

# Medical Hypothesis: Bifunctional Genetic–Hormonal Pathways to Breast Cancer

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As inherited germ line mutations, such as loss of *BRCA1* or *AT*, account for less than 5% of all breast cancer, most cases involve acquired somatic perturbations. Cumulative lifetime exposure to bioavailable estradiol links most known risk factors (except radiation) for breast cancer. Based on a series of recent experimental and epidemiologic findings, we hypothesize that the multistep process of breast carcinogenesis results from exposure to endogenous or exogenous hormones, including phytoestrogens that directly or indirectly alter estrogen metabolism. Xenohormones are defined as xenobiotic materials that modify hormonal production; they can work bifunctionally, through genetic or hormonal paths, depending on the periods and extent of exposure. As for genetic paths, xenohormones can modify DNA structure or function. As for hormonal paths, two distinct mechanisms can influence the potential for aberrant cell growth: compounds can directly bind with endogenous hormone or growth factor receptors affecting cell proliferation or compounds can modify breast cell proliferation altering the formation of hormone metabolites that influence epithelial–stromal interaction and growth regulation. Beneficial xenohormones, such as indole-3-carbinol, genistein, and other bioflavonoids, may reduce aberrant breast cell proliferation, and influence the rate of DNA repair or apoptosis and thereby influence the genetic or hormonal microenvironments. Upon validation with appropriate *in vitro* and *in vivo* studies, biologic markers of the risk for breast cancer, such as hormone metabolites, total bioavailable estradiol, and free radical generators can enhance cancer detection and prevention. — *Environ Health Perspect* 105(Suppl 3):571–576 (1997)

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## Introduction

As the most common cancer among women in modern societies, breast cancer is a complex and important disease. The average patient who dies with the disease loses about two decades of life, so that nearly 2 million women-years of life are lost annually to breast cancer in the United States and Europe (1). Although about 87% of all cases survive for 5 years, nearly

half of all women die from to breast cancer by one decade after diagnosis (1–3).

About one-third of all cases of breast cancer can be attributed to recognized risk factors. Neither changes in established risk factors nor screening practices completely account for the persisting 1% annual increase in the incidence of breast cancer. Similarly, changes in risk factors or in

screening practices do not explain geographic variations in prevalence of the disease (1–4). Inherited germ cell mutations occur in about 5% of all cases and in about 30% of cases under 40 years of age (3–6).

The common tie linking most of the established risk factors, aside from these mutations, is greater cumulative exposure to bioavailable 17 $\beta$ -estradiol (E<sub>2</sub>) (4,7–11). Bioavailable E<sub>2</sub> is defined as a free hormone not bound to steroid hormone-binding globulin (SHBG) or weakly bound to albumin (9–12). Women with elevated levels of bioavailable E<sub>2</sub> have a 2- to 4-fold excess risk of breast cancer (10). Bioavailable E<sub>2</sub> can diffuse into cells and subsequently be taken into the nucleus where it can bind to the estrogen receptor (ER). The hormone also can be converted in the cytoplasm into other biologically active metabolites and free radicals (4,7). In addition, other hormones, such as androgens and progestagens, can influence the production and metabolism of E<sub>2</sub> (4,9–12). The hormone–SHBG complex bound to the cell surface receptor induces cAMP-mediated phosphorylation (12).

## Medical Hypothesis

We have previously suggested that compounds functioning as xenoestrogens affect the rate and type of estradiol metabolites formed. Xenoestrogens may also bind directly with the ER to modulate breast cell proliferation and thereby influence the development of breast cancer and other hormonally mediated diseases (9,11,12). In this report we expand the hypothesis to include a more detailed consideration of possible genetic–hormonal–environmental interactions (3,4), including the complex relationship among estrogens, androgens, their antagonists, and other hormones in breast cancer development.

Based on recent experimental and epidemiologic findings in this laboratory and elsewhere (4,9,11,13), we hypothesize that prenatal, adolescent, or midlife exposure to endogenous endocrine agents, xenohormones, or their metabolites can have bifunctional effects on the risk of developing breast cancer.

