Dual roles of estrogen metabolism in mammary carcinogenesis

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A female hormone, estrogen, is linked to breast cancer incidence. Estrogens undergo phase I and II metabolism by which they are biotransformed into genotoxic catechol estrogen metabolites and conjugate metabolites are produced for excretion or accumulation. The molecular mechanisms underlying estrogen-mediated mammary carcinogenesis remain unclear. Cell proliferation through activation of estrogen receptor (ER) by its agonist ligands and is clearly considered as one of carcinogenic mechanisms. Recent studies have proposed that reactive oxygen species generated from estrogen or estrogen metabolites are attributed to genotoxic effects and signal transduction through influencing redox sensitive transcription factors resulting in cell transformation, cell cycle, migration, and invasion of the breast cancer. Conjugation metabolic pathway is thought to protect cells from genotoxic and cytotoxic effects by catechol estrogen metabolites. However, methoxylated catechol estrogens have been shown to induce ER-mediated signaling pathways, implying that conjugation is not a simply detoxification pathway. Dual action of catechol estrogen metabolites in mammary carcinogenesis as the ER-signaling molecules and chemical carcinogen will be discussed in this review. [BMB reports 2011; 44(7): 423-434]

INTRODUCTION

Beatson's original observation on breast cancer regression after ovariecctomy provided the original insight into the estrogen-dependent nature of the disease (1). Estrogens are composed of a total of 9 chemically different steroid of which the three major ones are 17β-estradiol (E2), estrone (E1), and estriol (E3) (2). These estrogens are essential for the growth and maintenance of various reproductive and non-reproductive organs in which they elicit different growth responses in tissues depending on the cell-types, a type of estrogen receptor (ER) present, concentration and timing of exposure (3). Epidemiology and animal studies demonstrated the firm link between elevated exposure to estrogen and the development of breast cancer (4, 5). The longer females are exposed to estrogen either through early menarche, late menopause and/or estrogen replacement therapy (ERT) (6), the more increased is the risk of developing breast cancer. The high level of serum estrogen is associated with incidence of breast cancer in premenopausal women (7). Estrogen exposure through hormone replacement therapy (HRT) in postmenopausal women has been associated with cancers of the estrogen-dependent tissues such as breast, cervix, and endometrium (8, 9). Studies in animal models have demonstrated that estrogens are established breast carcinogens (10, 11). These accumulating data on the causative effects of estrogens led the International Agency for Research (IAR) and the National Toxicology Program of National Institute of Environmental Health Sciences (NIEHS) to declare that steroid estrogens, as both endogenous and exogenous sources, are "known to be human carcinogens" (12, 13).

The molecular mechanisms underlying the estrogen-induced carcinogenesis are not well understood and remains elusive (14, 15). Three major mechanisms are postulated to be involved in carcinogenic effects of estrogens: (1) stimulation of cell proliferation via ER-mediated hormonal activity, (2) genotoxic effects by the metabolites and/or ROS generated during a cytochrome P450 (CYP)-mediated estrogen metabolism leading to increased mutation rates or chromosome abnormalities, and (3) regulation of activities of enzymes or transcription factors involved in redox signaling by estrogen-induced ROS. All of these proposed mechanisms may lead to the conclusion that estradiol and its oxidative metabolites cause either tumor initiation or promotion.

Both endogenous estrogens and xenoestrogens such as equine estrogens are converted into catechol estrogens via CYP-catalyzed metabolism (16). The catechol estrogens are further metabolized by O-methylation, reaction with glutathione (GSH), glucuronidation, and sulfation (17, 18). Reactive oxygen species (ROS) are generated via the redox cycling between catechol estrogens and their quinone analogues. Catechol estrogen metabolites and ROS have been shown to modify the gene structure, number of chromosomes, and activities of proteins associated with redox signaling and contribute to initiating and/or promoting the cellular transformation (15, 19-21). In addition, the catechol estrogen metabolites display the ER-mediated estrogenic action (22, 23), implying that the catechol estrogens are attributed to both their direct genotoxic effects as well as ER-mediated...
The roles of estrogens and their metabolites in mammary carcinogenesis will be discussed. The direct or indirect modifications at the gene levels will be described as one of the prime mechanisms of tumor initiation by estrogen metabolites as well as estrogen-induced ROS. ER-mediated tumor promotion effects will be then discussed by the parent estrogen and catechol estrogens as well as conjugated metabolites which are conventionally envisioned as the detoxified ones. Prediction models for the breast cancer risk due to estrogen metabolism will be finally reviewed to fortify the concept that estrogen metabolism plays a critical role in development of breast cancer and provide a useful tool in risk prediction.

