatively enhances both the constitutive and ligand-inducible transcriptional activities of the *CYP1B1* gene. (B) Estradiol (E2) induces the transcriptional activity of the *CYP1B1* gene by the binding of ER to the putative ERE between −63 and −49 in the *CYP1B1* gene, leading to an enhancement of the E2 metabolism.

by TCDD may increase the metabolism of estradiol; (2) the activated AhR may inhibit the binding of estradiol to ER; (3) the activated AhR may inhibit binding of the estradiol/ER complex to an estrogen response element (ERE) in the promoter regulation of target genes [61]; (4) TCDD enhances the degradation of nuclear ER through the activation of proteasome [62]; (5) TCDD down-regulates the ER levels via a repressor site in the promoter region of the ER gene [63]. Thus, AhR-ER cross-talk has been suggested to have a role in the antiestrogenic activity of TCDD. In our recent study, we found that the antiestrogenic activity of TCDD was not observed in *Cyp1b1* knockout mice as well as *AhR* knockout mice [64]. These findings suggest that the induction of CYP1B1 by TCDD mediated via AhR is a key factor for the antiestrogenic activity of TCDD.

Furthermore, we found an interesting and novel mechanism involved in the transcriptional regulation of the human *CYP1B1* gene. Many CYP isoforms are likely to be induced by the substrates themselves. CYP1B1 is a key enzyme for estrogen metabolism. In addition, a computer-assisted homology search identified a potential ERE on the human *CYP1B1* promoter (between −63 and −49). These findings prompted us to investigate whether the human CYP1B1 is induced by estradiol. Real-time RT-PCR analysis revealed that treatment with estradiol induced CYP1B1 mRNA expression in ER-positive MCF-7 cells. Luciferase reporter assays using MCF-7 cells showed a significant transactivation by estradiol with a reporter plasmid containing a region from −152 to +25 of the human *CYP1B1* gene. Specific binding of ER to the putative ERE was demonstrated by chromatin immunoprecipitation assays and gel shift analyses. These findings clearly indicated that the human *CYP1B1* is regulated by estrogen via ER (Fig. 2B; [65]). Since endometrial tissue is highly regulated by estrogens, the expression pattern of CYP1B1 protein in human endometrial specimens was examined by immunohistochemistry. The staining of CYP1B1 was stronger in glandular epithelial cells during a proliferative phase than those during a secretory phase, consistent with the pattern of estrogen secretion. Since 4-hydroxylation of estrogen by CYP1B1 leads to a decrease of the estrogenic activity but the produced metabolite is toxicologically active, the findings suggest a clinical significance in

the estrogen-regulated CYP1B1 expression for the homeostasis of estrogens as well as for estrogen-dependent carcinogenesis.

The induction of CYP3A isoforms is known to be regulated by some nuclear receptors such as the pregnane X receptor (PXR) and constitutive androstane receptor (CAR) via heterodimerization with the retinoid X receptor (RXR) [66]. Steroid hormones and metabolites such as pregnenolone, progesterone, and 5β-pregnane-3,20-dione bind to PXR and CAR [67,68]. The liganded-PXR/RXR heterodimer and CAR/RXR heterodimer interact with the everted repeat sequence separated by 6 bp (ER6) or direct repeat sequence separated by 3 bp (DR3) in the promoter region of the *CYP3A4* gene [69]. Thus, the *CYP3A4* gene appears to be regulated by these nuclear receptors with cross-talk through the same *cis*-elements. It is possible that the steroid hormones regulate CYP3A transcription, resulting in steroid catabolism and elimination from the body. Recently, Williams et al [70] examined the possible role of estrogen in regulating the expression of the human CYP3A subfamily, since the CYP3A4 mRNA expression in the liver was lower in females than in males [71]. It was shown that CYP3A4 and CYP3A43 are down-regulated by estrogen, while CYP3A5 is expressed at higher levels during the secretory phase in endometrium. Estrogens may be important for the tissue-specific expression of the CYP3A isoforms.

