Focus

A Holistic Approach to Breast Cancer Research

The incidence of breast cancer in the United States has more than doubled over the last three decades, from 1 in 20 in 1960 to 1 in 9 today. And though that 1 in 9 does not refer to any woman's chance of breast cancer at any given time but rather to the average lifetime risk of a woman who lives to age 85, it is nonetheless a formidable and frightening statistic, especially in light of its relentless climb and its deadly legacy.

The National Women's Health Network ranks breast cancer as second only to health care access, exceeding hormone replacement therapy on its list of priorities. Breast cancer is no less a nemesis to scientists grappling with still elusive causes, prevention, and treatment. As breast cancer rates rise and congressional interventions intensify, lawmakers demand to know why prevention efforts have failed and why certain geographic areas seem to spawn breast cancer.

Environmental Studies

The site of highest breast cancer incidence in the United States lies along the upper east coast. Alerted by his state's place among the 10 leading jurisdictions, Congressman Bernie Sanders (I-Vermont) introduced a bill in 1991 to establish uniform statewide breast cancer registries and earmark money for research to discern why the northeast and mid-Atlantic regions are especially prone to develop breast cancer. That bill was enacted late last year.

Even more recently, Congressman Henry Waxman (D-California) appended an amendment to the 1993 National Institutes of Health reauthorization bill directing the National Cancer Institute and NIEHS to launch a study to assess biological markers of environmental and other risk factors contributing to the incidence of breast cancer in four northeastern counties, including the two with the highest breast cancer mortality rates over a 5-year period during the last decade. The legislation passed in both houses and is not expected to encounter any obstacles to enactment.

According to Ripley Forbes, a Waxman aide involved in putting the legislation together, the focus of this study is somewhat different from the 10-state investigation mandated in the Sanders legislation in that it is "thoroughly local and individual and is intended to provide a total look," accounting on a case–control basis for all risk factors (endogenous, dietary, and environmental), with emphasis on the environmental factors. Forbes noted that the National Research Council and NIH collaborated on specifics of the proposed study.

Factor Analysis

According to Richard DiAugustine, head of the NIEHS hormones and cancer group, ovarian sex steroids play an obligatory role in the evolution of breast cancer. Women whose ovaries have been removed at an early age, he observes, "have about the same rate of breast cancer as men do." Breast cancer in men is not nonexistent but the rate is quite low. Last year breast cancer contributed to an estimated 46,000 deaths among women and 300 among men.

Estrogen is the singular moving force in mammary gland development until puberty, after which estrogen and progesterone are the critical factors in breast cell division. "Normal cyclic cell division occurs every month; breast tissue is constantly dynamic, which puts the tissue at risk," DiAugustine says, pointing to the need for more research into how sex steroids converge to stimulate growth factors in the breast. "We need to understand the normal pathway and then investigate what may be working to exacerbate it. In animals, the system doesn't have much tolerance for insult," he says.

As to other factors that may be instrumental in the playing upon the vulnerability of breast tissue, DiAugustine comments that "all doors must stay open," especially in the face of recent publicity implicating pesticides. He offers total calorie intake and exercise as factors affecting estrogen metabolism that are worthy of closer scrutiny. The fact that Japanese women living in Japan have a much lower breast cancer rate than their own siblings living in the United States or American women is an important question, he says. One obvious question is the relative importance of the typical Japanese diet, replete in soya-based products and about 25% lower in calories than the American diet, compared to the levels of chemical residues in the breast tissue of Japanese women.

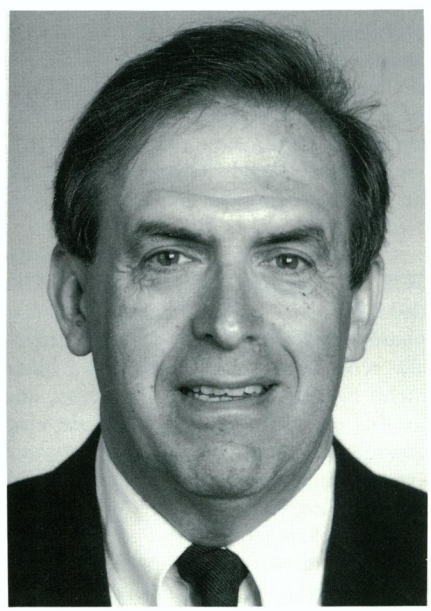
The United States is not the only country that has experienced increased breast cancer rates and mortality. According to the findings of David Hoel, head of the Department of Biostatistics, Epidemiology and Systems Science at the Medical University of South Carolina, breast cancer mortality has been rising in industrialized nations throughout the world over the last two decades. Most scientists believe that breast cancer involves a complex interaction of internally and externally introduced factors, all played upon by the element of time.

