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ROLE OF ESTROGEN METABOLISM AND OXIDATIVE STRESS IN ESTROGEN-INDUCED CARCINOGENESIS

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REVIEW

ABSTRACT. OXIDATIVE STRESS resulting from metabolic breakdown products of estrogens is suggested to play an important role in estrogen-induced carcinogenic process. Estrogens can be activated by cytochrome P450 enzymes to hydroxy estrogens (catechol estrogens). The tumorigenic estrogen metabolites, such as those of 17β-estradiol (βE2) and diethylstilbestrol (DES), are capable of redox cycling that results in the formation of reactive oxygen species (ROS) and free radicals, and consequently leads to oxidative stress. It has been shown that carcinogenic estrogens are capable of producing higher levels of oxidative stress than poorly carcinogenic or noncarcinogenic estrogens in vivo as well as in vitro. Data also suggest that the oxidant stress potential of estrogens depends on their ability to form catechol estrogens, and this ability is correlated with their carcinogenic potential. It is suggested that the oxidative stress caused by estrogens may act in concert with the estrogen receptor (ER)-mediated signaling pathways, leading to DNA damage, altered expression of genes critical to the control of cellular proliferation and defense against oxidative stress, and thus contribute to the development of estrogen-dependent tumors. Based on the available scientific data, the US federal government has added estrogens to the list of cancer-causing agents. Thus, studies aimed at understanding of the mechanisms of estrogen-induced carcinogenesis have important implications in the understanding and treatment of not just estrogen-, but in general, hormone-induced neoplasia. This review will attempt to summarize the importance of oxidative stress in estrogen-induced carcinogenesis.

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1. INTRODUCTION

In vivo studies using rodent models of estrogen-induced carcinogenesis, in vitro studies using breast cancer cell lines and human epidemiological data provide strong evidence that estrogens contribute to the development of breast cancers [1-17]. These data have prompted the US government to add steroidal estrogens to the list of known human carcinogens [18]. Because of increased breast cancer risk and lack of overall benefit to the patients, the clinical trials of estrogen plus progestin treatment therapy had to be terminated [19]. A large numer of epidemiological studies strongly suggest that estrogenic hormones are involved in the development of a variety of human cancers [1-12]. Elevated breast cancer risk in women has been associated with increased total life-time exposure of such women to estrogens [1,5,9,11,12]. A lack of an association between serum estrogen levels and breast cancer risk was observed in early cohort studies [20,21]. This may probably be due to lack of proper detection methods. However, more recent epidemiological data suggest strong associations between breast cancer risk and plasma or urinary estrogen levels [22,23]. These supportive data implicating estrogens in breast cancer are consistent with increased risk observed in most large studies and in a meta-analysis of hormone replacement studies [5,11,24-26]. Breast cancer risk factors such as early menarche, late menopause, obesity, and high mean values of serum estrogen support the concept of tumor induction by estrogens [8,9]. Postmenopausal women who are currently using estrogen either alone or in combination with progestin are suggested to have a significantly increased risk of breast cancer compared with postmenopausal women who have never used hormones [5,11,12]. Oral contraceptives containing estrogen medications have been estimated to increase breast cancer risk by approximately 3% per year of intake [27]. Conversely, late menarche, early menopause, and pregnancy at a young age all, in theory, decrease the risk of breast cancer by reducing the life-time exposure to estrogens [28]. Similarly, reduced breast cancer risk is reported in women who undergo ovariectomy before 35 years of age probably because of reduced estrogen levels following ovariectomy [29].

The literature data mentioned above suggest an association and a link between estrogens and cancer. The International Agency for Research on Cancer has now recognized the carcinogenic potential of estrogenic compounds and classified estrogens as human carcinogens [30,31]. The mechanisms of estrogen-induced carcinogenesis are far from being conclusively established and many aspects remain controversial. Investigations to understand this mechanism is in progress using rodent models of hormonal carcinogenesis and using relevant cell lines. Natural female sex hormone βE2 and synthetic estrogen DES induce tumors in rats, mice, and hamsters [13-17]. Some estrogens are tumorigenic in rodent models while others are not [32-34]. Many studies however suggest that carcinogenic and noncarcinogenic estrogens differ in their metabolic activation patterns and/or the extent to which they can be metabolically activated with carcinogenic estrogens having significantly higher potential to produce oxidative stress after metabolic activation to catechol estrogens [33-35]. It is predicted therefore that estrogen metabolism may play a predominant role in the development of estrogen-induced tumors.

2. ANIMAL MODELS OF ESTROGEN-INDUCED CARCINOGENESIS

Several models are being used to understand the mechanism of estrogen-induced breast carcinogenesis. One of them that has been well established, used for a long time and provided useful insights into the mechanism of hormonal carcinogenesis is the estrogen-induced hamster renal tumor model. One of the significant advantages of this model has been that estrogen alone is needed for the induction and promotion of tumors (FIG. 1). In this rodent model, subcutaneous implantation of βE2 for about six months induces target-organ-specific kidney tumors with ~80-100% tumor incidence [13,14,32]. Scientific data obtained using this model shares several characteristics with human breast and uterine cancers, and thus point to a

