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Invited Mini Review

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. Conjuguation 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 ovariectomy 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 (E_2), estrone (E_1), and estriol (E_3) (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 steroidal 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

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tumor promoter. Conjugation metabolic pathways are thought to protect cells from mitogenic and genotoxic effects by the parent estrogen as well as the catechol estrogen metabolites (24). *O*-Methoxylated conjugates are known to have very low binding affinity to the ER but to activate both genomic and non-genomic ER-signaling pathways (25-27). These findings imply that methoxylation may not be a deactivation pathway and make the hypothesis plausible that various types of estrogen metabolites as well as the parent estrogen may play a critical role in breast tumor promotion via the ER-mediated pathway.

As the recent research results define the estrogens and its metabolites as a prime breast cancer risk, prediction models for breast cancer risks have been developed on the basis of estrogen exposure as well as estrogen metabolism and metabolizing enzyme variants. For a more precise and individualized prediction, the kinetic effects of genetic variants of the major estrogen metabolizing enzymes are taken into account in the recent prediction model (28, 29).

In this review, 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

E_2 and E_1 are interconvertible by the 17β-hydroxysteroid dehydrogenase (17β-HSD) (30). Phase I metabolism of estrogen converts E_2 and E_1 to catechol estrogens and 16α-hydroxyestrogens (Fig. 1). The catechol estrogens are 2-hydroxy (2-OH) or 4-hydroxyestrone/estradiol (4-OHE_1/E_2) 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-OHE_2 was shown to be carcinogenic in the animal kidney tumor models whereas 2-hydroxycatechol estrogens were

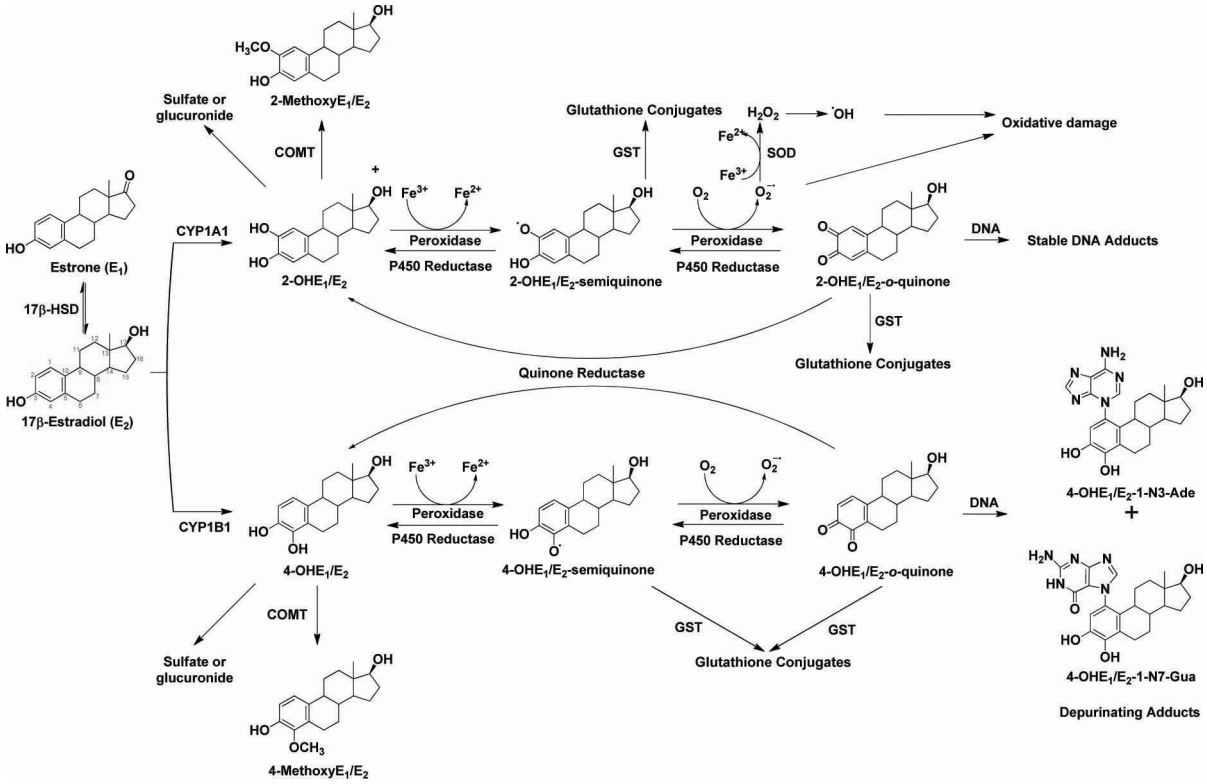

Fig. 1. Scheme showing metabolism of the endogenous estrogens, estrone (E_1) or 17β-estradiol (E_2), generation of reactive oxygen species during the redox cycling, and reaction of quinones with DNA bases. Production of hydroxyl radical from 4-OHE_1/E_2 is not shown here and occur in the same fashion to those in 2-OHE_1/E_2. 2-OHE, 2-Hydroxyestrogen; 4-OHE, 4-hydroxyestrogen; COMT, catechol *O*-methyltransferase; GST, glutathione S-transferase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; CYP, cytochrome P450; SOD, superoxide dismutase. Numbers in red denote the numbering in the steroid skeleton.

not (32, 33). Treatment of 4-OHE_2 induced higher incidence of uterine tumors in CD-1 mice compared to 2-OHE_1/E_2 or E_2 (34). Although ACI rat model was not successful to show the carcinogenic effects by 4-OHE_2, DNA adducts of catechol estrogens have been detected in the mammary glands of ACI rats treated with 4-OHE_2 or its quinone (35, 36). In addition, it has been shown that 4-OHE_2 is similar to E_2 in its ER binding affinity and activating the classical ER signaling pathway leading to its uterotropic potency in animals (37, 38). Various study results suggest that 4-OHE_2 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