We also hypothesize that some xenohormonal exposures can, through redox cycling between estrogens and their corresponding quinones, yield reactive oxygen species that can cause structural oxidative damage to DNA and increase rates of oxidative DNA base modifications (7,8,14–16).

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Abbreviations used: *ATM*, ataxia telangiectasia, mutated; *DMBA*, 7,12-dimethylbenz[*a*]anthracene; E<sub>2</sub>, 17 $\beta$ -estradiol; ER, estrogen receptor; Fapy-A, 4,6-diamino-5-formamidopyrimidine; <sup>•</sup>OH, hydroxyl; 2-OHE<sub>1</sub>, 2-hydroxyestrone; 16 $\alpha$ -OHE<sub>1</sub>, 16 $\alpha$ -hydroxyestrone; PI-3-kinases, phosphatidylinositol-3-kinases; SHBG, sex hormone-binding globulin.

Lipid oxidation products may also function as endogenous DNA damaging-agents (17). In addition, other types of reactive functions, such as methylation or phosphorylation, can affect key functional regions of DNA, including cell cycle genes critical for cell proliferation, development, and growth. Exposure to xenohormones through diet, pharmaceuticals, and environmental chemicals can alter the parenchymal environment, either by promoting already initiated breast cells into relatively rapid proliferation or by impeding such growth, if the xenohormones are antioxidants, hormone antagonists, or antiangiogenics (18).

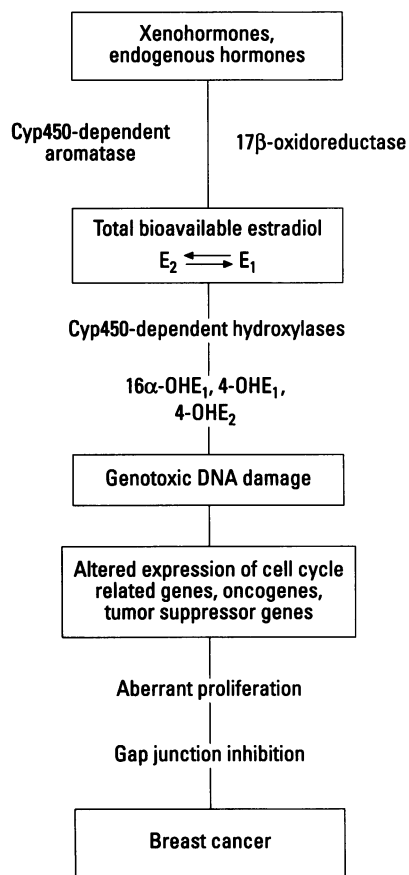
Figures 1 to 3 show the impact of steroid hormones on interacting genetic and hormonal pathways critical for breast

carcinogenesis and its prevention. Naturally occurring or synthetic xenohormones may affect the process of tumorigenic transformation at both genotoxic (initiation) or epigenetic (postinitiation) levels. Central to the bifunctional effect of bioavailable E<sub>2</sub> is its enzymatic conversion to products with distinctive biological activity. The microsomal enzymes, aromatase, 17β-oxidoreductase and steroid hydroxylases Cyp450 1A1, 1A2, 1B1, and 2B2 are essential enzymes for steroid hormone metabolism (4,7-9, 11,13). The metabolites themselves or their oxidative products may directly induce genotoxic DNA damage and modulation of oncogene, tumor suppressor gene, or cell-cycle control gene expression. Additionally, hormones, their metabolites, and xenoestrogens may exert epigenetic, paracrine