**METABOLISM OF ESTROGEN**

E2 and E1 are interconvertible by the 17β-hydroxysteroid dehydrogenase (17β-HSD) (30). Phase I metabolism of estrogen converts E2 and E1 to catechol estrogens and 16α-hydroxyestrogens (Fig. 1). The catechol estrogens are 2-hydroxy (2-OH) or 4-hydroxyestrone/estradiol (4-OHE1/E2) through catalysis by CYP1A1 in the liver or CYP1B1 in the extrahepatic target tissues such as breasts, ovaries, and uterus (31). It is noteworthy that 4-OHE2 was shown to be carcinogenic in the animal kidney tumor models whereas 2-hydroxy catechol estrogens were...
not (32, 33). Treatment of 4-OHE2 induced higher incidence of uterine tumors in CD-1 mice compared to 2-OHE1/2 or 2-OHE2 (34). Although ACI rat model was not successful to show the carcinogenic effects by 4-OHE2, DNA adducts of catechol estrogens have been detected in the mammary glands of ACI rats treated with 4-OHE2 or its quinone (35, 36). In addition, it has been shown that 4-OHE2 is similar to E2 in its ER binding affinity and activating the classical ER signaling pathway leading to its uterotropism in animals (37, 38). Various study results suggest that 4-OHE2 displays dual roles as both chemical and hormonal carcinogen.

Increasing unsaturation in the B ring in xenoestrogens alters the preference of aromatic hydroxylation of the A ring from 2-hydroxylation to 4-hydroxylation (16, 39). Phase I metabolism of equine estrogens present in hormone replacement therapy (HRT) leads to formation of much more 4-hydroxycatechol

![Diagram](http://bmbreports.org)

**Fig. 2.** (A) Primary Phase I metabolism of the equine estrogens present in HRT, equilenin and equilin, (B) Metabolism of 4-OHEN to form quinoids, reactive intermediates, and conjugates and reaction of 4-OHEN-o-quinone with DNA.
equine estrogen metabolites than 2-hydroxymerabolites and it is, therefore, reasonable to hypothesize that formation of 4-hydroxycatechol estrogen is attributed to the major carcinogenic pathway for equine estrogens (Fig. 2).

16α-Hydroxylation is catalyzed presumably by CYP2D6 or 3A4 (40). The roles of 16α-OHE1/E2 in terms of physiological function and carcinogenic effect still remain ambiguous. For this reason, this review will limit the discussion to only catechol estrogen metabolites.

The catechol estrogens can be oxidized by any oxidative enzyme or metal ions such as Cu2+ or Fe3+ to give rise to semiquinones and α-quinones (41-43) (Fig. 1). Reduction of α-quinones back to semiquinones and catechols provides an opportunity to generate ROS including superoxide anion radicals and hydroxyl radicals. Metal ions and hydroxyl radicals are thought to be responsible for oxidative damage to macromolecules such as DNA or lipids (44, 45).

Conjugation of estrogens makes them water soluble to be readily excreted or much more lipophilic to confer longer half-lives than the parent estrogens (46-48). It has been reported that these metabolic conjugation reactions play a critical role in deactivation of the redox active estrogen metabolites and marked reduction of the classical estrogenic activities of the parent estrogens (49, 50).

These results suggest that the conjugation pathway is considered as the protection mechanism against damage caused by reactive metabolites of endogenous estrogens and xenestrogens (17). Catechol O-methyl transferase (COMT) catalyzes the methoxyla-
tion of either 2-OHE1/E2 or 4-OHE1/E2 to form its O-methylxestro-
gens. Since the O-methylation reduces the circulate catechol estrogens thereby preventing biotransformation of catechol estrogen to quinones and generation of ROS, this pathway is regarded as the detoxification pathway. Furthermore, it has been found that 2-methoxyestradiol (2-MeOE2) possesses antitumor activity (51-53).

