Chapter 3: Endogenous Estrogens as Carcinogens Through Metabolic Activation

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A common thread linking the main risks for developing breast cancer in women is cumulative, excessive exposure to estrogen. The standard paradigm to account for this association focuses on increased cell proliferation caused by estrogen through estrogen receptor-mediated signal transduction accompanied by increased probability for mutation to occur during DNA synthesis. This chapter provides an overview of the mounting evidence, provided from cell culture and whole animal experimental studies, in support of a role for the oxidative metabolites of estrogen, in particular, the catechol estrogens, in the development of estrogen carcinogenesis. This provides a paradigm for how estrogens may contribute to the development of human breast cancer. The chapters that follow will fill in the details. Evidence shows that the catechols themselves are signaling molecules that work through the estrogen receptor. In addition, upon further oxidation, the catechols can give rise to reactive quinones capable of forming direct adducts with glutathione and purines in DNA and of redox cycling to generate reactive oxygen species that can cause oxidative damage. Estradiol and estrone, as well as their 4-hydroxy catechols, are carcinogenic in the Syrian golden hamster kidney, and ethinyl estradiol is a strong promoter of hepatocarcinogenesis in the rat. Increased oxidative DNA damage has been detected in target tissues after estrogen treatment in both animal model systems. Furthermore, several recent molecular epidemiologic studies have found that a polymorphism associated with a low-activity form of catechol-*O*-methyltransferase, an enzyme involved in the inactivation of catechol estrogens, is associated with an increased risk for developing breast cancer. The increased risk is observed in certain women, although the studies are not consistent on which subgroup of women (e.g., premenopausal or postmenopausal) is at increased risk, and one study detected no increased risk. Reasons for such discrepancies are discussed in light of factors, such as genetic polymorphisms and environmental/lifestyle susceptibility factors, which control the tissue-specific balance within cells among the estrogen metabolites. It is concluded that such factors will have to be identified through additional mechanistic studies and that, as they are identified, they can be incorporated into future molecular epidemiologic studies designed to determine their actual impact on cancer risk in human populations. [J Natl Cancer Inst Monogr 2000;27:67–73]

For a substantial fraction of breast cancer cases in women, well-established risk factors, revealed by epidemiologic studies, include early age at menarche, late first full-term pregnancy, nulliparity, late menopause, family history of breast cancer, socioeconomic status, and perhaps estrogen replacement therapy *(1–5).* A common thread linking these factors is cumulative, excessive lifetime exposure to estrogen, suggesting that this exposure has an important role in the cause of breast cancer. Although a number of environmental chemicals are suspected of contributing to breast cancer, no single environmental chemical has been identified as a strong "smoking gun" for causing breast cancer *(6).* However, a consequence of excessive estrogen exposure may include unwanted cell division. The standard paradigm providing a general mechanistic explanation for the association of cumulative, excessive estrogen exposure and breast cancer risk was aptly stated by Feigelson and Henderson *(4)* and is shown in Fig. 1. The notion is that the proliferative stimulus provided by 17β-estradiol (E_2) leads to the appearance of spontaneous mutations; thus, the key contribution of E_2 is the stimulation of breast epithelial cell proliferation (Chapter 8). However, an important aspect of estrogen toxicology is its tissue-specific, cellular oxidative metabolism by several specific cytochrome P450 isoforms and various peroxidases *(7–10).* Mounting evidence suggests that the oxidative metabolites may contribute to estrogen carcinogenesis (Chapters 4 and 5).

Among the metabolites formed during the process of estrogen biotransformation and elimination (Fig. 2), some are estrogenic *(11)* and some may be protective through their antioxidant properties and/or growth and angiogenesis inhibitory activities *(12–14).* On the other hand, the more reactive quinone metabolites are able to form direct adducts with DNA *(15)* and/or can cause oxidative damage to lipids *(16)* and DNA through redox cycling processes that produce reactive oxygen species (ROS) [*(7, 8);* Chapter 4]. Increased production of ROS could also lead to disruption of cellular redox homeostasis and, as a consequence, could alter transcription factor function, causing inappropriate alterations in the regulation of gene expression *(17).*

The possible contribution of these metabolites to estrogen carcinogenesis has received relatively little attention compared with that given to estrogen receptor-mediated processes. However, accumulating evidence, much of which was presented in this symposium, supports an expansion of the standard mechanistic paradigm for the causal association of estrogen exposure and breast cancer (Fig. 1). Thus, while estrogen-induced cell proliferation undoubtedly has an important role in estrogen carcinogenesis, complementary pathways involving indirect and/or direct genotoxicity originating from estrogen metabolites, in particular, the 4-hydroxy catechol metabolite, are also likely to make important contributions. Furthermore, since other metabolites, such as 2-methoxyE_2, may have protective effects, a balance among these metabolites is likely required to maintain homeostasis.

