Pregnancy and breast cancer: The other side of the coin

Early full-term pregnancy is thought to be one of the most effective means of decreasing lifetime breast cancer risk. Paradoxically, young women diagnosed with breast cancer shortly after giving birth have a higher risk of dying from their disease. These seemingly opposing effects have been largely attributed to mammary epithelial stem cell differentiation and precancerous cell proliferation, respectively, induced by pregnancy-associated hormonal changes. However, recent studies suggest that remodeling of the cellular microenvironment and extracellular matrix during pregnancy and involution may contribute to the enhanced invasive and metastatic potential of breast carcinomas and thus lead to their worse clinical outcome.

Breast cancer is one of the most commonly diagnosed neoplasms and a leading cause of cancer associated-death in women worldwide. Besides family history, lifetime cumulative exposure to reproductive hormones is the most significant and well-characterized risk factor for breast cancer. Conversely, early full-term pregnancy and multiparity decrease lifetime breast cancer risk in women of all ethnic groups with the exception of mutation carriers of high-penetrance breast cancer susceptibility genes (Cullinane et al., 2005). Supporting this observation, animal studies demonstrate that pregnancy or short-term treatment of virgin rats with pregnancy-associated hormones protects against chemically induced mammary carcinogenesis (Russo et al., 2005a, 2005b). One potential mechanism for this protective effect is that the terminal differentiation of mammary stem cells following pregnancy decreases the number of cells that could be targeted by oncogenic events. In addition, recent comprehensive genomic studies of isolated mammary epithelial cells have demonstrated permanent alterations in gene expression patterns induced by pregnancy (Russo et al., 2005a).

Based on molecular profiling studies, it is generally accepted that breast cancer is not a single disease with a single tumorigenesis pathway, but rather a heterogeneous group of diseases with each subtype having its own stable phenotype maintained during tumor progression. The most important determinants of these subtypes found to date are the presence or absence of estrogen receptor (ER) and progesterone receptor (PR), the amplification and overexpression of the HER2 oncogene, and histologic grade. Based on these features, breast tumors are divided into luminal A, B, and C, HER2+, and basal subtypes, and this classification correlates with clinical outcome. It is hypothesized that tumor subtypes reflect the cell of origin of the tumor with ER-negative and -positive tumors initiating from the earliest progenitors and more committed transit-amplifying cells, respectively. Correlating with the proposed existence of subtype-specific tumor progression pathways, it is increasingly evident that risk factors are also different for each tumor type. The protective effect of full-term pregnancy at an early age is only observed for ER+/PR+ tumors, while folate intake and alcohol consumption only influence the risk of ER-negative tumors (Colditz et al., 2004; Ursin et al., 2005).

In contrast to its long-term protective effect, in young women (under age 35 or 45 depending on the study) pregnancy is associated with a transient increase in breast cancer risk (Albrektsen et al., 2006). Furthermore, breast cancer diagnosed shortly (within 2 or 5 years depend-