It is of special interest that 4-hydroxyequilenin (4-OHEN), one of major catechol equine estrogen metabolites, can inhibit the activities of detoxification metabolizing enzymes such as gluta-
thione S-transferase P1-1 (GSTP1) and COMT (54-56). COMT variant with a low activity due to Val/Met polymorphism was more sensitive to 4-OHEN-mediated inhibition than the wild-type COMT (57). This implies that equine estrogen metabolites contrib-
ute to breast carcinogenesis by inhibition of protective drug metab-
olizing enzymes and supports the hypothesis that women with ho-
mozygous for the polymorphic variant COMT with a low activity could be exposed to higher risk when taking HRT formulations con-
taining equine estrogens such as Premarin®.

Endogenous estrogens and equilenin are subject to sulfation via catalysis of the steroid sulfotransferases to form sulfate conjugate. Although sulfation leads to decrease in hormonal activities of the parent estrogens by facilitating their excretion, a metabolic clearance rate of a sulfonated steroid is very slow (58, 59). In addition, sulfonated estrogens themselves display almost no ER binding affinity whereas estrogens released from sulfon-
ated ones may contribute to high levels of estrogen in target tis-
ues (46, 60). Indeed, E3-3-sulfate is a major circulating metabo-
lite and thought to be an important precursor of the active estro-
gen in postmenopausal women (60, 61). These findings cast a question whether sulfation truly represents a deactivation meta-

colic pathway.

Formation of glucuronide conjugate is catalyzed by UDP-glucuronosyltransferase and hydrolysis of estrogen glucuronide is by β-glucuronidase (17). The roles of these opposing metabolic reactions are similar to those described above in estrogen sulfate formation and sulfate hydrolysis. It has been shown that the es-

trogen glucuronide acts as one of the precursor of E2 in vivo and inhibition of β-glucuronidase-mediated hydrolysis of estrogen glucuronides suppressed the estrogen-dependent mammary tu-
mor promotion in Sprague-Dawley rat models (62, 63). It should be noted that estrogen sulfates or glucuronides can also be hy-

droxylated in steroid A-ring in liver as well as extrahepatic target cells (64). Deconjugation of hydroxylated conjugates may lead to catechol estrogen metabolites, which are partly responsible for estrogen-mediated toxic effects. Taken together, the regu-
lation of conjugation and hydrolysis of sulfates or glucuronides would be the attractive drug target for breast cancer prevention or treatment (65, 66).

ESTROGEN RECEPTOR SIGNALING-MEDIATED CARCI-

NOCESIS

Binding of E2 to its receptor, ERα/β, amplifies signals in either ge-
nomic, nongenomic, or mitochondrial ER-mediated signaling pathways that lead to increased cell proliferation and inhibition of apoptosis in uncontrolled cell division or growth and tumor pro-
motion (67).

The classical ER signaling pathway as one of the genomic path-
ways means by direct binding of E2-bound ER homodimers to the estrogen-response elements (ERE) in the regulatory regions of es-

trogen-responsive genes whose transcription is altered depending on binding basal transcription factors, coactivators, or core-

pressors. Indirect or non-classical action of ER on DNA is mediated via protein-protein interaction of the ER with transcription factors such as Sp-1, AP-1, or GATA1 that would bind to the specific DNA sites for regulation of target gene transcription (42, 68, 69). When genes involved in cell growth are altered upon estrogen exposure, increases in estrogenic activity may lead to in-
creased cell proliferation leading to estrogen-mediated tumor promotion and/ or progression.

Tyrosine-kinase receptors (TKR) can activate ER through phos-
phorylation in the absence of ligand and this pathway represents one of the nongenomic ER signaling pathways. Rapid activation of various protein kinases including mitogen-activated protein kinases (MAPKs) and increase in second messengers such as cy-

cyclic AMP (cAMP) have been reported via E2/ER-mediated activa-
tion (70, 71). These types of transcriptional effects do not involve direct activity of ER as a nuclear transcription factor, therefore, it is called as the nongenomic type of ER signaling pathway. Membrane-bound form of ERα, ERβ, or both is suggested to be asso-
related with nongenomic pathway which may provide the plat-
form for the crosstalk with growth factor-mediated signaling transduction pathways (72, 73). It is thought that either ER or growth factor-mediated signaling pathway is converged on activation of MAPK pathways that play a critical role in regulation of apoptosis, cell proliferation, and cell-cycle control, thereby, leading to growth of tissue and/or tumor (67, 72). Crosstalk between the genomic ER signaling cascades and kinase transduction pathways has a significant implication as a valuable therapeutic target to control ER-mediated cell proliferation and tumor growth.

