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Journal of Steroid Biochemistry & Molecular Biology 86 (2003) 477–486

The Journal of Steroid Biochemistry & Molecular Biology

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Genotoxic metabolites of estradiol in breast: potential mechanism of estradiol induced carcinogenesis☆

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Abstract

Long term exposure to estradiol increases the risk of breast cancer in a variety of animal species, as well as in women. The mechanisms responsible for this effect have not been firmly established. The prevailing theory proposes that estrogens increase the rate of cell proliferation by stimulating estrogen receptor-mediated transcription and thereby the number of errors occurring during DNA replication. An alternative hypothesis proposes that estradiol can be metabolized to quinone derivatives which can react with DNA and then remove bases from DNA through a process called depurination. Error prone DNA repair then results in point mutations. We postulate that these two processes, increased cell proliferation and genotoxic metabolite formation, act in an additive or synergistic fashion to induce cancer. If correct, aromatase inhibitors would block both processes whereas anti-estrogens would only inhibit receptor-mediated effects. Accordingly, aromatase inhibitors would be more effective in preventing breast cancer than use of anti-estrogens.

Our studies initially demonstrated that catechol estrogen (CE) quinone metabolites are formed in MCF-7 human breast cancer cells in culture. Measurement of estrogen metabolites and conjugates involved utilization of an HPLC separation coupled with an electrochemical detector. We then utilized an animal model that allows dissociation of estrogen receptor-mediated function from that of the effects of estradiol metabolites. Wnt-1 transgenic mice harboring a knock-out of ERα provides a means of examining the effect of estrogen deprivation in the absence of the ER in animals with a high incidence of breast tumors. ERβ was shown to be absent in the breast tissue of these animals by RNase protection assay. In the breast tissue of these estrogen receptor alpha knock-out (ERKO)/Wnt-1 transgenic mice, we demonstrated formation of genotoxic estradiol metabolites. The ERKO/Wnt-1 breast extracts contained picomole amounts of the 4-catechol estrogens, but not their methoxy conjugates nor the 2-CE or their methoxy conjugates. The 4-CE conjugates with glutathione or its hydrolytic products (cysteine and *N*-acetylcysteine) were detected in picomole amounts in both tumors and hyperplastic mammary tissue, demonstrating the formation of CE-3,4-quinones. These results are consistent with the hypothesis that mammary tumor development is primarily initiated by metabolism of estrogens to 4-CE and, then, to CE-3,4-quinones, which may react with DNA to induce oncogenic mutations.

The next set of experiments examined the incidence of tumors formed in Wnt-1 transgenic mice bearing wild type ERα (ER+/+), the heterozygous combination of genes (ER+/ER−) or ERα knock-out (ER−/−). To assess the effect of estrogens in the absence of ER, half of the animals were oophorectomized on day 15 and the other half were sham operated. Castration reduced the incidence of breast tumors in all animal groups and demonstrated the dependence of tumor formation upon estrogens. A trend toward reduction in tumor number (not statistically significant at this interim analysis) occurred in the absence of functional ER since the number of tumors was markedly reduced in ERKO animals which were castrated early in life. In aggregate, our results support the concept that metabolites of estradiol may act in concert with ER mediated mechanisms to induce breast cancer.

Keywords: Breast cancer; Catechol estrogen; Estrogen receptor; Carcinogenesis

1. Introduction

A variety of data support the concept that estrogens contribute to the development of breast cancer [1]. Inhibition of estrogen production with aromatase inhibitors abrogates the development of spontaneous breast tumors in aging

☆ Presented at the VI International Aromatase Conference: AROMATASE 2002, Kyoto, Japan, 26–30 October 2002.

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doi:10.1016/S0960-0760(03)00377-7

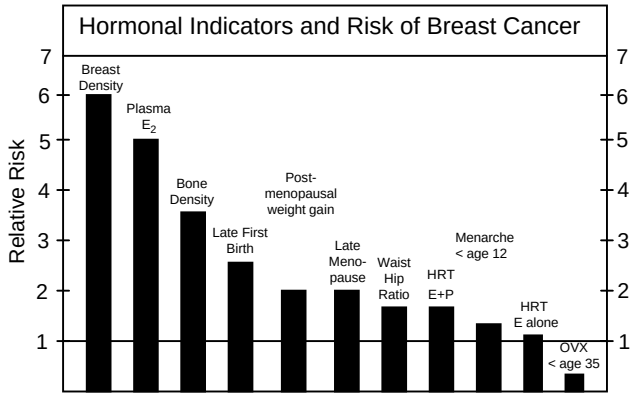

Fig. 1. Hormonal indicators and risk of breast cancer. Each bar represents the relative risk of breast cancer with respect to factors reflecting exposure to estradiol. Reproduced from [42].

Sprague–Dawley rats [2]. In women, bilateral oophorectomy before the age of 35 reduces the lifetime risk of breast cancer by nearly 75% [3,4]. Clinical factors reflecting an increased exposure to estrogen such as early menarche, late menopause, use of hormone replacement therapy, weight gain of 20 kg or more after age 21, bone density in the top quartile, and enhanced breast density are associated with increased relative risks of breast cancer (Fig. 1). Recent evidence, derived from pooling data from nine prospective studies, indicates an increased relative risk of breast cancer with each subsequent quintile of free estrogen level (Fig. 2) [5]. A pooled data and a meta-analysis from several studies suggest that anti-estrogens reduce the incidence of breast cancer by 50–75% in high-risk women [6,7]. Taken together, these data provide compelling evidence of a relationship between breast cancer risk and estrogens.