In this chapter, I will provide a brief overview of some evidence in support of a role for estrogen metabolites in estrogen

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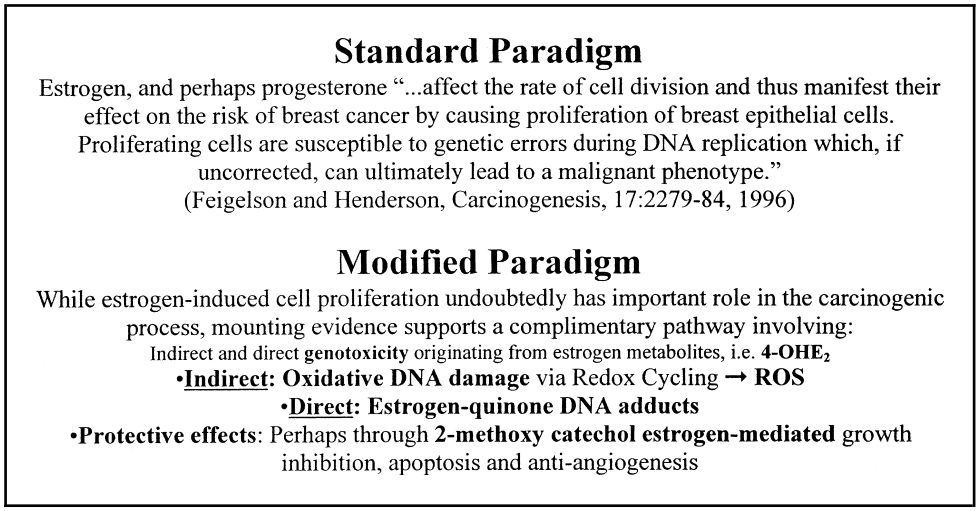

Fig. 1. Standard and modified paradigms for estrogen carcinogenesis.

carcinogenesis. I will place particular emphasis on their potential for causing oxidative DNA damage in association with the carcinogenic process [*see also* two reviews *(7,8)*].

Estrogen Oxidative Metabolism

A more specific scheme for the oxidative metabolism of E_2 is shown in Fig. 3. The chemical structures of the estrogen catechols, semiquinones, quinones, and DNA adducts are presented in Chapter 4. The oxidative metabolites that have been shown to exhibit estrogenic and genotoxic effects include 16α-hydroxyestrone [which will not be considered further in this monograph, but *see (18–20)*] and the 2-hydroxy- and 4-hydroxyE_2/estrone (E_1) catechols *(7,8).* The formation of the catechols is catalyzed by specific cytochrome P450 isoforms, including CYP1A1/1A2, CYP1B1, and CYP3A4 *(7,8,21–24).* The tissue specificity of estrogen metabolism results from the tissue-specific basal expression and inducibility of these enzymes and from differences among the P450 isoforms in their kinetic parameters for E_2 *(23).* This will be discussed in detail for CYP1A1 and CYP1B1 in Chapter 5 of this monograph, along with a discussion of the potential role for estrogen overproduction by aromatase in breast carcinogenesis.

Estrogen Carcinogenesis in the Syrian Golden Hamster Kidney

Extensive evidence for a role for catechol estrogen (CE) metabolites in estrogen carcinogenesis has come from work done with the use of the male Syrian golden hamster kidney carcinogenesis model, principally in the laboratories of Liehr et al. *(25)* and Li and Li *(26).* The data in Table 1 demonstrate that E_2, E_1, and their 4-hydroxy CE (4-OHE_2 and 4-OHE_1), but not their 2-OH catechols, are carcinogenic in this model. Additional support for a role for the CEs was provided by the finding that quercetin, which is both a competitive and a noncompetitive inhibitor of the phase II enzyme catechol-*O*-methyltransferase (COMT), increased the number of large renal tumors and the incidence of abdominal metastases in E_2-treated hamsters *(27,28).* Much additional data support a role for CE metabolites in estrogen carcinogenesis in this model. For example, hamster kidney microsomes have been shown to biotransform estrogens to their 2-OHE_2 and 4-OHE_2 metabolites *(29,30),* whereas *in vivo* treatment caused the appearance of DNA strand breaks *(31)* and increased the levels of 8-hydroxy-deoxyguanosine (8-OHdG) *(32).* Han and Liehr *(33)* also reported that, using ham-

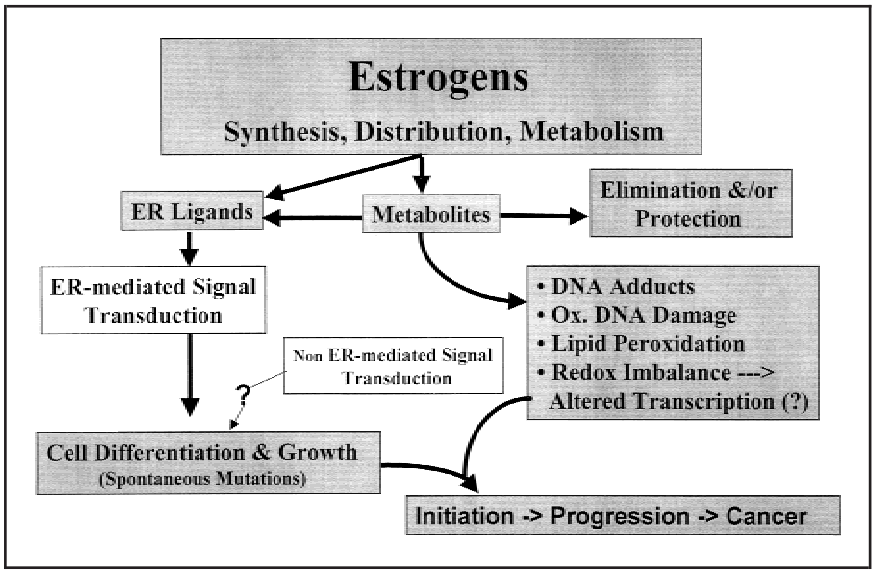

Fig. 2. Estrogens as carcinogens. ER = estrogen receptor; Ox = oxidative.