Multistage Mechanisms

The development of breast cancer, like most other cancers, probably requires several steps. Exposures to environmental chemicals may increase risk of breast cancer by facilitating one or more of those steps. For example, some chemicals may bind irreversibly to DNA, forming a DNA adduct. If a cell containing this DNA adduct divides, the damage may be fixed into the genome as a cellular mutation. The chance for a chemically induced cellular mutation is greatest between the ages of 12 and 20, during the time of rapid mammary cell division. Estrogens provide the stimulus for genetically altered breast cells to divide more rapidly than normal cells, a process that eventually causes a tumor. Therefore, estrogenically active chemicals (such as DDE, PCBs, and oral contraceptives) could increase breast cancer risk by an exaggerated stimulus of cell division.

Environmental Carcinogens

Opinions differ on how much each factor contributes to breast cancer. Former NIH Director Bernadine Healy told a congressional subcommittee that "pollution doesn't seem to be a major factor in breast cancer; even cigarette smoking, which causes so much cancer, doesn't seem to figure in this kind. Hormones and diet



Richard DiAugustine. All doors must stay open in breast cancer research.

appear to be the most compelling factors." She spoke at a hearing convened to air a Government Accounting Office report that concluded, "there has been no progress in preventing" breast cancer in the 20 years since the national "war against cancer" was launched in 1971.

At the same hearing, National Research Council Scholar-in-Residence Devra Lee Davis pointed to "broad environmental factors that can stimulate estrogen" as the place to look for clues to the other 70% or more of breast cancer cases not predicted by known risk factors. She likened the skepticism toward the suggestion that breast cancer could be caused by certain environmental carcinogens to that which greeted early reports that tobacco smoking causes lung cancer. "A number of serious scientists," she said, "argued that lung cancer could not possibly be caused by smoking because there was no mechanism that could be imagined. Moreover, others contended that the recorded increases in lung cancer were chiefly due to improvements in medical technology that made it easier to detect."

Davis and "cancer establishment" critic Samuel Epstein, professor of Occupational and Environmental Medicine at the University of Illinois Medical Center in Chicago, have suggested pesticides and other organic chemicals that concentrate in fat as targets for scrutiny. In a wide-ranging challenge to NCI priorities and practices, printed earlier this year in the *International Journal of Health Sciences,* Epstein decries the current preoccupation with dietary fat as a breast cancer risk factor while the "carcinogenic contaminants in dietary fat, particularly pesticides, polychlorinated biphenyls, and estrogens" (added to animal feed to promote growth) are ignored.

Davis and Epstein cite two recent studies that bolster the argument for more research in this area: one published last year in the *Archives of Environmental Health* by University of Michigan investigator Frank Falck showing that some women with breast cancer had markedly higher levels of pesticides and PCB residues in their breast tissue than women with benign fibrocystic breast conditions and another, published in 1990 in the *Annals of the New York Academy of Sciences,* in which Israeli investigators documented a 30% decrease in breast cancer deaths among premenopausal Israeli women after allowable pesticide levels in dietary fats were reduced, despite an overall increase in fat consumption.

The literature on chemical residues in breast fat tissue is "confused," comments NIEHS epidemiologist Walter Rogan. Different studies uncover higher levels of one or another agent in the breast tissue of breast cancer patients, but findings are not consistent. And though lactation has been associated with a weak protective effect in the nursing mother, presumably through lowering her pesticide burden, there has been no increased risk imposed on the daughter attributable to having been breastfed. The daughters of women who later develop breast cancer are at no greater or lesser risk of breast cancer for having been nursed or not.