The mechanisms whereby estrogens cause breast cancer have not been conclusively established and certain aspects remain controversial. The most commonly held hypothesis is that estrogens bind to ERα or ERβ and stimulate the transcription of genes involved in cell proliferation [8,9] (Fig. 3A). With each cycle of new DNA synthesis during mitosis, there is a chance for an error in DNA replication. If not repaired, these errors in replication result in point mutations. As the process continues, several mutations accumulate [10,11]. When these mutations involve critical regions needed for cellular proliferation, DNA repair, and apoptosis, neoplastic transformation results [12]. This mechanistic construct would explain why anti-estrogens reduce the risk of development of breast cancer.

A more controversial hypothesis is that estradiol can be metabolized to genotoxic metabolites and directly damage DNA [10,11,13,14]. Cytochrome P450 1B1 catalyzes the hydroxylation of estradiol to 4-OH-estradiol which is then further converted to the estradiol-3,4-quinone. As shown in Fig. 3, this compound can bind covalently to guanine or adenine and result in destabilization of the glycosyl bond. The 4-OH-estradiol-1-*N*7-guanine (Fig. 3B) and 4-OH-estradiol-1-*N*3-adenine (or their estrone analogues) adducts are released from the DNA and leave behind an abasic site on DNA. Through the process of error prone DNA repair, these sites now form point mutations, which serve as potential initiators of neoplastic transformation [14]. Our working hypothesis is that estradiol acts on both pathways shown in Fig. 3A in an additive or synergistic fashion to induce breast cancer.

Recent experimental data provide indirect evidence for the importance of the genotoxic pathway. The enzyme catechol-*O*-methyltransferase (COMT) serves to shunt estradiol metabolites away from the formation of the estradiol-3,4-quinones and lessens the potential genotoxicity of estradiol [11]. The COMT enzyme contains isoforms with low (L) and high (H) activity and phenotypes are divided into the LL, HL, and HH subtypes. In women with low COMT activity and consequently, a lower rate of inactivation of the catecholestrogens, one might expect an increase in breast cancer prevalence or incidence. Twelve

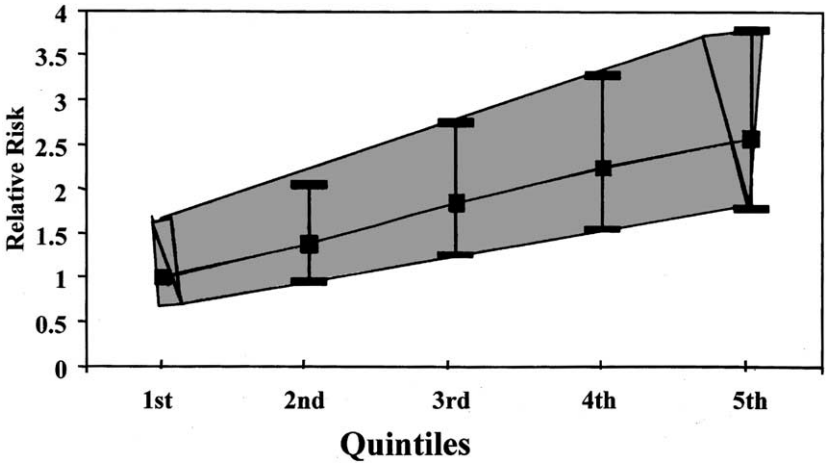

Fig. 2. Free estradiol levels and risk of breast cancer. Adapted from the pooled analysis of nine studies relating the risk of breast cancer to free estradiol levels in post-menopausal women [5].

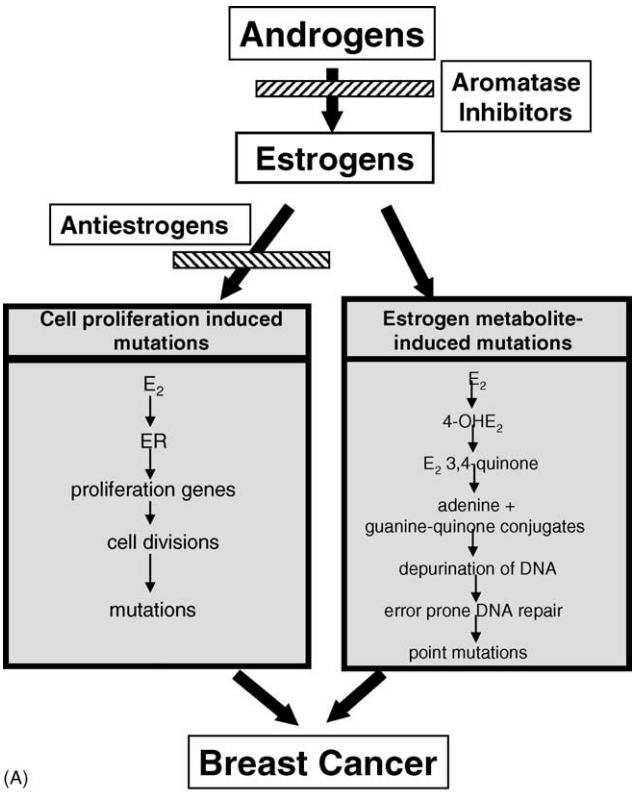

Fig. 3. (A) Diagrammatic representation of the two pathways by which estradiol is postulated to cause breast cancer. The anti-estrogens block only the ER mediated pathway, whereas the aromatase inhibitors block the formation of genotoxic metabolites as well as the ER mediated pathway. (B) Structure of 4-OH-estradiol-1-*N*7-guanine.

epidemiologic studies have examined this relationship and seven of them found a statistically significant increased risk of breast cancer in phenotypes with low COMT (Table 1) [15–24]. These findings remain controversial since not all studies found an increased risk, but the positive results are best interpreted by the genotoxic estradiol hypothesis.

