Homeostasis in the breast: It takes a village

Progression from normal to malignant phenotype involves aberrations in the reciprocal interactions of multiple cell types with each other and with other components of the microenvironment. In this issue of *Cancer Cell*, Allinen et al. (2004) demonstrate that progression to breast cancer involves genotypic as well as gene expression changes that are cell type-specific, suggesting that targeted therapies delivered to the tumor may need to include drugs targeted not only to the tumor cells, but also to the other cell types in the tumor microenvironment.

Maintaining homeostasis within any tissue, including breast, exhibits striking similarities to keeping a society civilized. In both cases, fostering balanced and functional relationships among members is crucial to achieve harmony. The principal function of the normal breast is to produce milk when needed. To achieve this, multiple cell types communicate and alter each other's behavior within a microenvironment with which they share a reciprocal relationship. Milk produced by the layer of luminal epithelial cells is secreted into the ductal lumen and is transferred along the duct with the help of contractile myoepithelial cells that surround the luminal epithelial cells. The double-layered ductal or acinar structures are encapsulated within a basement membrane (BM) in the normal breast. The compartment outside the ductal tree contains stromal-type extracellular matrix (ECM) components such as collagen I and elastin, adipose cells, fibroblasts, immune cells such as leukocytes and mast cells, and blood vessels. The confined nature of the "double-layered tube" within the surrounding BM, and the exquisitely choreographed interactions among the epithelial cells, the BM itself, and the surrounding stroma, are all necessary for the organization and architecture of the breast, which in turn are necessary for its functional integrity (Bissell et al., 2003).

In a society, loss of values that keep cohesive interactions can lead to breakup of the infrastructure and development of rogue elements that escape societal boundaries. Similarly, perturbations in stromal-epithelial or cell-ECM interactions and changes in hormonal and cytokine milieu can initiate cascades of events and responses, the end result of which could be loss of polarity and disruption of the epithelial compartment within the BM, leading to invasion, a hallmark of malignancy. The progression from normal to malignant breast is defined by pathological stages as determined by histological and cellular characteristics of the tissue. Hyperplasia (usual or atypical), ductal carcinoma in situ (DCIS), and invasive carcinoma are some of the easily decipherable stages of breast cancer progression (Figure 1). Acquisition of malignancy is accompanied by changes in cell morphology, function, and genome integrity, as well as metaplastic changes in cell behavior such as epithelial-to-mesenchymal transition and fibroblast-to-myofibroblast conversions in the stroma (Ronnov-Jessen et al., 1995). Most malignant tumors are a disorganized mass of cells and stromal components constantly reinventing themselves as "new organs" (Bissell and Radisky, 2001).

Defining the processes by which a functional, hormone-responsive gland, wired to produce life-promoting milk, turns into a malignant time bomb requires a complete understanding of both the functions of the individual cell types and how they interact with each other and with their microenvironment. Since the majority of breast tumors express luminal epithelial cell markers, most of the work done in delineating the molecular determinants of breast cancer progression has so far focused on this epithelial cell type. However, it is becoming increasingly clear that all cell types and stromal components will need to be studied in order to get a complete and accurate picture of malignant transformation. For example, myoepithelial cells have been emerging as a crucial component of breast function, and were shown to have a gene expression profile which implicated them as possible architectural tumor suppressors of the breast (Barsky, 2003; Gudjonsson et al., 2002; Jones et al., 2004).

A comprehensive study in this issue of *Cancer Cell* by Allinen et al. (2004) has taken this approach to determine the molecular alterations that accompany progression from normal to malignant phenotype in the luminal epithelial, myoepithelial, and stromal components of the breast. In order to study all cell

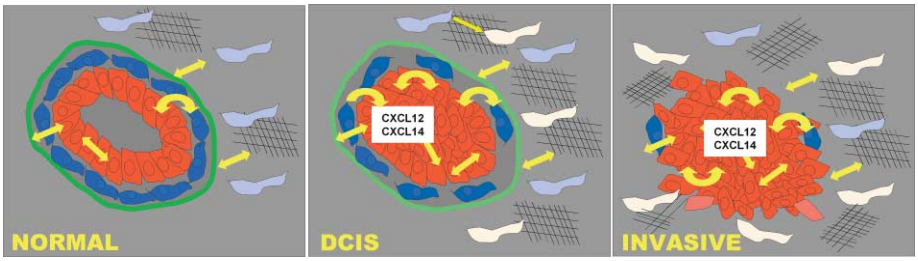

Figure 1. Genetic and gene expression changes in breast cancer progression are cell type-specific