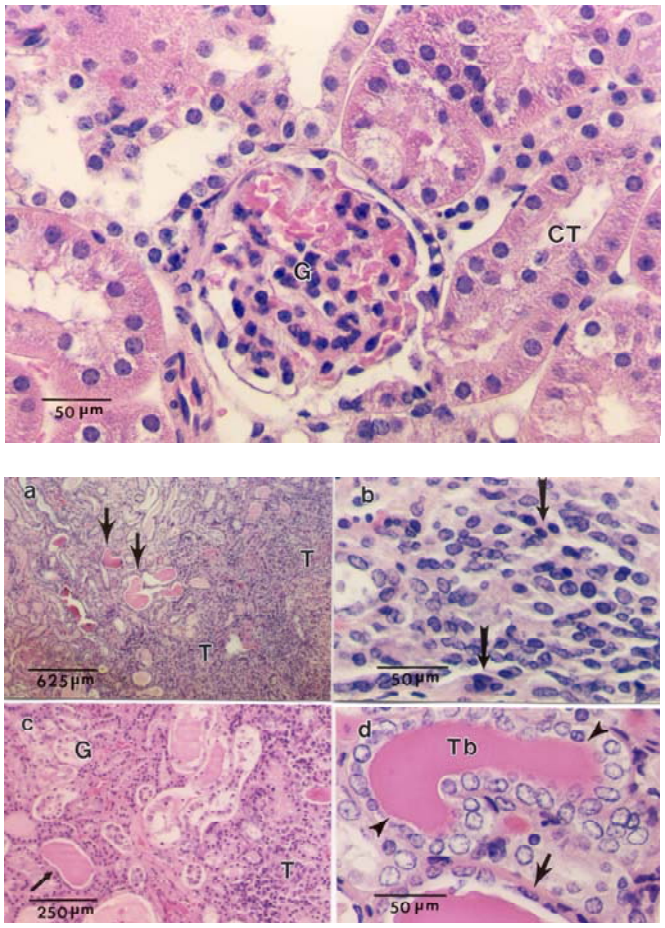

FIGURE 1. A. PARAFFIN SECTION OF AN UNTREATED MALE SYRIAN HAMSTER KIDNEY STAINED WITH HEMATOXYLIN AND EOSIN. Normal kidney architecture with normal convoluted tubules (CT) and glomerulus (G) is observed. Magnification = 40X (courtesy of Proc. Natl. Acad. Sci. USA, 100, 3913-3918, 2003). B. Paraffin section of a tumor-bearing kidney stained with hematoxylin and eosin. The tumors were induced by treatment of male Syrian hamsters with βE2 for 7 months. (a) An abnormal kidney architecture with tumor nodules (T) and congestion of scattered convoluted tubules (arrow) within the tumor nodules can be observed. (b and c) The tumor nodules are composed of a combination of round to spindled hyperchromatic cells (b, arrows), and in some of the tumor nodules (T), entrapped and atrophic glomeruli (G) are present (c). Many of the congested tubules (Tb) are filled with pink eosinophilic deposits (arrow in c, arrow head in d) and are lined by somewhat flattened epithelial cells (d, arrow). Magnification: a = 4X; b = 40X; c = 10X; d = 40X (courtesy of Proc. Natl. Acad. Sci. USA, 100, 3913-3918, 2003).

common mechanistic origin. Some of these shared characteristics include: (i) increased covalent binding of estrogen-quinone metabolites to DNA, (ii) enhancement of endogenous DNA adducts by chronic estrogen exposure, and (iii) chromosomal damage/aberration induced by estrogens or by reactive estrogen quinone metabolites [33,36-53]. In organs prone to estrogen-induced hyperplasia or cancer, e.g., rat pituitary, mouse uterus, and hamster kidney, a specific estradiol-4-hydroxylase activity has been identified [54-56]. However, this enzyme activity could not be detected in organs that are not prone to estrogen-induced cancers like livers of these species (54-56). Increased estradiol-4-hydroxylase activity has also been identified in human myometrium [39] and in human breast cancer cell line MCF-7 [57]. Based upon physiological studies in the mouse uterus, benign tumor growth formation and blastocyst implantation has been linked to increased 4-hydroxyesradiol (4-OHE2) formation in myometrial precursor cells [58]. Liehr and Ricci have shown predominant 4-hydroxylation of βE2 by microsomes of neoplastic human breast tissue compared with non-neoplastic breast tissue [40]. A number of studies have indicated that 4-hydroxylation of βE2 plays an important role in estrogen-induced carcinogenesis [33,34,39,40]. 4-OHE2 has been shown to be as carcinogenic as the parent estrogen, βE2, in the hamster kidney tumor model [33,34,59]. Synthetic or natural steroid hormones induced genomic instability in a variety of species (e.g., hamster, mouse, rat) at different tissue sites [43,60], closely resembling genomic instability found in hormone-

related/associated human cancers [60].

In the hamster renal tumor model, mesenchymal cells are postulated to play an important role in the origin of the estrogen-induced and estrogen-dependent renal neoplasm [13,62,63]. It is worth noting that mesenchymal cells of the human breast are the major source of estrogen, either as such or after the conversion of androgens to estrogens by aromatase in postmenopausal women [61]. The usefulness of the hamster tumor model is thus evident from a strong similarity between this rodent model of hormonal carcinogenesis and hormone-associated human cancers. These data suggest a common mechanistic origin in human breast cancers and the estrogen-induced hamster renal tumor model.

Because estrogen use is not associated with renal tumors in humans, the estrogen-induced hamster renal tumor model is sometimes criticized as an inaccurate model system to mimic human breast or uterine carcinogenesis. The mechanism of estrogen-induced carcinogenesis is now being studied in the female ACI rat model of breast cancer, a rodent model that seems to be more relevant to human breast cancers. This βE2-induced rat model of breast cancer is now being used as a representative model system to study the mechanism of estrogen-induced breast carcinogenesis [16,17]. Shull reported in 1997 that mammary tumors can be induced in female ACI rats with βE2, although the propensity of the ACI rats to develop mammary carcinomas has been recognized for a long time [16,64,65]. Subchronic treatment of female ACI rats with βE2 results in 100% mammary tumor incidence in about 6 months [16,66]. The first palpable tumors have been observed between 143 and 145 days. All mammary tumors have been classified as carcinomas, and invasive features have been observed. The majority of tumors show features of intraductal carcinoma of the comedo type. The disadvantage of the ACI rat model is that pituitary tumors have also been observed in ~100% of βE2-treated rats (in contrast with the hamster model where βE2 causes only target organ-specific kidney tumors). Despite this drawback, it is probably prudent to use the rat model of breast carcinogenesis in order to characterize the mechanism of hormone-induced breast carcinogenesis.

3. MECHANISMS OF ESTROGEN-INDUCED CARCINOGENESIS

3.1. ESTROGEN METABOLISM AND RESULTANT OXIDATIVE STRESS IN ESTROGEN-INDUCED CANCER

A large number of published studies on the mechanisms of estrogen-induced carcinogenesis indicate that tumor induction may be dependent on the generation of βE2 metabolites [51-58,67-71]. In estrogen-induced and estrogen-dependent carcinogenesis, continuous supply of hormones like βE2 is considered essential; therefore, it is postulated that hormonal activity of estrogens is necessary but not sufficient to induce tumors. In rodent models if estrogen is withdrawn, tumors will regress; therefore, these tumors are considered to be estrogen-induced and estrogen-dependent. Estrogens and their metabolic products are considered to play an important role in tumor development. The natural female sex hormone, βE2 is mainly metabolized to 2- and 4-OHE2 (generally known as catechol estrogens) through cytochrome P450-mediated enzymes in target organs of estrogen-induced cancer [51,54-56]. Increased estradiol-4-hydroxylase activity has been shown in organs prone to estradiol-induced hyperplasia or cancer in rodents, humans, and human breast cancer cell lines [39,40,54-58].