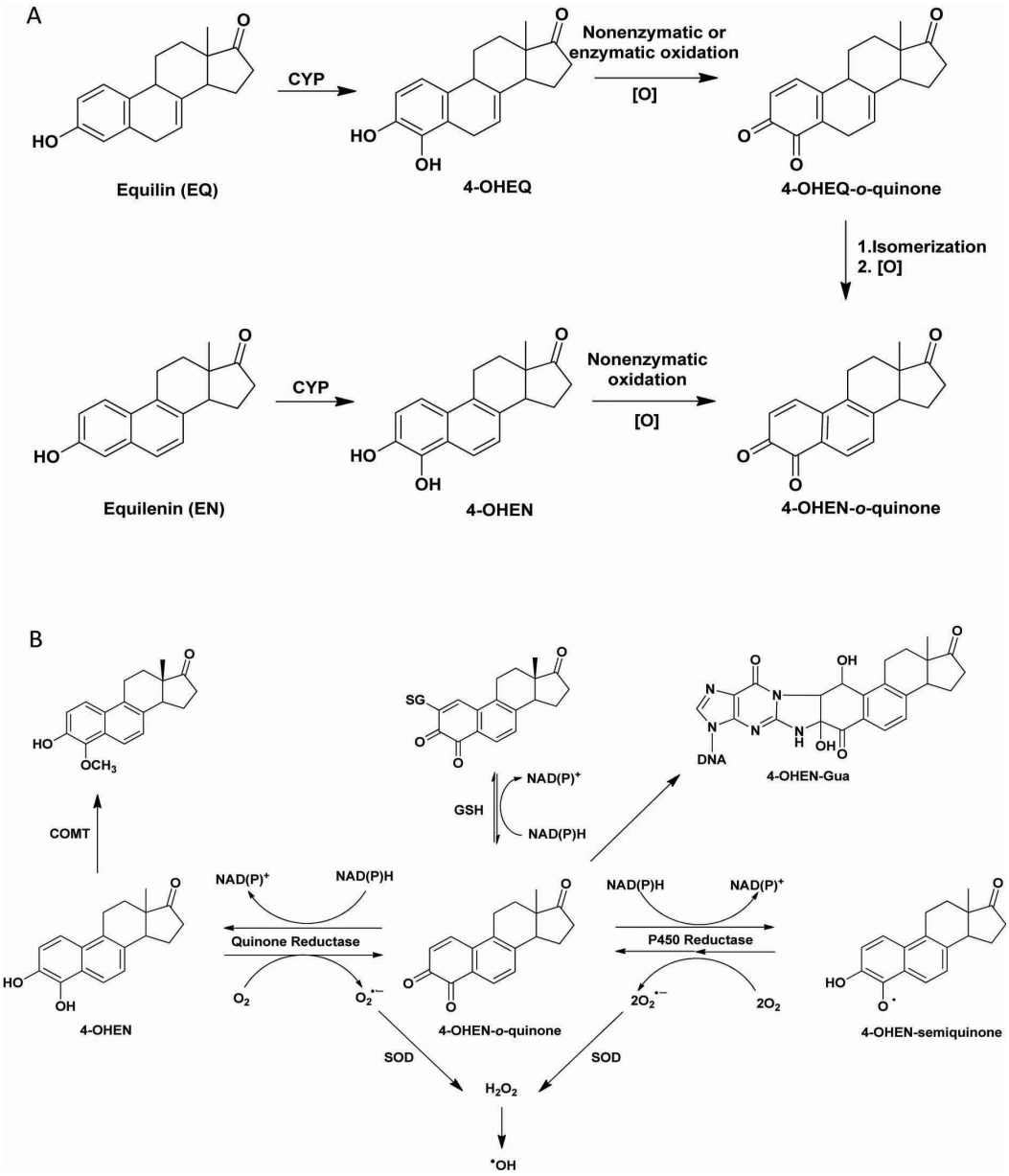

Fig. 2. (A) Primary Phase I metabolism of the equine estrogens present in HRT, equilenin and equilin, (B) Metabolism of 4-OHEN to form quinoids, reactive intermediates, and conjugates and reaction of 4-OHEN-*o*-quinone with DNA.

equine estrogen metabolites than 2-hydroxymetabolites 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-OHE_1/E_2 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 Cu^{2+} or Fe^{3+} to give rise to semiquinones and o-quinones (41-43) (Fig. 1). Reduction of o-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 xenoestrogens (17). Catechol *O*-methyl transferase (COMT) catalyzes the methoxylation of either 2-OHE_1/E_2 or 4-OHE_1/E_2 to form its *O*-methyoxyestrogens. Since the *O*-methylation reduces the circulating 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-$MeOE_2$) 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 glutathione 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 contribute to breast carcinogenesis by inhibition of protective drug metabolizing enzymes and supports the hypothesis that women with homozygous for the polymorphic variant COMT with a low activity could be exposed to higher risk when taking HRT formulations containing 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 sulfonated ones may contribute to high levels of estrogen in target tissues (46, 60). Indeed, E_1-3-sulfate is a major circulating metabolite and thought to be an important precursor of the active estrogen in postmenopausal women (60, 61). These findings cast a question whether sulfation truly represents a deactivation metabolic 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 estrogen glucuronide acts as one of the precursor of E_2 *in vivo* and inhibition of β-glucuronidase-mediated hydrolysis of estrogen glucuronides suppressed the estrogen-dependent mammary tumor promotion in Sprague-Dawley rat models (62, 63). It should be noted that estrogen sulfates or glucuronides can also be hydroxylated 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 regulation 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 CARCINOGENESIS

Binding of E_2 to its receptor, ERα/β, amplifies signals in either genomic, nongenomic, or mitochondrial ER-mediated signaling pathways that lead to increased cell proliferation and inhibition of apoptosis t uncontrolled cell division or growth and tumor promotion (67).

The classical ER signaling pathway as one of the genomic pathways means by direct binding of E_2-bound ER homodimers to the estrogen-response elements (EREs) in the regulatory regions of estrogen-responsive genes whose transcription is altered depending on binding basal transcription factors, coactivators, or corepressors. 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 increased cell proliferation leading to estrogen-mediated tumor promotion and/ or progression.