More direct evidence regarding estradiol genotoxicity derives from in vitro studies. Liehr and co-workers, using the V-79 cell carcinogenicity assay, found that low doses of estradiol in the 10^{-11} and 10^{-12} molar ranges causes a 3.8–4.2-fold increase in rate of genetic mutations [25]. In other experiments, Russo et al. administered estradiol to benign MCF-10F breast cells in vitro in doses ranging from 0.007 nM to 1 μM [26,27]. They found that even the very low estradiol concentrations induced loss of heterozygosity (LOH) at chromosomal sites (11q23.3, 11q23.1-25, 3p21, 3p21-21.2, 3p21.1-14.2, 3p14.2-14.1) at which human breast cancers commonly exhibit LOH. They also documented the neoplastic transformation of these cells by demonstrating an increase in anchorage independent colony formation and loss of duct differentiation. Taken together, these recent data provide additional support for the genotoxic hypothesis.

Table 1
COMT polymorphism and breast cancer risk

Population	OR	95% CI	COMT	Menopause	Reference
Maryland	2.2	0.9–5.1	LL	Post	[19]
New York	2.4	1.4–4.3	L–	Pre	[20]
N. Carolina	0.7	0.4–1.2	L	Pre	[18]
	0.8	0.5–1.4	L	Post	
China	2.5	1.0–6.1	LL	Pre	[16]
Taiwan	9.3	1.3–19.3	LL	Post	[21]
Korea	1.7	1.0–2.8	L–	Both	[22]
Finland	0.6	0.3–1.0	L–	Post	[23,24]
	0.4	0.2–0.9		Pre	
	4.0	1.13–14.3	L–	Post/HRT	
Japan	0.99	0.5–2.02	L–	Both	[17]
Sweden	0.86	0.37–2.0	L–	Pre	[15]

In the studies described in this manuscript, we sought further evidence of the validity of the estrogen genotoxic hypothesis. Initially we wished to demonstrate that human breast cancer cells contain the enzymes necessary for conversion of estradiol to estradiol quinones and that depurination of 4-CE-DNA adducts can occur. For these studies we utilized MCF-7 cells containing a stably transfected aromatase gene and measured genotoxic products after incubation with estrogen substrates [13,28,29]. In addition, we used an estrogen receptor alpha knock-out (ERKO) animal model [30–33]. These animals express no ERβ in breast tissue as demonstrated by RNase protection assay. In this model system, estradiol therefore, would not act via receptor-mediated effects on breast, and any neoplastic changes induced by estrogens must work through estrogen receptor independent pathways. Taken together, these studies demonstrated that breast cancer cells can convert estradiol to genotoxic metabolites and that non-receptor-mediated mechanisms involving estradiol can modulate the process of breast cancer development.

2. Materials and methods

For the measurements of metabolites, we utilized MCF-7 cells stably transfected with the aromatase gene, which our laboratory has used extensively in previous studies [28]. In prior publications, we have described the precise methods for cell culture, counting, determination of viability, and assessment of aromatase activity in these cells [28,29].

Estradiol metabolites, conjugates and depurinating DNA adducts were measured by HPLC with a 12-channel

electrochemical detector [13,34,35]. Confirmation with mass spectrometry was utilized to verify key measurements. These methods have been extensively described previously and will be only briefly described here. Samples were suspended in 50 mM ammonium acetate and incubated for 6 h with glucuronidase/sulfatase to cleave the sulfate or glucuronide conjugates. Following this step, methanol was added to bring the concentration to 60%. The mixture was then extracted with 8 ml of hexane to remove lipids. The aqueous phase was then diluted with 50 mM ammonium acetate, pH 4.0, (containing 2 mg/ml ascorbic acid to minimize oxidation of catechol estrogens and their conjugates) and a concentration of 25% methanol was obtained. This extract was applied to a Certify II Sep-Pak (200 mg cartridge, Varian, Palo Alto, California) and subjected to sequential elutions with 2 ml of 20, 40 and 70% methanol as previously described [34]. The three fractions were analyzed by HPLC. Details of the choice of oxidation potentials, the acetonitrile/methanol/water elution gradient, the methods for peak height ratios between the dominant peak and preceding and trailing peaks in adjacent channels have been described in detail [34]. Synthesized standards for each of the compounds to be analyzed were used for identification and quantification of various compounds. Data analysis utilized Coul-Array software. The system is sufficiently sensitive to detect 1 pmol of metabolites and conjugates injected into the column.

ERKO/Wnt-1 and Wnt-1/ER−/+ heterozygous mice were obtained from the National Institute of Environmental Health Sciences, Research Triangle Park [30]. Initially animals were bred from a stock of wild type Wnt-1+/− heterozygotes. Later, Wnt-1 wild type animals were obtained from the Jackson laboratories to continue our breeding colony.