The presence of ERα, ERβ, or both in mitochondria of various cells and tissues has been reported (74). EREs have been found in the promoter regions of certain genes and estrogen was able to increase transcription of mitochondrial DNA-encoded genes in ER-mediated fashion (75, 76). Further research is required to elucidate how ER is imported to mitochondria, how ER functions to induce transcription of mitochondrial DNA, and what are the ultimate effects of E2-stimulated gene transcription in mitochondria on cellular proliferation and growth.

The catechol estrogen metabolites have high binding affinities to the ER at a comparable level to E2 itself and induce estrogen-responsive gene expression via classical ER-mediated pathways (23, 77). It is of our special interest that O-methoxylated catechol estrogens also displayed the proliferative effects via genomic ER signaling pathways and enhanced tumor growth in animal models (26, 27). These findings would contradict the notion that O-methoxylolation represents a favorable biotransformation, since 2-MeOE2 possess antitumor activity in ER-independent mechanisms and it is generally accepted that O-methoxylolation prevents cells from genotoxic effects by the reactive intermediates through catechol estrogen formation. Activation of ER signaling pathways by catechol estrogens as well as methoxylated estrogens implies that estrogen metabolites have the potential to promote tumor an ER agonist and O-methoxylolation metabolic pathway may not fully protect cellular damage from reactive intermediates generated during oxidative estrogen metabolism.

As far as equine estrogen metabolites are concerned, a major catechol metabolite of equine estrogens present in HRT formulation, 4-hydroxyequilenin (4-OHEN; Fig. 2) and 4-methoxyequilenin (4-MeOEN; Fig. 2) were full ER agonists and induced cell proliferation depending on the redox state of cells (22, 25).

Both of the equine estrogen metabolites were shown to have the classical ER signaling effects despite of its extremely low binding affinity to the ER protein (22). In addition, treatment of MCF-7 cells with these metabolites resulted in activation of ERK within 5 minutes, implying that equine estrogen metabolites are involved in activation of nongenomic ER signaling pathway (25). More studies are guaranteed to determine the effects of the equine estrogen metabolites on the tumor promotion and progression in human breast tissues.

GENOTOXIC EFFECTS VIA OXIDATIVE ESTROGEN METABOLISM

Mutation in critical regulatory genes triggers cancer. This type of carcinogenesis process is called as tumor initiation since mutation would result in abnormal DNA repair, DNA replication, and cell proliferation and therefore provide an initial opportunity for cells to lose normal cell cycle control (78). Substantial evidence supports that the estrogen metabolites react with DNA leading to the mutations responsible for the initiation of cancer. Quinoids and hydroxyl radicals generated during the oxidative estrogen metabolism are known to induce either oxidative DNA damage such as formation of 8-hydroxyguanosine (8-OHdG) or stable or apurinic DNA adducts. Balance between bioactivation of estrogen and supposedly deactivating conjugation pathways would determine whether estrogen metabolites cause DNA damage. In addition, specific types of DNA damage and DNA repair mechanisms would also affect the ultimate tumor initiation effects by estrogen metabolites and reactive intermediates.

The strong oxidizing agent hydroxyl radicals play a major role in oxidative damage to DNA bases. It has been shown that treatment of E2 in hamsters induces various free radical-mediated oxidative damage including DNA single strand breaks (44, 45), formation of 8-OHdG (79), and chromosome abnormalities (80). It should be noted that the level of 8-OHdG has been utilized as a biomarker of oxidative damage or carcinogenesis because this lesion is relatively easily formed and is mutagenic. Mutations that may arise from formation of 8-OHdG involve GC → TA transversions (81). Treatment of 4-OH-E2 induced both oxidative stress and apoptosis in ER-human mammary epithelial MCF-10A cells (82). Tumor initiation effects by catechol estrogens in the absence of major forms of ER in this cell line suggest that ER is not an essential molecular determinant for estrogen-induced carcinogenesis and estrogen metabolism plays a viral role in carcinogenesis.