A number of studies provide strong evidence for the role of oxidative stress in tumor formation [33,34,72-75]. For example, treatment with DES in the presence of an exogenous metabolic activation system, of Syrian hamster embryo cells, enhances the frequency of morphological transformation of cells; furthermore, this treatment elicits unscheduled DNA synthesis and mutations [41]. When Syrian hamster embryo cells are treated with catechol estrogens, it not only induces a higher frequency of morphological transformation than βE2, but it also causes chromosome aberrations [41-44]. Several kinds of oxidative stress-mediated changes have been reported in the target organs of hamsters and rats in vivo, after treatment with DES or with βE2 and its carcinogenic metabolite 4-OHE2. These

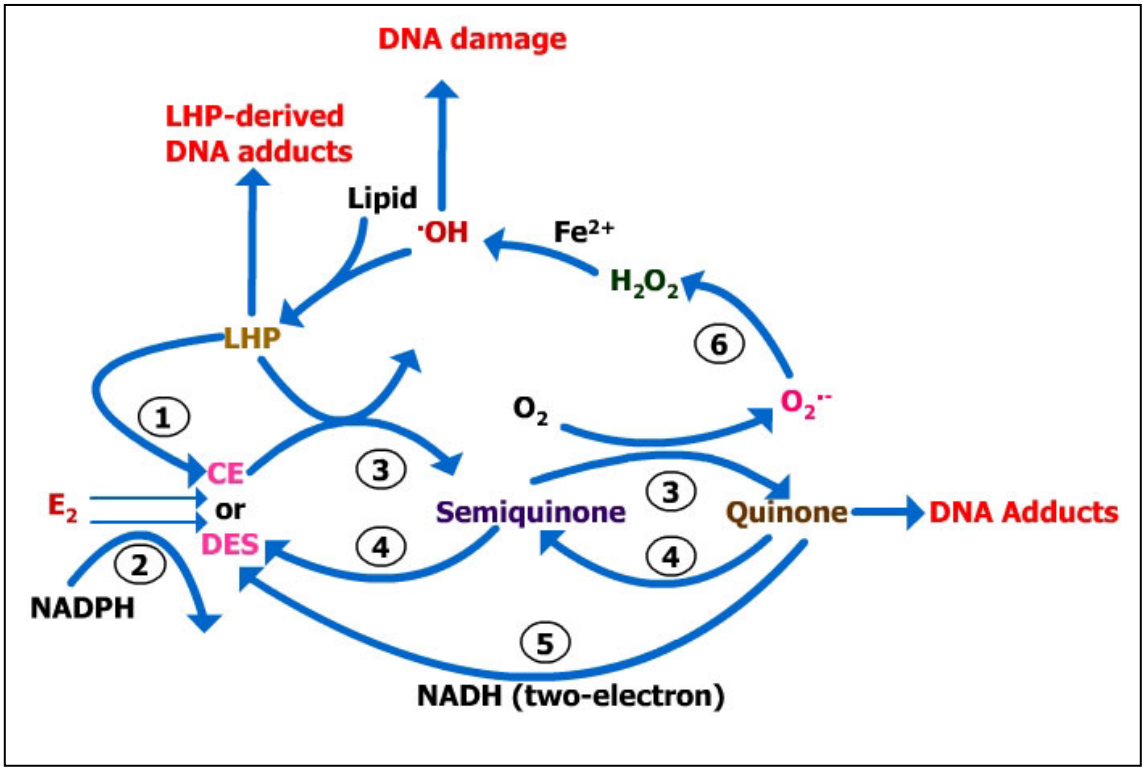

FIGURE 2. PROPOSED MECHANISM OF THE FORMATION OF REACTIVE OXYGEN SPECIES BY STEROID ESTROGENS. Steroid estrogens (E2) are converted to catechol estrogens (CE) by hydroperoxide or NADPH-dependent cytochrome P450-mediated oxidation [1,2]. CEs and DES are oxidized by organic hydroperoxide-dependent microsomal enzymes to semiquinones and quinones [3]. Quinone metabolites may be reduced by NADPH or NADH-dependent cytochrome P450 reductase [4,5]. This metabolic redox cycling may generate superoxide radicals. Hydrogen peroxide, formed from superoxide by superoxide dismutase [6] may be reduced by Fe^{2+} to hydroxyl radicals. Thus potentially harmful reactive oxygen species may be generated that may result in diffeent types of DNA, protein or lipid damage.

changes include: (i) increase in 8-hydroxylation of guanine residues [36,46]; (ii) increase in formation of single strand breaks [47]; (iii) increases in levels of lipid hydroperoxides and lipid hydroperoxide-induced DNA adducts (48-50); and (iv) increased damage to mitochondrial DNA [53]. Similar reports of free radical-mediated genotoxicity have been reported in human breast cancer cell line MCF-7 after treatment with estrone-3,4-quinone [76]. That both renal tumor incidence and the number of tumor nodules are increased in hamsters treated with a combination of iron-enriched diet and βE2 provide support for the role of oxidative stress in tumor formation as iron is known to participate in the generation of hydroxy radicals [77].

Analogous to the requirement of metabolic activation of hydrocarbons and other non-steroidal estrogen carcinogens in the carcinogenic process, estrogen intermediary metabolism is considered to be an essential requirement for the carcinogenic process [33-35,46-50,74-79]. βE2 can be metabolically activated to catechol estrogens, and catechol estrogens have the potential of undergoing metabolic redox cycling between catechol estrogens and their corresponding quinones [67-72]. Metabolic redox cycling between catechol estrogens and their corresponding quinones generates oxidative stress and potentially harmful free radicals (FIG. 2). In the female ACI rat mammary gland, catechol quinone DNA adducts of 4-OHE2 are formed after injection