3. Results

3.1. MCF-7 aromatase transfected cell culture experiments

Our initial experiments examined whether the enzymes responsible for formation of estrogen metabolites and conjugates were present in human breast cancer cells. The MCF-7 cells were incubated with 10 μM 4-OH-estradiol for 24 h before collecting media for later measurement of the various metabolites and conjugates. Fig. 4A and B shows in diagrammatic form the experimental design of our experiments and measurements made. As shown in Fig. 5A, we detected large amounts of 4-methoxy-estradiol as well as substantial amounts of the quinone conjugates and the depurinating 4-OH-estradiol-1-*N*7-guanine adduct and its estrone analogue. Note that these amounts are considered large since they represent levels much higher than in human breast tissue which (as shown below) are in the range of 1.5 pg/ml (i.e. 5 pM). We next determined whether these cells could aromatize a sufficient amount of testosterone to estradiol to result in formation of the depurinating species. As shown in Fig. 5B, we detected 131 pg/ml of estrogen (92 pg/ml of estradiol and 39 pg/ml of estrone in the media) indicating the production of estrogens from aromatization. The 4-OH-estradiol-1-*N*7-guanine adduct (and its estrone analogue) was also present at a total concentration of 0.92 pg/ml as were the glutathione, cysteine, and *N*-acetyl-cysteine conjugates of estradiol-3,4-quinone and its estrone analogue. Finally, the aromatase inhibitor letrozole was capable of inhibiting the formation of the estrogens from a total of 131 pg/ml of E_1 and E_2 (Fig. 5B) to 2.8 pg/ml (Fig. 5C) and their downstream metabolites to undetectable levels in most cases.

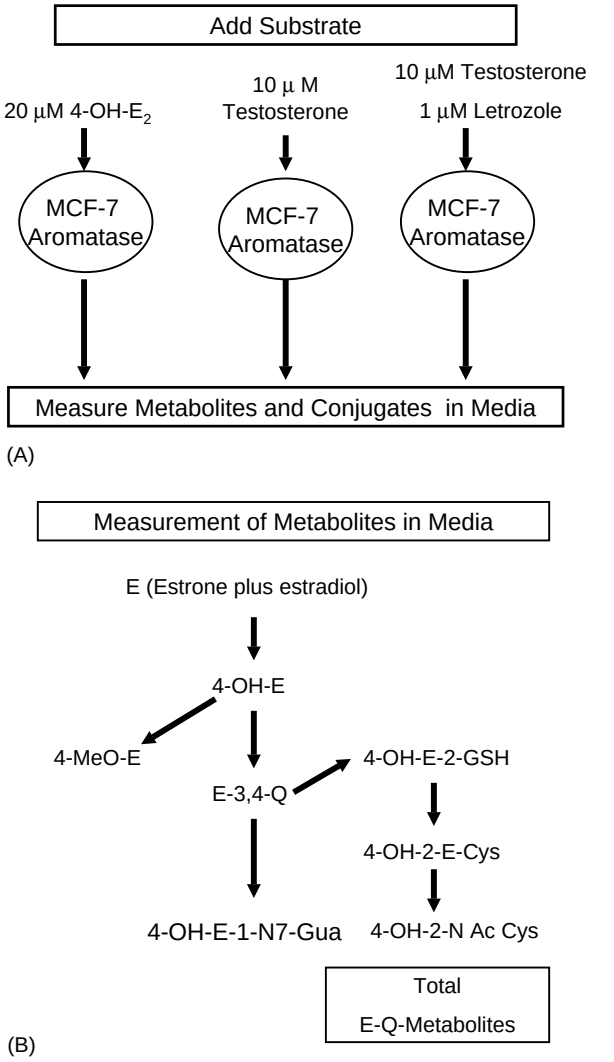

Fig. 4. (A) Diagrammatic representation of the experimental design of the studies demonstrating formation of metabolites in aromatase transfected MCF-7 cells. (B) Diagrammatic representation of the metabolites measured.

3.2. Measurements in human breast tissue

Breast tissue samples were collected at the time of surgery from women with histologically confirmed breast cancer