Progression from the normal tissue architecture to ductal carcinoma in situ (DCIS) to invasive cancer involves changes in and interactions among (shown by yellow arrows) the double-layered epithelium of luminal epithelial cells on the inside (red) and myoepithelial cells on the outside (blue) enclosed within a basement membrane (green); there are changes also in the stromal component which contains fibroblasts (light blue), adipose, immune, and endothelial cells (not shown), and stromal ECM (black mesh). DCIS contains disorganized, hyperproliferating luminal epithelial cells and an aberrant stroma with conversion of fibroblast to myofibroblasts (white), but an apparently intact basement membrane. Invasive carcinoma is defined by loss of BM integrity, loss of myoepithelial cells, and additional cell type conversions such as epithelial-to-mesenchymal transition (from a red cell to a pink cell). Luminal epithelial cells are prominent in invasive cancers and are genomically unstable, whereas myoepithelial cells show the most significant gene expression changes in progression but no apparent genomic instability. Paracrine interactions between myoepithelial and luminal epithelial cells that involve chemokines CXCL12 and CXCL14 enhance cell proliferation, migration, and invasion, starting at the DCIS stage.

types involved in breast cancer progression, and to do so without altering their properties during the process of isolation from the breast, Kornelia Polyak's group improved existing protocols (Page et al., 1999; Pechoux et al., 1999) to obtain not only purified luminal epithelial and myoepithelial cells but also endothelial cells, leukocytes, and myofibroblasts. The remaining fraction was used as a source of semipurified fibroblasts, since no specific fibroblast cell surface marker is yet available. Cells isolated from normal, in situ carcinoma, or invasive carcinoma were examined by array CGH and SNP arrays to determine genetic alterations, and by SAGE to determine gene expression profiles. Results for both the genomic integrity and the gene expression analysis clearly demonstrate the utility of studying each cell type separately, with particular emphasis on the significance of myoepithelial cells in tumor progression. Interestingly, Alinnen et al. found that unlike luminal epithelial cells, myoepithelial cells displayed no detectable genomic abnormalities. However, the most dramatic gene expression changes in tumor progression occurred in myoepithelial cells already at the DCIS stage.

Of the gene classes that are altered , Allinen et al. focus on chemokines for further analysis, and find that expression of CXCL12 and CXCL14 is highly upregulated in myoepithelial cells and myofibroblasts in DCIS and invasive carcinomas. By immunohistochemistry, CXCL14 was found also in luminal epithelial cells in a subset of invasive tumors and malignant breast cancer cell lines. Allinen et al. demonstrate that CXCL14 can bind to both normal and malignant luminal epithelial cells and cell lines, suggesting the presence of a receptor on luminal cells. CXCL12 and CXCL14 could also increase cell proliferation, migration, and invasion of luminal epithelial cell lines in culture. These findings are consistent with previous studies that implicate altered chemokine expression levels as an indicator of progression to tumorigenicity (Porter et al., 2001, 2003) and metastatic capacity (Muller et al., 2001), and suggest a paracrine mechanism of action. Inhibition of CXCR4, the receptor for CXCL12, has been shown to prevent breast cancer metastasis (Liang et al., 2004). Allinen et al. raise the possibility of using such inhibitors to treat early-stage breast cancers as well as the advanced metastatic cancers.

Another important conclusion of Allinen et al.'s studies is the demonstration that sufficient material can be obtained to study gene expression and changes in genotype in most cell types within the breast tissue, using the technical improvements introduced. Even though the number of tissue samples was small, it was possible to discover cell type-specific markers of breast cancer progression. Such studies would have enormous implications for targeted therapies should a large enough number of samples become available. A comprehensive profiling of the molecular determinants of breast tumor progression would also require access to tissues from the intermediate stages of tumor progression from the same patient, given the known heterogeneity of the tumorigenic process. These, however, are hard to come by in human tumors. Indeed, we have very few studies where such a requirement has been fulfilled for breast tumors. The Polyak group now provide the valuable proof-of-principle study to motivate large scale efforts to accomplish this goal at a statistically significant manner for person-specific, as well as cell type-specific, therapies. To thoroughly understand breast cancer with all its cell type-specific and other microenvironmental changes, joint efforts and multi-institutional collaborations to pool tissues are necessary. We now know that conquering cancer will also take a village!

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Selected reading

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