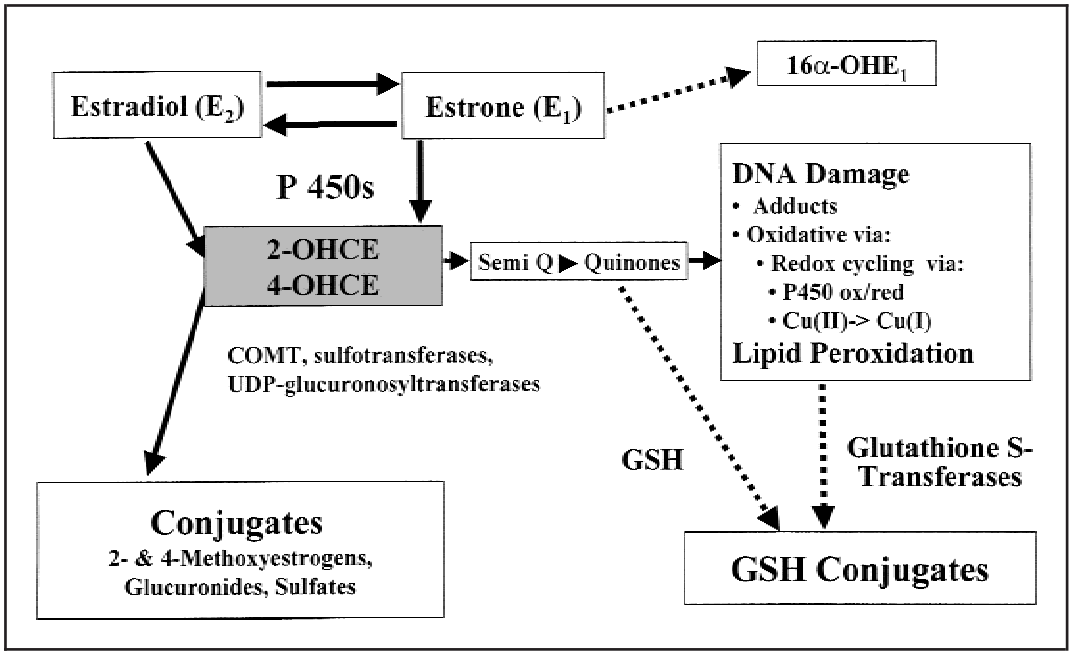

Fig. 3. Oxidative metabolism of estrogens. 2-OHCE and 4-OHCE = 2-hydroxy and 4-hydroxy catechol estrogens, respectively; COMT = catechol-*O*-methyltransferase; GSH = glutathione.

Table 1. Syrian golden hamster renal carcinogenicity of catechol estrogens

	% animals with renal carcinomas	
Estrogen*	Liehr et al. *(25)*	Li and Li *(26)*
None	0	0
E_2	80	100
4-OHE_2	80	100
2-OHE_2	0	0
E_1	—	80
4-OHE_1	—	33
2-OHE_1	—	0

*E_2 = 17β-estradiol; 4-OHE_2 = 4-hydroxyE_2; 2-OHE_2 = 2-hydroxyE_2; E_1 = estrone; 4-OHE_1 = 4-hydroxyE_1; 2-OHE_1 = 2-hydroxyE_1.

ster liver microsomes 4-OHE_2 but not 2-OHE_2 caused increased 8-OHdG levels. Similarly, Liehr and co-workers *(34–36)* demonstrated formation of 4-OHE_2 by microsomes from normal and tumor tissues of human breast, uterus, cervix, and ovary, whereas other investigators *(37,38)* have detected increased levels of 8-OHdG in human breast tumor tissue. These associations support the hypothesis that ROS generated through redox cycling processes involving CE metabolites may contribute to estrogen carcinogenesis of the human breast and perhaps other tissues.

Estrogen Carcinogenesis in Rat Liver

A role for oxidative DNA damage originating from estrogen metabolism is also supported by results from studies on the mechanisms of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and ethinyl estradiol (EE) carcinogenesis in rat liver. TCDD is a potent carcinogen in certain laboratory animals *(39).* Tritscher et al. *(40)* reported detecting higher levels of 8-OHdG in nuclear DNA from livers of TCDD-treated intact rats than in nuclear DNA from livers of TCDD-treated ovariectomized rats. Intact female rats are more sensitive to TCDD-induced hepatocarcinogenesis than are ovariectomized female rats or male rats *(39,40).* Since TCDD is a potent inducer of the P450s involved in the oxidative metabolism of E_2, these results are consistent with a contribution of endogenous estrogens and increased oxidative DNA damage arising from estrogen metabolites in TCDD carcinogenesis in female rat liver, although other unknown mechanisms could be contributing to or could be responsible for this carcinogenic process in this experimental model.