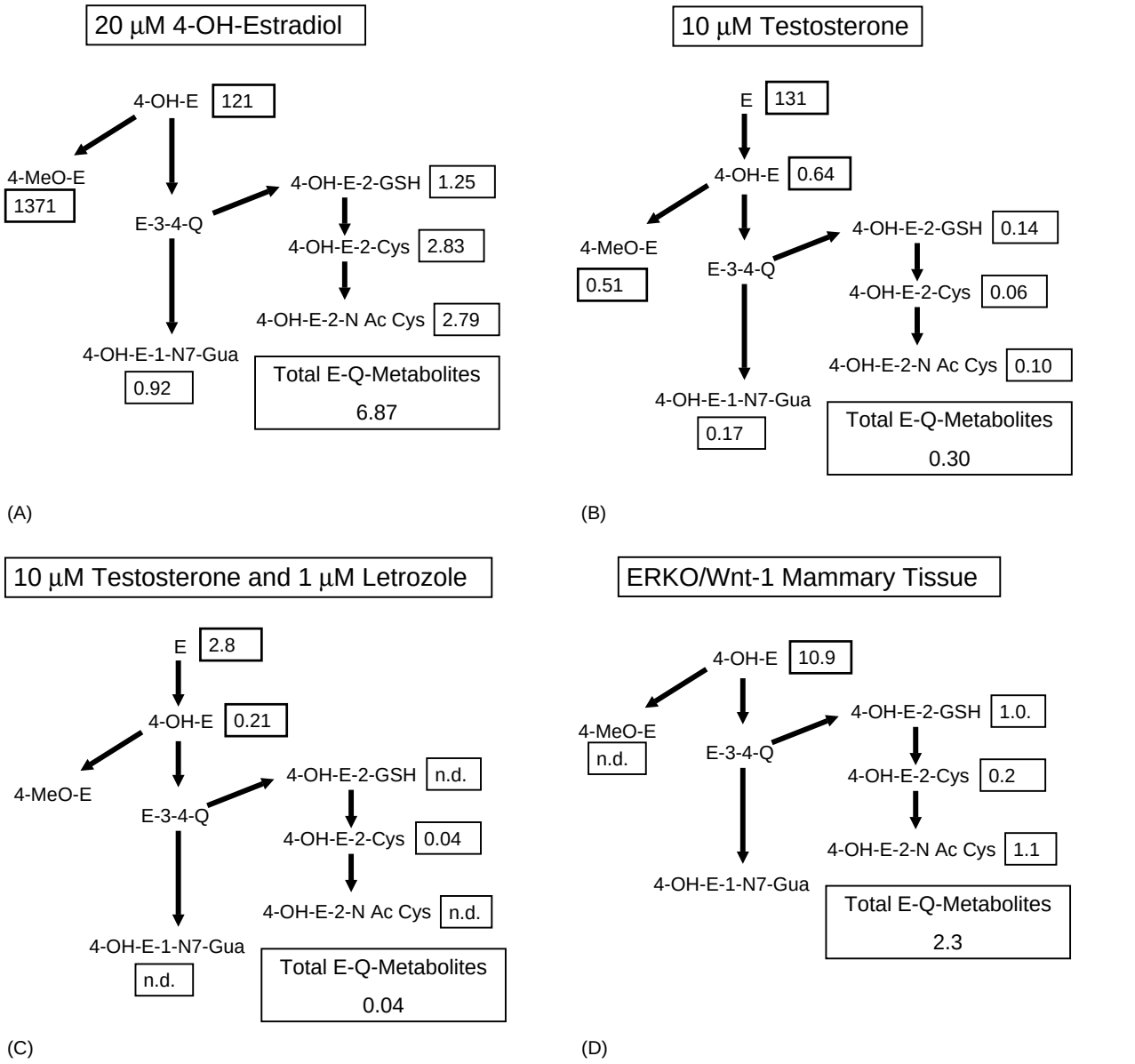

Fig. 5. (A) Formation of metabolites when 20 μM 4-OH-estradiol are added. (B) Formation of metabolites when 10 μM testosterone are added. (C) Formation of metabolites when 1 μM letrozole and 10 μM testosterone is added. (D) Measurement of metabolites in the hyperplastic mammary tissue of ERKO/Wnt-1 double transgenic mice. The results in the boxes represent pg/ml of metabolites. The results in tumor tissue are expressed as pg/g.

[35]. The tissues analyzed were those which contained the breast cancer. For controls, breast samples were taken at the time of surgical excision of benign lesions. These included the benign fatty breast tissue surrounding lesions excised and tissue from lesions called benign fibrocystic changes after histologic examination. As previously reported and shown in Fig. 6A and B, the levels of estradiol plus estrone in breast tissue from women with breast carcinoma approximated 5 pmol/g of tissue and in breast from women without carcinoma, 2 pmol/g [35]. Expressed as pg/ml these values would represent 1650 and 660 pg/ml. These values are similar to those found by other investigators and represent levels one to two orders of magnitude higher than found in the plasma of post-menopausal women [36]. The levels of the 4-OH-, 2-OH-, and 16α-OH-estrogen metabolites are similar in magnitude. As evidence that the human breast can synthesize the catechol estrogen quinones, the total E_1 and E_2 conjugates of the estrogen quinones are in the range of 2 pmol in tissue from women without breast carcinoma and 8 pmol in breast tissue from women with breast carcinoma [35]. These preliminary measurements provide the first direct evidence that the estradiol quinones are present in high concentrations in human breast tissue.

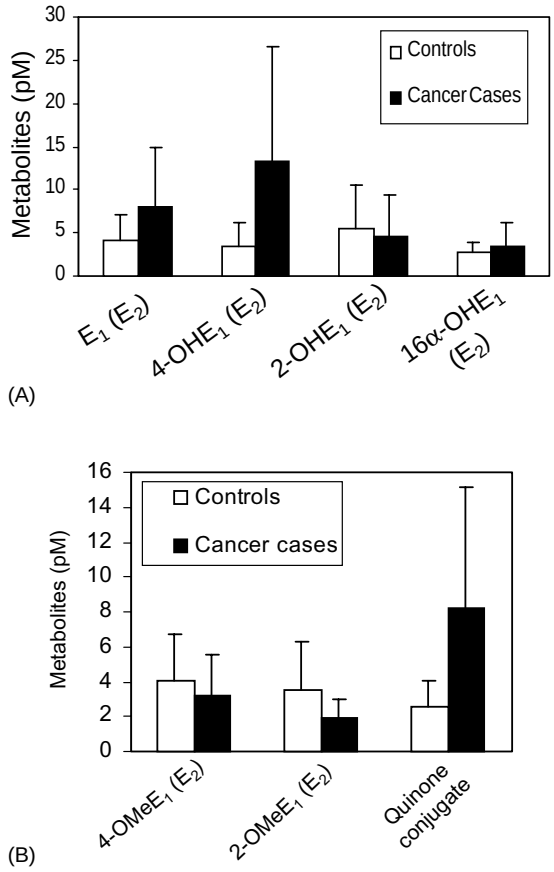

Fig. 6. (A) This part depicts the levels of estrone and estradiol, 2-OH- and 4-OH-estrone and estradiol, and 16α-OH-estrone and 16α-OH-estradiol in 77 breast tissues from women with (cancer cases) and from women without (controls) breast carcinoma. (B) This illustrates the levels of 2- and 4-methoxy-estrone and estradiol and the quinone conjugates in these same samples. The values on the vertical scale represent pM of metabolites. This figure has been reprinted from the original article (Ref. [35]) with the permission of the authors and publisher.

