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Stromal Effects in Breast Cancer

Edison T. Liu, M.D.

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Investigations into the interaction between epithelial cancer cells and adjacent stroma have revealed paracrine interactions that enhance the tumorigenic characteristics of the cancer cell.¹ Tumor-associated stroma have been reported to have increased motility and invasive potential and are thought to secrete distinct, abnormal molecules into the extracellular matrix. Although we once assumed that all fibroblasts are alike, we now know that stromal fibroblasts from different tissues have different gene-expression profiles, even when cultivated under the same culture conditions. All the data suggest that even fibroblasts of similar appearance have markedly different characteristics. Still, the primary assumption is that tumor-associated fibroblasts are genetically normal and are different from the cells of epithelial cancers with which they interact, since the two types of cells are from distinct embryonic lineages.

For this reason, the work by Patocs et al. in this issue of the *Journal* (pages 2543–2551) will come as a surprise to many. The investigators used laser-capture microdissection to examine the stromal and epithelial compartments of primary breast cancers and found that the stromal compartment harbors genetic changes — mutations and loss of heterozygosity of the tumor-suppressor gene *TP53* — that differ from the

genetic changes in the epithelial compartment. In over one fourth of the tumors, the stromal cells appeared to harbor *TP53* mutations in the absence of any perturbations of that gene in the cancer cells. Moreover, the presence of *TP53* mutations in the stroma was significantly associated with lymph-node metastases.

The first response to these results might be disbelief and the suggestion that the findings are artifactual. However, the methods followed appear to have been precise and meticulous. Moreover, the results seem to be consistent with mechanisms that we already understand. For example, the finding that 74% of the tumors from *BRCA1* carriers had *TP53* mutations, as compared with 42% of the tumors from noncarriers, is consistent both with previous findings and with the mechanistic framework of the *BRCA1*–*p53* interaction. My colleagues and I have found that *BRCA1* and *p53* cooperatively regulate the induction of 14-3-3 σ (a protein that binds to numerous signaling proteins) after genotoxic challenge and that the loss of this checkpoint regulator may contribute to mammary carcinogenesis. We speculated, on the basis of this result, that carriers of the *BRCA1* mutation would have breast cancers that show an associated loss of *p53*.² Patocs et al. also found that loss of heterozygosity was more frequent in tumors with a

mutant *TP53* than in those with wild-type *TP53*, which is consistent with the role of *p53* in maintaining genomic stability. Moreover, there was no overlap in the loss-of-heterozygosity profile between the epithelial cancer and the tumor-associated stroma, which suggests that different pathways of clonal expansion are involved.

How can tumor-associated stromal fibroblasts have oncogenic mutations? Patocs et al. do not address this question, but it deserves speculation. One possible explanation is that stromal fibroblasts surrounding the tumor are actually derived from the same epithelial progenitor cell as the tumor. Indeed, epithelial–mesenchymal transition has been implicated in the generation of tumor-associated myofibroblasts,³ but in that case, the cancer cells and the myofibroblasts are of the same genetic lineage and so should have mutations in common. In the study by Patocs et al., the epithelial cancers and associated stromal fibroblasts did not share common mutations. So where did the mutations come from?

Cancer cells or the cancer milieu, which includes tumor-associated immune cells, can generate high levels of reactive oxygen species. These, in turn, can damage DNA and cause mutations. Also, tissues recovering from cytotoxic insults can undergo oligoclonal expansion, and a mutant clone of stromal fibroblasts could

therefore emerge along with its cancer “partner,” each feeding the other through mechanisms that include paracrine signaling.

The “field effect” in carcinogenesis, in which a seemingly normal epithelium is the result of the clonal expansion of a genetically abnormal clone, has been documented in colon and lung cancers.⁴ The literature is dotted with reports of the harboring in normal breast tissue of mutations, including *TP53* mutations, *Her2* amplification, loss of heterozygosity, and mitochondrial mutations. There is even some evidence that these mutations are associated with susceptibility to disease.⁵ Frequently dismissed as experimental oddities, these results should probably be reexamined in light of the more definitive evidence presented by Patocs et al.

On a practical level, does this work mean that marker studies in breast cancer must now use laser-capture microdissection to separate epithelial cells from stromal cells and that biomarkers must be analyzed in each compartment? Such a standard might be appropriate for detailed studies of disease mechanisms, but I think not for routine clinical care.

In addition to the problem of laser-capture microdissection's being time-consuming and expensive, entirely new standards would have to be formulated for the quality and quantity of cells need-

ed for the compartmental analysis. These implications render such an approach impractical. Moreover, there may not be any need for such refined separation for clinical purposes. Certain mutations in either compartment, stromal or epithelial, are associated with a poor prognosis. The debate over whether or not to dissect has been running through the expression-array community for a long time, with purists agitating for precise determinations of where somatic mutations reside and the “lumpers” saying it doesn't matter.

In the realm of mechanistic science, I am a purist, but in clinical practice, I am a lumpster. All the expression-array studies that investigate the association between the outcome of breast cancer and the presence of transcript-based biomarkers use whole tumors, not microdissected tissue. The molecular and correlative findings are firm and reproducible — so here I am guided by Voltaire's dictum that “the perfect is the enemy of the good.”

What are the implications of this work for breast cancer research? It should spur more studies that analyze normal breast stromal tissues for possible mutations, with the use of precise and multiplex genomic technology. Epigenetic and transcriptional profiling will allow us to further analyze the molecular differenc-

es between fibroblasts from various tissues and various cancers. Better growth mediums and substrates may assist in the expansion of such tissue-associated fibroblasts. Developmental biologists can help by better assessing the characteristics of fibroblasts from various embryonic anlagen and their molecular correlates. Such work could open the door for an alternative approach to cancer prevention, since the specific intervention of stromal paracrine secretion might substantially suppress the progression of cancer. All of this augurs well for breast cancer research.

Dr. Liu is the executive director of the Genome Institute of Singapore at the Agency for Science, Technology, and Research, Singapore.

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