Tyrosine-kinase receptors (TKR) can activate ER through phosphorylation 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 cyclic AMP (cAMP) have been reported via E_2/ER-mediated activation (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 associated 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 E_2-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 E_2 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*-methoxylation represents a favorable biotransformation, since 2-MeOE_2 possess antitumor activity in ER-independent mechanisms and it is generally accepted that *O*-methoxylation prevents cells from genetoxic 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*-methoxylation 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 E_2 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-OHE_2 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 N^6 amino group of adenine or N^2 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-OHE_1/E_2 (4-OHE_1/E_2-*o*-quinone; Fig. 1) and to much lesser extent, 2-OHE_1/E_2-o-quinone were shown to react with DNA and generated the critical mutations (85). In

particular, the carcinogenic 4-OHE_1/E_2 are oxidized to form predominantly the depurinating adducts, 4-OHE_1/E_2-1-N3-Ade and 4-OHE_1/E_2-1-N7-Gua (86, 87). Reaction with 2-OHE_2-quinone methide produced stable Ade or Gua adducts. Considering the fact that the redox potentials of 2-OHE_1/E_2 and 4-OHE_1/E_2 are similar, the greater carcinogenicity of the 4-OHE_1/E_2 would come from the experimental results in which 4-OHE_1/E_2 forms the carcinogenic depurinating DNA adducts than 2-OHE_1/E_2 at higher levels and 2-OHE_1/E_2 forms stable adducts. It is important to acknowledge that stable bulky Gua adducts of 4-OHE_1/E_2 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 *o*-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 biotrasformation 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 cysteines 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 E_2 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 E_2-treated samples and that cotreatment of antioxidant with E_2 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/Abl-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 E_2 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 E_2 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 E_2 or 4-OHE_2 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

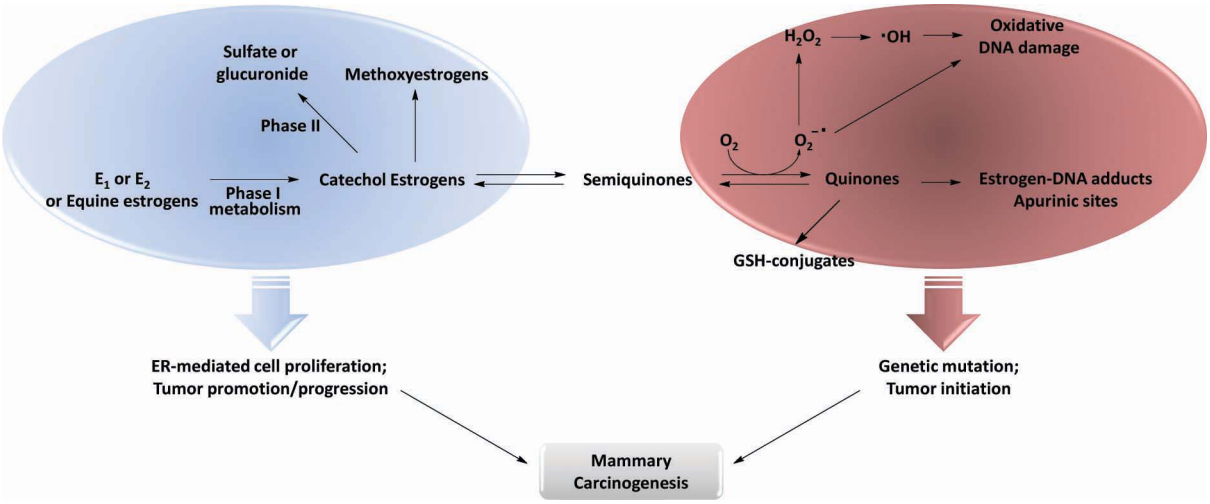

Fig. 3. Proposed mechanisms of estrogen-induced mammary carcinogenesis.

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 ERα 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 present in HRT produce carcinogenic metabolites. Further research on the dual roles of equine estrogen metabolites is deserved to assess the risk and benefit facets of HRT and to develop a safer formulation.

