Does Microenvironment Contribute to the Etiology of Estrogen Receptor–Negative Breast Cancer?

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Abstract

What dictates the prevalence of certain types of breast cancer, which are classified by markers, particularly estrogen receptor (ER), expression profiles such as basal or luminal, and genetic alterations such as HER2 amplification, in particular populations is not well understood. It is increasingly evident that microenvironment disruption is highly intertwined with cancer progression. Here, the idea that microenvironment shapes the course of carcinogenesis, and hence breast cancer subtype, is discussed.

Aggressive, basal-like, ER-negative breast tumors occur in younger women, African-American women, and women who carry BRCA1 mutation, and women exposed to ionizing radiation. Recent experimental studies using ionizing radiation, a well-documented environmental exposure, suggest that certain processes in the microenvironment strongly favor the development of ER-negative tumors. Understanding the contribution of tissue microenvironment during carcinogenesis could lead to prevention strategies that are personalized to age, agent, and exposure to reduce the risk of aggressive breast cancer.

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Introduction

Breast cancer is a heterogeneous disease based on histology, marker expression, imaging features, and molecular profiles. Molecular portraits of breast cancer built from high content analysis of mRNA, microRNA, protein, copy number, and sequencing support a minimum of 4 (1), or up to 10 (2) intrinsic classes. Amplification of HER2 provides a distinct molecular target and is sufficient to identify a specific breast cancer subtype, whereas the presence of estrogen receptor (ER) is one of the most important clinical markers in breast cancer. An ER-positive tumor is well managed with antiestrogen treatments and is associated with distinct risk factors, pathologic features, and clinical behavior (3). For example, ER-positive tumors are more frequent in postmenopausal women and have a relatively good prognosis for 5-year survival but a substantial risk of recurrence after that period. ER-negative tumors are more frequent in younger women (<45 years) and have a short-term worse prognosis (4) but are less likely to recur after 5 years. Among ER-negative breast cancers there are several diseases. ER-negative and progesterone receptor (PR)–negative cancers tend to be poorly differentiated and aggressive. Triple-negative breast cancer (TNBC) invasive ductal carcinomas lack all 3 markers: ER, PR, and HER2. TNBC can be further subdivided by those tumors that express basal markers, cytokeratin 5/6 or α-smooth muscle actin, which are called basal-like, and those that do not (5).

Molecular typing using expression profiles identified several distinct breast cancer subtypes that are enriched for, yet not synonymous with, particular markers (reviewed in ref. 6). Expression profiling has divided ER-positive tumors into luminal A and luminal B subtypes that have better and worse prognosis, respectively. Similarly, expression profiles of TNBC can be classified into 2 subtypes, claudin-low or basal-like, that have distinct features and clinical behaviors. For example, claudin-low tumors are enriched in functional tumor-initiating cells (7).

Although our appreciation of the variety of breast cancers is much more detailed, what determines the prevalence of a particularly subtype of tumor in a population is not well understood (8). The observation that Ashkenazi Jewish women have a high risk for breast cancer led to identification of the BRCA1 gene, and recognition that these women usually develop TNBC led to new understanding of the biology of these cancers (reviewed in ref. 9). Young women are at a high risk of developing ER-negative breast cancer, and TNBC in particular, as these cancers are predominantly classified as the basal-like intrinsic molecular subtype (10). African-American women have lower age-adjusted incidence rates of breast cancer diagnoses (95.4 vs. 111.8 per 100,000) yet higher age-adjusted breast cancer mortality rates compared with European-American women (31.4 vs. 27.0 per 100,000; ref. 11). Racial differences remain after adjustment for sociodemographic and pathologic variables, which points to differences in the nature of the disease itself. This supposition is further supported by the greater prevalence of high-grade, ER-negative tumors in African-
American women (12–15). TNBC account for 40% of breast cancer cases in African-American women compared with 15% to 20% in European-American women.