Formation of DNA adducts is referred to direct DNA damage via chemical reaction of DNA with by quinoid estrogen metabolites such as semiquinones, quinone methids, or quinones. Two types of DNA adducts are formed: stable ones and depurinating ones. Stable ones are obtained when carcinogens react with the exocyclic N6 amino group of adenine or N2 amino group of guanine and they remain in the DNA until they are removed by the DNA repair machinery. Depurinating adducts are formed when carcinogens covalently bind at the N3 or N7 of adenine (Ade) or the N7 of guanine (Gua). They are lost from the DNA by destabilization of the glycosyl bond leaving apurinic sites in the DNA that can generates the mutations. Evidence from the studies performed with estrogens as well as polycyclic aromatic hydrocarbon (PAH)-DNA adducts suggests that depurinating adducts play a major role in tumor initiation compared to stable adducts (83). Furthermore, only the N3-Ade adduct is likely to induce mutations since this adduct depurinates instantaneously, whereas the N7-Gua adduct takes hours to hydrolyze (84).

Quinones from the 4-OHE2/E2 (4-OHE2/E2-α-quinone; Fig. 1) and to much lesser extent, 2-OHE2/E2-α-quinone were shown to react with DNA and generated the critical mutations (85). In
particular, the carcinogenic 4-OHE/E2 are oxidized to form predominantly the depurinating adducts, 4-OHE/E2-1-N3-Ade and 4-OHE/E2-1-N7-Gua (86, 87). Reaction with 2-OHE2-quinone methide produced stable Ade or Gua adducts. Considering the fact that the redox potentials of 2-OHE/E2 and 4-OHE/E2 are similar, the greater carcinogenicity of the 4-OHE/E2 would come from the experimental results in which 4-OHE/E2 forms the carcinogenic depurinating DNA adducts than 2-OHE/E2 at higher levels and 2-OHE/E2 forms stable adducts. It is important to acknowledge that stable bulky Gua adducts of 4-OHE/E2 have been detected in human breast tumor tissue (88).

Reactive intermediates generated from the redox cycling between catechol equine estrogens and their quinones were shown to induce variety of DNA lesions in vitro, in vivo, and even in human. Reactions of 4-OHEN-o-quinone and ROS produced oxidative DNA damage (89, 90), a depurinating Gua adduct (91), and stable bulky cyclic adducts (91, 92) in cells and animals treated with 4-OHEN or Premarin. Finally, oxidative DNA damage was detected in peripheral leukocytes of postmenopausal women receiving HRT containing conjugated equine estrogens (93). In addition, cyclic stable dC, dG, and dA adducts of 4-OHEN were detected for the first time in part of samples of women with a known history of Premarin-based HRT (88).

Detection of stable adducts through reaction with the α-quinones of both endogenous and equine estrogens in human samples raises a question whether the depurinating adducts play a more causative role in estrogen-induced genetic mutation compared to stable ones; however, these data strongly implied that ultimate mutation potential might be altered by DNA repair activities and its kinetics. Finally, various animal model studies as well as investigation done in human samples suggest that the estrogen metabolism plays a significant role in carcinogenic process and 4-hydroxylation is a more carcinogenic biotransformation pathway than 2-hydroxylation.

**ROS-MEDIATED REDOX SIGNALINGS**

A low level of ROS is beneficial to normal cellular process including signal transduction, apoptosis, cell differentiation, and regulation of transcription factors (94, 95). However, excess ROS could chemically modify cellular macromolecules including DNA, proteins, carbohydrates, or lipids, thereby disrupting normal physiological functions of these biomolecules (96).

Oxidative estrogen metabolism produces ROS levels enough to alter ROS homeostasis in cells. These "estrogen-induced ROS" may directly affect the redox-sensitive transcription factors such as nuclear factor-erythroid-2-related factor 2 (Nrf2), activating protein 1 (AP-1), or NF-κB transcription factor, all of which are involved in mediating inflammatory responses and key players in carcinogenesis (97). General concepts in outcomes and mechanisms of activation of these redox sensitive transcription factors by ROS are reviewed elsewhere (98).