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REFERENCES

1. Beatson, G. T. (1896) On the treatment of inoperable cases of carcinoma of the mamma: suggestions for a new method of treatment with illustrative cases. *Lancet* **2**, 104-107.
2. Simpson, E. R. (2003) Sources of estrogen and their importance. *J. Steroid Biochem. Mol. Biol.* **86**, 225-230.
3. Boukari, K., Ciampi, M. L., Guiochon-Mantel, A., Young, J., Lombes, M. and Meduri, G. (2007) Human fetal testis: source of estrogen and target of estrogen action. *Hum. Reprod.* **22**, 1885-1892.
4. Colditz, G. A. (1998) Relationship between estrogen levels, use of hormone replacement therapy, and breast cancer. *J. Natl. Cancer I.* **90**, 814-823.
5. Feigelson, H. S. and Henderson, B. E. (1996) Estrogens and breast cancer. *Carcinogenesis* **17**, 2279-2284.
6. Xu, W. H., Xiang, Y. B., Ruan, Z. X., Zheng, W., Cheng, J. R., Dai, Q., Gao, Y. T. and Shu, X. O. (2004) Menstrual and reproductive factors and endometrial cancer risk: results from a population-based case-control study in urban Shanghai. *Int. J. Cancer* **108**, 613-619.
7. Kaaks, R., Berrino, F., Key, T., Rinaldi, S., Dossus, L., Biessy, C., Secreto, G., Amiano, P., Bingham, S., Boeing, H., Bueno de Mesquita, H. B., Chang-Claude, J., Clavel-Chapelon, F., Fournier, A., van Gils, C. H., Gonzalez, C. A., Gurrea, A. B., Critselis, E., Khaw, K. T., Krogh, V., Lahmann, P. H., Nagel, G., Olsen, A., Onland-Moret, N. C., Overvad, K., Palli, D., Panico, S., Peeters, P., Quiros, J. R., Roddam, A., Thiebaut, A., Tjonneland, A., Chirlaque, M. D., Trichopoulou, A., Trichopoulos, D., Tumino, R., Vineis, P., Norat, T., Ferrari, P., Slimani, N. and Riboli, E. (2005) Serum sex steroids in premenopausal women and breast cancer risk within the European Prospective Investigation into Cancer and Nutrition (EPIC). *J. Natl. Cancer Inst.* **97**, 755-765.
8. Zucchetto, A., Serraino, D., Polesel, J., Negri, E., De Paoli, A., Dal Maso, L., Montella, M., La Vecchia, C., Franceschi, S. and Talamini, R. (2009) Hormone-related factors and gynecological conditions in relation to endometrial cancer risk. *Eur. J. Cancer Prev.* **18**, 316-321.
9. Russo, J., Hu, Y. F., Yang, X. and Russo, I. H. (2000) Developmental, cellular, and molecular basis of human breast cancer. *J. Natl. Cancer Inst. Monogr.* **27**, 17-37.
10. Castagnetta, L., Granata, O. M., Cocciadiferro, L., Saetta, A., Polito, L., Bronte, G., Rizzo, S., Campisi, I., Agostara, B. and Carruba, G. (2004) Sex steroids, carcinogenesis, and cancer progression. *Ann. N.Y. Acad. Sci.* **1028**, 233-246.
11. Bocchinfuso, W. P. and Korach, K. S. (1997) Mammary gland development and tumorigenesis in estrogen receptor knockout mice. *J. Mal. Gland Biol. Neoplasia* **2**, 323-334.
12. The National Toxicology Program (NTP). (2002) Federal Report on Carcinogens. 177283-177285.
13. WHO (1999) Hormonal Contraception and Post-Menopausal Hormonal Therapy; in *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, pp. 399-530, IARCPress, Lyon, France.
14. Yager, J. D. and Davidson, N. E. (2006) Estrogen carcinogenesis in breast cancer. *N. Engl. J. Med.* **354**, 270-282.
15. Russo, J. and Russo, I. H. (2006) The role of estrogen in the initiation of breast cancer. *J. Steroid Biochem. Mol. Biol.* **102**, 89-96.
16. Sarabia, S. F., Zhu, B. T., Kurosawa, T., Tohma, M. and Liehr, J. G. (1997) Mechanism of cytochrome P450-catalyzed aromatic hydroxylation of estrogens. *Chem. Res. Toxicol.* **10**, 767-771.
17. Raftogianis, R., Creveling, C., Weinshilboum, R. and Weisz, J. (2000) Estrogen metabolism by conjugation. *J. Natl. Cancer Inst. Monogr.* **27**, 113-124.