Prolonged exposure of women to EE in the form of oral contraceptives has been associated with increased risk for developing hepatic tumors *(41).* A number of laboratories have been involved in studies on the mechanisms of EE hepatocarcinogenesis. The effects of EE on rat liver are summarized in Fig. 4 *(7).* EE is a strong promoter of hepatocarcinogenesis initiated by diethylnitrosamine, enhancing the development of altered hepatic foci, nodules, and carcinomas *(7).* In non-nitrosamine-initiated rats, EE alone is a weak carcinogen. Associated with this carcinogenic process, EE causes a transient increase in DNA synthesis, followed by growth inhibition, which provides a period of negative selective pressure during which resistant hepatocytes (initiated) begin clonal expansion (promotion) *(42).* In addition, increased oxidative DNA damage occurs during EE treatment, as shown by data (Table 2) compiled from those presented in a report by Ogawa et al. *(43).* In that study, female Wistar rats were treated with EE at the doses shown, which were sufficient to cause the development of hepatocellular carcinomas after 12 months. These results show an association between increased 8-OHdG and increased incidence of carcinomas. Furthermore, simultaneous treatment with each of three antioxidant vitamins inhibited tumor development and reduced the levels of oxidative DNA damage.

Support From Molecular Epidemiologic Studies for a Role of Endogenous CE Metabolites in Human Breast Cancer Development

Evidence from model experimental systems mentioned above and described in the following chapters supports a role for CE metabolites in estrogen carcinogenesis. In humans, however, the growing body of available evidence is indirect and, thus, is only suggestive. As mentioned above, Liehr and Ricci *(35)* found 4-hydroxylase activity in normal and tumor breast tissues, Sutter

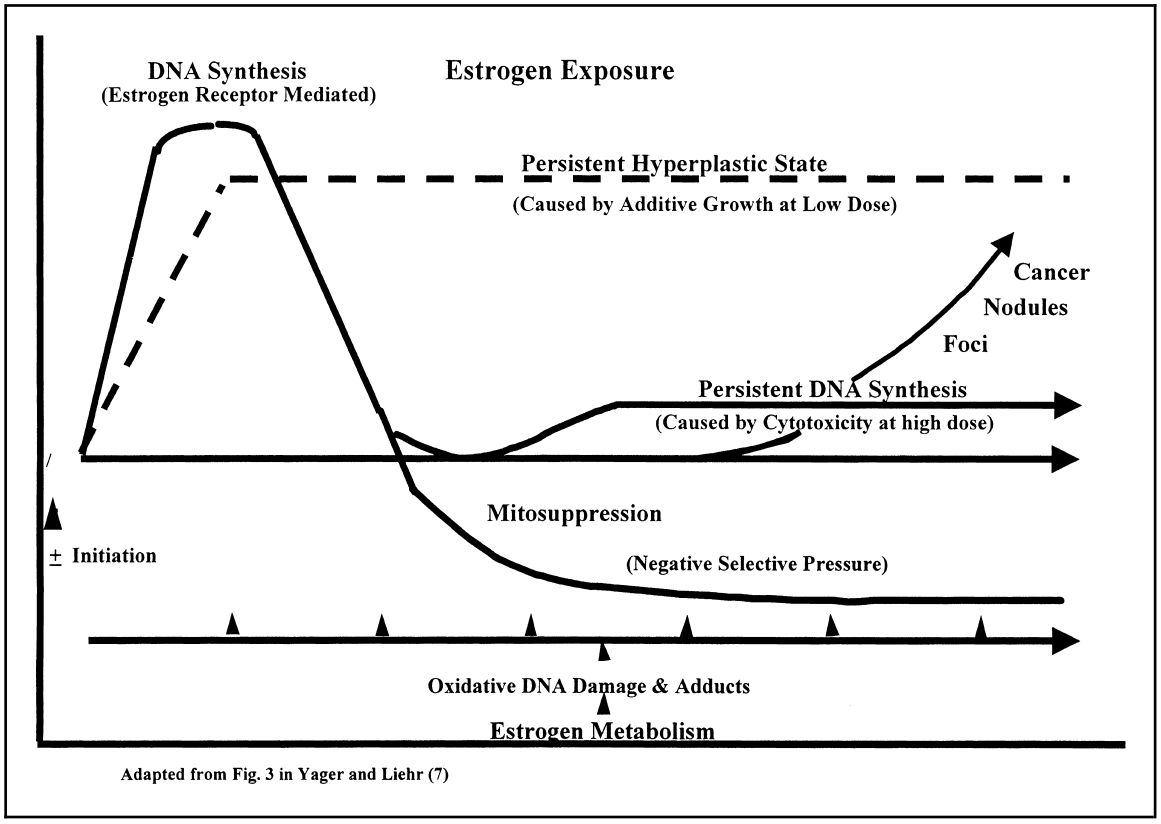

Fig. 4. Effects of ethinyl estradiol on rat liver. Adapted from Fig. 3 in *(7).* Reprinted with permission from the *Annual Review of Pharmacology and Toxicology,* Vol. 36, ©1996 by Annual Reviews www.AnnualReviews.org.

Table 2. Enhanced oxidative DNA damage in liver nuclear DNA from ethinyl estradiol (EE)-treated rats*

Treatment	8-oxodG/10^6 dG, 1 mo, mean ± standard deviation	Hepatocellular carcinoma incidence, 12 mo, % (No./total No.)
Control	3.5 ± 0.7	0 (0/24)
EE, 75 μg/day	7.1 ± 0.9†	8.7 (2/23)†
EE, 750 μg/day	8.4 ± 0.7†	38.5 (10/26)†
EE, 75 μg/day + vitamin C, 1g/kg diet	6.0 ± 1.7	0 (0/19)
Vitamin E, 500 mg/kg diet	5.4 ± 1.9	4.5 (1/22)‡
β-Carotene, 250 mg/kg diet	5.5 ± 1.8	4.8 (1/21)‡

*Adapted from Ogawa et al. *(43)*. oxodG = oxodeoxyguanosine; dG = deoxyguanosine.