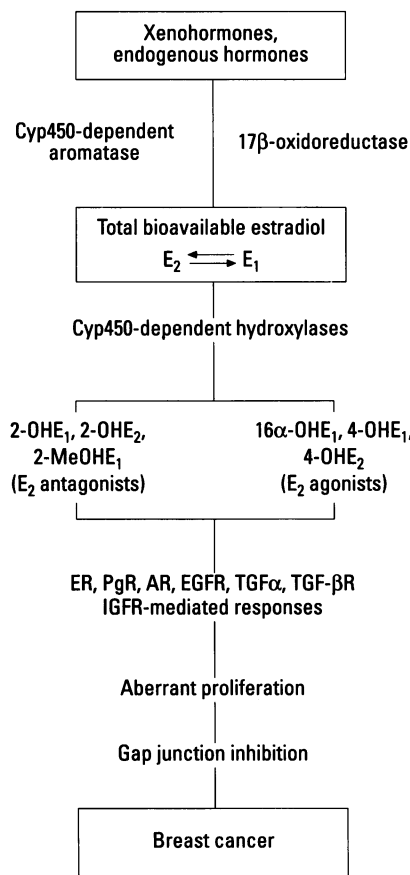
effects on preinitiated cells via hormone and growth factor receptors or via intercellular gap junctional communication. These indirect effects predominantly operate during the postinitiation (promotional) events of carcinogenic transformation.

Beneficial xenoestrogens, such as genistein and other bioflavonoids that occur in vegetables, fruits, and grain products, may reduce aberrant breast cell proliferation and inhibit angiogenesis, and may increase DNA repair processes and enhance cytodifferentiation and apoptosis. Some phytoestrogens such as genistein also influence aberrant cell proliferation. At the pharmacological levels these compounds operating via receptor-independent mechanisms may inhibit kinases and DNA topoisomerases and thereby affect other intracellular biochemical targets (14-16).

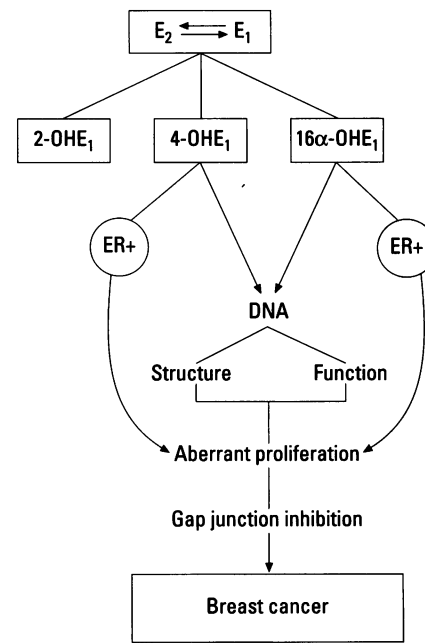
About 15% of women who are carriers of mutated *BRCA1* apparently do not develop breast cancer (5,6). In these cases, beneficial xenohormones or other exogenous factors may play a positive role by promoting enzymatic detoxification of potential carcinogens, DNA repair processes, or



**Figure 1.** Impact of steroid hormones on genotoxic pathways of breast carcinogenesis. In the genotoxic pathway, bioavailable 17β-estradiol is converted via Cyp450-dependent hydroxylases to 16α-OHE<sub>1</sub>, 4-OHE<sub>1</sub>, or 4-OHE<sub>2</sub>. These metabolites, by virtue of their direct effect on DNA, cause genotoxic DNA damage. This damage alters the expression of cell cycle related genes, oncogenes, and tumor suppressor genes leading to aberrant proliferation and breast cancer development.



**Figure 2.** Impact of steroid hormones on hormonal pathways of breast carcinogenesis. In the hormonal pathway, the E<sub>2</sub> metabolites having estrogenic properties (16α-OHE<sub>1</sub>, 4-OHE<sub>1</sub>, or 4-OHE<sub>2</sub>) or antiestrogenic properties (2-OHE<sub>1</sub>, 2-OHE<sub>2</sub>, and 2-MeOHE<sub>1</sub>) exert their growth modulatory effects indirectly via receptor-mediated mechanisms. These alterations lead to modulation of aberrant proliferation and of breast cancer development.