Less well understood are life events and exposures that are associated with breast cancer subtypes. Although an early pregnancy confers a life-long protective effect (reviewed in ref. 16), breast cancer risk increases within 5 to 10 years of a pregnancy. Lyons and colleagues have shown that this transient increase in breast cancer is a function of the remodeling of the mammary gland during involution, which provides the specific context to promote cancer progression (17).

Mammographic density is a physiologic feature that is associated with breast cancer risk (18). At the most fundamental level, mammographic density reflects tissue composition, that is, epithelial and stroma relative to fat. Analysis of mammographic breast density in the Canadian National Breast Screening Study and other study cohorts indicates that density is an independent risk factor for invasive breast cancer (19–23). Women who have greater than 50% of total breast area that is mammographically dense have a 3- to 5-fold greater risk of being diagnosed with breast cancer than women with less than 25% mammographically dense breasts. Boyd and colleagues proposed that the radiologic features of breast tissue provide an index of cumulative exposure to the current and past hormonal and reproductive events that influence breast cancer incidence (24). Pregnancy at an early age has a permanent beneficial effect of decreasing breast cancer risk as well as mammographic breast density (25), whereas older age at birth of a first child is associated with a greater likelihood of mammographically dense breasts (26, 27). With age, menopause accounts for some of the decrease in breast density, but increasing age is also an independent contributor to the decrease in mammographic density (28). Hormone replacement therapy is associated with increased mammographic breast density in 16% to 20% of menopausal women within the first year of starting therapy (29, 30), and greater increases in mammographic density are associated with estrogen and progestin regimens compared with estrogen therapy alone (31). Taken together, these findings suggest that mammographic breast density is influenced by endogenous and exogenous estrogen exposure. Interestingly, the risk for developing ER-positive or -negative breast cancers is comparable in women with high mammographic density (32, 33). It is unclear what biology underlies the association between mammographic density and breast cancer, but it is likely due to factors besides estrogen exposure.

Ionizing radiation is one of a very few environmental exposures known to increase breast cancer risk (34). An analysis by Preston and colleagues of pooled data from 8 studies of irradiated women, which included atomic bomb survivors, girls treated for scoliosis or tuberculosis, and women treated for mastitis, showed that increased risk of breast cancer is inversely correlated with age at irradiation, with the greatest risk conferred by exposure before the age of 20 years (35). A singular study by Castiglioni and colleagues revealed that tumors from women exposed to therapeutic radiation for childhood/young adult cancers were more likely to be ER negative, specifically TNBC, compared with tumors in age-matched controls (36). Brooks and colleagues reported that gene expression profiles in radiation-preceded breast cancer were consistent with basal-like intrinsic subtype, were more aggressive, and could be clustered compared with those occurring in women diagnosed at the same age without radiation treatment (37). These data suggest that an exogenous agent not only may increase the risk of cancer but may also ultimately affect the type of cancer.

**Breast cancer diversity is rooted in the mammary hierarchy**

Two conceptual models are currently used to explain the diversity of cancer. One holds that tumor phenotype is a function of the specific set of genetic alterations; the other is that the specific cell in which transformation is the source of tumor heterogeneity (reviewed in ref. 38). Importantly, these models are not mutually exclusive. The “cell of origin” hypothesis postulates that the heterogeneity of cancer is determined by the cell of origin’s position within the epithelial hierarchy and that upon transformation its fundamental programming remains evident in the biology, behavior, and signature of the cancer subtype (38, 39). This hypothesis postulates that stem cells give rise to tumors that are less differentiated and more aggressive. It is thought that transformation of mammary stem or progenitor cells and aberrant differentiation processes result in generation of the phenotypic heterogeneity found in human and rodent breast cancers. Consistent with this, malignant transformation with the same genetic elements of specific human breast cell types results in tumor xenografts exhibiting major differences in histopathology, tumorigenicity, and metastatic behavior (40).