†$P<.05$ versus control.

‡$P<.05$ versus EE alone.

(*see* Chapter 5) has observed expression of a 4-hydroxylase, CYP1B1, in human breast tissue, and Malins *(37);* Chapter 9) has detected increased oxidative DNA damage in breast tumor tissue. In addition, the results from some molecular epidemiology studies also provide support for a contribution of CE metabolites to the development of breast cancer. A number of studies, guided by the hypothesis that increased breast cancer risk is associated with exposures to certain environmental chemicals, have examined the association between risk and genetic polymorphisms in several genes encoding biotransformation enzymes. These include genes encoding CYPlAl *(44–46), N*-acetyltransferase 2 *(47),* and glutathione-*S*-transferase (GST) isoforms Ml (null), Tl (null), and Pl (low-activity allele) *(45,46,48).* The results have been mixed, depending on the subject cohort. COMT is a gene involved in the phase II metabolism of catechols, such as catecholamines and flavanoids. However, COMT also catalyzes the *O*-methylation of both 2-OH- and 4-OH-catechols formed from the oxidative metabolism of endogenous E_2 and E_1. *O*-Methylation of CEs inactivates their estrogenic potential and blocks their ability to undergo further oxidation to more reactive semiquinone and quinone metabolites that can directly adduct DNA and/or participate in redox cycling to produce superoxide, as described above. COMT is polymorphic, and 25% of Caucasians are homozygous for an allele encoding a low-activity form of the enzyme. The ability of CE metabolites to contribute to estrogen carcinogenesis, suggested by the experimental studies mentioned above and in Chapters 4 and 5 of this monograph, led to the hypothesis that women homozygous for the low-activity COMT allele would be at increased risk for breast cancer. At the time of this conference, two studies, one published *(49)* and another one that was in press but is now published *(50),* presented evidence that the gene encoding a low-activity form of COMT was associated with an increased risk for developing breast cancer in certain women. These data will be discussed in greater detail in Chapter 7.

Briefly, in a prospective, nested, case–control study from a large western Maryland cohort, Lavigne et al. *(49)* found that the risk for developing postmenopausal breast cancer associated with the low-activity COMT allele was increased in postmenopausal women who were also either heavy (body mass index >24.27 kg/m^2) or GSTMl null or GSTP1 low activity. In a hospital-based case–control study of subjects from western New York, Thompson et al. *(50)* found an increased breast cancer risk associated with the low-activity COMT allele, but only in premenopausal women, and they found that the risk was increased further in those women who had increased body mass indices (>23 kg/m^2). Two additional studies have been published. In one study *(51),* no increase in risk was detected; in contrast, in the

other study *(52),* an increased breast cancer risk associated with the low-activity COMT allele was found in postmenopausal women.

Why might there be such differences in the risk conferred by the same genetic polymorphism in different cohorts? One possibility is that these studies simply detected random findings. At this point, this possibility cannot be ruled out, for the number of cases and controls in these studies was small. On the other hand, the studies were hypothesis driven, and an increased risk associated with the low-activity allele has now been detected in three of four studies, one in premenopausal and two in postmenopausal women. Another possibility to account for the differences among the various cohorts may relate to the strength of the effect. According to a classification by Rebbeck et al. *(53),* mutations (detected as genetic polymorphisms) in some genes, such as BRCA1, confer a high risk for an individual. However, because these allele frequencies are low, the overall attributable risk that they represent is small. On the other hand, mutations in phase I and II enzyme genes involved in xenobiotic (but also endobiotic, i.e., endogenous molecules) metabolism might confer a low relative cancer risk for an individual. But, because these mutations seem to be common among individuals, they represent a high attributable risk category of genes. In addition, the specificities of many of these enzymes are overlapping, their activities within the cells can be altered by environmental agents, their polymorphic allele frequencies within a population can differ depending on ethnicity *(54),* and they function in somewhat redundant pathways. Thus, the balance in the cell of the metabolite products of these enzymes could be differentially affected by interactions among various genetic and environmental factors, as illustrated in Fig. 5. The intent of this scheme is to show that both genetic and environmental/lifestyle factors can act either as susceptibility or as protective influences with regard to risk of developing a particular disease. With regard to the oxidative metabolism and conjugation of estrogens, several enzymes are involved, including specific cytochrome P450 isoforms, sulfotransferases, COMT, and GST (for the products of oxidative damage). The levels of these enzymes in a given individual or population may be influenced (induced or inhibited) by xenobiotic exposures. In addition, these enzymes are polymorphic, and the distribution of polymorphisms may vary among different populations as a result of ethnic compositions. Since the oxidative metabolites of estrogen appear to contribute to and protect from disease, it is, therefore, not surprising to detect differences in single-gene genetic polymorphism/disease associations among different study populations.

