# Unifying mechanism in the initiation of breast cancer by metabolism of estrogen (Review)

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**Abstract.** Excessive exposure to estrogen is associated with increased risk of breast cancer. The mechanisms of carcinogenesis in the breast caused by estrogen metabolism include formation of depurinating adducts which are released from DNA to generate apurinic sites, and production of reactive oxygen species (ROS). Excess ROS not only exerts genotoxicity by indirectly increasing genomic instability, but also stimulates progression of mammary carcinogenicity by inducing a redox-associated signaling pathway. Estrogen metabolism enzymes serve an important role in estrogen metabolism. Alterations in the expression and activity of estrogen metabolism enzymes may influence estrogen metabolism homeostasis. The present review discusses the process of estrogen metabolism, the role of estrogen metabolites and ROS in breast carcinogenesis, and the effect of metabolism enzyme polymorphisms on generation of pro-carcinogens and breast cancer susceptibility.

**Contents**



## 1. Introduction

Breast cancer is one of the most common malignancies among females worldwide (1). Estrogens have been implicated in the etiology of breast cancer. Epidemiological and clinical evidence has indicated that factors associated with elevated estrogen levels throughout the lifetime of a female, including the early onset of menstruation, late menopause, use of oral contraceptives, late first full-term pregnancy, and hormone replacement therapy, are associated with an increase in breast cancer risk among pre- and postmenopausal women (2). Estrogens are generally considered to cause proliferation of breast cancer cells via the estrogen receptor (ER), and serve as a transcription factor to regulate the expression of target genes encoding proteins with important biological functions (3). The ER-mediated signaling pathway may have an important role in the development of cancer; however, they do not serve a crucial role in cancer initiation (4). Compared with ER-mediated processes, substantial evidence suggests that the oxidative metabolism of estrogens serves a major role in the initiation of breast cancer (5,6). Specific estrogen metabolites, predominantly catechol estrogens-3,4-quinones (CE-3,4-Q), have the potential to initiate the cancer process by binding to DNA and forming depurinating adducts, 4-OHE$_1$($E_2$)-1-N3Ade and 4-OHE$_1$($E_2$)-1-N7Gua (6,7). These depurinating DNA adducts are rapidly lost from DNA by cleavage of the glycosyl bond, leaving apurinic sites in DNA that may generate mutations that initiate cancer (8). In addition, redox cycling of quinone and semiquinone metabolites results in the generation of free radicals and reactive oxygen species (ROS). Excessive ROS not only exerts genotoxicity by indirectly increasing genomic instability, but also stimulates progression of mammary carcinogenicity by transducing redox-associated signal pathways (9,10).

Estrogen metabolism and elimination can be characterized by two steps. Phase is the conversion of estrogen into CEs by cytochrome P450 (CYP) 450 enzymes. The major P450 enzymes involved in estrogens metabolize include CYP1A1 and CYP1B1. Phase II is the inactivation and detoxification pathways of CEs, including O-methylation by catechol-O-methyltransferase (COMT). The present review discusses the process of estrogen metabolism, the role of estrogen metabolites and ROS in breast carcinogenesis, and the effect of metabolism enzyme polymorphisms on generation of procarcinogens and breast cancer susceptibility.

## 2. Estrogen metabolism

Endogenous estrogen mainly refers to estrone ($E_1$) and 17β-estradiol ($E_2$). $E_1$ and $E_2$ are interconverted by

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17β-hydroxy steroid dehydrogenase (17β-HSD; Fig. 1) (11). Endogenous estrogens are metabolized by two main pathways: Formation of the 2-OH- and 4-OH-estrogens, which are known as CEs, and to a lesser extent, 16a-$OHE_1(E_2)$ (11). CYP3A5, CYP3A7and CYP1A1 have catalytic ability for 16a-$OHE_1(E_2)$ (11). In extrahepatic tissues, estrogens are transformed into 2-$OHE_1(E_2)$ by CYP1A1, whereas 4-$OHE_1(E_2)$ is catalyzed primarily by CYP1B1 (11). Since CYP1B1 has been demonstrated to be expressed in healthy human breast ductal tissue and over-expressed in invasive ductal carcinomas, estrogen-quinone depurinating adducts levels are greater in women with breast cancer compared with healthy women (4,12-14).