**Figure 3.** Bifunctional pathways to breast cancer. In the bifunctional pathway, the E<sub>2</sub> metabolites affect cell proliferation and breast cancer development either directly via receptor-independent mechanisms involving structural/functional alterations in DNA, or indirectly via receptor-dependent mechanisms involving phenotypic growth regulation. Both mechanisms eventually upregulate aberrant proliferation and development of breast cancer.

antioxidant formation, or by otherwise enhancing the ability of the cell to override signals that would produce uncontrolled growth. Recently several researchers reported that the average age of onset of the disease has fallen in carriers of *BRC1* as well (5,6). Thus exogenous factors appear to affect the timing and expression of breast cancer in those with predisposing germ cell mutations. Under this hypothesis, xenohormonal-genetic interactions could account for variations in gene expression.

## Hormone Metabolism and Breast Cancer

Many steroid hormones are involved in the development of the human breast. The mammatropic steroids estrogen and progesterone are as crucial for breast-cell proliferation as the lactogenic hormones prolactin and glucocorticoids are for cytodifferentiation (3,4,11,13,19). Thus hormone-mediated alterations in proliferative and cytodifferentiative status may regulate carcinogenesis. Receptors for most of the steroid hormones are reported to be upregulated in clinical breast cancer (20–22). The complex interacting influences of steroid hormones on breast carcinogenesis, however, remain to be elucidated.

Although a role of androgens in breast cancer remains to be unequivocally established, researchers have long observed that the higher the cumulative levels of bioavailable  $E_2$ , the greater the endogenous rate of breast-cell division and the cumulative risk of breast cancer (4,11). Endogenous  $E_2$  and most natural plant estrogens (phytoestrogens) are metabolized and excreted relatively rapidly and readily bind to SHBG, whereas most xenoestrogens do not appear to have this binding capacity (11,12,23–25). Moreover, the half-life of some lipophilic xenoestrogens, such as the organochlorine pesticides, can extend over several decades, in contrast to most natural estrogens, which are metabolized completely within several minutes or hours.

We and others (9–13,23–24) have suggested that two competing, mutually exclusive enzymatic pathways can alter the production of bioavailable estradiol. Pathway 1 inserts a hydroxyl (OH) function at the C2- position and yields the catechol estrogen 2-hydroxyestrone (2-OHE<sub>1</sub>), a weakly estrogenic metabolite. Pathway 2 adds an OH at the C16 $\alpha$  position and yields 16 $\alpha$ -hydroxyestrone (16 $\alpha$ -OHE<sub>1</sub>); this creates a fully potent estrogenic metabolite that can covalently bind to the ER (Figure 4).

Our studies on murine mammary epithelial cell cultures have shown that initiators of rodent mammary carcinogenesis (chemical carcinogens, oncogenes, and transforming retroviruses) upregulate pathway 2 at the expense of pathway 1 (3,26–29). Exposure of immortalized but nontumorigenic murine mammary epithelial cells to 16 $\alpha$ -OHE<sub>1</sub> results in genotoxic DNA damage and increased cellular proliferation in anchorage dependent and anchorage independent growth conditions in a manner similar to that induced by treatment with the complete carcinogen 7,12-dimethylbenz[*a*]anthracene (DMBA) (25). In contrast, 2-OHE<sub>1</sub> lacks these activities and downregulates the effects of DMBA (28–29). In carcinogen initiated and in tumor-derived cells, 16 $\alpha$ -OHE<sub>1</sub> enhances, while 2-OHE<sub>1</sub> inhibits, the expression of transformed phenotype (11,13,24,26). Similar changes have been reported in the human mammary explant and cell culture models (13,28). It is therefore conceivable that the cellular metabolism of  $E_2$  is altered during rodent and human mammary carcinogenesis and that individual metabolites may exert distinct biological effects during initiation or promotion of carcinogenesis. Furthermore, experiments on laboratory models of mammary carcinogenesis have shown that the naturally occurring phytochemical indole-3-carbinol and omega-3 polyunsaturated fatty acid prevent carcinogenic transformation, largely due to enhancement of the C2-hydroxylation of  $E_2$  that leads to increased formation of antiproliferative 2-OHE<sub>1</sub> (11,24,31).