3.3. Measurements in ERKO/Wnt-1 mammary tissue

As previously reported, the ERKO/Wnt-1 mammary tissue appears to exhibit an altered metabolic balance [37]. Formation of 4-OH-estrogen metabolites is favored over those of the 2-OH species and the catechol-*O*-methyl-transferase pathway appears to be relatively inactive (Fig. 5D). As summarized in Fig. 5, we detected 10.9 pmol/g of 4-OH-E_2 and 4-OH-E_1 in mammary tissue, as well as a total of 2.3 pmol/g of conjugated estrogen quinone. No 4-methoxy-estrogen metabolites were present. We have not as yet been able to detect the 1-*N*7-guanine adducts in this tissue but are now making additional measurements on newly bred animals.

3.4. Tumor incidence in ERKO/Wnt-1 animals

Bocchinfuso et al. had previously shown that ERKO/Wnt-1 mice still exhibit a 100% incidence of mammary tumors with a delayed progression even though ERα has been knocked out and they lack detectable ERβ in breast tissue [30,31]. Preliminary data from this study suggested the possibility that early castration reduced the incidence of tumors and delayed the onset of tumors that did develop. To confirm and extend these results, we bred a large number of animals and performed castration on day 15 in one group. We reasoned that demonstration of a reduced incidence of tumors would provide strong support for the principle that the genotoxic metabolites of estradiol contribute to breast tumor formation. Accordingly, we castrated one group of 60 ERKO/Wnt-1 animals and left another group of 21 animals intact. After 12 months of observation, 50% of the intact and only 20% of the castrate animals have developed tumors (Fig. 7). At the time of this interim analysis, the differences are not statistically significant ($P = 0.2$). We anticipate that the tumors will continue to appear at the same differential rate in the two groups over the next few months. If that does occur, we expect (but cannot be certain) that with a larger number of tumors, the differences will become statistically significant.

To confirm the prior results of Bocchinfuso and Korach, we also compared tumor incidence in the ER+/+ Wnt-1+/− animals with those in the ER−/− group (Fig. 8, top and bottom) [30,31]. After the end of 6 months, 50–65% of animals in the ER+/+ homozygous and ER+/− heterozygous groups had developed tumors. This is nearly identical to the 50% tumor incidence found by Bocchinfuso et al. in these groups at 6 months [30]. As shown previously by them, the ER−/− Wnt-1 animals exhibited an incidence of only 35% at this time point. We then extended their observations by examining the effect of early castration on tumor formation in these ER positive animals (Fig. 8, top and bottom). Castration before day 15 reduced the incidence of tumors in these two groups to 10% at 6 months. These data provide evidence that estradiol works both through an ERα dependent pathway as well as an ER independent pathway to produce breast tumors.

4. Discussion

Studies in vitro, in experimental animals, and in women provide compelling evidence that estrogens contribute to the development of breast cancer [1]. The commonly held mechanism of carcinogenesis is that estrogens stimulate cell proliferation, increase the number of genetic mutations in proportion to the number of mitotic divisions, and promote the propagation of these mutations by stimulating growth [9,38]. An alternate hypothesis suggests that estrogens may be metabolized directly to genotoxic compounds [10,11,13,14,39]. Our working hypothesis is that these two pathways act in concert in an additive or synergistic fashion to cause breast cancer (Fig. 3A). Our studies sought to provide evidence in support of the genotoxic hypothesis by demonstrating the formation of genotoxic metabolites in cell culture and in human breast tissue. In addition, we