In extrahepatic tissues, the most common pathway of conjugation of CEs is O-methylation inactivation via COMT, which prevents their conversion to estrogen semiquinones and quinines (15). COMT catalyzes 2-$OHE_1(E_2)$ at the 2-OH and 3-OH positions, and 4-$OHE_1(E_2)$ at the 4-OH position, with the methyl group derived from S-adenosylmethionine (16). Inhibition of COMT increases the amount of oxidative DNA damage and 4-OH quinine depurinating adduct levels, which means that COMT is responsible for preventing the oxidative metabolism of CEs to genotoxic quinone metabolites (17,18). Previous studies have suggested that 2-methoxyestradiol, the major O-methylation metabolite of 2-$OHE_1(E_2)$, has anticancer activities by growth inhibitory effects (19).

Unless detoxified, 2-$OHE_1(E_2)$ and 4-$OHE_1(E_2)$ are further oxidized to the corresponding quinones, $E_1(E_2)$-2,3-Q and $E_1(E_2)$-3,4-Q. Of the two estrogen quinones, $E_1(E_2)$-3,4-Q is believed to be a critical metabolite which reacts with DNA to form depurinating adducts. Quinones also undergoes a two-electron reduction to form corresponding hydroquinones, which are transformed by NAD(P)H-Quinone oxidoreductase 1 (NQO1) (20). NQO1 transforms CE-Q back to CEs, thus making quinone metabolites unavailable for reaction with DNA and oxidative stress (20).

### 3. Role of estrogen and its metabolites in carcinogenesis

Chemical carcinogens covalently bind to DNA to generate two types of adducts: stable ones and depurinating ones. $E_1(E_2)$-3,4-Q produces much higher levels of depurinating adducts and smaller amounts of stable adducts (7). Depurinating estrogen-DNA adducts serve an important role in cancer initiation. The depurinating adducts are rapidly lost from DNA by cleavage of the glycosyl bond, and then produce apurinic sites that may lead to cancer (21). Mounting experiments on estrogen metabolism, formation of DNA adducts, carcinogenicity, mutagenicity and cell transformation has identified that estrogen metabolites, especially CE-3,4-Q, react with DNA to form predominantly depurinating adducts, 4-$OHE_1(E_2)$ -1-N3Ade and 4-$OHE_1(E_2)$-1-N7Gua, leading to the accumulation of mutations and potentially cell transformation (6-8,22). 2-$OHE_1(E_2)$ is transformed to $E_1(E_2)$-2,3-Q;$E_1(E_2)$-2,3-Q is much less reactive with DNA than $E_1(E_2)$ -3,4-Q, because different mechanisms of adduction are responsible for different reactivity. $E_1(E_2)$-3,4-Q reacts via a proton-assisted 1,4-Michael addition; however, reaction of $E_1(E_2)$-2,3-Q with Ade results in the generation of 2-$OHE_1(E_2)$-6-N3Ade by 1,6-Michael addition (23,24). The greater carcinogenic activity of 4-$OHE_1(E_2)$ is associated with a higher amount of depurinating DNA adducts formed by $E_1(E_2)$-3,4-Q, compared with $E_1(E_2)$-2,3-Q (25). According to previous studies, 2-$OHE_1(E_2)$ methylation by COMT may have an inhibitory effect on cell proliferation (19). This may be another reason for the reduced genotoxic of 2-$OHE_1(E_2)$.

Accumulating evidence for the initiation of cancer by estrogen-DNA adducts has been identified by using human breast epithelial cell lines such as MCF-10F, which is an immortalized, non-transformed ER-a-negative cell line. Treatment of these cells with $E_2$ or 4-$OHE_2$ produces depurinating estrogen-DNA adducts (26-28). These adducts induce colony formation in soft agar, the expression of which are indicative of neoplastic transformation ability. (29-31). The cells are transformed by estrogens even in the presence of the anti-estrogen tamoxifen or ICI-182,780 (31). The results further indicate that transformation occurs via the genotoxic effects of the estrogen metabolites. The 2-$OHE_2$ metabolite induces these alterations to a much smaller extent. Implantation of estrogen-transformed MCF-10F cells, selected by their invasiveness, into severely compromised immune-deficient mice, produces tumors (30). Female ERKO/Wnt-1 mice were ovariectomized at 15 days old and implanted with $E_2$. Breast tumors developed in a dose-dependent manner (32). Tumors were induced even following implantation of $E_2$ plus the anti-estrogen ICI-182,780 (33). These results support the hypothesis that estrogen metabolism is a crucial event in the initiation of estrogen-induced cancer.