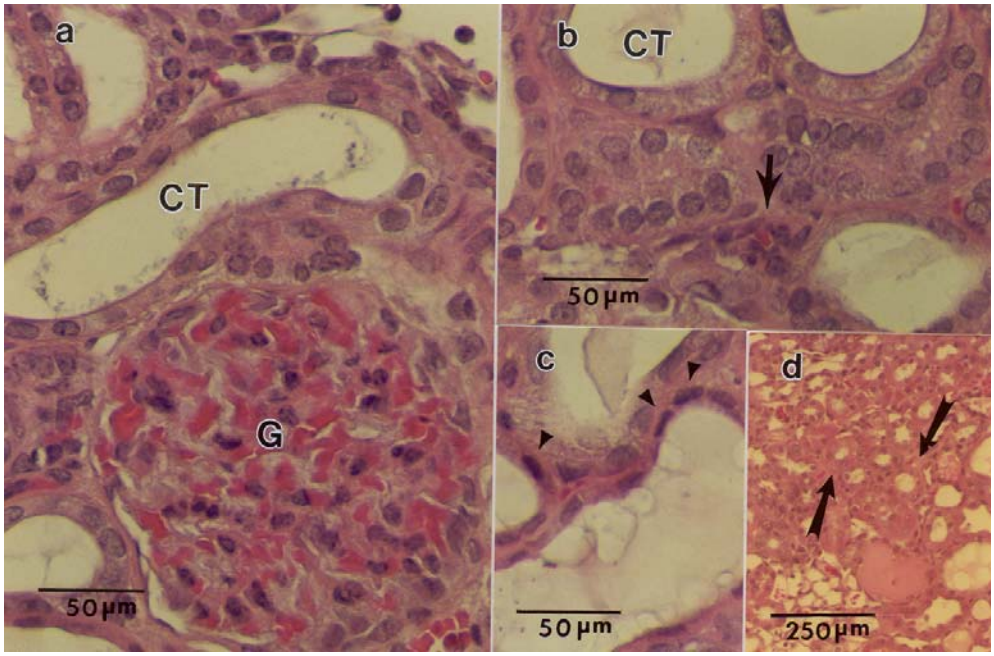

FIGURE 3. PARAFFIN SECTION OF A MALE HAMSTER KIDNEY STAINED WITH HEMATOXYLIN AND EOSIN. Male Syrian hamsters were treated with a combination of αEE and αE2 for 7 months. (a) Vascular congestion of glomeruli (G) and convoluted tubules (CT) is observed. (b) Kidneys contain foci where there are atypical collections of both interstitial cells (arrow) and convoluted tubules (CT). (c) Some of the tubules contain cuboidal cells that have scant cytoplasm and irregular, slightly pleomorphic hyperchromatic nuclei (arrowheads). (d) Some slightly crowded renal collecting tubules (arrows) are also observed in kidneys of hamsters treated with a combination of αEE and αE2 for 7 months. Magnification: a-c = 40X; d = 10X (courtesy of Proc. Natl. Acad. Sci. USA, 100, 3913-3918, 2003).

of 4-OHE2 into the rat mammary gland and excision of the mammary gland 1 hour after treatment [80]. Different estrogens differ in the catechol estrogen forming potential. βE2 is a good catechol progenitor; its use results in ~ 80-100% tumor incidence in the hamster kidney and rat breast [13,14,16,17]. 17α-Ethinylestradiol (αEE), although a potent estrogen as reflected by receptor activation, uterine wet weight growth and other measures of estrogenic potential, is a very weak catechol progenitor and its use results in only up to 10% tumor incidence in the hamster model after 9 months of continuous exposure [32-34,81,82]. Low doses of βE2 (in the 10^{-10} and 10^{-12} molar range) cause a 3.8 - 4.2 fold increase in the rate of genetic mutations in V79 cells [83]. Even very low doses of βE2 (ranging from 0.007 nM to 1µM) resulted in loss of heterozygosity in non-neoplastic human breast cell line MCF-10, at chromosomal sites at which human breast cancers commonly exhibit LOH [84,85]. Neoplastic transformation in these MCF-10 cells was also demonstrated by anchorage-independent colony formation and loss of duct differentiation [84,85]. Induction of 8-hydroxydeoxyguanine (8-OHdG), both in vitro and in vivo, in mammary gland epithelia has been demonstrated, thereby suggesting a role of estrogen-mediated oxidative DNA damage and/oxidative stress in the initiation and/or progression of breast neoplasia [36,72,86-92]. Damage to mtDNA has also been demonstrated in βE2-induced hamster renal tumors [53]. The induction of kidney tumors in Syrian hamsters has been shown to be decreased by using inhibitors of estrogen metabolism or with the use of free radical scavengers [93,94] indicating the role of oxidative stress mediated by estrogen metabolism in estrogen-induced cracinogenesis. These data support the concept that in addition to a hormonal stimulus, oxidative stress resulting from metabolic activation of carcinogenic estrogens

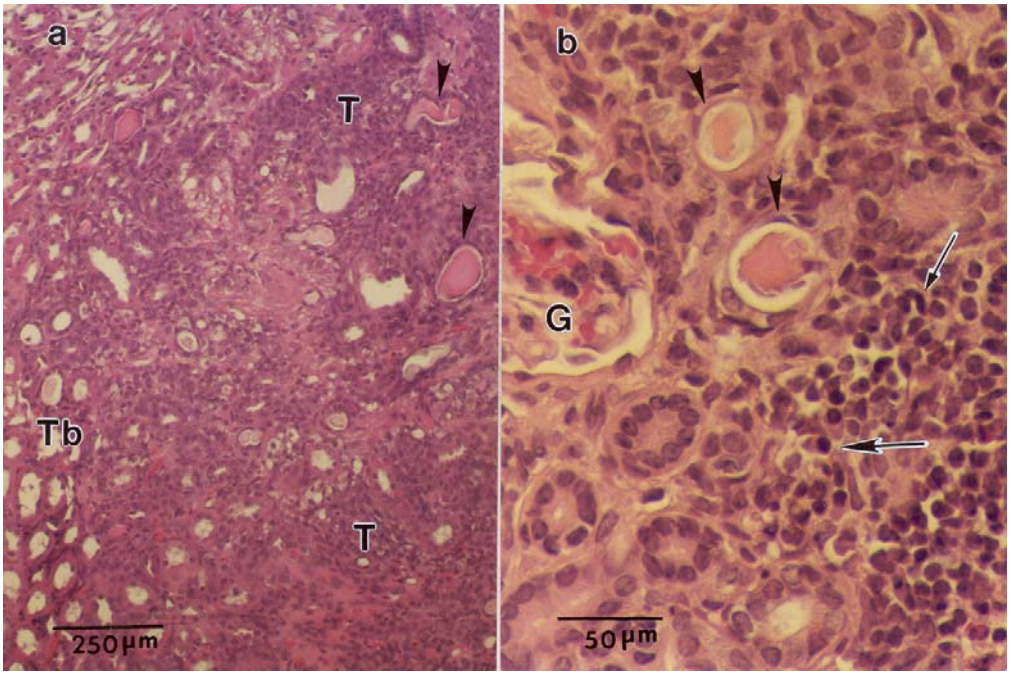

FIGURE 4. PARAFFIN SECTION OF A TUMOR-BEARING KIDNEY STAINED WITH HEMATOXYLIN AND EOSIN. The tumors were induced by treatment of male Syrian hamsters with a combination of αEE and menadione for 7 months. (a) Sections of the kidney demonstrate foci of tumor (T) with congested renal tubules (Tb and arrowheads). (b) The tumor is comprised of hyperchromatic cells with nuclear crowding (arrows). Congested renal tubules (arrow heads) and atrophied glomeruli (G) are also seen entrapped within the tumor nodule. Magnification: a = 10X, b = 40X (courtesy of Proc. Natl. Acad. Sci. USA, 100, 3913-3918, 2003).

plays a critical role in tumor development.