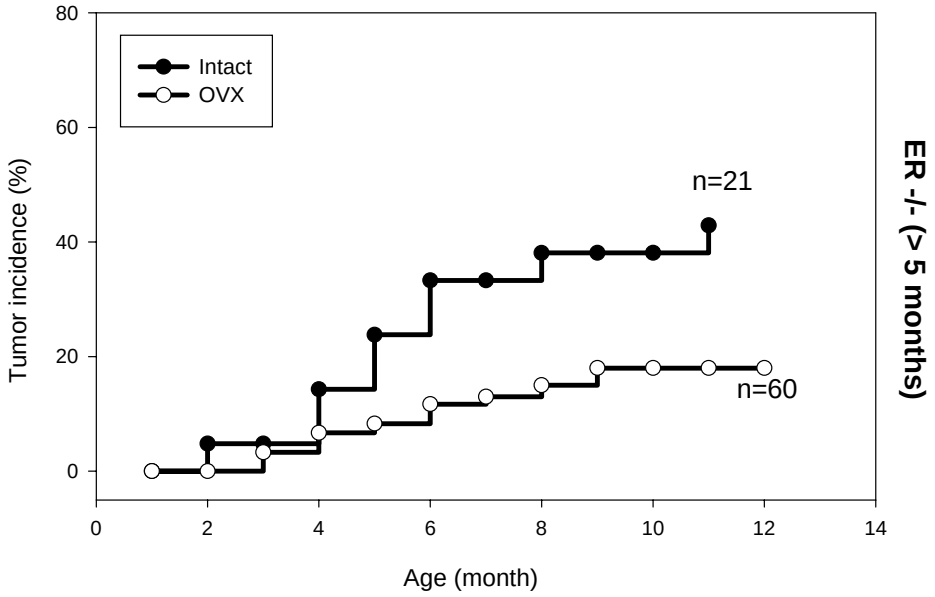

Fig. 7. Incidence of tumors in intact and castrate ERKO/Wnt-1 mice observed over a period of 12 months. The number of animals in each group is shown.

utilized a double gene targeted transgenic mouse model to provide proof of the principle that estrogens can influence breast tumor development in the absence of functioning estrogen receptors.

Our results demonstrated that MCF-7 cells can convert estradiol and testosterone both to catechol estrogen quinones that react with DNA to form the 4-OH-estradiol-1-*N*7-guanine depurinating adducts and their estrone analogues. We also demonstrated that the incidence of breast tumor development in ERKO/Wnt-1 transgenic animals could be diminished by early oophorectomy. These animals have been shown by Bocchinfuso et al. to lack ERβ in breast tissue when studied by RNase protection assay [30,31]. It is possible however, that minimally detectable levels of ERβ are present in the gland. Taken together, these new data support the possibility of the biological importance of the genotoxic pathway.

Our first aim was to demonstrate that human breast cancer cells could convert testosterone or estradiol to the genotoxic products. We clearly demonstrated this in an MCF-7 cell model system by using a highly sensitive and specific assay for measurements of estrogen metabolites and conjugates. Each of the enzymes involved in catechol-estrogen formation and further metabolism was found to be present using this methodology. These experiments were designed merely to demonstrate the presence of these enzymes and not their relative abundance. Accordingly, we utilized large amounts of substrate (10–20 μM) in these experiments.

A commonly expressed criticism of the genotoxic hypothesis is that investigators have needed supra-physiologic amounts of estrogen to demonstrate formation of genotoxic metabolites of estradiol [11]. Our in vitro experiments can be criticized on the same basis. However, we think that biologic endpoints of this process provide a higher level of sensitivity than do biochemical measurements. This reasoning is supported by studies which examined the biologic effects of estrogen under similar in vitro conditions. Russo et al. have shown that 0.007 nM estradiol can induce neoplastic transformation as evidenced by increased colony formation in benign MCF-10F cells which lack a functional ER [26,27]. This treatment also reduced the formation of ducts, another parameter indicative of neoplastic transformation. Similar concentrations induce loss of heterozygosity in benign, non-ER breast cells at hot spots for LOH in breast cancer tissue. Such low concentrations can also induce mutations in V-79 cells [25]. As further evidence of the ability of physiologic amounts of estrogen to serve as substrate for these genotoxic metabolites, human breast tissues from women with and without breast cancer contain large amounts of these metabolites [35]. Taken together, the tissue measurement data and the findings from incubated cells in vitro clearly demonstrate that human breast tissue can form substantial amounts of the genotoxic metabolites of estradiol.

Our studies with the ERKO/Wnt-1 animals are preliminary and will require additional time to determine if more tumors will be formed over an extended period. An increased number of tumors will increase the statistical power for detecting differences. Not-withstanding these statistical considerations, our data comparing intact with castrate ERα wild type (ER+/+), heterozygotes (ER+/−) and ERKO (ER−/−) animals clearly show the effect of estradiol on tumor formation. All animals castrated on day 15 had fewer tumors than the intact animals. More importantly, estradiol depletion reduced the incidence of tumors even in the absence of a functioning ERα. These data strongly support the genotoxic hypothesis.

The ERKO/Wnt-1 animal is a powerful model for studying the effect of estrogen in the absence of a functioning