The ratio of 16 $\alpha$ -hydroxyestrone to 2-hydroxyestrone has been found to be elevated in women and experimental animals with high rates of mammary tumors (13,30,32). In human mammary carcinoma cell cultures, some organochlorine pesticides activate the type of Cyp450 that is responsible for 16 $\alpha$ -hydroxyestrone formation and produce elevated metabolite ratios, comparable to that induced by the known rodent carcinogen DMBA (24).

A number of studies indicate that 16 $\alpha$ -hydroxylation of  $E_2$  plays a bifunctional role in the development of breast cancer. The estrogen receptor as a recognized transcription factor may be central to the process by which estrogen metabolites induce genomic changes. This process includes the ability of ER to bind to appropriate DNA response elements, enhance transcriptional activation, and initiate a cascade of events involving expression of

several estrogen-responsive genes such as *pS2*, *c-fos*, *c-jun*, and *c-myc*, which code for positive growth regulatory nuclear proteins (33). Other metabolites of estradiol exhibit reversible binding to ER. Work from this laboratory indicates that 16 $\alpha$ -OHE<sub>1</sub> has the unique capacity to bind covalently and irreversibly with the ER (13,34).

Natural variations in the levels of endogenous estrogen metabolism leading to the formation of metabolites with distinctive biological activity may explain some reported ethnic and geographic variations in breast cancer. Asian women, who have much lower rates of breast cancer and breast secretions, generally have higher levels of 2-OHE<sub>1</sub> and low levels of 16 $\alpha$ -OHE<sub>1</sub> (9,11,23,35). Diets rich in vegetables, fruits, and grain products could represent an additional source of phytoestrogens capable of modifying breast carcinogenesis.

The relative importance of various metabolites of  $E_2$  in the process of carcinogenesis has been addressed in several models. The catechol estrogens 2-OHE<sub>1</sub>, 4-OHE<sub>1</sub>, 2-OHE<sub>2</sub>, and 4-OHE<sub>2</sub> have documented pleiotropic effects on organ-site carcinogenesis. For example, the catechol estrogens 4-OHE<sub>2</sub> and 4-OHE<sub>1</sub> are reported to function as genotoxic agents in the hamster kidney model, in part due to their ability to induce oxidative DNA damage via free radical generation (7,8,36–38). Microsomes from human fibroadenoma and adenocarcinoma exhibit higher levels of 4-OHE<sub>2</sub> than of 2-OHE<sub>2</sub> compared to normal breast tissue (7,8). *In vivo* and *in vitro* studies from our laboratory have shown that during mammary carcinogenesis, formation of 2-OHE<sub>1</sub> is decreased while that of 16 $\alpha$ -OHE<sub>1</sub> is increased, and agents that increase 2-OHE<sub>1</sub> inhibit carcinogenesis (26,28,29,39). Thus positive regulation of growth by 16 $\alpha$ -OHE<sub>1</sub>, 4-OHE<sub>1</sub>, and 4-OHE<sub>2</sub> and negative regulation by 2-OHE<sub>1</sub> and 2-OHE<sub>2</sub> may be consistent with the estrogenic or antiestrogenic properties of specific metabolites of  $E_2$ . Despite the pleiotropic effects of  $E_2$  metabolites, the general consensus is that bioavailable  $E_2$  has an important role in the risk for breast cancer (4,9,11,23,35). The role of other hormones in breast carcinogenesis, however, remains to be elucidated.

## Structural And Functional Damage To DNA

Distinct endogenous processes can alter the structure or function of DNA, including oxidation, methylation, deamination, phosphorylation, and depurination.