Regulation of these transcription factors is also mediated via activation of kinase signaling pathways and activities of the kinases are modulated through cysteine-based phosphatases (CBPs). Reversible oxidation and reduction of cysteine residues present in CBPs is a major regulation mechanism by which estrogen-induced ROS ultimately regulate signaling pathways at various levels and contributes to carcinogenic processes in estrogen-dependent breast tumors. This mechanism involves oxidative modification of critical cysteine in phosphatases that catalyze the dephosphorylation of protein kinases involved in kinase signaling pathways, such as MAPKs, followed by activation of redox sensitive transcription factors. The gene regulation via these redox transcription factors will eventually affect the expression of genes involved in cell transformation or growth. Therefore, the estrogen-induced ROS ripple their oxidative properties cross the gene regulation leading to cancer promotion or progression.

CBPs regulated by estrogen-induced ROS include protein tyrosine phosphatases (PTPs), dual-specificity phosphatases such as Cdc25s, low molecular weight PTPs, and the lipid phosphatase such as phosphatase and tensin homolog (PTEN). Inter or intramolecular disulfide bonds due to oxidation of cysteines in the catalytic sites lead to dramatic changes in structural conformation and prevent the enzymes from normal activities. It has been reported that E2 at physiological levels caused a rapid decrease in Cdc25A activity in ERα+ human breast cancer cells, MCF-7 (99). The same study showed that a lower level of free thiol present in Cdc25A was observed in E2-treated samples and that treatment of antioxidant with E2 prevented oxidation of thiol residues, implying that estrogen-induced ROS is attributed to inactivation of Cdc25A. Other phosphatases such as mitogen-activated protein kinase phosphatase 3 (MKP3), PTEN, PTP1B1, and PTP2A have been shown to respond to ROS and regulate estrogen-mediated signaling. For example, MKP3, ERK-specific phosphatase, is regulated upon improper ROS levels and may upregulate ERK-1/2 pathway leading to phosphorylation at serine 118 position of ERα (100).

Conformational changes in CBPs lead to upregulation of signaling cascades including src/Abi-dependent, MAPK-dependent, and phosphoinositol 3 kinase (PI3K)-dependent pathways. All of these kinase signaling pathways are known to activate redox sensitive transcription factors. Phosphorylation of A-Raf localized in mitochondria was stimulated upon E2 exposure to MCF-7 cells and as a result, cell cycle progression was increased (101). A-Raf/MEK/MAPK signaling cascade is thought to play a crucial role in cell cycle control by estrogen-induced ROS. One of the redox sensitive kinases is c-Jun N-terminal Kinase (JNK) family that is involved in stress responses, apoptosis, and cell proliferation. Increased level of ROS triggers the detachment of JNK associated GSTP1 as well as a knock down of a JNK phosphatase, facilitating the JNK activation (102). These data suggest that the A-Raf/JNK signaling is a major pathway that responds to estrogen-induced ROS and mediates oncogenic signals leading to cell proliferation.

Mechanisms of rapid activation of the MAPKs, in particular...
ERK-1 and -2, in response to E2 are suggested to involve the actions via membrane bound ERα/β or G-protein coupled receptor 30 (GPR30). Since it has been shown that hydrogen peroxide is able to activates ERK-1/2 and inactivate Cdc25A in MCF-7 cells and both proteins form a protein complex, it is possible that ERK may be activated by inhibiting its association with Cdc25A or inactivating Cdc25A by estrogen-induced ROS (103).

PI3K/Akt pathway is another signaling event regulated by estrogen-induced ROS. Activation of Akt by E2 or 4-OHE2 is either ER-independent or dependent (104, 105). The exact mechanism of estrogen-mediated Akt activation is unclear; however, it is possible that the reversible inactivation of Cdc25A or PTEN by estrogen-induced ROS plays a key role in the activation of Akt.

A common feature of CBPs-targeted kinase signaling pathways such as A-Raf/MEK/MAPK and PI3K/Akt is that they affect the activities of redox sensitive transcription factors. Estrogen-induced ROS inactivate CBPs and then upregulate various kinase pathways resulting in regulation of AP1, Nrf-1, or NF-κB transcription factors and estrogen carcinogenesis.