The epithelium of both human breast and rodent mammary gland consists of histologically distinct cell types that include hormone receptor–positive and –negative luminal cells and myoepithelial cells. The mammary gland contains a well-documented stem cell, the function of which is assayed using mammary repopulation or serial transplantation capacity and undergoes postnatal morphogenesis (41). The fact that the epithelial anlagen can be surgically removed before ductal morphogenesis, which is initiated at puberty, provides a means to examine what regulates stem cells, how host or stromal factors or exposures affect mammary morphogenesis, or how engineered genetic alterations contribute to tissue function (Fig. 1). As shown by the pioneering studies of Young and colleagues, transplantation of donor mammary cells or tissue fragments reinitiates ductal morphogenesis and can be used to demonstrate the presence of robust tissue-specific stem cell. Functional assays suggest that stem cells and several progenitors exist, but unequivocal in situ markers of mammary stem or early progenitor cells have yet to be identified (reviewed in ref. 42). Rather, researchers have used a combination of properties (e.g., quiescence), functions (e.g., repopulation) and marker exclusion (e.g., lineage negative) or inclusion
(e.g., CD49f, CD44) to infer which subpopulation is most consistent with a putative stem cell phenotype (i.e., multipotency, proliferative potential, and self-renewal). More detailed understanding of the mammary epithelial hierarchy has provided new tools and insights into the carcinogenic process. Lim and colleagues isolated 4 mammary cell subpopulations from mouse mammary glands and human breasts based on cell surface markers and showed that the respective gene signatures of each population show a remarkable degree of overlap in humans and mice (43). Notably, intrinsic breast cancer subtypes are enriched for particular signatures, which is thought to be due to transformation of specific cell types (7, 44).

Another key idea is that deregulation of normal self-renewal programs can prime the tissue to generate particular tumors. Compelling support for this concept in humans has recently come from analysis of breast tissue from BRCA1 mutation carriers, who almost always develop ER-negative, basal-like breast cancer (45, 46). This distinctive biology led to the hypothesis that BRCA1 functions as a stem cell regulator in the breast (47). Aberrant lineage commitment is a unique feature of the BRCA1-deficient breast epithelium as shown by several laboratories using different approaches, including marker characteristics, molecular analysis, cell surface antigens, and functional assays (48–53). Knockdown of BRCA1 in primary breast epithelial cells increases cells displaying aldehyde dehydrogenase (AldH) and decreases cells expressing luminal epithelial markers and ER (52). Moreover, in breast tissue from BRCA1 mutation carriers, loss of BRCA1 heterozygosity was seen in AldH-positive lobules but not in adjacent AldH-negative lobules, supporting the idea that loss of BRCA1 blocks epithelial carcinogenesis.

Figure 1. Mammary chimera models. The mammary gland provides an opportunity to manipulate the host and stroma independently of the mammary epithelium because mammary morphogenesis occurs postnatally under the hormones of puberty. The mammary chimera consists of surgically removing the mammary epithelium of a 3-week-old mouse to create a parenchyma-free gland, the so-called “cleared” fat pad. At any point thereafter the mammary fat pad can be transplanted with isolated cells or a fragment of syngeneic donor tissue, which will undergo ductal morphogenesis. By exposing the host and donor differently, the mammary chimera can be used to dissect the relative contribution of cell-intrinsic biology from that determined by supporting host cells. One use of the chimera is to transplant the cleared fat pad with mammary epithelium from donor mice that have been manipulated (left). For example, the donor mice might have a defined genetic alteration or might have been exposed to a carcinogen or undergone a defined physiologic event (e.g., pregnancy, aging). The donor tissue can also be manipulated in culture (e.g., lentivirus infection) before transplantation. An alternative use of the chimera is to subject the host to different manipulations before transplantation (middle). For example, the radiation chimera consists of host mice in which the fat pad is cleared and mice are irradiated and subsequently transplanted with mammary tissue fragment. In this model, the epithelium is oncogenically primed by deletion of p53; thus, it is a genetic-radiation chimera. Comparison of the tumor development between controls and irradiated hosts provides a means to assess the action of radiation on host biology. These chimeras can be compared with effects in intact mice, for example, following radiation exposure (right). In all cases, mice can be monitored externally for palpable tumor development, morphogenesis analyzed in whole-mount preparations, or the tissue and tumors analyzed by a variety of means. LN, lymph nodes.
lineage commitment of ER-positive cells. Stem cell surface marker analysis of cells isolated from tissue from BRCA1 mutation carriers showed substantially fewer cells in the mammary stem cell–enriched subset but an increase in the luminal progenitor cell fraction relative to age-matched normal breast tissue (51). Targeted deletion of Brca1 in mammary luminal progenitors results in basal-like murine tumors (50), consistent with the observation that the intrinsic subtype of breast cancer from BRCA1 carriers is predominantly basal-like (54). Proia and colleagues specifically showed that genetically engineered transformation of nonmalignant BRCA1-mutant breast epithelium preferentially generate ER-negative, basal-differentiated breast tumors, perhaps the most definitive test of this hypothesis to date (49).