18. Hachey, D. L., Dawling, S., Roodi, N. and Parl, F. F. (2003) Sequential action of phase I and II enzymes cytochrome p450 1B1 and glutathione S-transferase P1 in mammary estrogen metabolism. *Cancer Res.* **63**, 8492-8499.
19. Yue, W., Santen, R. J., Wang, J. P., Li, Y., Verderame, M. F., Bocchinfuso, W. P., Korach, K. S., Devanesan, P., Todorovic, R., Rogan, E. G. and Cavalieri, E. L. (2003) Genotoxic metabolites of estradiol in breast: potential mechanism of estradiol induced carcinogenesis. *J. Steroid Biochem. Mol. Biol.* **86**, 477-486.
20. Russo, J., Fernandez, S. V., Russo, P. A., Fernbaugh, R., Sheriff, F. S., Lareef, H. M., Garber, J. and Russo, I. H. (2006) 17-Beta-estradiol induces transformation and tumorigenesis in human breast epithelial cells. *FASEB J.* **20**, 1622-1634.
21. Russo, J., Hasan Lareef, M., Balogh, G., Guo, S. and Russo, I. H. (2003) Estrogen and its metabolites are carcinogenic agents in human breast epithelial cells. *J. Steroid Biochem. Mol. Biol.* **87**, 1-25.
22. Peng, K. W., Chang, M., Wang, Y. T., Wang, Z., Qin, Z., Bolton, J. L. and Thatcher, G. R. (2010) Unexpected hormonal activity of a catechol equine estrogen metabolite reveals reversible glutathione conjugation. *Chem. Res. Toxicol.* **23**, 1374-1383.
23. Schutze, N., Vollmer, G. and Knuppen, R. (1994) Catecholestrogens are agonists of estrogen receptor dependent gene expression in MCF-7 cells. *J. Steroid Biochem. Mol. Biol.* **48**, 453-461.
24. Zhu, B. T. and Conney, A. H. (1998) Functional role of estrogen metabolism in target cells: review and perspectives. *Carcinogenesis* **19**, 1-27.
25. Chang, M., Peng, K. W., Kastrati, I., Overk, C. R., Qin, Z. H., Yao, P., Bolton, J. L. and Thatcher, G. R. (2007) Activation of estrogen receptor-mediated gene transcription by the equine estrogen metabolite, 4-methoxyequilenin, in human breast cancer cells. *Endocrinology* **148**, 4793-4802.
26. Tsutsui, T., Tamura, Y., Hagiwara, M., Miyachi, T., Hikiba, H., Kubo, C. and Barrett, J. C. (2000) Induction of mammalian cell transformation and genotoxicity by 2-methoxyestradiol, an endogenous metabolite of estrogen. *Carcinogenesis* **21**, 735-740.
27. Sutherland, T. E., Schuliga, M., Harris, T., Eckhardt, B. L., Anderson, R. L., Quan, L. and Stewart, A. G. (2005) 2-Methoxyestradiol is an estrogen receptor agonist that supports tumor growth in murine xenograft models of breast cancer. *Clin. Cancer Res.* **11**, 1722-1732.
28. Parl, F. F., Egan, K. M., Li, C. and Crooke, P. S. (2009) Estrogen exposure, metabolism, and enzyme variants in a model for breast cancer risk prediction. *Cancer Inform.* **7**, 109-121.
29. Crooke, P. S., Justenhoven, C., Brauch, H. B., Dawling, S., Roodi, N., Higginbotham, K. S., Plummer, W. D., Jr., Schuyler, P. A., Sanders, M. E., Page, D. L., Smith, J. R., Dupont, W. D. and Parl, F. F. (2011) Estrogen metabolism and exposure in a genotypic-phenotypic model for breast cancer risk prediction. *Cancer Epidemiol. Biomarkers Prev.* **20**, 1502-1515.
30. Williamson, D. G. (1979) The biochemistry of the 17-hydroxysteroid dehydrogenases; in *Steroid Biochemistry*. (Hobkirk, R., ed.), pp. 83-110, CRC Press, Boca Raton, FL.
31. Suchar, L. A., Chang, R. L., Rosen, R. T., Lech, J. and Conney, A. H. (1995) High-performance liquid chromatography separation of hydroxylated estradiol metabolites: formation of estradiol metabolites by liver microsomes from male and female rats. *J. Pharmacol. Exp. Ther.* **272**, 197-206.
32. Liehr, J. G., Fang, W. F., Sirbasku, D. A. and Ari-Ulubelen, A. (1986) Carcinogenicity of catechol estrogens in Syrian hamsters. *J. Steroid Biochem. Mol. Biol.* **24**, 353-356.
33. Li, J. J. and Li, S. A. (1987) Estrogen carcinogenesis in Syrian hamster tissues: role of metabolism. *Fed. Proc.* **46**, 1858-1863.
34. Newbold, R. R. and Liehr, J. G. (2000) Induction of uterine adenocarcinoma in CD-1 mice by catechol estrogens. *Cancer Res.* **60**, 235-237.
35. Li, K. M., Todorovic, R., Devanesan, P., Higginbotham, S., Kofeler, H., Ramanathan, R., Gross, M. L., Rogan, E. G. and Cavalieri, E. L. (2004) Metabolism and DNA binding studies of 4-hydroxyestradiol and estradiol-3,4-quinone in vitro and in female ACI rat mammary gland in vivo. *Carcinogenesis* **25**, 289-297.
36. El-Bayoumy, K., Ji, B. Y., Upadhyaya, P., Chae, Y. H., Kurtzke, C., Rivenson, A., Reddy, B. S., Amin, S. and Hecht, S. S. (1996) Lack of tumorigenicity of cholesterol epoxides and estrone-3,4-quinone in the rat mammary gland. *Cancer Res.* **56**, 1970-1973.
37. Franks, S., MacLusky, N. J. and Naftolin, F. (1982) Comparative pharmacology of oestrogens and catechol oestrogens: actions on the immature rat uterus in vivo and in vitro. *J. Endocrinol.* **94**, 91-98.
38. Van Aswegen, C. H., Purdy, R. H. and Wittliff, J. L. (1989) Binding of 2-hydroxyestradiol and 4-hydroxyestradiol to estrogen receptors from human breast cancers. *J. Steroid Biochem.* **32**, 485-492.
39. Spink, D. C., Zhang, F., Hussain, M. M., Katz, B. H., Liu, X., Hilker, D. R. and Bolton, J. L. (2001) Metabolism of equilenin in MCF-7 and MDA-MB-231 human breast cancer cells. *Chem. Res. Toxicol.* **14**, 572-581.
40. Yamazaki, H., Shaw, P. M., Guengerich, F. P. and Shimada, T. (1998) Roles of cytochromes P450 1A2 and 3A4 in the oxidation of estradiol and estrone in human liver microsomes. *Chem. Res. Toxicol.* **11**, 659-665.
41. Zhang, F. and Bolton, J. L. (1999) Synthesis of the equine estrogen metabolites 2-hydroxyequilin and 2-hydroxyequilenin. *Chem. Res. Toxicol.* **12**, 200-203.
42. Roy, D., Bernhardt, A., Strobel, H. W. and Liehr, J. G. (1992) Catalysis of the oxidation of steroid and stilbene estrogens to estrogen quinone metabolites by the beta-naphthoflavone-inducible cytochrome P450 IA family. *Arch. Biochem. Biophys.* **296**, 450-456.
43. Markides, C. S., Roy, D. and Liehr, J. G. (1998) Concentration dependence of prooxidant and antioxidant properties of catecholestrogens. *Arch. Biochem. Biophys.* **360**, 105-112.
44. Roy, D. and Liehr, J. G. (1999) Estrogen, DNA damage and mutations. *Mutat. Res.* **424**, 107-115.
45. Rajapakse, N., Butterworth, M. and Kortenkamp, A. (2005) Detection of DNA strand breaks and oxidized DNA bases