3.2. ESTROGENS, ESTROGEN METABOLISM AND HUMAN BREAST CANCERS

Target sites that metabolize estrogen to hydroxy metabolites may be prone to the development of cancer. These hydroxylated estrogens may elicit biological activities distinct from βE2, most notably an oxidant stress response induced by free radicals generated by metabolic redox cycling reactions. Whereas levels of both 2- and 4-OHE2 have been shown to be increased, levels of 4-OHE2, a carcinogenic metabolite of βE2 in animal models, have been shown to be increased ~20-fold in human breast tumor tissue compared with normal or non-tumorigenic breast tissue [95]. No differences in the levels of O-methylated estrogens have been demonstrated between the tumor and control groups [95]. Similar findings have been reported by Rogan et al [96]. 4-OHE2 may undergo metabolic redox cycling between its catechol and quinone metabolites and generate potentially harmful free radicals and oxidative stress [33-35,39,40,73,74] that can cause cellular DNA damage, induce cell proliferation, and initiate tumorigenesis [72,74]. Cytochrome P4501B1 (Cyp1B1) mRNA levels have been found to be highest in the human breast tissue among all the Cyps analyzed [97]. This is consistent with the fact that Cyp1B1 is primarily an extrahepatic enzyme and is the main enzyme responsible for the conversion of βE2 into 4-OHE2, a catechol metabolite of βE2 [98-100]. The presence of Cyp1B1 in the breast tissue will result in the formation of 4-OHE2 from βE2. Although not confirmed to be carcinogenic in the human breast, 4-OHE2 has been observed to have a strong carcinogenic activity in a Syrian hamster kidney tumor

model and induces uterine tumors in mice [15,59]. Local activation of estrogen to potentially reactive metabolites in the breast tissue may play a role in initiating and promoting the carcinogenic process. Redox cycling between quinone and unstable semiquinone form causes hydroxyl radical formation, the most ROS that when formed at the target site can lead to hydroxylated nucleotide bases e.g., 8-OHdG formation and permanent mutations if not repaired. It is possible that the reactive oxygen metabolites produced in the liver and other organs, and transported via the blood could be responsible for damage in the breast. This is unlikely, though, because concentration of unconjugated catechol estrogens is very low in systemic circulation [101,102]. Therefore it is unlikely that these 2- and 4-hydroxy estrogen metabolites will have any significant interaction with ERs or DNA in tissues distant from where they are produced. Rather, their effects are likely to be exerted in the tissue in which they are synthesized.

3.3. RECEPTOR-MEDIATED HORMONAL EFFECTS OF ESTROGENS AND ESTROGEN-INDUCED PROLIFERATION

A predominant school of thought believes in the epigenetic mechanism of carcinogenesis through hormone receptor-mediated events. Estrogens primarily elicit their responses by binding to ERα (or ERβ), their cognate receptors and then through the interaction of the receptor-ligand complex with the estrogen response elements (EREs) on estrogen-responsive genes [103-108]. There is a chance of an error in DNA replication with each cycle of new DNA synthesis during mitosis. These errors in replication can result in point mutations if not repaired. As the replication process continues,

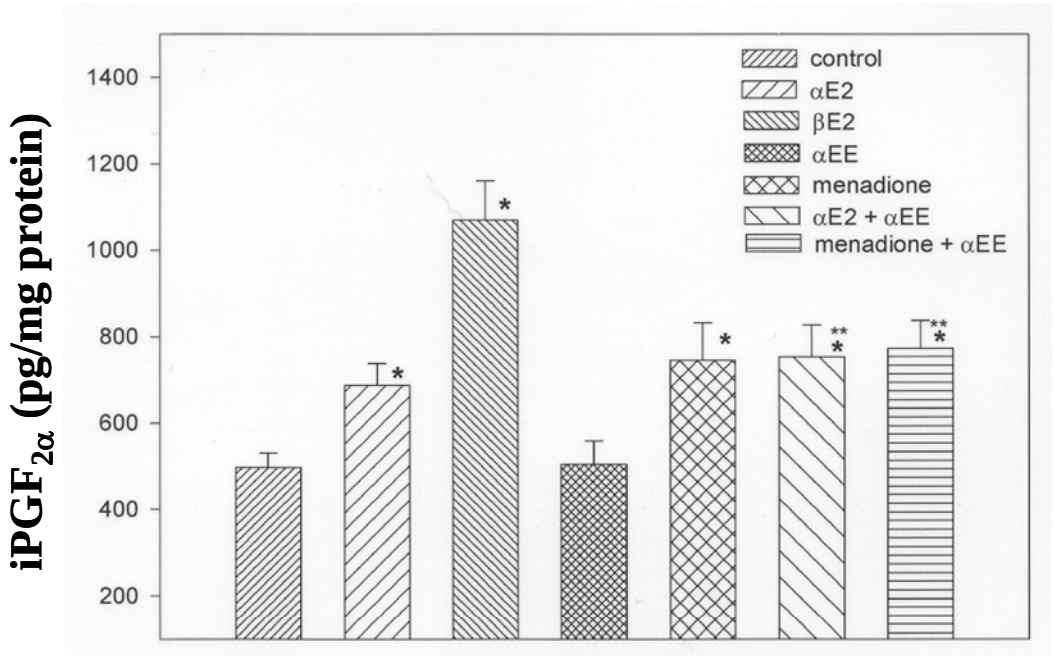

FIGURE 5. 8-ISO-PROSTAGLANDIN F_{2A} ($iPGF_{2A}$) LEVELS IN KIDNEY HOMOGENATES OF HAMSTERS TREATED WITH AE2, BE2, AEE, MENADIONE, AE2 + AEE, OR MENADIONE + AEE FOR 7 MONTHS. A > 2-fold increase in iPGF2α is detected in βE2-treated tumor-bearing kidneys of hamsters. The fold increases in iPGF2α are 1.38, 1.50, 1.52 and 1.55, respectively, for kidneys of hamsters treated with αE2, menadione, αE2 + αEE, and menadione + αEE compared with untreated controls. iPGF2α and protein were analyzed from 10 kidney homogenates from each group, and data are expressed as mean iPGF2α pg/mg protein ± s.e.m. *P < 0.05 compared with untreated controls by an unpaired t-test, **P < 0.05 compared to αEE treated group by an unpaired t-test (courtesy of Proc. Natl. Acad. Sci. USA, 100, 3913-3918, 2003).