In summary, most of the data from the studies cited above and to be discussed in the following chapters that implicate metabolites of endogenous estrogens in estrogen carcinogenesis are from *in vitro* studies and *in vivo* studies in which rodent models were used. The results have provided important insight that has led to the development of a new paradigm (*see* Fig. 1) for the contribution of CE metabolites to estrogen carcinogenesis. It predicts that the level of and balance among the parent hormone and the catechol metabolites should be determined by the level of expression and activity of certain key enzymes including aromatase (Chapter 5), several cytochrome P450s, particularly CYP1B1 (Chapter 5), and various protective enzymes, such as COMT (Chapters 6 and 7). Expression of these enzymes is likely to be tissue specific as well as developmental stage specific and to be affected by both environmental and endogenous factors. These parameters need to be thoroughly defined. Expression levels should be determined, along with the levels of the estrogen metabolites and selected biologic end points for their potential effects, e.g., gene expression, DNA damage, and mutagenesis. For several of these enzymes, genetic polymorphisms that affect activity have been discovered; e.g., the COMT polymorphism decreases enzyme activity. The effects of these polymorphisms on estrogen metabolite levels and on the biologic end points also require thorough investigation. However, an important question pertains to the appropriate experimental model systems to use. One can envision using cultured human cells originating from various human tissues, e.g., breast, prostate, and ovary, along with cells genetically engineered to express these enzymes or their polymorphic forms alone and in combination. One can also envision using knockout mice to determine the effects of the absence of particular genes, e.g., aromatase, CYP1B1, and COMT, on tissue estrogen metabolite levels and on the biologic end points including cancer. From the knockout mice, it should be possible to create transgenics expressing the human genes. By use of bacterial artificial chromosome (bac) vectors that accept up to 300-kilobase inserts, it is

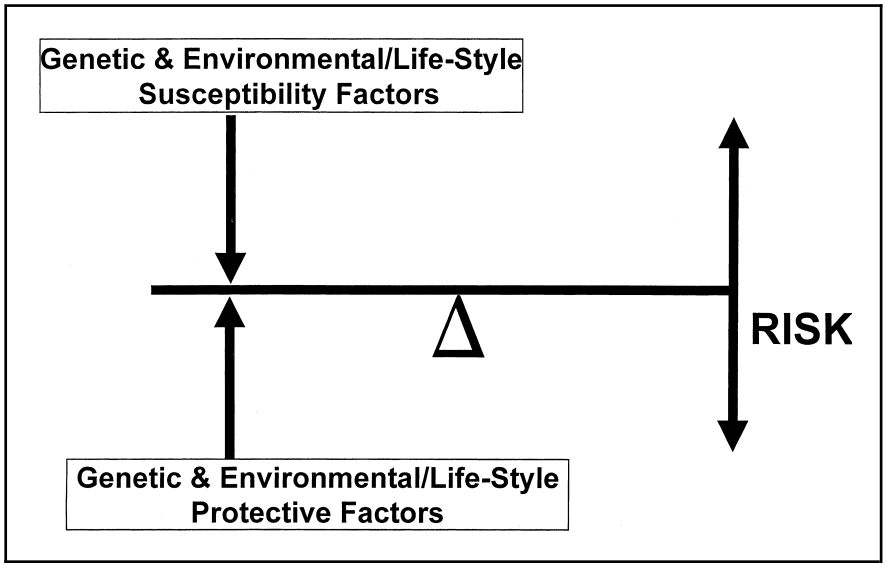

Fig. 5. Genetic, environmental, and lifestyle factors affect risk.

possible to include most of the regulatory regions of these genes and, thus, perhaps achieve their tissue-specific and developmental stage-specific expression. Results from studies such as these should more directly test the paradigm for the role of the CE metabolites. However, data obtained from human tissues will ultimately be required to be certain that the conclusions drawn from the model systems apply. Thus, efforts should be devoted to obtaining normal and tumor human tissues, e.g., breast and prostate, for simultaneous analysis of estrogen metabolites and the relevant biologic end points, e.g., DNA damage, CYP1B1 genotype and expression, and COMT genotype and expression. It will only be through such future in-depth mechanistic studies that we will be able to dissect the genetic and environmental "factors" affecting the pathways that provide adverse or protective "states," characterized by a correct or incorrect balance of oxidative metabolites impacting cancer risk. Identification of these factors should also allow more focused, mechanistically based molecular epidemiologic studies to be conducted in the future.

References

(1) Harris JR, Lippman ME, Veronesi U, Willett W. Breast cancer (1). N Engl J Med 1992;327:319–28.

(2) Madigan MP, Ziegler RG, Benichou J, Byrne C, Hoover RN. Proportion of breast cancer cases in the United States explained by well-established risk factors. J Natl Cancer Inst 1995;87:1681–5.

(3) Henderson BE, Pike MC, Bernstein L, Ross RK. Breast Cancer. In: Schottenfeld D, Fraumeni JF Jr, editors. Cancer epidemiology and prevention. New York (NY): Oxford University Press; 1996. p. 1022–39.

(4) Feigelson HS, Henderson BE. Estrogens and breast cancer. Carcinogenesis 1996;17:2279–84.

(5) Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer [published erratum appears in Lancet 1997; 350:1484]. Lancet 1997;347:1047–59.

(6) Wolff MS, Collman GW, Barrett JC, Huff J. Breast cancer and environmental risk factors: epidemiological and experimental findings. Ann Rev Pharmacol Toxicol 1996;36:573–96.

(7) Yager JD, Liehr JG. Molecular mechanisms of estrogen carcinogenesis. Ann Rev Pharmacol Toxicol 1996;36:203–32.

(8) Zhu BT, Conney AH. Functional role of estrogen metabolism in target cells: review and perspectives. Carcinogenesis 1998;19:1–27.

(9) Levin M, Weisz J, Bui QD, Santen RJ. Peroxidatic catecholestrogen production by human breast cancer tissue *in vitro.* J Steroid Biochem 1987; 28:513–20.