The mutagenicity of $E_1(E_2)$-3,4-Q was first studied in female SENCAR mice by determining the H-ras mutations induced, and the estrogen-DNA adducts formed (34). Equal amounts of the depurinating 4-$OHE_1(E_2)$-1-N3Ade and 4-$OHE_1(E_2)$-1-N7Gua adducts were identified in the skin, representing >99% of the total adducts formed (34). Mutations were observed in the H-ras oncogene within 6-12 h after treatment. The rapid appearance of mutations indicated that they arose by error-prone repair of the apurinic sites generated by the depurinating estrogen-DNA adducts. In a second study, female ACI rats, which are susceptible to estrogen-induced mammary tumors, were treated with $E_1(E_2)$-3,4-Q by intramammillary injection. Depurinating N3Ade and N7Gua adducts as well as H-ras mutations were detected in mammary skin tissue. These results demonstrate the mutagenic activity of these estrogen metabolites (35). With multiple treatments of the mammalian cells with 4-$OHE_1(E_2)$, a dose-dependent, statistically significant increase in mutant fraction was observed (36). The reactive quinone formed from 4-$OHE_1(E_2)$, $E_1(E_2)$-3,4-Q, was similarly mutagenic. However, no mutagenicity was detected when the cells were treated with 2-$OHE_1(E_2)$ (36).

To date, three studies have been conducted in women at normal or high risk for breast cancer. The risk of developing breast cancer is measured as the ratio of estrogen-DNA adducts to their respective estrogen metabolites and conjugates. High levels of estrogen-DNA adducts have been seen in analyses of urine and serum from women that are at high risk of breast cancer (14,37,38). Observation of higher levels of estrogen-DNA adducts in women at high risk for breast cancer would suggest that formation of these adducts is a causative factor in the etiology of breast cancer rather than a consequence of the disease (4).