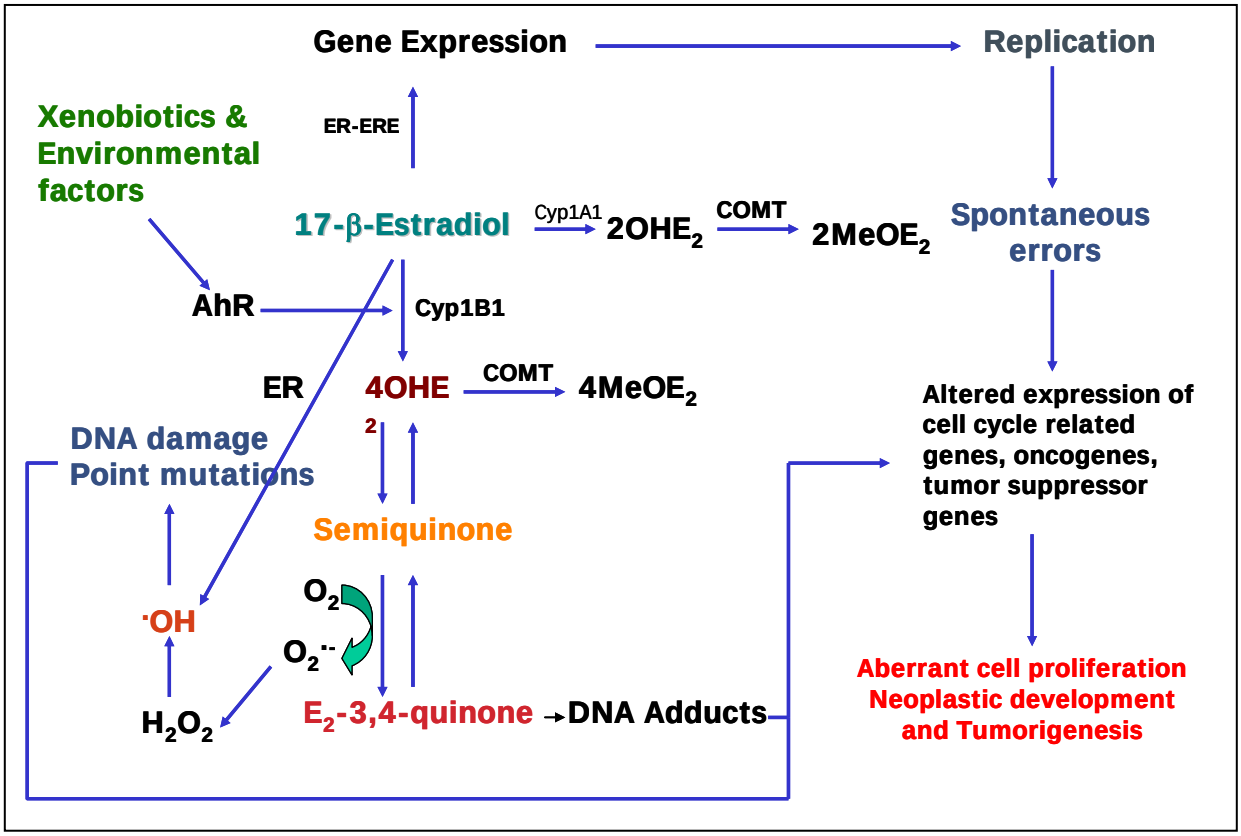

FIGURE 6. PROPOSED MECHANISM OF ESTROGEN-INDUCED CARCINOGENESIS. The mechanism of estrogen-induced carcinogenesis seems to be complex. βE2 can be metabolically activated to 2-OHE2 and 4-OHE2 by Cyp1A1 and Cyp 1B1 respectively. 4-OHE2 (and 2-OHE2) can be converted into their respective quinones via semiquinone intermediates. This redox cycling can lead to the formation of superoxide radical. 2-OHE2 and 4-OHE2 can be methylated by catechol-O-methyltransferase (COMT) and thus not available for redox cycling. H_2O_2 formed from superoxide radical by superoxide dismutase can be further converted into hydroxyl radical by an iron-catalyzed Fenton reaction. The hydroxyl radical can also be produced by an ER-dependent pathway. The hydroxyl radical produced can damage DNA and cause point mutations. E2-quinones can also lead to DNA adduct formation. DNA damage as a result of adduct formation, point mutations etc is suggested to lead to altered expression of cell cycle-related genes, oncogenes, and tumor suppressor genes. βE2 can induce gene expression via ER-ERE-dependent mechanisms. Spontaneous errors during replication can result in altered expression of genes. Xenobiotics and other environmental factors can also alter estrogen metabolizing enzyme CYP1B1 via aryl hydrocarbon (AhR) receptor-mediated processes. Thus, oxidative stress created as a result of the metabolic activation of estrogens to catechol estrogens and subsequent redox cycling of catechol estrogens to quinone metabolites generates reactive oxygen species and free radicals, which act in concert with receptor-mediated processes to produce altered expression of genes critical in the cellular control of proliferation, and finally results in aberrant cell proliferation, neoplastic development and tumorigenesis.

several mutations may accumulate [33,71]. If these mutations involve critical regions of genes needed for control of cellular proliferation and DNA repair, then neoplastic transformation may occur [109]. That is why anti-estrogens may reduce the risk of breast cancer development by interfering with ligand-receptor activation pathway.

In other proposed mechanisms of breast tumor development 16α-hydroxyestrone, a metabolite of estrone, has been proposed to be involved in the induction of breast cancer by a covalent modification of ER [51,52]. It is suggested that this covalent binding of 16α-hydroxyestrone with ER would result in a permanent uncontrolled stimulation of cell proliferation by receptor-mediated processes [51,52,110]. Thus, high levels of 16α-hydroxyestrone in some studies have been associated with the induction of breast cancer. This hypothesis

however did not receive much support over the years and has not been substantiated.

During the last decade, a new school of thought, based on accumulated experimental evidence, suggests that ER-stimulated processes may not be the only contributors to estrogen-mediated tumor growth. It has been reported that in both normal and malignant mammary glands, cells that express proliferation markers do not express ERα [111-117]. Because more than 90% of the ERβ-bearing mammary cells do not proliferate, and because 55-70% of the dividing cells have neither ERα nor ERβ, it is clear that the presence of these receptors in the epithelial cells is not a pre-requisite for estrogen-mediated proliferation [112]. The fact that Syrian hamster embryo cells (used for mechanistic studies of hormonal carcinogenesis) do not express measurable levels of ERs [118-120] implies that the induction of aneuploidy and cell transformation by estrogens in these cells may not solely depend on hormone receptors. In the target organ of estrogen-induced carcinogenesis, the male hamster kidney, only a limited number of cells express ERα [13,121]. ERα in this tissue is up-regulated after sub-chronic exposure to βE2 [13,121]. In the cross-bred ER knockout/Wnt-1 expressing mice, the onset of mammary cancer was delayed but not eliminated suggesting that Wnt-1 proto-oncogene expression in these animals induces mammary cancer regardless of ER status [122]. It is speculated that the direct mitogenic effect of estrogens on mammary epithelial cells may be acquired during breast cancer development [123].