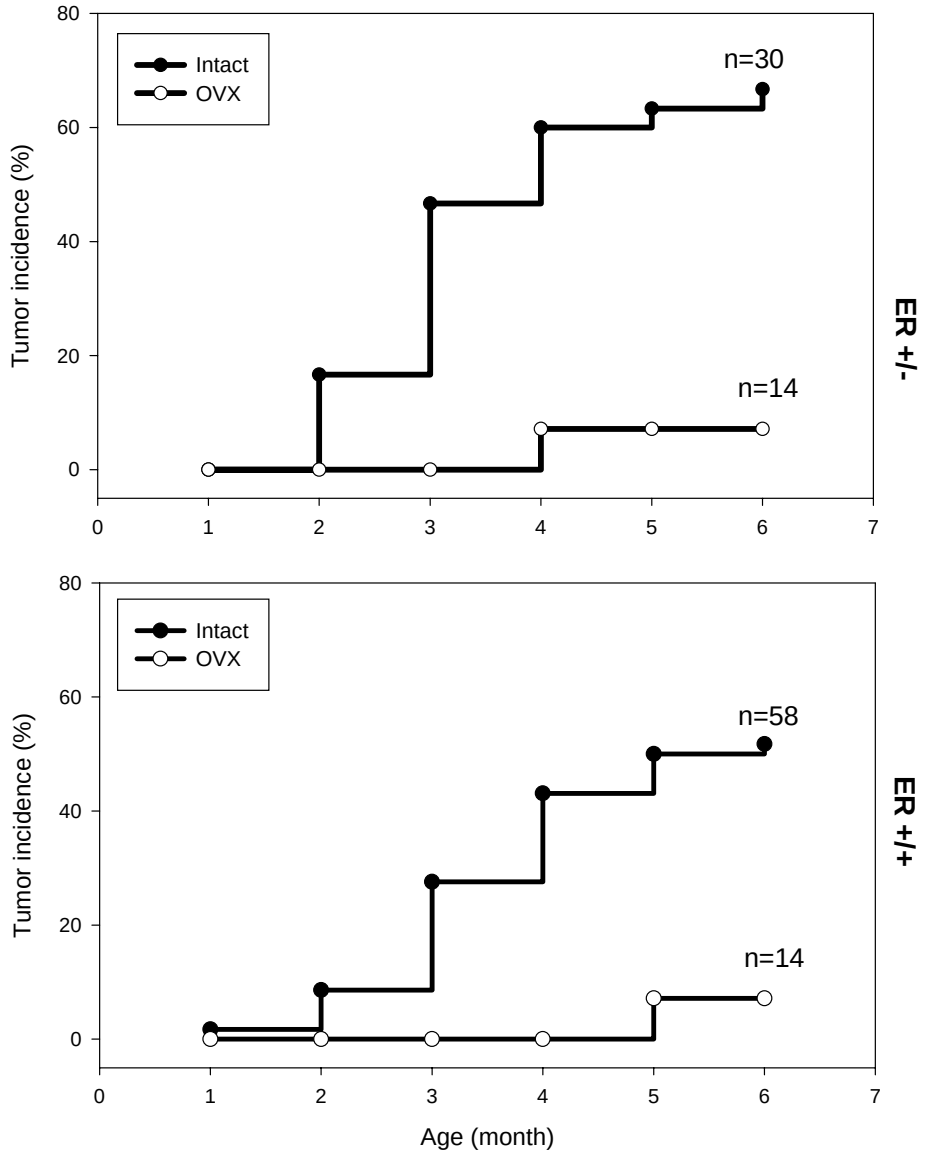

Fig. 8. Incidence of tumors in intact and castrate ER+/− Wnt-1 (top panel) animals and in ER+/+ Wnt-1 (bottom panel) animals. The horizontal scale represents months of observation.

ERα. These animals have circulating estradiol levels in the range of 325 pg/ml (personal communication, Dr. Kenneth Korach). This is approximately 3–5-fold higher than normal as a consequence of the absence of estradiol negative feedback on the pituitary and the resultant rise in LH levels. In addition, the breast tissue from these animals appears to convert little 4-OH-estradiol to 4-methoxy-estadiol [37], a metabolite which is thought to be inactive and to obviate further conversion to genotoxic metabolites. In addition, most of the catechol estrogen adducts formed are depurinating ones from *E*-3,4-quinones rather than the stable DNA adducts from *E*-2,3-quninones. In a prior report, we called this metabolic pathway unbalanced because of the propensity for estrogens to be converted to the genotoxic estrogen-3,4-quinones [37].

We are not certain at the present time whether the metabolic imbalance reported reflects effects of the lack of ERα, of the Wnt-1 gene, or the combination of the two. We have now obtained breast tissue systematically in wild type, ERKO, and ERKO/Wnt-1 animals for measurement of all metabolites. These measurements, when completed, should allow conclusions regarding this issue.

Several potential pitfalls must be considered when interpreting the ERKO/Wnt-1 data. First, these animals may possess some degree of residual ERβ in breast tissue, even though this could not be detected by RNase protection assay [30,31]. Second, the effects of castration could relate to the deprivation of other products produced by the ovary such as inhibin or progesterone [40] since it has been shown that progesterone is mitogenic in the mouse mammary gland.

Later studies are planned to add back estradiol and progesterone by silastic implant to assess the effects of these substances on tumor formation. Recombinant inhibin could also be injected chronically to assess its direct effect. During estradiol add back experiments in castrate animals, we will pay close attention to precise dose response effects. It should be noted that ERKO animals have estradiol levels that are supra-physiologic (i.e. 325 pg/ml) and thus it will be necessary to examine the effect of add back of physiologic doses of estradiol. In this way, the genotoxic effects of physiologic levels of estradiol can be studied. Finally, the ERKO animals will be treated with the pure anti-estrogen, fulvestrant, to ensure blockade of all remaining ER.

We consider it highly relevant with respect to prevention of breast cancer to determine whether or not the genotoxic pathway is biologically important. Anti-estrogens act only to block ER mediated function whereas the aromatase inhibitors reduce estradiol levels and consequently block both ER mediated as well as genotoxic pathways. Theoretically, aromatase inhibitors would then be much more efficacious for prevention of breast cancer than the anti-estrogens [8]. Data from the recently reported ATAC trial can be interpreted in light of the genotoxic hypothesis [41]. In this trial, the aromatase inhibitor, anastrozole, resulted in a 50% greater reduction of contralateral breast cancer ($P < 0.05$) at 3 years than did the anti-estrogen, tamoxifen. While there are other explanations for this difference, the magnitude of greater effect of the aromatase inhibitor is substantial. This observation, when taken together with the biologic data presented in this manuscript, highlight the compelling need to determine conclusively whether or not the genotoxic hypothesis of estradiol induced carcinogenesis is operative.

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