Mouse models in which factors affecting stem cells are manipulated also support the importance of the relationship between stem cell regulation and cancer. Stem cells must be able to switch between self-renewal and differentiation in response to damage (55). Expansion of stem cells during chronic inflammation specifically contributes to the development of gastric cancer (56). Overexpression of β-catenin transcriptional activity increases stem cells, which in turn reduces tumor latency and increases tumor display of cell markers associated with progenitor cells (57, 58). Mammary stem cells lacking p53 exhibit very high self-renewal mediated by Notch, which is postulated to contribute to the high rate of malignant transformation in this model (59, 60). Unbalancing the epidermal cell clock through deletion of Bmal1 resulted in a progressive accumulation of stem cells, which increased the predisposition to undergo neoplastic transformation (61). Thus, activities that affect stem cell frequency result in a tissue property that contributes to cancer frequency.

If mammary stem cells are the likely cell of origin for ER-negative breast cancer, the corollary is that such tumors retain fundamental programming that remains evident in the tumor biology, behavior, and signature (62). Accordingly, a stem cell–like expression profile is specifically enriched in claudin-low breast cancers (63). Similarly, murine mammary tumors arising in MMTV-Wnt1 and p53−/− mice were enriched for mammary stem cell–subset genes, whereas the gene profiles of MMTV-Neu and MMTV-PyMT tumors were most concordant with the luminal progenitor cell signature (43). This hypothesis is further supported by the demonstration that engineered transformation of specific human breast cell types gives rise to tumors that display specific histopathologic features (44).

**Microenvironment shapes the course of carcinogenesis**

Most models of carcinogenesis focus on disruption of either DNA sequence or proliferation, even though it has become increasingly evident that the composition and character of the microenvironment and cell dysfunction are highly interdependent during cancer development (64, 65). The carcinogenic action of ionizing radiation is a case in point. Although cancer risk following radiation is widely thought to be due to misrepaired DNA damage resulting in mutations, radiation exposure alters signaling and results in persistent microenvironment changes (reviewed in ref. 66). Mancuso and colleagues provided an impressive example of the systemic action of radiation on carcinogenesis in which mice exposed to radiation developed brain tumors even when the brain was shielded from exposure (67).

To evaluate the contribution of the irradiated microenvironment in breast cancer, we developed the radiation chimera model that consists of surgically removing the endogenous mammary parenchyma, irradiating the mouse, and then transplanting the cleared mammary fat pads several days later with syngeneic, oncogenically primed mammary tissue (Fig. 1). Host irradiation dramatically promoted tumor development from cells that gave rise to mostly normal outgrowths in unirradiated hosts (68). A second series of experiments used Trp53 null epithelium transplanted to syngeneic wild-type hosts (69). This genetic chimera model, developed by Medina and colleagues, showed that Trp53 null epithelium transplanted to a wild-type host mimics essential biologic and genetic attributes of human breast cancer (70). Trp53 null epithelium undergoes broadly normal morphogenesis followed by the appearance of ductal carcinoma in situ and genomic instability before giving rise to diverse tumor types that can be ER-positive or -negative (71). Expression profiles of Trp53 null mammary tumors can be classified into at least 4 intrinsic molecular subtypes (7).

