Homing In on Mechanisms Linking Breast Density to Breast Cancer Risk

By Vicki Brower

In probably the best-known finding from the Women’s Health Initiative, postmenopausal hormone therapy with estrogen and progesterin was linked to a substantial increase in breast cancer risk. Now researchers think that the increase may be explained by an associated rise in breast density.

Among women taking hormone therapy in the large, prospective women’s health study, both the density found on baseline mammograms and the change in density were statistically significantly associated with breast cancer risk, according to Celia Byrne, Ph.D., assistant professor at Georgetown University’s Lombardi Comprehensive Cancer Center. Byrne discussed the results of this new analysis of Women’s Health Initiative data at the 2010 American Association of Cancer Research meeting in April.

Researchers now consider mammographic density—the amount of white, radiodense area compared with the amount of gray, radiolucent area on a mammogram—one of the strongest independent risk factors for breast cancer (see “Breast Density Gains Acceptance as Breast Cancer Risk Factor,” J. Natl. Cancer Inst. 2010;102:374–5). But researchers do not fully understand just how breast density increases breast cancer risk. Research into the biological mechanisms underlying breast density and linking it to breast cancer risk has been an understudied area, according to Paolo Provenzano, Ph.D., a research associate at Seattle’s Fred Hutchinson Cancer Research Center.

That situation is changing as Provenzano and others have begun to identify some of the complex biological processes that account for breast density and to explain how they affect breast tissue.

“Direct studies to begin to address a causal relationship between breast tissue density and cancer have only started to emerge,” Provenzano said. “While there is still much work to be done, I believe that high breast tissue density is causal for the increasing risk of developing breast cancer.”

Hormones, Growth Factors

One line of research on mechanisms that may link density to breast cancer focuses on hormones and growth factors. Some studies have suggested that blood levels of estrogen and testosterone are related to breast cancer risk in pre- and postmenopausal women. However, the findings have not consistently shown a statistical correlation between hormone levels and risk or between hormone levels and density. “This [finding] suggests that these hormones may influence risk via other pathways unrelated to density,” said Norman Boyd, M.D., Ph.D., senior scientist at the Ontario Cancer Research Institute in Toronto.

Breast density is a heritable factor, and girls with dense breasts have higher density as women, Boyd and others have shown. According to Boyd’s proposed model of risk, cumulative “exposure” to density is an important determinant of breast cancer incidence.

The risk for breast cancer associated with mammographic density may be explained, he said, by the combined effects of mitogens and mutagens. Mitogens, such as human growth hormone and insulin-like growth factor 1 (IGF-1), cause cell proliferation, whereas mutagens, such as radiation, cause genetic damage to proliferating cells.

In a recent study of hormones and growth factors, Boyd’s team found that, after accounting for a variety of factors, only human
growth hormone was statistically significantly associated with density. But other research suggests that blood levels of IGF-1 are associated with greater cancer risk. IGF-1 is a known mitogen for breast epithelium, which the breast stroma and the liver produce in response to growth hormone. Rulla Tamimi, Sc.D., of Harvard Medical School has compared common genetic variations in the IGF-1 gene and found that certain variants are associated with increased density.

The hormone prolactin, which increases breast cell proliferation and reduced apoptosis, or programmed cell death, has also been correlated with breast density in post-menopausal women. Also, urinary levels of melatonin, a marker of oxidative stress and a mutagen, have been correlated with dense breast tissue, as have genetic polymorphisms of certain antioxidant enzymes and IGF-1.

**Stroma, Collagen, and Density**

Other studies veer away from genetic and hormonal factors and focus on interaction among epithelial cells, fibroblasts, adipocytes (fat cells), and the breast microenvironment. In this camp, researchers are examining the mechanical effect of tissue rigidity, or stiffness, on epithelial cells, on surrounding stroma, and on stromal–epithelial interactions.

“While transformation of breast epithelial cells is necessary for breast cancer, stroma surrounding them has been suspected to be involved in epithelial transformation,” said Provenzano. Tumors must access fibroblasts, inflammatory cells, and other cells to form stroma in order to grow.