at the single-cell level resulting from exposure to estradiol and hydroxylated metabolites. *Environ. Mol. Mutagen.* **45**, 397-404.
46. Hobkirk, R. (1985) Steroid sulfotransferases and steroid sulfate sulfatases: characteristics and biological roles. *Can. J. Biochem. Cell Biol.* **63**, 1127-1144.
47. Roy, A. K. (1992) Regulation of steroid hormone action in target cells by specific hormone-inactivating enzymes. *Proc. Soc. Exp. Biol. Med.* **199**, 265-272.
48. Ball, P. and Knuppen, R. (1990) Formation, metabolism, and physiologic importance of catecholestrogens. *Am. J. Obstet. Gynecol.* **163**, 2163-2170.
49. Merriam, G. R., MacLusky, N. J., Picard, M. K. and Naftolin, F. (1980) Comparative properties of the catechol estrogens, I: methylation by catechol-O-methyltransferase and binding to cytosol estrogen receptors. *Steroids* **36**, 1-11.
50. Yue, T. L., Wang, X., Louden, C. S., Gupta, S., Pillarisetti, K., Gu, J. L., Hart, T. K., Lysko, P. G. and Feuerstein, G. Z. (1997) 2-Methoxyestradiol, an endogenous estrogen metabolite, induces apoptosis in endothelial cells and inhibits angiogenesis: possible role for stress-activated protein kinase signaling pathway and Fas expression. *Mol. Pharmacol.* **51**, 951-962.
51. Schumacher, G. and Neuhaus, P. (2001) The physiological estrogen metabolite 2-methoxyestradiol reduces tumor growth and induces apoptosis in human solid tumors. *J. Cancer Res. Clin. Oncol.* **127**, 405-410.
52. Schumacher, G., Kataoka, M., Roth, J. A. and Mukhopadhyay, T. (1999) Potent antitumor activity of 2-methoxyestradiol in human pancreatic cancer cell lines. *Clin. Cancer Res.* **5**, 493-499.
53. Pribluda, V. S., Gubish, E. R., Jr., Lavallee, T. M., Treston, A., Swartz, G. M. and Green, S. J. (2000) 2-Methoxyestradiol: an endogenous antiangiogenic and antiproliferative drug candidate. *Cancer Metastasis Rev.* **19**, 173-179.
54. Yao, J., Li, Y., Chang, M., Wu, H., Yang, X., Goodman, J. E., Liu, X., Liu, H., Mesecar, A. D., Van Breemen, R. B., Yager, J. D. and Bolton, J. L. (2003) Catechol estrogen 4-hydroxyequilenin is a substrate and an inhibitor of catechol-O-methyltransferase. *Chem. Res. Toxicol.* **16**, 668-675.
55. Yao, J., Chang, M., Li, Y., Pisha, E., Liu, X., Yao, D., Elguindi, E. C., Blond, S. Y. and Bolton, J. L. (2002) Inhibition of cellular enzymes by equine catechol estrogens in human breast cancer cells: specificity for glutathione S-transferase P1-1. *Chem. Res. Toxicol.* **15**, 935-942.
56. Chang, M., Shin, Y. G., van Breemen, R. B., Blond, S. Y. and Bolton, J. L. (2001) Structural and functional consequences of inactivation of human glutathione S-transferase P1-1 mediated by the catechol metabolite of equine estrogens, 4-hydroxyequilenin. *Biochemistry* **40**, 4811-4820.
57. Li, Y., Yao, J., Chang, M., Nikolic, D., Yu, L., Yager, J. D., Mesecar, A. D., van Breemen, R. B. and Bolton, J. L. (2004) Equine catechol estrogen 4-hydroxyequilenin is a more potent inhibitor of the variant form of catechol-O-methyltransferase. *Chem. Res. Toxicol.* **17**, 512-520.
58. Loriaux, D. L., Ruder, H. J., Knab, D. R. and Lipsett, M. B. (1972) Estrone sulfate, estrone, estradiol and estriol plasma levels in human pregnancy. *J. Clin. Endocrinol. Metab.* **35**, 887-891.
59. Ruder, H. J., Loriaux, L. and Lipsett, M. B. (1972) Estrone sulfate: production rate and metabolism in man. *J. Clin. Invest.* **51**, 1020-1033.
60. Carlstrom, K., Bergqvist, A. and Ljungberg, O. (1988) Metabolism of estrone sulfate in endometriotic tissue and in uterine endometrium in proliferative and secretory cycle phase. *Feril. Steril.* **49**, 229-233.
61. Santen, R. J., Leszczynski, D., Tilson-Mallet, N., Feil, P. D., Wright, C., Manni, A. and Santner, S. J. (1986) Enzymatic control of estrogen production in human breast cancer: relative significance of aromatase versus sulfatase pathways. *Ann. N. Y. Acad. Sci.* **464**, 126-137.
62. Zhu, B. T., Evaristus, E. N., Antoniak, S. K., Sarabia, S. F., Ricci, M. J. and Liehr, J. G. (1996) Metabolic deglucuronidation and demethylation of estrogen conjugates as a source of parent estrogens and catecholestrogen metabolites in Syrian hamster kidney, a target organ of estrogen-induced tumorigenesis. *Toxicol. Appl. Pharmacol.* **136**, 186-193.
63. Walaszek, Z. (1990) Potential use of D-glucaric acid derivatives in cancer prevention. *Cancer Lett.* **54**, 1-8.
64. Watanabe, K., Takanashi, K., Imaoka, S., Funae, Y., Kawano, S., Inoue, K., Kamataki, T., Takagi, H. and Yoshizawa, I. (1991) Comparison of cytochrome P-450 species which catalyze the hydroxylations of the aromatic ring of estradiol and estradiol 17-sulfate. *J. Steroid Biochem. Mol. Biol.* **38**, 737-743.
65. Purohit, A., Reed, M. J., Morris, N. C., Williams, G. J. and Potter, B. V. (1996) Regulation and inhibition of steroid sulfatase activity in breast cancer. *Ann. N. Y. Acad. Sci.* **784**, 40-49.
66. Duncan, L., Purohit, A., Howarth, N. M., Potter, B. V. and Reed, M. J. (1993) Inhibition of estrone sulfatase activity by estrone-3-methylthiophosphonate: a potential therapeutic agent in breast cancer. *Cancer Res.* **53**, 298-303.
67. Bjornstrom, L. and Sjoberg, M. (2005) Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes. *Mol. Endocrinol.* **19**, 833-842.
68. Marino, M., Galluzzo, P. and Ascenzi, P. (2006) Estrogen signaling multiple pathways to impact gene transcription. *Curr. Genomics* **7**, 497-508.
69. Safe, S. and Kim, K. (2008) Non-classical genomic estrogen receptor (ER)/specificity protein and ER/activating protein-1 signaling pathways. *J. Mol. Endocrinol.* **41**, 263-275.
70. Levin, E. R. (2002) Cellular functions of plasma membrane estrogen receptors. *Steroids* **67**, 471-475.
71. Stoica, G. E., Franke, T. F., Moroni, M., Mueller, S., Morgan, E., Iann, M. C., Winder, A. D., Reiter, R., Wellstein, A., Martin, M. B. and Stoica, A. (2003) Effect of estradiol on estrogen receptor-alpha gene expression and activity can be modulated by the ErbB2/PI 3-K/Akt pathway. *Oncogene* **22**, 7998-8011.
72. Levin, E. R. (2003) Bidirectional signaling between the estrogen receptor and the epidermal growth factor