3.4. OXIDATIVE STRESS AND RECEPTOR MODIFICATION

It is suggested that oxidative stress may modify the ability of ERα to bind to EREs and thereby modify subsequent gene expression. Reported data suggest that in one third of human breast cancer patients, ERα is unable to bind to its cognate ERE [124,125]. The fact that treatment with a thiol reducing agent can partially rectify this problem suggests that ER-DNA interaction may be subject to redox modulation [124]. Similar results have been reported for other zinc-finger type proteins and transcriptional activators [126]. Treatment of recombinant ERα DNA-binding domain or ERα-enriched extracts from CHO and MCF-7 cells with oxidative stress-inducing chemicals such as H_2O_2 and menadione produces a dose dependent loss of ER-DNA-binding capacity [124]. These results suggest that DNA-binding and transactivation are highly sensitive intracellular ERα functions that can be impaired by oxidative stress in some ERα-positive human breast tumors. These studies further suggest that oxidative stress can modify estrogen-dependent gene expression. In fact, 4-hydroxyequilenin, a major metabolite of equine estrogens present in estrogen replacement formulations, has been shown to induce 8-OHdG formation in ERα-containing cell lines [127]. This damage has been shown to be increased by agents that catalyze redox cycling or deplete glutathione, and decreased by an ERα antagonist tamoxifen, suggesting that the mechanism of DNA damage induced by equine catechol estrogens could involve oxidative stress, and ERs may play a role in this process [127]. Free radical formation in human breast cancer cells MCF-7 has been shown after the addition of estrone 3, 4-quinone, the o-quinone form of 3,4-catechol estrogen [128]. Patel and Bhat have shown that physiological levels of βE2 can increase 8-iso Prostaglandin $F_{2\alpha}$ ($iPGF_{2\alpha}$) (a known marker of oxidative stress) levels in MCF-7 cells [129]. It has also been demonstrated that co-treatment of tumor cells with βE2 and α-naphthoflavone (an inhibitor of metabolic activation of βE2) results in the inhibition of βE2-induced increase in $iPGF_{2\alpha}$ levels, which suggests that oxidative stress is created as a result of metabolic activation of estrogens [129]. Additionally, Mobley and Brueggemier have recently shown in human breast cancer cell lines that βE2 is capable of inducing an increase in sensitivity to oxidative DNA damage through an ER-mediated mechanism [130]. It appears therefore that oxidative stress may be produced in an ER-dependent or independent manner. Oxidative stress may alter expression of genes and cause loss of normal cellular control. Reactive oxygen species

and metabolic activation have been known to modulate gene expression [131-135].

3.5. INVOLVEMENT OF BOTH OXIDATIVE STRESS AND RECEPTOR-MEDIATED EFFECTS IN ESTROGEN-INDUCED CARCINOGENESIS

Bhat et al. used an estrogen-dependent hamster renal tumor model, a well established animal model of hormonal carcinogenesis that shares many biochemical and molecular characteristics with human breast and uterine cancers, in an attempt to show that both oxidative stress and receptor-mediated effects are required in estrogen-induced carcinogenesis [136,137]. Hamsters were implanted with 25 mg pellets of βE2, 17α-estradiol (αE2), αEE, menadione, a combination of αE2 and αEE, or a combination of αEE and menadione for 7 months. Different estrogens used in this study differed in their carcinogenic, metabolic activation and hormonal potential [32,54,138,139]. Menadione was used a test chemical to produce oxidative stress which has been shown to induce oxidative stress both in vitro as well as in vivo [140-142]. As expected, the group treated with βE2 developed target organ specific kidney tumors (FIG. 1). The kidneys of hamsters treated with αE2, αEE, or menadione alone did not show any gross evidence of tumors. Kidneys of hamsters treated with a combination of αE2 and αEE showed vascular congestion of glomeruli, foci with abnormal tubules, and first signs of proliferation in the interstitial cells (FIG. 3). Kidneys of hamsters treated with a combination of menadione and αEE showed foci of tumors with congested tubules and atrophic glomeruli (FIG. 4). Chronic treatment of hamsters with a combination of a chemical known to produce oxidative stress, and a poorly carcinogenic estrogen that is metabolized to catechol estrogens to a lesser extent than βE2 resulted in tumor formation. Thus, these data provide evidence that oxidant stress plays a crucial role in estrogen-induced carcinogenesis. No evidence of hemosiderin was observed in kidney sections of hamsters treated with menadione or menadione plus αEE, suggesting that the histopathological effects of menadione are not through its effects on iron homeostasis [136,143]. Tumors were not detected in the groups of animals that were treated with αE2, αEE, or menadione alone. These chemicals either lack or have weak estrogenic potential or are poor catechol progenitors [138,139]. It appears that both estrogenic potential and oxidative stress caused by metabolic redox cycling of estrogen metabolites are essential for estrogen-induced carcinogenesis since, if estrogen is withdrawn, tumors regress [14]. The hormonal effects of estrogens may promote the development of tumors. In the hamster renal tumor model, chronic treatment with αEE results in poor tumor incidence (~10%) after a prolonged treatment of 9-10 months, compared with βE2, which takes only about 6 months to induce tumors with incidence rates near 100%. αEE has about 30% of the potential to form catechol estrogens compared to βE2 [139]. This means that αEE still has some, albeit low, potential to produce oxidative stress. Thus αEE is a poor carcinogen in the hamster model.

A more than 2-fold increase in 8-iso-Prostaglandin $F_{2\alpha}$ ($iPGF_{2\alpha}$), an established marker of oxidative stress in vitro and in vivo [144,145], has been detected in tumor-bearing kidney homogenates of hamsters treated with βE2 for 7 months compared with untreated controls (FIG. 5) [136]. Kidney homogenates of hamsters treated with αEE for 7 months did not show any increase in $iPGF_{2\alpha}$ levels compared with untreated controls (FIG. 5). As expected, menadione treatment resulted in an increase in kidney levels of $iPGF_{2\alpha}$ compared with untreated controls. There were no significant differences in $iPGF_{2\alpha}$ levels among the αE2-, menadione-, and menadione + αEE-treated groups.