We found that when host mice that were irradiated with doses (10–100 cGy) in the range associated with increased breast cancer in women (35) developed aggressive tumors. Tumors appeared sooner and grew faster as compared with nonirradiated hosts (69). Unexpectedly, the ER status of tumors was affected by host irradiation. Only 35% of tumors that arose in sham-irradiated hosts were ER-negative compared with 65% in irradiated hosts. Thus, the radiation chimera model recapitulates the important features of radiation-preceded breast cancer described above: decreased latency and increased frequency of ER-negative tumors, and aggressive tumors (69). This seems to be the first demonstration that mammary tumor ER status can be affected by host biology.

Moreover, expression profiles of the tumors arising in irradiated hosts are distinct from those that arose in control mice. Supervised analysis of genes using significance of analysis of microarray methodology and permutation analysis under a leave-one-out bootstrap scheme resulted in 156 gene signatures enriched in tumors that developed in irradiated hosts (69). A second signature was able to cluster the ER-negative tumors arising in the irradiated host from those in controls, consistent with multiple subtypes of ER-negative tumors. Several pathways were enriched: leukocyte chemotraction and binding, monocyte maturation, and proliferation.

As described above, ER-negative breast cancers are thought to arise from pluripotent stem/progenitor cells (reviewed in ref. 38). Notably, the mammary stem cell signature but not the progenitor or mature luminal signature, as reported by
induced by radiation act via the microenvironment could enable to manipulation. For example, defining which signals than genetic integrity because it is imminently more ame-

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development of ER-negative cancer may be primed by experimental radiation chimera data (69) suggest that TNBC compared with age-matched controls (36), our hood cancers have a greatly increased risk of developing immunobiology that may provide clues to the cancer association associated with repopulating activity compared with sham-irradiated mice. Consistent with this observation, limiting dilution experiments showed twice the functional mammary repopulating activity in irradiated mice compared with sham-irradiated mice. These data are consistent with the idea that deregulation of stem cell self-renewal may itself be a factor in cancer development (74–76).

When considered together, these data lead to the hypoth-

a significant increase in the population associated with repopulating activity compared with sham-irradiated mice. Consistent with this observation, limiting dilution experiments showed twice the functional mammary repopulating activity in irradiated mice compared with sham-irradiated mice. These data are consistent with the idea that deregulation of stem cell self-renewal may itself be a factor in cancer development (74–76).

Implications for cancer prevention

The idea that development of an ER-negative tumor may represent neither a random event nor an immutable path-

way is thought provoking. Identification of breast cancer predisposition genes, BRCA1 and BRCA2, and use of phar-

maceutical agents such as tamoxifen to reduce recurrence and second breast cancers transformed the approach for those women who are at high risk for developing breast cancer. ER-negative breast cancer poses a particular chal-

lenge because there are no targeted therapies. Gene expres-

sion profiles of tumor versus stroma in African-American patients with breast cancer identified intrinsic differences in pathways related to chemotaxis, tumor angiogenesis, and immunobiology that may provide clues to the cancer health disparity (78). Because women irradiated for child-

hood cancers have a greatly increased risk of developing TNBC compared with age-matched controls (36), our experimental radiation chimera data (69) suggest that development of ER-negative cancer may be primed by specific host tissue processes.

Tissue integrity, that is, microenvironment, inflamma-
tion, and stem cell regulation, is arguably more important than genetic integrity because it is imminently more ame-
nable to manipulation. For example, defining which signals induced by radiation act via the microenvironment could provide avenues to protect susceptible populations. We tested this using genetic ablation of Tgfβ1 in the irradiated hosts, which showed unexpectedly that radiation-induced TGFB activity is tumorigenic (69). Such data could motivate short-term therapeutic strategies to reduce cancer risk in populations at risk of developing ER-negative breast cancer, perhaps by using TGFB-inhibitors that are in phase I and II study for other diseases (79).