Another theory is that background levels of environmental chemicals that act as estrogens accumulate in breast tissue and ultimately cause cancer. Rogan calls this speculation, but adds, "there is evidence for some degree of hormonal activity from exposure" to such chemicals as DDE, the stable DDT metabolite, which persists in fat tissue for decades, unlike endogenous estradiol with a half-life of 12 hours. "If you give tiny doses of DDE to a mouse every day, eventually it will become fully estrogenized. And women with higher levels of DDE lactate for shorter periods of time than women with low levels. The DDE acts like estrogen in opposing prolactin," he says. Indeed, DDE blood levels were significantly elevated in a dose–response manner in breast cancer patients in a study published last April that also demonstrated a trend toward a PCB association with breast cancer. The age pattern related to increased breast cancer rates, according to Mary S. Wolff, of New York's Mount Sinai School of Medicine, is "consistent with the historical pattern of accumulation of organochlorine residues in the environment. However, NIEHS's DiAugustine cautions that if pesticides were potent enough estrogens to increase breast cancer rates, endometrial cancer rates would be soaring.

Devra Lee Davis. Estrogen stimulators hold key to breast cancer.

Endogenous Estrogen

Aside from a genetic propensity for breast cancer, which accounts for perhaps 5% to 10% of breast cancer in the United States, endogenous estrogen is the most important intrinsic factor that propels breast cell growth, both normal and abnormal. Animal studies have shown that rats exposed to breast cancer-causing chemicals do not develop tumors in the absence of estrogen; similarly, chemically induced oncogenes are dormant in rat mammary tissue until estrogen is added.

Working with human breast cancer cell lines, called MCF-7, investigators have found that estrogen accelerates cell growth and stimulates the secretion of growth factors such as transforming growth factor-α (TGF-α) that are overexpressed in cancerous breast tissue, while inhibiting the secretion of TGF-β, which suppresses mammary epithelial cell growth. In fact, enhanced secretion of TGF-β is one plausible mechanism whereby the antiestrogen tamoxifen lowers the risk of cancer in the contralateral breast of breast cancer patients.

Estrogen production is a central component of many known breast cancer risk factors. Early age at menarche and late age at menopause, both of which prolong exposure to ovarian sex steroids in general, are associated with a higher risk of breast cancer; conversely, early menopause and late menarche are linked to lower risk. Postmenopausal obesity, wherein breast adipose tissue serves as an estrogen factory, is also associated with a higher risk of breast cancer .

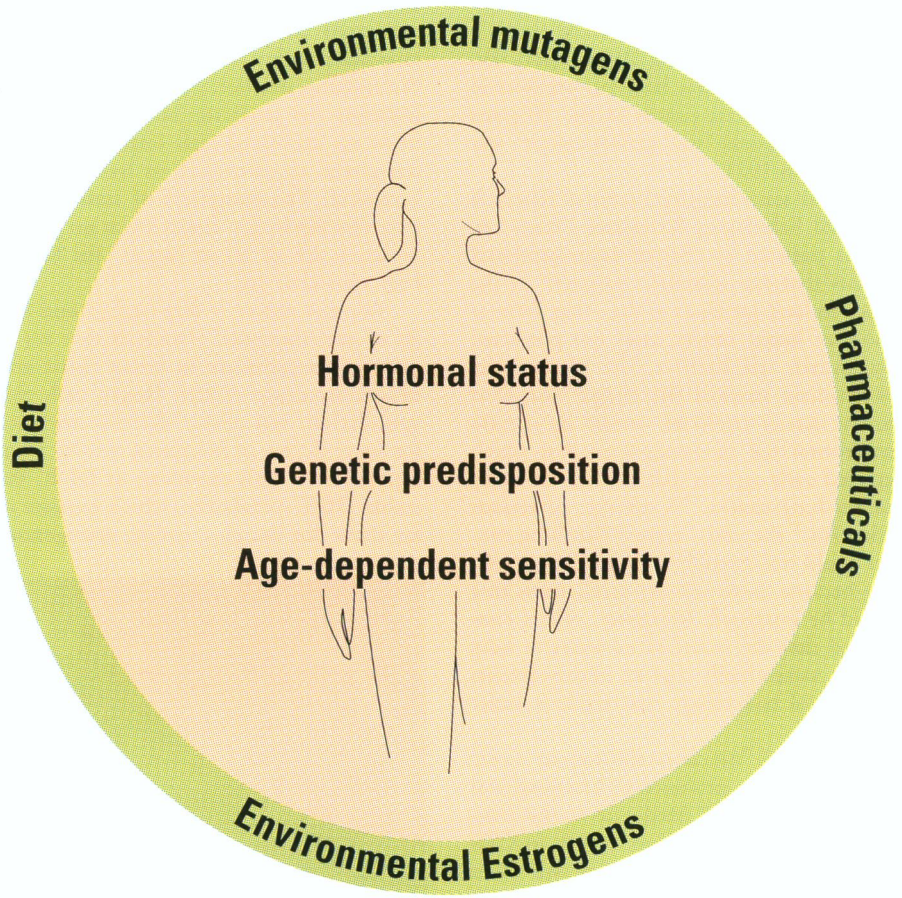
According to Jeanne Petrek, assistant attending surgeon at the Memorial Sloan-Kettering Cancer Center in New York, there's been a rise in pregnancy-associated breast cancer among the growing numbers of women who delay childbearing until their late thirties or early forties, but she believes this observation reflects an age-related increased risk of breast cancer rather than a pregnancy-induced phenomenon. Some scientists speculate that the older a woman is, the more likely she is to harbor tumor cells whose growth would be stimulated by a pregnancy-enhanced hormonal environment. Another mechanism might involve enhanced breast epithelial cell division, increasing the chances of oncogene generation or loss of tumor-suppressor genes, both of which are implicated in breast cancer.