PREDICTION OF BREAST CANCER RISK

Cancer risk prediction models provide an important approach to assessing risk and susceptibility by identifying individuals at high risk, facilitating the design and planning of clinical chemoprevention trials, and allowing the evaluation of interventions. Conventional breast cancer risk model includes the cumulative estrogen exposure data such as age, age at menarche and menopause, age at first live birth, and use of HRT in risk calculation, since it is well recognized that estrogens are the prime risk factor for mammary carcinogenesis (106). As discussed throughout this review, more recent data confirm that estrogen metabolism plays an essential role in initiation, promotion, and/or progression of breast cancer. Current models do not indicate any of factors associated with estrogen metabolism and a more complete risk model is required to reflect metabolic and genotypic components in estrogen metabolism in risk calculations. Several models attempted to include the enzyme kinetics of a single enzyme which is the major Phase I or conjugation metabolizing enzymes such as CYP1A1, CYP1B1, COMT, or GSTP1 in a qualitative manner (107, 108). However, estrogen metabolic pathways are interconnected and complex. Furthermore, each of the metabolizing enzymes contains genetic polymorphisms that could result in alteration of catalytic activity of the enzyme. The fact that genetic variation does not quantify the functional consequences of the enzyme activities makes it more complicated to develop a quantitative model. The models could reflect the factors associated with estrogen metabolism into risk calculations only in a qualitative manner. It is, therefore, necessary to develop a refined model that utilizes a pathway-based functional and quantitative approach. The most recent risk prediction model proposed by Parl and his colleagues is developed to incorporate estrogen exposure parameters, individual phenotypic factors such as body mass index or family history, and the functional effects of genetic variants of CYP1A1, CYP1B1, and COMT (29, 109). Consideration of phenotypic factors and genetic polymorphism will allow researchers to predict the exposure to carcinogenic catechol estrogen metabolites at more accurate and quantitative levels in this novel genotypic-phenotypic model. As with many other in silico models based on computer-assisted calculations, this model has the limitation in that actual estrogen metabolites cannot be measured. Taking into account that it is not impractical to obtain a sufficient number of samples to provide the accurate measures for estrogen exposure time or amounts and to measure the estrogen metabolites in

Fig. 3. Proposed mechanisms of estrogen-induced mammary carcinogenesis.
tumor samples, integration of traditional estrogen risk factors and phenotypic factors with genetic variation in estrogen metabolism would provide the plausible risk prediction model.

CONCLUSIONS AND FUTURE DIRECTIONS

The estrogenic action through ER signaling pathways plays a critical role in normal development of mammary gland as well as promoting growth of ER+ breast cancer cells. The fact that mammary tumor can develop in ER-α knockout mice suggests that the estrogen-induced activation of ER signaling pathway is neither the prime nor only factor in breast carcinogenesis (11, 110). Reactive intermediates and ROS generated during oxidative estrogen metabolism are attributed to DNA damage and chromosome abnormalities leading to cancer initiation (Fig. 3). Conjugation metabolic pathways such as O-methylation and sulfate or glucuronide conjugation have been regarded as deactivation pathways against estrogen metabolite-induced toxicities. However, accumulating data suggest that methoxylated catechol estrogens possess ER agonist-like properties that could result in cell transformation and tumor growth. Sulfate/glucuronide conjugates seem to play a role as cellular reservoir for free estrogen (Fig. 3). These findings suggest that conjugation metabolism may not represent detoxification. The most recent risk prediction model utilizes the functional and genetic effects of COMT or GST, although it is controversial that this type of conjugation mitigates the catechol estrogen-mediated carcinogenic properties. Therefore, more extensive research on the interaction between estrogen metabolic pathways, polymorphism of estrogen metabolizing enzymes, and carcinogenic potentials of each of estrogen metabolites are guaranteed to evaluate the dual roles of estrogen metabolism on mammary carcinogenesis. The equine estrogens are guaranteed to evaluate the dual roles of estrogen metabolism, polymorphism of estrogen metabolizing enzymes, and carcinogenic potentials of each of estrogen metabolic pathways, conjugation metabolic pathways against estrogen metabolite-induced toxicities, and phenotypic factors with genetic variation in estrogen metabolism.

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