5. Genetic polymorphisms of CYP enzymes that catalyze estrogen metabolism and the association with cancer risk

For the human *CYP1A1* gene, several variant alleles including *CYP1A1*1B* to *CYP1A1*11* have been identified (http://www.imm.ki.se/CYPalleles/). Among them, *CYP1A1*2A* giving rise to an *Msp* I restriction site in the 3′-noncoding region at T3801C and *CYP1A1*2C* with an amino acid substitution at codon Ile462Val have been reported to significantly elevate the inducible enzymatic activity compared with the wild-type genotype. Some studies reported that *CYP1A1*2A* and **2C* variants increase the risk of breast cancer [72,73]. The *CYP1A1*2C* allele also appears to be associated with the risk of prostate and ovarian cancer [74,75]. Endometrial cancer was also related to *CYP1A1* polymorphisms [76,77], and a minor or negative association of cervical cancer with the *CYP1A1* genotype was reported [78].

Concerning the human *CYP1A2* gene, variant alleles including *CYP1A2*1B* to *CYP1A2*14* have been identified. It has been reported that the enzyme activity is decreased with the *CYP1A2*7* causing a splicing defect and *CYP1A2*11* causing an amino acid substitution [79,80]. However, no evidence that *CYP1A2* polymorphism is associated with a cancer risk has been published.

For the human *CYP1B1* gene, variant alleles including *CYP1B1*2* to *CYP1B1*26* have been identified. Two polymorphisms with an amino acid substitution at codon Ala119Ser and codon Leu432-Val of *CYP1B1* have been reported to have an association with breast or endometrial cancer risk [81,82]. It has been shown that the 4-hydroxylase activities of estradiol by these variant enzymes are 2–4 fold higher than those by the wild-type enzyme [83,84]. Thus, genetic polymorphisms of CYP1B1 lead to alterations in estrogen metabolism and a risk for estrogen-associated cancers.

For the *CYP3A4* gene, variants alleles including *CYP3A4*1B* to *CYP3A4*19* have been identified. The *CYP3A4*1B* allele, with a mutation in the promoter region, may be associated with the risk of prostate cancer or the high clinical stage of tumours [85]. In contrast, no association between the *CYP3A4*1B* allele and breast or ovarian cancer risk, or myeloid leukemia has been reported [86,87]. Thus, associations between *CYP3A4* genetic polymorphisms and cancer risk remain unclear. In *CYP3A5* genetic polymorphisms, *CYP3A5*1B* to *CYP3A5*10* have been reported. The *CYP3A5*3* allele has decreased enzyme activity because of a splicing defect, but no relationship with the risk of developing acute myeloid leukemia has been reported [87]. For *CYP3A7* gene, four variant alleles containing single nucleotide polymorphisms have been described [88]. None of them has been studied in relation to cancer risk.

6. Conclusion

Biotransformation of steroid hormones constitutes a dynamic network of control for the bioavailability of steroid hormones to their corresponding target

receptors. The liver is the most important organ for steroid hormone catabolism, and other local organs also contribute to the catabolism. Recently, the concept of intracrinology has become important for the estrogen activity in local tissues [89]. Inactive steroids in plasma are locally converted to bioactive estrogens in the reproductive tract as an intracrine activity.

One of the important steroid-metabolizing enzymes is the CYP family. Generally, many CYP isoforms are induced by the substrates themselves, resulting in enhanced metabolism and elimination from the body. The same induction mechanism works on estradiol for CYP enzymes. As discussed above, we clearly showed that the expression level of CYP1B1 is regulated by estradiol via ER. There is also some evidence that CYP1A1 and CYP1A2 might be induced by estradiol [53,57], although the induction mechanism has not been clarified. In addition, CYP3A isoforms may be regulated by estrogens. The regulation of CYP enzymes, which are responsible for estrogen metabolism by estrogen itself, would be important for the homeostasis of estrogens in local organs.

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