(10) Sipe HJ Jr, Jordan SJ, Hanna PM, Mason RP. The metabolism of 17β-estradiol by lactoperoxidase: a possible source of oxidative stress in breast cancer. Carcinogenesis 1994;15:2637–43.

(11) Schutze N, Vollmer G, Tiemann I, Geiger M, Knuppen R. Catecholestrogens are MCF-7 cell estrogen receptor agonists. J Steroid Biochem Mol Biol 1993;46:781–9.

(12) Fotsis T, Zhang Y, Pepper MS, Adlercreutz H, Montesano R, Nawroth PP, et al. The endogenous oestrogen metabolite 2-methoxyestradiol inhibits angiogenesis and suppresses tumour growth. Nature 1994;368: 237–9.

(13) Yue TL, Wang X, Louden CS, Gupta S, Pillarisetti K, Gu JL, et al. 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 1997; 51:951–62.

(14) Zhu BT, Conney AH. Is 2-methoxyestradiol an endogenous estrogen metabolite that inhibits mammary carcinogenesis? Cancer Res 1998;58: 2269–77.

(15) Cavalieri EL, Stack DE, Devanesan PD, Todorovic R, Dwivedy I, Higginbotham S, et al. Molecular origin of cancer: catechol estrogen-3,4-quinones as endogenous tumor initiators. Proc Natl Acad Sci U S A 1997;94: 10937–42.

(16) Wang MY, Liehr JG. Induction by estrogens of lipid peroxidation and lipid peroxide-derived malonaldehyde–DNA adducts in male Syrian hamsters: role of lipid peroxidation in estrogen-induced kidney carcinogenesis. Carcinogenesis 1995;16:1941–5.

(17) Li Y, Zhu H, Stansbury KH, Trush MA Role of reactive oxygen species in multistage carcinogenesis. In: Thomas CE, Kalyanaraman B, editors. Oxygen radicals and the disease process. Amsterdam (The Netherlands): Harwood Academic Publishers; 1997. p. 237–77.

(18) Swaneck GE, Fishman J. Covalent binding of the endogenous estrogen 16α-hydroxyestrone to estradiol receptor in human breast cancer cells: characterization and intranuclear localization. Proc Natl Acad Sci U S A 1988;85:7831–5.

(19) Davis DL, Telang NT, Osborne MP, Bradlow HL. Medical hypothesis: bifunctional genetic–hormonal pathways to breast cancer. Environ Health Perspect 1997;105(Suppl 3):571–6.

(20) Telang NT, Suto A, Bradlow HL, Wong GY, Osborne MP. Genotoxic damage and aberrant proliferation in mouse mammary epithelial cells. Recent Prog Horm Res 1993;48:481–8.

(21) Brian WR, Sari MA, Iwasaki M, Shimada T, Kaminsky LS, Guengerich FP. Catalytic activities of human liver cytochrome P-450 IIIA4 expressed in Saccharomyces cerevisiae. Biochemistry 1990;29:11280–92.

(22) Stresser DM, Kupfer D. Catalytic characteristics of CYP3A4: requirement for a phenolic function in ortho hydroxylation of estradiol and mono-*O*-demethylated methoxychlor. Biochemistry 1997;36:2203–10.

(23) Hayes CL, Spink DC, Spink BC, Cao JQ, Walker NJ, Sutter TR. 17β-estradiol hydroxylation catalyzed by human cytochrome P450 1B1. Proc Natl Acad Sci U S A 1996;93:9776–81.

(24) Martucci CP, Fishman J. P450 enzymes of estrogen metabolism. Pharmacol Ther 1993;57:237–57.

(25) Liehr JG, Fang WF, Sirbasku DA, Ari-Ulubelen A. Carcinogenicity of catechol estrogens in Syrian hamsters. J Steroid Biochem 1986;24:353–6.

(26) Li JJ, Li SA. Estrogen carcinogenesis in Syrian hamster tissues: role of metabolism. Fed Proc 1987;46:1858–63.

(27) Zhu BT, Liehr JG. Inhibition of catechol *O*-methyltransferase-catalyzed *O*-methylation of 2- and 4-hydroxyestradiol by quercetin. Possible role in estradiol-induced tumorigenesis. J Biol Chem 1996;271:1357–63.

(28) Zhu BT, Liehr JG. Quercetin increases the severity of estradiol-induced tumorigenesis in hamster kidney. Toxicol Appl Pharmacol 1994;125:149–58.

(29) Li SA, Klicka JK, Li JJ. Estrogen 2- and 4-hydroxylase activity, catechol estrogen formation, and implications for estrogen carcinogenesis in the hamster kidney. Cancer Res 1985;45:181–5.

(30) Zhu BT, Bui QD, Weisz J, Liehr JG. Conversion of estrone to 2- and 4-hydroxyestrone by hamster kidney and liver microsomes: implications for the mechanism of estrogen-induced carcinogenesis. Endocrinology 1994;135:1772–9.

(31) Han X, Liehr JG. DNA single-strand breaks in kidneys of Syrian hamsters treated with steroidal estrogens: hormone-induced free radical damage preceding renal malignancy. Carcinogenesis 1994;15:997–1000.

(32) Han X, Liehr JG. 8-Hydroxylation of guanine bases in kidney and liver DNA of hamsters treated with estradiol: role of free radicals in estrogen-induced carcinogenesis. Cancer Res 1994;54:5515–7.