receptor. *Mol. Endocrinol.* **17**, 309-317.
73. Liao, J. K. (2003) Cross-coupling between the oestrogen receptor and phosphoinositide 3-kinase. *Biochem. Soc. Trans.* **31**, 66-70.
74. Chen, J. Q. and Yager, J. D. (2004) Estrogen's effects on mitochondrial gene expression: mechanisms and potential contributions to estrogen carcinogenesis. *Ann. N. Y. Acad. Sci.* **1028**, 258-272.
75. Chen, J. Q., Delannoy, M., Cooke, C. and Yager, J. D. (2004) Mitochondrial localization of ERalpha and ERbeta in human MCF7 cells. *Am. J. Physiol. Endocrinol. Metab.* **286**, E1011-1022.
76. Demonacos, C. V., Karayanni, N., Hatzoglou, E., Tsiriyiotis, C., Spandidos, D. A. and Sekeris, C. E. (1996) Mitochondrial genes as sites of primary action of steroid hormones. *Steroids* **61**, 226-232.
77. Zhu, B. T., Han, G. Z., Shim, J. Y., Wen, Y. and Jiang, X. R. (2006) Quantitative structure-activity relationship of various endogenous estrogen metabolites for human estrogen receptor alpha and beta subtypes: Insights into the structural determinants favoring a differential subtype binding. *Endocrinology* **147**, 4132-4150.
78. Vincent, T. L. and Gatenby, R. A. (2008) An evolutionary model for initiation, promotion, and progression in carcinogenesis. *Int. J. Oncol.* **32**, 729-737.
79. Lavigne, J. A., Goodman, J. E., Fonong, T., Odwin, S., He, P., Roberts, D. W. and Yager, J. D. (2001) The effects of catechol-O-methyltransferase inhibition on estrogen metabolite and oxidative DNA damage levels in estradiol-treated MCF-7 cells. *Cancer Res.* **61**, 7488-7494.
80. Banerjee, S. K., Banerjee, S., Li, S. A. and Li, J. J. (1994) Induction of chromosome aberrations in Syrian hamster renal cortical cells by various estrogens. *Mutat. Res.* **311**, 191-197.
81. Valko, M., Izakovic, M., Mazur, M., Rhodes, C. J. and Telser, J. (2004) Role of oxygen radicals in DNA damage and cancer incidence. *Mol. Cell. Biochem.* **266**, 37-56.
82. Chen, Z. H., Na, H. K., Hurh, Y. J. and Surh, Y. J. (2005) 4-Hydroxyestradiol induces oxidative stress and apoptosis in human mammary epithelial cells: possible protection by NF-kappaB and ERK/MAPK. *Toxicol. Appl. Pharmacol.* **208**, 46-56.
83. Cavalieri, E., Chakravarti, D., Guttenplan, J., Hart, E., Ingle, J., Jankowiak, R., Muti, P., Rogan, E., Russo, J., Santen, R. and Sutter, T. (2006) Catechol estrogen quinones as initiators of breast and other human cancers: implications for biomarkers of susceptibility and cancer prevention. *Biochim. Biophys. Acta* **1766**, 63-78.
84. Saeed, M., Zahid, M., Gunselman, S. J., Rogan, E. and Cavalieri, E. (2005) Slow loss of deoxyribose from the N7deoxyguanosine adducts of estradiol-3,4-quinone and hexestrol-3',4'-quinone. Implications for mutagenic activity. *Steroids* **70**, 29-35.
85. Zhao, Z., Kosinska, W., Khmelnitsky, M., Cavalieri, E. L., Rogan, E. G., Chakravarti, D., Sacks, P. G. and Guttenplan, J. B. (2006) Mutagenic activity of 4-hydroxyestradiol, but not 2-hydroxyestradiol, in BB rat2 embryonic cells, and the mutational spectrum of 4-hydroxyestradiol. *Chem. Res. Toxicol.* **19**, 475-479.
86. Saeed, M., Rogan, E., Fernandez, S. V., Sheriff, F., Russo, J. and Cavalieri, E. (2007) Formation of depurinating N3Adenine and N7Guanine adducts by MCF-10F cells cultured in the presence of 4-hydroxyestradiol. *Int. J. Cancer* **120**, 1821-1824.
87. Zahid, M., Kohli, E., Saeed, M., Rogan, E. and Cavalieri, E. (2006) The greater reactivity of estradiol-3,4-quinone vs estradiol-2,3-quinone with DNA in the formation of depurinating adducts: implications for tumor-initiating activity. *Chem. Res. Toxicol.* **19**, 164-172.
88. Embrechts, J., Lemiere, F., Van Dongen, W., Esmans, E. L., Buytaert, P., Van Marck, E., Kockx, M. and Makar, A. (2003) Detection of estrogen DNA-adducts in human breast tumor tissue and healthy tissue by combined nano LC-nano ES tandem mass spectrometry. *J. Am. Sco. Mass Spectr.* **14**, 482-491.
89. Liu, X., Yao, J., Pisha, E., Yang, Y., Hua, Y., van Breemen, R. B. and Bolton, J. L. (2002) Oxidative DNA damage induced by equine estrogen metabolites: role of estrogen receptor alpha. *Chem. Res. Toxicol.* **15**, 512-519.
90. Chen, Y., Liu, X., Pisha, E., Constantinou, A. I., Hua, Y., Shen, L., van Breemen, R. B., Elguindi, E. C., Blond, S. Y., Zhang, F. and Bolton, J. L. (2000) A metabolite of equine estrogens, 4-hydroxyequilenin, induces DNA damage and apoptosis in breast cancer cell lines. *Chem. Res. Toxicol.* **13**, 342-350.
91. Zhang, F., Swanson, S. M., van Breemen, R. B., Liu, X., Yang, Y., Gu, C. and Bolton, J. L. (2001) Equine estrogen metabolite 4-hydroxyequilenin induces DNA damage in the rat mammary tissues: formation of single-strand breaks, apurinic sites, stable adducts, and oxidized bases. *Chem. Res. Toxicol.* **14**, 1654-1659.
92. Okahashi, Y., Iwamoto, T., Suzuki, N., Shibutani, S., Sugiura, S., Itoh, S., Nishiwaki, T., Ueno, S. and Mori, T. (2010) Quantitative detection of 4-hydroxyequilenin-DNA adducts in mammalian cells using an immunoassay with a novel monoclonal antibody. *Nucleic Acids Res.* **38**, e133.
93. Ozcagli, E., Sardas, S. and Biri, A. (2005) Assessment of DNA damage in postmenopausal women under hormone replacement therapy. *Maturitas* **51**, 280-285.
94. Kamata, H. and Hirata, H. (1999) Redox regulation of cellular signalling. *Cell Signal.* **11**, 1-14.
95. Sauer, H., Wartenberg, M. and Hescheler, J. (2001) Reactive oxygen species as intracellular messengers during cell growth and differentiation. *Cell Physiol. Biochem.* **11**, 173-186.
96. Circu, M. L. and Aw, T. Y. (2010) Reactive oxygen species, cellular redox systems, and apoptosis. *Free Radic. Biol. Med.* **48**, 749-762.
97. Surh, Y.-J., Kundu, J. K., Na, H.-K. and Lee, J.-S. (2005) Redox-Sensitive Transcription Factors as Prime Targets for Chemoprevention with Anti-Inflammatory and Antioxidative Phytochemicals. *J. Nutr.* **135**, 2993S-3001S.
98. Roy, D., Cai, Q., Felty, Q. and Narayan, S. (2007) Estrogen-induced generation of reactive oxygen and nitrogen species, gene damage, and estrogen-dependent cancers. *J. Toxicol. Environ. Health B. Crit. Rev.* **10**, 235-257.
99. Foster, J. S., Henley, D. C., Bukovsky, A., Seth, P. and Wimalasena, J. (2001) Multifaceted regulation of cell cy-