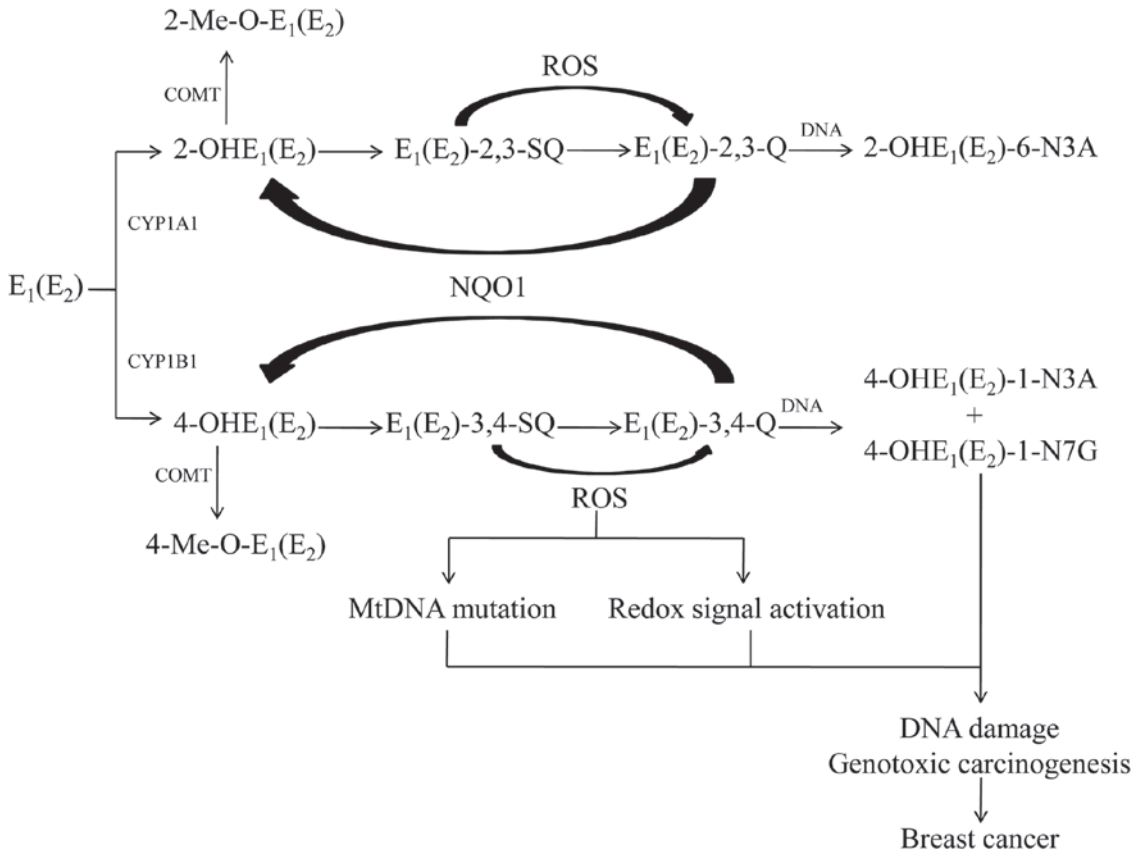


Figure 1. Metabolic pathway for estrogen carcinogenesis. ROS, reactive oxygen species; $E_1$, estrone; $E_2$, 17β-estradiol; NQO1, NAD(P)H-Quinone oxidoreductase 1; MtDNA, mitochondrial DNA; COMT, catechol-O-methyltransferase; CYP, cytochrome P450.

## 4. Role of ROS in estrogen carcinogenesis

Redox cycling via reduction of CE-Q to semiquinones, catalyzed by CYP reductase, and subsequent oxidation back to CE-Q by $O_2$, forms super-anion radicals and then $H_2O_2$ (39). Estrogen-mediated high ROS accumulation serves a key role in driving carcinogenesis (40). Excessive ROS serves as an important effector to increase genomic instability and activate the redox-associated signaling pathway. Physiologically available concentrations of estrogens or estrogen metabolites directly acting on the mitochondria of mammary epithelial cells produces ROS, which subsequently enhances the phosphorylation of kinases to activate redox-sensitive transcription factors (41,42). Therefore, ROS serves an important role in estrogen-induced cancer.

2-$OHE_1(E_2)$ and 4-$OHE_1(E_2)$ are highly redox active and generate ROS in breast epithelial cells (9). The prolonged exposure to estrogen aggravates mutations in mitochondrial DNA (mtDNA) and mitochondrial protein damage by inducing ROS overproduction. Previous studies have identified that instability of mtDNA induces cancer cell metastasis and triggers cancer malignant transformation, whereas ROS scavengers suppress the metastatic potential in mice via alleviation of mtDNA mutation (43). Furthermore, mutations in mtDNA altering expression and function of the mitochondrial respiratory chain was observed in breast cancer cells, blocking of estrogens attenuated the respiratory and metabolic responses and superoxide accumulation (44,45). These results indicated that ROS-triggered mtDNA mutations may contribute to cancer malignant transformation. Furthermore, overproduction of ROS induced by alteration of mitochondrial metabolism is also involved in estrogen-mediated carcinogenesis via induction of oxidative DNA damage (40).

A recent study identified that ROS induced by 4-$OHE_1(E_2)$ causes malignant transformation of MCF-10A cells, and co-treatment with 4-$OHE_1(E_2)$ and biological or chemical ROS scavengers prevents the tumorigenic conversion of MCF-10A cells (41). It appears that oxidant-mediated activation of redox-sensitive phosphatidylinositol 3-kinase/protein kinase B (AKT) pathways serves a pivotal role in tumor malignant transformation of healthy breast epithelial cells by estrogens (41). In addition to AKT, the nuclear factor-κB (NF-κB) family is another important redox transcription factor activated by ROS that has been observed during neoplastic transformation of mammary epithelial cells (46). ROS overproduced by CEs accelerates the nuclear translocation of NF-κB by induction of IκB kinase (IKK) α and -β activities (47). At the same time, inhibition of NF-κB activation by antioxidants has demonstrated a positive associated between NF-κB-associated neoplastic transformation and ROS overproduction (47,48). Excess ROS generated by repeated exposure to 4-$OHE_1(E_2)$ causes malignancy of human mammary epithelial cells in nude mice (41).

In conclusion, estrogen induces the overproduction of ROS, which subsequently initiates multiple biological functions, including mtDNA mutation, alteration of mitochondrial metabolism, and activation of the redox-associatedsignaling pathway, thereby accelerating cell proliferation involved in tumor progression.