Oncogenes have been considered molecular targets whose gain of function is associated with cancer. An array of tumor suppressor genes such as *BRCA1*, *Rb*, *DCC*, *p53*, and ataxia telangiectasia, mutated (*ATM*) allow cancer to proceed undisturbed when their editing and review functions are lost through phosphorylation or other changes (5,6,26,33). In addition, growth factors and hormone receptors can also independently stimulate cell proliferation or activate hormone-responsive genes without causing structural damage to DNA (3,4,17,26,33).

Metabolism of oxygen involving a chain of one-electron reductions and the formation of DNA-reactive free radicals plays a pivotal role in the cause of extensive structural or functional damage to DNA. One-electron oxidation of the catechol estrogen 4-OHE<sub>2</sub> leads to the formation of a semiquinone that is a reactive species. Semiquinones can be further oxidized to quinones. Alternatively, semiquinones can react to give superoxide ions, which in turn yield H<sub>2</sub>O<sub>2</sub>. The hydrogen peroxide thus formed then yields the reactive ·OH moiety. Free radicals produced from semiquinones can directly damage the phosphodiester bonds or alter the DNA nucleotide base sequence, thereby impairing normal transcriptional processes (7,8,14–16,36–38). Free-radical induced damage may also cause the loss of tumor suppressor gene function, reduce natural killer cells, impair enzymatic detoxification processes, or alter growth factor synthesis.

Several investigations demonstrate that hydroxyl (·OH) radicals and oxidative metabolites of aromatic hydrocarbons can irreparably and specifically modify the structure of DNA, leading to mutagenesis and carcinogenesis (7,8,14–16,40). Distinctive types of ·OH-induced modifications in DNA bases have been detected in women with breast cancer and in those at risk for the disease, compared to controls, who have undergone reduction mammoplasty. DNA from mammoplasty patients had relatively higher proportions of ring opening product of adenine, 4,6-diamino-5-formamidopyrimidine (Fapy-A), whereas cancer patients had markedly lower levels of Fapy-A and higher levels of 8-hydroxyquinine (16). It has been suggested that the relevance of oxidative DNA damage in breast cancer reduction is predominantly due to H<sub>2</sub>O<sub>2</sub> generation. H<sub>2</sub>O<sub>2</sub> can cross the nuclear membrane, where it is converted by the iron- or copper-catalyzed Fenton reaction to the free radical ·OH. Some xenoestrogens and estrogen

metabolites may promote the production of free radicals by redox cycling of H<sub>2</sub>O<sub>2</sub> mediated by cytochrome P450 oxidase and reductase, leading to production of DNA-damaging reactive oxygen species.

Another critical pathway to breast cancer can arise from agents that affect genes for regulatory proteins, such as phosphatidylinositol-3-kinases (PI-3-kinases). These proteins are essential for detecting DNA damage. PI-3-kinase may function by halting improper cell growth and division until damage is repaired. Persons who have inherited *ATM* and are lacking this key regulatory gene are unable to recover from radiation and possibly other DNA-damaging exposures. Consequently, they accumulate harmful mutations much more readily than healthy persons. Some experimental and epidemiologic evidence suggests that women who inherit a single defective copy of *ATM* are at increased risk of breast cancer compared with those with normal *ATM*. Experimental cell culture studies reveal that cells containing a single faulty copy of *ATM* incur a higher death rate following radiation exposure.

## Discussion

Development of cancer is the consequence of complex and subtle interactions between the environment and the genome. As 95% of human breast cancer is not due to inherited mutations, the question becomes one of what causes people who have inherited a healthy array of genes to acquire the disease. It appears likely that a wide variety of xenobiotic compounds may alter bioactive estrogen, androgen, or progesterone in breast cancer cases, in part via Cyp450-mediated enzymatic reactions. Persons who have inherited genetic susceptibility to breast cancer might be especially sensitive to the proliferative effects of some xenohormones from the environment. Persons with these defects who do not develop the disease may have had greater exposure to beneficial xenohormones or to other protective factors. Both genetic and hormonal pathways that act in a bifunctional manner may induce aberrant proliferation leading to the development of breast cancer. Some xenoestrogens affect the production and metabolism of estradiol and thereby regulate cumulative levels of the bioavailable hormone. Some xenohormones directly modify DNA structure or function. Structural damage can result from genotoxic E<sub>2</sub> metabolites and from redox cycling of harmful xenoestrogens or other agents that may produce reactive oxygen species.