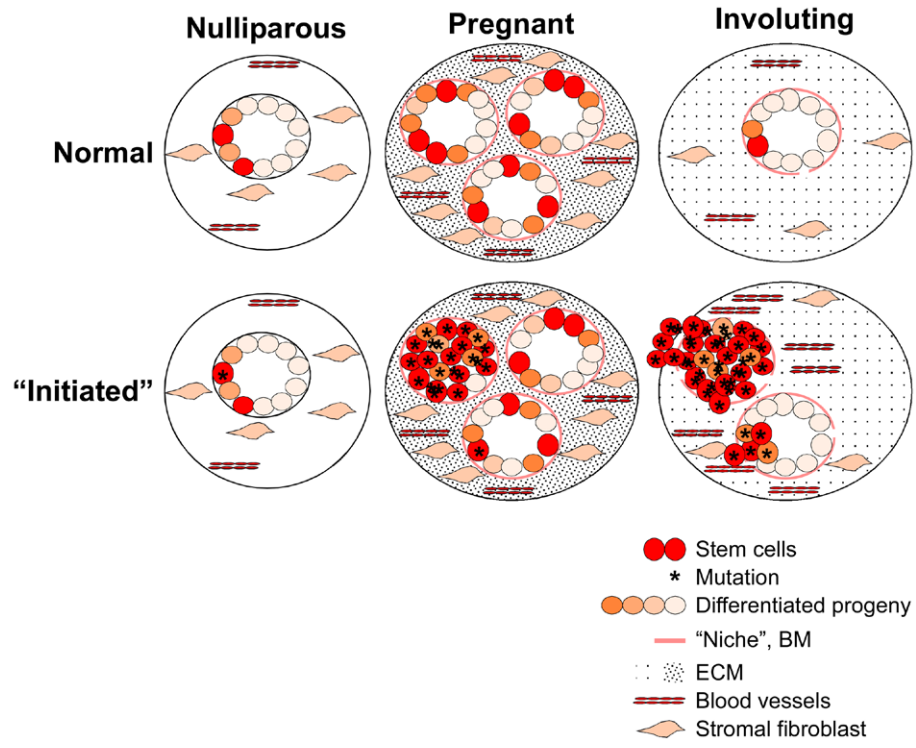

Figure 1. Hypothetical model depicting the potential contribution of pregnancy-induced changes in the cellular microenvironment to breast tumor progression

During pregnancy, the number of mammary epithelial cells is dramatically increased, potentially at least in part due to the expansion of the stem and/or transit-amplifying cell population. This dramatic epithelial cell proliferation is accompanied by an increase in angiogenesis and the number of stromal fibroblasts, as well as changes in ECM. Pregnancy and involution may also alter the stem cell niche and basement membrane (BM). Following lactation and involution, the number of mammary epithelial cells, including stem cells, is decreased, together with breast density due to degradation of ECM components. If pregnancy occurs in a woman whose breast already contains an "initiated" stem cell that has acquired a tumor-initiating genetic alteration (*), or such an initiating event occurs during pregnancy, then the number of these cells can also increase, allowing for the acquisition of additional genetic alterations and clonal selection due to expanded population size. Increased proteolysis leading to ECM degradation and potential focal disruption of the BM during involution could promote the further progression of these early-stage tumors, resulting in invasion and metastatic spreading.

ing on the study) after a woman gives birth has a worse clinical outcome than tumors not associated with a recent pregnancy, even after adjusting for known prognostic determinants (Daling et al., 2002). Multiple hypotheses have been formulated to explain this apparent paradox. Women in this age group are not routinely screened by mammography; thus, there could be a delay in diagnosis leading to the detection of tumors at a more advanced stage. Decreased immune surveillance due to a transient immunosuppressive state, relative insulin resistance, and associated high circulating insulin levels, and altered melatonin levels induced by pregnancy and childbirth, have also been suggested as factors contributing to the worse outcome of tumors diagnosed shortly after pregnancy. Pregnancy-associated hormones, particularly prolactin, have been implicated as promoters of tumor cell growth and progression. Supporting this idea, a large fraction of breast tumors express prolactin and its receptor; thus, in addition to its systemic effects, prolactin may also act as a local autocrine/paracrine factor.

Almost all epidemiological and molecular studies have focused on factors affecting breast epithelial cells, while the potential contribution of the microenvironment and extracellular matrix (ECM) composition to breast cancer risk and disease progression has not been thoroughly investigated. Many lines of evidence suggest that these components may play important roles in breast cancer. High mammographic breast density is a significant risk factor for breast cancer, but the molecular mechanism underlying this observation is unknown. Polymorphism in CXCL12 (SDF-1), a chemokine that is overexpressed in breast tumor stroma and promotes tumor cell invasion and metastasis, has recently been demonstrated to be associated with increased breast cancer risk. Data obtained in numerous model systems and human cancer types describing alterations in stromal cells and ECM composition during tumor progression suggest that changes in the microenvironment and tissue architecture contribute to tumorigenesis (Bissell et al., 2003). Correlating with this, a gene expression signature reflecting activated stroma that is characteristic of tumors and wound healing is associated with shorter overall and disease-free survival. Despite all this evidence indicating that pathologic changes in the microenvironment influence tumor progression and clinical outcome, the role of physiologic alterations in breast tissue architecture in breast tumorigenesis has not been analyzed.