Radiation also induces Notch, both in normal tissues and in tumors (80, 81). Transient treatment with Notch inhibitors might preclude stem cell expansion in children treated with radiation for childhood cancers, if the activity profile was sufficiently safe. Radiation also induces a gene signature of chronic inflammation, enriched in genes involved in leukocyte chemoattraction, transendothelial migration, and monocyte maturation (69). Gonda and colleagues proposed that chronic inflammation, whether induced by chronic infection or carcinogen exposure, results in a myriad of effects that produce an environment conducive for the emergence of cancer (65). Agents that suppress inflammation, such as aspirin, are thought to reduce cancer risk.

The questions of how and why certain women develop aggressive breast cancers is critical to prevention efforts before disease onset in women at higher risk and to manage disease once it manifests (82). For example, Lyons and colleagues proposed that nonsteroidal anti-inflammatory drugs may reduce risk of postpartum breast cancer due to physiologic consequences of involution (17). Women diagnosed with breast cancer within 5 years of pregnancy have a poor prognosis. During involution secretory mammary epithelial cells that undergo apoptosis provoke other mam-

mary epithelial cells to become phagocytic and engulf the shed apoptotic cells. This, in turn, induces Th2-like cyto-
kines and growth factor production by the phagocytic mammary epithelial cells, which promotes alternative acti-

vation of macrophages and inhibition of cytotoxic T cells. These events, together with extracellular matrix remodeling, contribute to immunosuppression and a protumorigenic microenvironment. It is important to consider whether relatively benign prophylactic therapies could prevent this process.

Dairkee and colleagues found that bisphenol A treatment of breast epithelial cells cultured from premalignant biop-
sies elicited an expression profile associated with large, high-histologic-grade breast tumors (83). They proposed a biologic “fingerprint” of probable prior exposure to endo-

crine-disrupting agent. Our preliminary bioinformatic analysis suggests that the gene expression signature derived from host irradiation clusters radiation-preceded cancer in humans (77), consistent with this idea that a microenvi-

ronment “meta-profile” could be used to evaluate probable etiology. It is possible that such profiles could identify whether ER-negative breast cancer arises because lifestyle or external events deregulate stem cells or affect the com-

position of the microenvironment.

In summary, the concept of microenvironmental drivers of cancer susceptibility has important implications for...
cancer prevention. A chemoprevention that targets a stochastic epithelial event, such as mutation of p53, amplification of HER2, or loss of PARP sensitivity, is unlikely to be at high enough frequencies to warrant widespread use. However, a root cause, like proliferation, can be generally suppressed, as with antiestrogen or oophorectomy for women with high risk factors. Arguably, aspirin may prove to be the prototype of low-cost, low-risk therapy that provides benefit via its effect on the microenvironment rather than the target epithelium. The prominent COX-2 involvement in progression of ductal carcinoma in situ has prompted the suggestion that its pharmaceutical inhibition could be beneficial (17, 84).

The possibility of personalized prevention, specific to the exposure, age, and genotype, cannot be achieved without considering that cancer results from a mutant epithelial cell that is supported and promoted by a specific stroma. If the action of environmental exposures or physiologic events on critical microenvironmental signals is sufficiently understood to be predictable, it is highly likely that cancer risk can be reduced in vulnerable populations. Women treated with radiation as children have a very high risk of developing TNBC (36). Could transient inhibition of a key signal translate into risk reduction for children after exposure? New studies show that stromal cells from mammographically dense breast executing a CD36 dependent program that is permissible for cancer provide a novel target for reducing cancer risk via the microenvironment (85). Even established disease can be suppressed or normalized by rewiring the signals from the microenvironment (86), thus it is likely that normalizing the microenvironment after an exposure or event could restore control of aberrant cells. As cancer theory evolves to encompass the microenvironment, there is new opportunity to develop personalized prevention by identifying those systemic processes that support the growth of aberrant cells.

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References


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