cle progression by estrogen: regulation of Cdk inhibitors and Cdc25A independent of cyclin D1-Cdk4 function. *Mol. Cell. Biol.* **21**, 794-810.
100. Cui, Y., Parra, I., Zhang, M., Hilsenbeck, S. G., Tsimelzon, A., Furukawa, T., Horii, A., Zhang, Z. Y., Nicholson, R. I. and Fuqua, S. A. (2006) Elevated expression of mitogen-activated protein kinase phosphatase 3 in breast tumors: a mechanism of tamoxifen resistance. *Cancer Res.* **66**, 5950-5959.
101. Weinstein-Oppenheimer, C. R., Burrows, C., Steelman, L. S. and McCubrey, J. A. (2002) The effects of beta-estradiol on Raf activity, cell cycle progression and growth factor synthesis in the MCF-7 breast cancer cell line. *Cancer Biol. Ther.* **1**, 256-262.
102. Adler, V., Yin, Z., Fuchs, S. Y., Benezra, M., Rosario, L., Tew, K. D., Pincus, M. R., Sardana, M., Henderson, C. J., Wolf, C. R., Davis, R. J. and Ronai, Z. (1999) Regulation of JNK signaling by GSTp. *EMBO J.* **18**, 1321-1334.
103. Kar, S., Wang, M., Yao, W., Michejda, C. J. and Carr, B. I. (2006) PM-20, a novel inhibitor of Cdc25A, induces extracellular signal-regulated kinase 1/2 phosphorylation and inhibits hepatocellular carcinoma growth in vitro and in vivo. *Mol. Cancer Ther.* **5**, 1511-1519.
104. Banerjee, S., Saxena, N., Sengupta, K. and Banerjee, S. K. (2003) 17alpha-estradiol-induced VEGF-A expression in rat pituitary tumor cells is mediated through ER independent but PI3K-Akt dependent signaling pathway. *Biochem. Biophys. Res. Commun.* **300**, 209-215.
105. Lee, Y. R., Park, J., Yu, H. N., Kim, J. S., Youn, H. J. and Jung, S. H. (2005) Up-regulation of PI3K/Akt signaling by 17beta-estradiol through activation of estrogen receptor-alpha, but not estrogen receptor-beta, and stimulates cell growth in breast cancer cells. *Biochem. Biophys. Res. Commun.* **336**, 1221-1226.
106. Rockhill, B., Spiegelman, D., Byrne, C., Hunter, D. J. and Colditz, G. A. (2001) Validation of the Gail et al. model of breast cancer risk prediction and implications for chemoprevention. *J. Natl. Cancer Inst.* **93**, 358-366.
107. Cavalieri, E. L., Stack, D. E., Devanesan, P. D., Todorovic, R., Dwivedy, I., Higginbotham, S., Johansson, S. L., Patil, K. D., Gross, M. L., Gooden, J. K., Ramanathan, R., Cerny, R. L. and Rogan, E. G. (1997) Molecular origin of cancer: catechol estrogen-3,4-quinones as endogenous tumor initiators. *Proc. Natl. Acad. Sci. U. S. A.* **94**, 10937-10942.
108. Yager, J. D. and Liehr, J. G. (1996) Molecular mechanisms of estrogen carcinogenesis. *Annu. Rev. Pharmacol. Toxicol.* **36**, 203-232.
109. Parl, F. F., Dawling, S., Roodi, N. and Crooke, P. S. (2009) Estrogen metabolism and breast cancer: a risk model. *Ann. N. Y. Acad. Sci.* **1155**, 68-75.
110. Bocchinfuso, W. P., Hively, W. P., Couse, J. F., Varmus, H. E. and Korach, K. S. (1999) A mouse mammary tumor virus-Wnt-1 transgene induces mammary gland hyperplasia and tumorigenesis in mice lacking estrogen receptor-alpha. *Cancer Res.* **59**, 1869-1876.