Some researchers in this area are scrutinizing increased levels of collagen, produced by stroma and a hallmark of dense breast tissue. While at the University of Wisconsin–Madison, Provenzano investigated a causal connection among dense tissue, stroma, and breast cancer; to do so, he first tried to determine whether increased collagen density could directly promote growth of mammary epithelial cells without stromal cells. He cultured human mammary cells within 3-dimensional collagen gels and measured proliferation. The cells cultured in low-density matrices formed well-differentiated structures, as in normal tissue. But those that formed in high-density collagen matrices were larger, less organized, and marked by cellular proliferation, similar to cancerous tissue. This study appeared in 2008 in *BioMed Central Medicine.*

Provenzano then took live images of tumor–stroma interactions in transgenic mice bred to have more collagen in their mammary glands. Using multiphoton laser-scanning microscopy, he found that tissue with more stromal collagen increased tumor formation threefold and resulted in a more invasive cancer with about three times more metastases to the lung. He also saw that stromal collagen facilitated local invasion. Mice with more stromal collagen had increased mammary tumors, and these were more metastatic and invasive.

Tumor growth increases collagen production, so inhibiting collagen synthesis is one possible therapeutic approach to density, according to Thea Tlsty, Ph.D., professor of pathology at the University of California, San Francisco. “Type I collagen is an important binding protein for a wide variety of growth factors that influence carcinogenesis,” Tlsty said. That means that targeting collagen metabolism might be a way to reduce density and breast cancer risk.

In a study looking at the role of epithelial–stromal interaction, Tlsty noted that epithelial cells may initiate incorrect stromal signaling, whereas stromal cells—in response to a local stress—can signal to local epithelial cells to proliferate or move into a new developmental pathway. Fibroblasts, which produce collagen, may produce abnormal or fibrotic stroma even before epithelial changes occur.

And in new, unpublished research, Tlsty identified a gene that controls the process by which fibroblasts normally become adipocytes. “If this gene is defective, however, the cells develop along a different, abnormal pathway, and become stromal cells,” she said. So normal breast development means more fat and epithelial cells and fewer fibroblasts, stroma, and collagen. Dense breasts consist largely of stromal cells, which deposit more collagen into the microenvironment, stretching and stiffening cells and tissues and setting off mechanosensory signaling, increasing cell proliferation and transformation, Tlsty said.

Increased collagen density may result from one of two mechanisms, according to Provenzano. First may be the influence of increased stromal collagen on breast fibroblasts, which in turn influence epithelial cells. Stromal fibroblasts can regulate epithelial cells through growth factors and chemokines such as transforming growth factor β, epidermal growth factor receptor, and IGF-1, all implicated in tumorigenesis and metastasis, he said. Higher density is also associated with stiffer extracellular matrix, which alters signaling; increases cell proliferation; and produces a less normal, more cancerous cell type.

**Tissue Stiffness**

To explore the link between density and the extracellular matrix, Provenzano tested whether higher collagen matrix density, or tissue stiffness, could promote cell proliferation and invasion without stromal cells. In a 2009 *Oncogene* study, he demonstrated that matrix stiffness increased formation of abnormal 3-dimensional groups of cells, and certain elevated enzyme levels and signaling, which were necessary to develop and maintain cells prone to cancer and metastasis. This signaling hyperactivated the Ras–MAPK (mitogen-activated protein kinase) oncogenic pathway, which promotes epithelial growth and proliferation.
Valerie Weaver, Ph.D., of the University of California, San Francisco, is also investigating the relationship of breast tissue stiffness and cancer. Last year she reported in *Cell* that tissue rigidity induces cancer by means of the enzyme lysyl oxidase (LOX). She demonstrated that LOX can transform abnormal but nonmalignant breast tissue into tumor and, conversely, that blocking LOX decreased stiffness and reduced the chance of tumor formation.

According to Weaver, LOX causes many structural changes in collagen through a process called cross-linking. High LOX levels in mammary glands increase collagen cross-linking, making tissue stiffer, and they correlate with a higher incidence of tumor invasiveness. Cross-linking also enhances integrin signaling, which is tied to tumor progression. Weaver, however, believes that the cross-linking of collagen, not its quantity, is the key to breast density and cancer risk.

“My gut says that mammographic density is going to be tricky and complicated with respect to understanding whether or not it is stiffer and if that stiffness could contribute to increased susceptibility to cancer,” she said.

Weaver is now measuring breast tissue of women with dense and nondense breasts with atomic-force microscopy, coupled with a comprehensive analysis of cross-linking status of matrix gene profiles, mechanosignaling data, and other criteria, including inflammation markers.

“We anticipate that we should have at least some indication of what defines density in a few years,” she said. “But until then, my belief is that all folks who claim that they are modeling breast density when they study the effect of increased collagen concentration on cell behavior ex vivo are overinterpreting and extending data that are not yet conclusive.”

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