## 5. Association of metabolism enzyme polymorphisms in breast cancer

Levels of 4-$OHE_1(E_2)$ in breast cancer are increased compared with healthy breast tissue (14). As CYP1B1 is the key enzyme for the formation of 4-$OHE_1(E_2)$, expression and genetic variations in CYP1B1 may influence breast cancer progression by increasing concentration of 4-$OHE_1(E_2)$. To date, >300 polymorphisms have been found in CYP1B1 (49). The most common polymorphisms of CYP1B1, including Arg48Gly, Ala119Ser, Val432Leu and Asn453Ser, lead to alterations in estrogen metabolism and may influence the risk of breast cancer (49). An *in vitro* study demonstrated that the 4-hydroxylase activities of estradiol by Ala119Ser and Asn453Ser variants of CYP1B1 are 2-4-fold higher compared with wild-types (49). The Val432Leu variant increases CYP1B1 catalytic ability, with a subsequent elevation in 4-$OHE_1(E_2)$ formation (50). The Asn453Ser polymorphism is associated with decreased levels of CYP1B1 cellular protein, which is associated with a reduced risk of breast cancer in postmenopausal women (51). However, the effect of CYP1B1 polymorphisms in breast cancer etiology remains controversial. It has been reported that there are no associations between Arg48Gly, Ala119Ser, Val432Leu and Asn453Ser and breast cancer risk in Polish population, but another study identified that Arg48Gly, Ala119Ser and Val432Leu variants were associated with increased breast cancer risk in the Polish population (52,53). A case-control study reported that Leu432Val and Val432Val genotypes significantly increased breast cancer risk (50). Jiao *et al* (54) also identified that CYP1B1 432 Val variants appear to be a factor for susceptibility to breast cancer.

In contrast to 4-$OHE_1(E_2)$, 2-$OHE_1(E_2)$ is weakly carcinogenic or has protective activity. An increase in CYP1A1 activity directs CEs toward 2-$OHE_1(E_2)$ and away from the genotoxic 4-$OHE_1(E_2)$ (11,19). T3801C, T3205C, A2455 G and C2453A variants in CYP1A1 have been studied in regard to their potential implication in breast cancer risk. The two former variants are located in the 3'-noncoding region, whereas the latter two lead to amino acid substitutions in exon 7 (Ile462Val and Thr461Asp, respectively), which increase CYP1A1 activity (55). Chen *et al* (56) demonstrated that the A2455 G G/G genotype is associated with increased breast cancer risk in East-Asians. Additionally, Caucasian subjects carrying the A2455 G allele also exhibited an elevated breast cancer risk (57). T3801C, T3205C and C2453A variants were not associated with breast cancer risk (57).

COMT is a phase II protective enzyme in that methylation of the catechol metabolites blocks oxidative metabolism to reactive quinones, and thus is protective against formation of the depurinating adducts and ROS. A widely studied single nucleotide polymorphism (SNP) in exon 4 results in the amino acid substitution of Val with Met, termed Val158Met (16). Compared with wild-types, the Met158 variant demonstrates thermo instability; thus, COMT activity is reduced in cells expressing Met158 (58). Previous studies have identified that the Val158Met variant affects protein stability, and altered conformation renders it more susceptible to recognition by the cellular protein degradation processes, thus reducing cell activity (58,59). Meta-analyses have examined the influence of COMT on breast cancer incidence in women. The results suggested that a Val158Met polymorphism in the COMT gene may be a risk factor for breast cancer in the Chinese population (60,61). However, Li *et al* (62) suggested that the COMT Val158Met polymorphism is not a risk factor for breast cancer in the Asian population.

However, numerous studies have been performed to investigate the association of these polymorphisms with susceptibility to breast cancer. However, the results of these studies remain conflicting. Together, these results demonstrate that without some measure of enzyme expression or activity, it is difficult to predict and interpret results from these types of SNP-association studies.

## 6. Conclusion

Increasing studies have identified that excessive exposure to estrogens is associated with increased breast cancer risk. Results from *in vivo* and *in vitro* studies have indicated that oxidative metabolism of estrogens serves a major role in the initiation of breast cancer. The oxidative metabolism of estrogens to reactive quinones causes both formation of depurinating adducts and production of ROS, which are associated with breast cancer progression. The regulation of metabolism enzymes, which are responsible for estrogen metabolism, are critical for the homeostasis of estrogen. SNPs in enzymes may influence the risk of breast cancer, but the results remain conflicting. Future investigations into the role of estrogen metabolism, phase I and phase II involvement in estrogen metabolism, and their SNPs are required to measure specific biomarkers of metabolites. This may involve determination of the levels of the specific adenine and guanine DNA adducts and markers of oxidative DNA damage, detectable in urine and plasma. Through this approach, the role of SNPs in the formation and inactivation of enzymes, including CYP1A1, CYP1B1 and COMT, maybe determined.

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