Functional damage can include altered transcriptional activities related to oncogenes and tumor-suppressor genes, which are responsible for positive and negative regulation of growth. Phosphorylation of *p53* gene product or of PI-3-kinase is also indicative of functional DNA damage that impedes cell repair by hindering recognition of damaged cells and allowing the accumulation of harmful mutations. The growth advantage of aberrant cells, possibly by activation of genes involved in cell-cycle progression, leads to the development of breast cancer (3,4,13,19,33).

The bifunctional genetic-hormonal hypothesis for breast cancer also provides a means by which to link the interactive influence of dietary factors on disease progression. The effect of dietary fat on breast cancer still remains equivocal. The type of dietary fat and the levels of xenoestrogenic contaminants in the diet may provide important leads to resolve the observed inconsistencies. Furthermore, such an analysis should also provide mechanism-based interpretation for experimental and clinical evidence about the role of dietary fat in breast cancer development. Polyunsaturated fatty acids are considered to modulate breast carcinogenesis in part by interfering with membrane-mediated gap-junctional intercellular communication (41). This process is critical for cellular homeostasis whose impairment may promote growth advantage of the cancer cell. Lipophilic organochlorine pesticides operating via synergistic interactions inhibit gap-junctional communication in human mammary epithelial cells derived from reduction mammoplasty (42). Xenobiotics such as the food colorant Red Dye No. 3 have been reported to stimulate cell-cycle progression in human mammary carcinoma T47D cells acting via the cyclin-dependent kinase 2 and ER binding (14,15). Xenobiotics that have a carboxylic acid function can be incorporated structurally into complex lipids such as triglyceride (fat) and phospholipids (membranes). When mobilized some of these metabolites may serve as signaling molecules similar to phorbol esters, which are well-established tumor promoters (41,43). We need to determine whether bioaccumulated lipophilic xenohormones, xenobiotics such as aromatic hydrocarbons, and organochlorines are elevated in women at risk for breast cancer.

This genetic-hormonal hypothesis also may account for one of the discrepancies in cancer patterns that was first noted by the distinguished Danish researcher

J. Clemmensen (44). Commonly referred to as Clemmensen's hook, this discrepancy occurs in the relationship between age and breast cancer incidence noted for several countries. Incidence of breast cancer rises progressively with age up to about 45 years of age, after which the rate of increase forms a hook and levels off or declines for about 10 years and then resumes an increasing, but more modest, slope. The ages of this plateau correspond to the period of perimenopause, when the ovaries begin to produce less estrogen and progesterin (44). It is tempting to speculate that the renewed surge in breast cancer after menopause, especially in obese women,

might be linked with xenoestrogens and with the production of endogenous estrogens that would be greatest in those with proportionally more body fat. Also, obese women would have higher rates of membrane lipid damage (9-11,23,35,42,45).

With respect to breast cancer, most of the confirmed risk factors, which relate to reproductive behavior and dietary factors, are not easily changed by social policy. Many of the proposed interventions to reduce breast cancer involve the lifelong use of pharmaceutical agents to change hormonal metabolism or the advocacy of radical changes in diet, lifestyle, or even reproductive behavior. As for the latter

point, a generation of women that has struggled long for reproductive freedom is unlikely to accept constraints on their reproductive choices.

This hypothesis has major implications for breast cancer screening, prevention, treatment, and management. Biologic markers of structural and functional damage to DNA and of estradiol metabolism could prove useful for identifying persons at risk of developing breast cancer, assist in prognostic predictions, and provide baselines to assess the efficacy of potential therapeutic and nutritional interventions for prevention, treatment, and management of the disease.

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