Tumors were clearly seen in kidneys of hamsters treated with a combination of αEE and menadione. This treatment group also showed a significant increase in $iPGF_{2\alpha}$ levels compared with the αEE-treated group and compared with untreated controls. αE2 treatment leads to $iPGF_{2\alpha}$ formation at reduced levels compared with βE2. This may indicate that αE2 catechols may be methylated faster than βE2 catechols, thus making them available at reduced levels for catechol-quinone redox cycling. Treatment of hamsters with αEE did not

result in increased $iPGF_{2\alpha}$ formation. αEE is known to inhibit cytochrome P450 activity and, therefore, catechol estrogen formation [146,147]. The reduced ability of αEE to form catechol estrogens has been suggested to be responsible for the poor carcinogenic potential of αEE [139].

In summary, it is now widely accepted that steroid hormones contribute to the development of certain malignancies, particularly that of the breast and the endometrium. However, the mechanism of tumorigenic transformation is not yet fully understood. This complex process may involve endocrine aspects, such as receptor-mediated cell proliferation as well as estrogen metabolism mediated oxidative stress events (FIG. 6).

4. CONCLUSION

Estrogens induce tumors in rodents, and estrogen use has been associated with human cancers [1-17]. Based on epidemiological data a strong correlation between estrogen use and incidence of breast cancer has been suggested [1-12]. The mechanisms involved in estrogen-dependent carcinogenesis have not been fully established. It is now generally believed that the mechanism of tumor formation by estrogens is more complex than previously considered. Some of the recent studies provide strong evidence that oxidative stress plays a critical role in estrogen-induced tumorigenesis, and that the oxidant potential of different estrogens is associated with their potential to form catechol estrogens. Oxidative stress is created as a result of the metabolic activation of carcinogenic estrogens to catechol estrogens and redox cycling between catechol estrogens and their corresponding quinones (FIG. 2).

Catechol estrogens 2-OHE2 and 4-OHE2 are produced in vivo from βE2 by cytochrome P450 mediated processes [57,98-100]. However, 2-OHE2 and 4-OHE2 differ in their carcinogenic potential. Catechol quinone DNA adducts of 4-OHE2 have been shown to be formed in the female ACI rat mammary gland [148]. 4-OHE2 levels have been shown to be significantly increased in human breast cancers compared to non-tumor tissue [39,95,96]. 4-OHE2 levels have also been reported to be elevated in human endometrial and breast cancers compared with normal tissue [39]. 4-OHE2 appears to play a role in tumorigenesis because it generates free radicals from reductive-oxidative cycling with the corresponding semiquinone and quinone forms, which can cause cellular damage [47,149] although it is not proven to be carcinogenic in human breast cancers. 4-OHE2 formation has been demonstrated in breast tumor samples derived from ER knock out (ERKO) mice harboring Wnt-1 oncogene [150]. Surprisingly, neither 2-OHE2 nor the methoxy conjugates of 2- or 4-OHE2 have been found in the mammary tumor tissue of ERKO/Wnt-1 mice [150]. These results are consistent with the hypothesis that in mammary tumor development metabolism of estrogens to 4-OHE2 (which can form of catechol estrogen quinones that can redox cycle to produce ROS and that may react with DNA to induce oncogenic mutations) plays an important role [150].

Contrary to the speculated role of 4-OHE2 in breast carcinogenesis, evidence for the role of 2-OHE2 in breast carcinogenesis is lacking and may even support a protective role for this metabolite in breast cancer risk [74]. Some studies suggest that treatment of rodents with certain inducers of estradiol 2-hydroxylation may decrease spontaneous tumorigenesis in estrogen-sensitive tissues [151]. 2-OHE2 has also been shown to have antiproliferative and anticarcinogenic effects [152-154]. In human breast cancer cells MCF-7, 2-OHE2 has been shown to have a growth inhibitory effect [155]. In addition, 2-OHE2 has little tumorigenic activity in the Syrian hamster tumor model [59]. 4-OHE2 and 2-OHE2 have been shown to affect gene regulation differently [156]. 4-OHE2 but not 2-OHE2 has been shown to induce hypoxia-inducible factor 1α (HIF-1α) and vascular endothelial growth factor A (VEGF-A) expression in ovarian cancer cell lines OVCAR-3 and A2780-CP70 [156]. Overexpression of HIF-1α occurs in most human cancers [157]. HIF-1α expression has been correlated with tumorigenicity and angiogenesis in nude mice [158]. In addition, certain estrogen-induced tumors have also been associated with an increase in the expres-

sion of VEGF-A and its receptors [159]. It has also been shown that only 4-OHE2 but not 2-OHE2 is capable of inducing 8-OHdG in MCF-7 cells [160]. It must be noted however that 2-OHE2 has been shown to induce uterine tumors in mice although only 12% compared to 66% with 4-OHE2 treatment [149]. This differential mechanism of 4-OHE2 vs. 2-OHE2-induced carcinogenesis needs to be explored.

It is also possible that 4-OHE2 may affect tumorigenesis via ER-mediated processes. Although 4-OHE2 has a lower affinity for ERα than βE2, it does however bind ERα [161]. It is also possible that 4-OHE2 has its own receptor different from βE2 as has been suggested recently [162]. Furthermore, it is known that 2-OHE2 is methylated faster that 4-OHE2 [139,163] and thus removed out of circulation before it has chance to produce ROS. It is also known that 2-OHE2 inhibits the methylation of 4-OHE2 [164], thus making it available for a longer time. It is important to point out that estrogenic effects, most likely through receptor-mediated events, cannot be discounted in estrogen-mediated carcinogenesis.

Oxidative stress may also modify the ability of ERα to bind to its ERE usually located within the promoter region of estrogen-responsive genes and thereby modify subsequent gene expression [105-108]. In some murine models, endogenous cleavage of 67 kD ER and formation of an ~50 kD nuclear receptor product has been suggested to be associated with the progression to hormonally-independent breast tumors [165]. It has been suggested that the resulting truncated DNA binding ER may compete for available target gene EREs, interfering with normal receptor regulated gene transcription. In one third of the human breast cancer patients, ERα has been shown to be unable to bind to its cognate ERE [124,125]. CHO or MCF-7 cells when exposed to oxidant chemicals like H_2O_2 or menadione in culture, impairs the ability of endogenous ER to bind to DNA and transactivate an ER-responsive reporter gene, demonstrating that extracellular redox stress can modulate intracellular ER function [124]. Treatment of MCF-7 cells with physiological levels of βE2 has been shown to increase the sensitivity to DNA damage as measured by 8-OHdG formation [130]. A 2-4-fold increase in iPGF2α levels has been shown in cancer cell lines following treatment with 10 nM βE2 [129]. Thus, it is concluded that oxidative stress generated as a result of metabolic redox cycling of carcinogenic estrogens between their catechol estrogen and quinone metabolites plays an important role in estrogen-induced carcinogenesis.

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