The mammary gland is a unique organ in that it undergoes dramatic tissue remodeling depending on endocrine status. Monthly fluctuations in hormone levels, pregnancy, lactation, and involution all induce dramatic changes in the composition of the breast microenvironment and ECM, similar to that observed during wound healing and inflammation. All these alterations could influence tumorigenesis and could potentially explain the worse outcome of breast tumors diagnosed shortly after pregnancy (Figure 1). Testing these hypotheses was the goal of a study by McDaniel et al. published in a recent issue of *American Journal of Pathology* (McDaniel et al., 2006).

McDaniel and colleagues used a three-dimensional in vitro culture assay and a xenograft model of breast cancer to investigate the role of physiologic changes in ECM composition on mammary epithelial and tumor cell behavior. First, they determined that ECM isolated from involuting rat mammary glands has increased fibrillar collagen, fragmented laminin, and fibronectin content and elevated MMP (matrix metallo-protease) activity compared to ECM purified from nulliparous glands. Next, they analyzed the growth of MCF-12A immortalized human mammary epithelial cells on matrix pads containing nulliparous or involuting matrix, or Matrigel (ECM produced by tumor cells). Cells grown on nulliparous matrix formed duct-like structures, while those plated on involuting matrix and Matrigel grew as spheroids, suggesting that only nulliparous matrix contains factors necessary for branching behavior. In contrast to the nontumorigenic MCF-12A cells, a highly tumorigenic variant of the MDA-MB-231 breast cancer cell line only grew as disorganized organoids invading the surrounding stroma in all three matrices tested. Analysis of the migratory and invasive behavior of both cell lines in transwell insert assays revealed that nontumorigenic MCF-12A cells were immotile and noninvasive with all matrices, while the invasion but not the migration of MDA-MB-231 cells was influenced by the matrix with the highest response observed to involuting matrix. To investigate if involuting matrix also promoted tumor invasion and metastasis in vivo, McDaniel and coworkers mixed MDA-MB-231 cells with various matrices, injected them orthotopically into the mammary fat pad of immunodeficient mice, and analyzed tumor growth and metastatic behavior. While primary tumor growth was not affected by the origin of the admixed matrix, metastasis to the lung, liver, and kidney was significantly promoted by involuting matrix. To begin dissecting the mechanisms by which the involuting matrix may exert this effect, McDaniel et al. observed increased angiogenesis and VEGF levels in primary tumors originating from cells mixed with involuting matrix. Additionally, these tumors contained higher numbers of smooth muscle actin-positive mouse myofibroblasts, cells characteristic of reactive stroma.

The study by McDaniel et al. highlights the importance of ECM in breast tumorigenesis and warrants further studies in this area. Specifically, the relevance of the results for human disease has to be evaluated, and the molecular processes underlying the observed effects have to be deciphered in order to move these findings closer to clinical relevance and application. Interestingly, prior studies from the same lab have demonstrated that the protumorigenic effects of the involuting ECM may be breast cancer subtype dependent, since it was only observed with the ER-negative MDA-MB-435 and MDA-MB-231 breast cancer cells, but not with the ER-positive cell line MCF-7, nor with a nonmetastatic variant of the MDA-MB-435 cells (Bemis and Schedin, 2000). Thus, once again it appears that tumorigenesis is the result of a symbiotic relationship between transformed tumor epithelial cells and their microenvironment. Changes in both tumor and stromal cells are necessary for breast tumor progression, therefore therapeutically targeting both at the same time may be the optimal approach to harness and potentially conquer this cancer.

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Kornelia Polyak[1,2,*]

[1]Department of Medical Oncology, Dana-Farber Cancer Institute, 44 Binney Street, Boston, Massachusetts 02115
[2]Department of Medicine, Harvard Medical School and Brigham and Women's Hospital, 44 Binney Street, Boston, Massachusetts 02115
*E-mail: kornelia_polyak@dfci.harvard.edu

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IRS-1: Auditing the effectiveness of mTOR inhibitors

Rapamycin analogs that inhibit mTOR signaling have antitumor activity against certain lymphomas, but treatment of solid tumors has been less encouraging despite inhibition of mTOR function. Two recent papers give insight into the potential use of mTOR inhibitors. O'Reilly et al. provide evidence that poor tumor response to rapamycins is the result of relieving mTOR-mediated feedback inhibition of insulin receptor substrate 1, and activation of Akt-mediated survival. In the second paper, Kaper et al. address the impact of pathway activation on hypoxia-mediated downregulation of mTOR signaling, raising the possibility that rapalogs could selectively inhibit hypoxic cells.