Interestingly, though early menarche does not confer a huge disadvantage (onset at age 11 carries a relative risk of breast cancer of 1.3 compared to age 16 among

American women), it represents a striking differential between American and Asian women, whose overall breast cancer risk is four times lower. The average age of menarche in the United States is 12.8; in China, for instance, it is 17.

Similarly, the impact of dietary fat intake on breast cancer risk in women whose lives are spent within U.S. borders has yet to be conclusively demonstrated, but at the international level, the collective eating habits of countries as a whole compared with other countries correlates with relative breast cancer risks. The dramatic rise in breast cancer rates among Asian women who emigrate to the United States has fueled the argument that diet is a powerful factor in breast cancer development. "Large variations in the rates of breast cancer among countries and over time within countries and large increases in the rates of breast cancer among populations migrating from nations with a low incidence to those with a high incidence indicate the existence of major nongenetic determinants of breast cancer and the potential for prevention," observe collaborating American and Italian breast cancer specialists in a review article published last year in the *New England Journal of Medicine.*

George Lucier, NIEHS



A complex interaction of endogenous and exogenous factors leads to breast cancer.

Time

The potential for prevention may revolve around the timing of the efforts, just as timing appears to be a pivotal aspect of breast carcinogenesis. Whatever burdens teen pregnancy imposes, a woman who carries a pregnancy to term before the age of 18 reduces her risk of breast cancer to one-third of a woman whose first birth occurs at age 35 or later. Some investigators predict a 9% increase in breast cancer rates attributable to the trend toward delayed childbearing.

The first term pregnancy is believed to hasten the completion of breast epithelial cell differentiation, rendering the breast less susceptible to disruptive growth forces and the opportunity for mutation. Indeed, scrutinizing environmental factors for potential breast carcinogenicity without considering the stage of breast development at the time of exposure could yield deceptive information, many scientists caution.

Animal and epidemiologic studies reveal an age-related sensitivity to carcinogenic exposures. For example, exposure to polycyclic aromatic hydrocarbons, a component of exhaust fumes, at a time of rapid division of immature breast epithelial cells virtually ensures the development of breast cancer in rats. Earlier or later exposure causes a much lower incidence.

The same age-related phenomenon has been documented in humans regarding exposures to a variety of potential breast cancer risk factors: cigarette smoking, alcohol, radiation, and oral contraceptives. Epidemiologic studies also suggest that diet may exert its greatest impact early in life.

One example of age-related sensitivity is illustrated by a study on smoking and breast cancer. Cigarette smoking started at age 25 had no influence on breast cancer risk, but heavy smoking that began in the early teens imposed a two- to threefold greater risk later in life. This effect possibly is due to the work of chemical carcinogens in the smoke acting on time-dependent cell susceptibility.

Among Japanese females who survived the atomic bomb, the risk of later breast cancer was greater with younger age at radiation exposure. Presumably radiation, like smoking, is damaging to the DNA. This damage can be readily fixed into the genome by the rapid cell division that occurs in young women. Estrogens stimulate these damaged cells to multiply, giving rise to a tumor.