(33) Han X, Liehr JG. Microsome-mediated 8-hydroxylation of guanine bases of DNA by steroid estrogens: correlation of DNA damage by free radicals with metabolic activation to quinones. Carcinogenesis 1995;16:2571–4.

(34) Liehr JG, Ricci MJ, Jefcoate CR, Hannigan EV, Hokanson JA, Zhu BT. 4-Hydroxylation of estradiol by human uterine myometrium and myoma microsomes: implications for the mechanism of uterine tumorigenesis. Proc Natl Acad Sci U S A 1995;92:9220–4.

(35) Liehr JG, Ricci MJ. 4-Hydroxylation of estrogens as marker of human mammary tumors. Proc Natl Acad Sci U S A 1996;93:3294–6.

(36) Somasunderam A, Liehr JG. 4-Hydroxylation of estradiol by microsomes of human neoplastic and normal ovarian and cervical tissues [abstract]. Proc Am Assoc Cancer Res 1997;38:451–2.

(37) Malins DC, Haimanot R. Major alterations in nucleotide structure of DNA in cancer of the female breast. Cancer Res 1991;51:5430–2.

(38) Musarrat J, Arezina-Wilson J, Wani AA. Prognostic and aetiological relevance of 8-hydroxyguanosine in human breast carcinogenesis. Eur J Cancer 1996;32A:1209–14.

(39) Lucier GW, Tritscher A, Goldsworthy T, Foley J, Clark G, Goldstein J, et al. Ovarian hormones enhance 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-mediated increases in cell proliferation and preneoplastic foci in a two-stage model for rat hepatocarcinogenesis. Cancer Res 1991;51:1391–7.
(40) Tritscher AM, Seacat AM, Yager JD, Groopman JD, Miller BD, Bell D, et al. Increased oxidative DNA damage in livers of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin treated intact but not ovariectomized rats. Cancer Lett 1996;98:219–25.
(41) Palmer JR, Rosenberg L, Kaufman DW, Warshauer ME, Stolley P, Shapiro S. Oral contraceptive use and liver cancer. Am J Epidemiol 1989;130: 878–82.
(42) Yager JD, Zurlo J, Sewall CH, Lucier GW, He H. Growth stimulation followed by growth inhibition in livers of female rats treated with ethinyl estradiol. Carcinogenesis 1994;15:2117–23.
(43) Ogawa T, Higashi S, Kawarada Y, Mizumoto R. Role of reactive oxygen in synthetic estrogen induction of hepatocellular carcinomas in rats and preventive effect of vitamins. Carcinogenesis 1995;16:831–6.
(44) Taioli E, Trachman J, Chen X, Toniolo P, Garte SJ. A CYP1A1 restriction fragment length polymorphism is associated with breast cancer in African-American women. Cancer Res 1995;55:3757–8.
(45) Bailey LR, Roodi N, Verrier CS, Yee CJ, Dupont WD, Parl FF. Breast cancer and CYP1A1, GSTM1, and GSTT1 polymorphisms: evidence of a lack of association in Caucasians and African Americans. Cancer Res 1998; 58:65–70.
(46) Ambrosone CB, Freudenheim JL, Graham S, Marshall JR, Vena JE, Brasure JR, et al. Cytochrome P4501A1 and glutathione *S*-transferase (M1) genetic polymorphisms and postmenopausal breast cancer risk. Cancer Res 1995;55:3483–5.
(47) Ambrosone CB, Freudenheim JL, Graham S, Marshall JR, Vena JE, Brasure JR, et al. Cigarette smoking, *N*-acetyltransferase 2 genetic polymorphisms, and breast cancer risk. JAMA 1996;276:1494–501.
(48) Helzlsouer KJ, Selmin O, Huang HY, Strickland PT, Hoffman S, Alberg AJ, et al. Association between glutathione *S*-transferase M1, P1, and T1 genetic polymorphisms and development of breast cancer. J Natl Cancer Inst 1998;90:512–8.
(49) Lavigne JA, Helzlsouer KJ, Huang HY, Strickland PT, Bell DA, Selmin O, et al. An association between the allele coding for a low activity variant of catechol-*O*-methyltransferase and the risk for breast cancer. Cancer Res 1997;57:5493–7.
(50) Thompson PA, Shields PG, Freudenheim JL, Stone A, Vena JE, Marshall JR, et al. Genetic polymorphisms in catechol-*O*-methyltransferase, menopausal status, and breast cancer risk. Cancer Res 1998;58:2107–10.
(51) Millikan RC, Pittman GS, Tse CK, Duell E, Newman B, Savita D, et al. Catechol-*O*-methyltransferase and breast cancer risk. Carcinogenesis 1998; 19:1943–7.
(52) Huang CS, Chern HD, Chang KJ, Cheng CW, Hsu SM, Shen CY. Breast cancer risk associated with genotype polymorphism of the estrogen-metabolizing genes CYP17, CYP1A1, and COMT: a multigenic study on cancer susceptibility. Cancer Res 1999;59:4870–5.
(53) Rebbeck TR, Walker AH, Phelan CM, Godwin AK, Buetow KH, Garber JE, et al. Defining etiologic heterogeneity in breast cancer using genetic biomarkers. Prog Clin Biol Res 1997;396:53–61.
(54) Garte S. The role of ethnicity in cancer susceptibility gene polymorphisms: the example of CYP1A1. Carcinogenesis 1998;19:1329–32.

NOTES

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