Signaling pathways upstream of mTOR are dysregulated in multiple human cancers, which might make them susceptible to mTOR inhibition (so-called "oncogene addiction"). For example, recent data from genomic sequencing of tumor samples identified mutations in *PIK3CA*, the gene encoding the p110α subunit of phosphatidylinositol 3′ kinase (PI3K) in approximately 30% of colon and breast cancers. Although not all of the mutations have been shown to activate PI3K, there is supporting evidence that this might be the case, since the *PIK3CA* and *PTEN* mutations within the analyzed samples were mutually exclusive. This is consistent with the idea that both events lead to an increase in the second messenger 3′ phosphorylated inositol membrane lipids required to activate the kinase signaling cascade. *PTEN* deletions, or inactivating mutations, are frequently present in carcinomas of the breast and prostate and also gliomas.

The insulin-like growth factors (IGF-I/II) are also implicated in numerous cancers. The IGF/insulin pathway is unique in that, upon ligand binding and receptor autophosphorylation, insulin receptor substrate 1 (IRS-1) associates with these receptors. Tyrosine phosphorylation of IRS-1 in turn leads to the binding and activation of PI3K. The phosphorylation of inositol membrane lipids at the 3′ position by PI3K is a critical step in the IGF-IR signaling pathway. A number of kinases have been identified that associate with these 3′ phosphorylated membrane lipids and subsequently participate in the kinase signaling cascade. Akt, one of the kinases in this activation cascade, has a distinct function in promoting cell survival by phosphorylating and blocking the proapoptotic activity of proteins such as BAD, FoxO transcription factors, and GSK-3α/β (reviewed in Plas and Thompson, 2005) (Figure 1). Akt also positively controls cell proliferation via phosphorylation and inactivation of TSC2, thereby blocking the inhibitory effects of the tuberous sclerosis complex (TSC) on the rapamycin-sensitive mTOR complex (mTORC1) (Inoki et al., 2002; Potter et al., 2002; Dan et al., 2002). The TSC complex, along with Rheb which is considered an activator of mTOR, facilitates mTORC1 sensing of the cellular environment. The activity of mTORC1 is negatively regulated by amino acid deprivation, elevated AMP, or low O_2 (Bjornsti and Houghton, 2004). Activated mTORC1 (comprised of mTOR, Raptor, and GβL/mLST8) phosphorylates 4EBP1 and S6K1. Phosphorylation of 4EBP1 results in its release from the translation initiation factor eIF4E, and the assembly of the preinitiation translation complex eIF4G (Pause et al., 1994). Activation of mTORC1 also leads to phosphorylation of S6K1, which is required for its full activation by PDK1, and assembly of the preinitiation complex required for efficient translation of RNAs with secondary 5′ structures (Holz et al., 2005). Importantly, activation of S6K1 represses upstream signaling through phosphorylation of IRS-1 and its subsequent proteasome-mediated degradation (Haruta et al., 2000).

Rationale for combining inhibitors in the mTOR pathway

Rapamycin, a macrocylic antibiotic in complex with a 12 kDa immunophillin (FKBP12) potently inhibits mTORC1 signaling, resulting in cytostatic or cytotoxic effects on cancer cells. Consistent with the "oncogene addiction" hypothesis, several studies have indicated that cells lacking PTEN function, and hence constitutive Akt activation, are hypersensitive to rapamycin (reviewed in Hay, 2005). However, there are clear exceptions, and reexpression of PTEN does not necessarily induce resistance to rapamycin. Other factors clearly modulate sensitivity to rapamycins. IGF-I and, less potently, insulin are unique among growth factors tested in their ability to overcome the inhibitory effects of rapamycin on proliferation and apoptosis (Hosoi et al., 1999). However, for most cancer cells under normal growth conditions with exogenous growth factors, the effect of rapamycin and its analogs is largely cytostatic, leading to decreased transit through G1 phase and