A similar pattern was documented for nearly 900 female survivors of Hodgkin's disease who underwent radiation therapy at Stanford between 1961 and 1990. Those who were treated between the ages of 10 and 19 had a nearly 40-fold increased risk of breast cancer between 4 and 22 years after treatment and on average at 15 years; the relative risk was about 15 for those treated between ages 20 and 29 and was not elevated at all for those treated after age 30. A high rate of breast cancer among young women given radiation therapy for Hodgkin's disease was also reported by physicians at Memorial Sloan-Kettering Cancer Center who also conducted research with MCF-7 breast cancer cell lines showing that manipulation of insulin and estrogen levels modifies cellular response to radiation.

Radiation treatment for cancer in one breast has been found to increase the already elevated risk of cancer in the other breast by 3%. The radiation dosage that accompanies lumpectomy procedures is the same as that used for Hodgkin's patients, but "you don't see a radiation-induced risk after menopause," a researcher at Sloan-Kettering notes.

A role for alcohol in breast cancer has been suggested by some studies. Several studies have found an increased risk among women with a history of having two or more drinks a day before age 30 or beginning consumption in adolescence. At the other end of the reproductive cycle, the possibility of a synergistic effect between alcohol and estrogen replacement therapy emerged in the Nurses' Health Study,

which found an increased risk of breast cancer among current estrogen replacement therapy users who also drank alcohol.

Of the different factors, aside from radiation, exogenous estrogen has received the most attention as a potential cause of elevated breast cancer risk due to medically prescribed regimens. In the case of oral contraceptives, age at first use and use prior to first term pregnancy have emerged as factors that may increase breast cancer risk in younger women. Epidemiologic studies suggestive of this age-related vulnerability, coupled with its biologic plausibility, have led to calls for caution and further research, especially into the lower-dose formulations that reflect oral contraceptives used today, as opposed to the higher dose products on the market when most of the studies were done.

Exogenous estrogen in the form of estrogen replacement therapy also moderately increases breast cancer risk after 10 years of use, a finding that led the FDA to revise estrogen replacement therapy labeling to alert long-term users to this possibility. Still unresolved, however, is the impact of adding a progestin to the estrogen replacement therapy regimen, a practice widely recommended to counter the estrogen-associated risk of endometrial cancer in postmenopausal women. A Scandinavian study that implicated adjunctive progestin in a fourfold increased breast cancer risk has been criticized for design flaws. But some investigators point out that the biology of breast cell mitotic activity, which peaks during the second half, or luteal phase, of the menstrual cycle after progesterone kicks in, supports the notion that estrogen and progesterone may stimulate mammary cell proliferation. On the other hand, timing breast cancer surgery during the luteal phase of the menstrual cycle has been found to improve survival in premenopausal patients in some studies, a phenomenon variously attributed to the hormonal milieu or immunologic state at that time. More studies are in progress on this issue.

The correlation of higher breast cancer incidence with earlier menarche and taller stature is widely invoked as reflecting the influence of nutrition during childhood and adolescence. An abundance of calories, fat, and protein hastens and enhances growth and maturation, thereby setting the stage for later breast cancer. (In animals, restricted food intake early in life yields a dramatic decrease in mammary tumors.) Consistent with this hypothesis are studies showing higher rates of breast cancer among Asian women who immigrated to the United States early in life. Subsequent generations of these immigrants were taller and also had higher incidences of breast cancer. However, this finding is being challenged by results of more recent studies among the same populations suggesting that breast cancer rates among immigrants increase regardless of age of arrival to the United States.

Dietary Fat

Pinpointing dietary elements that may potentiate breast cancer has been difficult. Animal and epidemiologic studies suggest a number of macronutrients and micronutrients for investigation, but results of case–control and cohort studies are ambiguous. In a report last year from the Nurses' Health Study, lower fat intake was not found to confer reduced breast cancer risk. This finding drew immediate responses from the National Women's Health Network and National Cancer Institute, both of which pointed out that fat intake in the study ranged from 27% to 50% of total calories, not the 20% that will be studied in NIH's massive Women's Health Initiative, which will also look at fiber, fruits, and vegetables. The women's health advocacy group observed "claiming that this research negates the relationship between dietary fat and breast cancer is tantamount to claiming that since smoking one pack or three packs of cigarettes per day both result in lung cancer, smoking is not related to lung cancer." Among the findings of an as-yet unpublished joint U.S.-Finnish study is a positive association between fat as a percentage of calories and level of daily cholesterol intake and breast cancer risk.

Though many have long demanded a government-sponsored low fat/breast cancer intervention trial and applaud its launching, some have said the focus may be too limited. Breast surgeon Susan Love, of the University of California-Los Angeles, observed at a congressional hearing that it would be a good idea to study the impact of a low-fat diet at puberty, when it might make more of a difference, something unaddressed in the Women's Health Initiative, which involves women 45 and older. Love, like others, questions whether it is the fat in the diet or the carcinogens in the fat that increase breast cancer risk.

Louise Brinton, chief of the NCI Environmental Epidemiology Branch, said she anticipates "interesting findings" from a case–control study in progress scrutinizing multiple risk factors, including alcohol intake, diet, oral contraceptive use, and genetic factors. The study seeks to gather information on adolescent as well as adult dietary patterns, but, Brinton said, "it's unclear that we'll get that data. It's difficult to ascertain adolescent diet. The unanswered question there is whether diet is having an effect on endogenous hormones."

The goal of risk factor identification clearly is "something we haven't yet been able to do very well—gain insights into the role of the preventable factors, like diet and medications, which lend themselves to intervention, unlike other identified factors such as age at menarche and early familial breast cancer," Brinton said. Currently, she added, the one possible mechanism for prevention in postmenopausal women is weight loss.

Body Fat

Whatever the significance of dietary fat, the significance of body fat in postmenopausal women has been demonstrated often enough for investigators to advise preventive actions, especially if the fat is distributed in the pattern of so-called android, or apple-shaped, obesity. Whether weight loss actually lowers breast cancer risk has yet to be proved, but it has been reported to reverse the high-risk hormonal patterns (increased levels of nonprotein-bound estrogen) associated with this type of obesity.

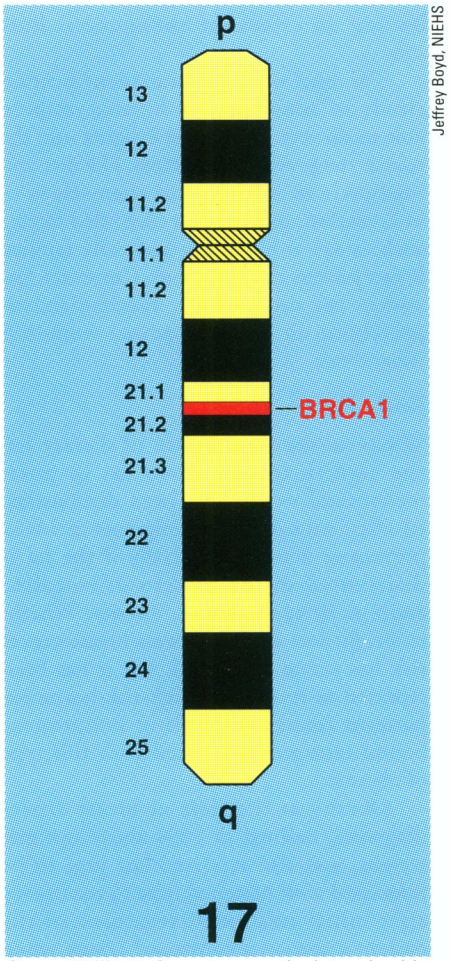
Obesity at the time of breast cancer diagnosis, defined as 25% above optimal weight, was also reported last year to be associated with hastened time to recurrence after initial treatment, regardless of the patient's age or menopausal status when diagnosed. The investigators proposed that further research be undertaken on the role that obesity might play in the lower survival rates among black breast cancer patients, and they advocated weight control as a general preventive measure.

Weight control, however, is notoriously difficult to achieve. Obesity is among a list of known risk factors Devra Davis says are really "not amenable to social policies to change them," however desirable that might be. Other factors include family history, age of childbearing, and timing of sexual maturation. Most investigators who comment on modifiable risk factors exclude these from serious consideration.

Hormones

The authors of a review article in the *New England Journal of Medicine* (30 July 1992) comment that "prevention may depend on artificial manipulation of hormones and growth regulators that underlie the known risk predictors, such as a woman's age at the birth of her first child and at menopause." At an NIH workshop on research opportunities in women's health, investigators discussed the idea of hormonal manipulation to delay menarche by a year or two to lower breast cancer risk.

Malcolm Pike, chairman of preventive medicine at the University of Southern



Jeffrey Boyd, NIEHS

Susceptibility to breast cancer is determined by gene *BRCA1* on chromosome 17.

California School of Medicine, and his colleagues have been evaluating the impact of gonadotropin-releasing hormone agonist in women between the ages of 25 and 40 at high risk for breast cancer. The regimen they use simulates the hormonal environment of a woman who has had her ovaries removed. Pike has also suggested using tamoxifen instead of standard hormone replacement therapy for postmenopausal women with a history of breast cancer or otherwise at higher risk of breast cancer. As an antiestrogen with weak estrogenic effects, tamoxifen could be expected to yield the cardiovascular and bone benefits of estrogen replacement while averting the potential cancer risks. The same rationale underlies the NCI-sponsored tamoxifen breast cancer prevention trial, which will involve 16,000 women at high breast cancer risk and represents the most ambitious attempt to manipulate hormonal breast cancer risk factors.

But some women's health advocates strongly oppose the trial, pointing to tamoxifen's documented estrogen-related side effects as an unjustifiable danger to otherwise healthy women. That tamoxifen has been found to reduce the incidence of cancer in the contralateral breast of women treated for breast cancer does not mean it will function the same way in a woman who has never had breast cancer, they say.

"Both breasts of a woman with breast cancer have been exposed to identical genetic, reproductive, hormonal, and environmental influences. Thus, there is no scientific basis for regarding the contralateral breast of a woman with breast cancer as a healthy control," said Adriane Fugh-Berman, a physician-consultant to the National Women's Health Network, in testimony before Congress and the FDA.

Genes

It is the confluence of genetic, reproductive, hormonal, and environmental influences that has commanded the interest of Congress and much of the breast cancer research community. But the one thread that appears to be removed from the rest of the weave is genetic predisposition.

Regarding the question of whether any other risk factors influence the expression of a breast cancer gene thought to be carried by 1 in 200 women, Sarah Rowell, an epidemiologist in the laboratory of Mary Clair King at the University of California at Berkeley School of Public Health, says, "to put it simply, no," at least not regarding those women in high-risk families who inherit the genotype that results in early breast cancer. Rowell says the gene, which is called *BRCA1* and is located within a very small region of the long arm of chromosome 17, is transmitted through autosomal dominant inheritance. "It could also be," she adds, "that the same gene is mutated in other women who develop breast cancer," in which case any circumstance that lends itself to acquired gene mutations could be responsible and therefore amenable to preventive measures.

Chromosome 17 is rife with genes associated with cancer in general and breast cancer in particular, including the *HER-2/neu* oncogene, which is overexpressed in breast cancer, the metastasis-supressor gene *NM23*, the gene that encodes the enzyme estradiol 17-β dehydrogenase, the gene encoding the retinoic acid receptor, and the gene encoding the tumor-suppressor protein p53.

The presence of mutant p53 in breast cancer patients is associated with lower postoperative survival. A number of environmental factors have been found to be associated with p53 mutation, at least in regard to several other cancers, including radon-induced mutations associated with lung cancer in uranium miners and virus-induced mutations associated with lymphoma in human immunodeficiency viral infection. Overexpression of the *HER-2/neu* oncogene, associated with more aggressive breast and ovarian cancer, is caused by cell damage and may be amenable to targeted therapy with monoclonal antibodies.

George Lucier, chief of the NIEHS Laboratory of Biochemical Risk Analysis, observes that some preventive efforts could be directed toward early recognition of genetic mutations and the development of molecular approaches to block their expression. This process would also identify those risk factors that induced the mutations and suggest avenues to avoid them.

Lucier adds that development of effective prevention and intervention strategies can only be achieved by increasing understanding of the hormonal, genetic, dietary, and environmental factors that affect the risk of breast cancer. That those risk factors tend not to act in isolation, but rather interact to produce an effect, is the premise from which leading-edge research into breast cancer prevention and intervention must now spring.

Fran Pollner

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