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Review

Cancer Research

Pten in the Breast Tumor Microenvironment: Modeling Tumor–Stroma Coevolution

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Abstract

Solid human tumors and their surrounding microenvironment are hypothesized to coevolve in a manner that promotes tumor growth, invasiveness, and spread. Mouse models of cancer have focused on genetic changes in the epithelial tumor cells and therefore have not robustly tested this hypothesis. We have recently developed a murine breast cancer model that ablates the *PTEN* tumor suppressor pathway in stromal fibroblasts. Remarkably, the model resembles human breast tumors both at morphologic and molecular levels. We propose that such models reflect subtypes of tumor–stromal coevolution relevant to human breast cancer, and will therefore be useful in defining the mechanisms that underpin tumor–stroma cross-talk. Additionally, these models should also aid in molecularly classifying human breast tumors on the basis of both the microenvironment subtypes they contain as well as on the tumor subtype. *Cancer Res; 71(4); 1203–7. ©2011 AACR.*

Tumor Fibroblasts Promote Tumor Progression

Over the past decade, the idea that the cells comprising the tumor microenvironment contribute to the initiation, growth, and spread of solid tumors has become generally accepted in cancer biology. Of note, stromal fibroblasts have drawn attention as a pivotal cell type capable of shaping both the architecture of the microenvironment and modulating communication between the various cell types present, through effects on the extracellular matrix (ECM) and by secretion of various growth factors and cytokines (1). Seminal studies using mouse genetic model systems showed the contribution of stromal fibroblasts to tumor initiation and progression (2, 3). For example, disruption of the *TGF-βRII* gene in stromal fibroblasts resulted in carcinoma of the forestomach (2), and loss of *p53* function in the stromal fibroblasts preceded *p53* inactivation in the epithelium in a prostate cancer model (3). Using a different approach to study the same underlying concepts, introduction of tumor-associated fibroblasts along with tumor cells into immune-compromised mice leads to more robust tumor growth (4, 5). Although these models highlight the critical function of fibroblasts in tumor progression, their relevance to the human tumor microenvironment has not been well established. Indeed, mouse models of cancer have incompletely recapitulated the histopathology of human tumors. In particular, breast cancer models lack the extensive ECM found in most human breast tumors.

Conversely, important studies using human breast cancer samples have shown that stromal fibroblasts acquire epigenetic changes in the tumor microenvironment, and perhaps even genetic changes in tumor suppressor genes like *TP53* and *PTEN* (6–8). Such human studies may identify potential genes that function from the stroma to promote mammary tumor growth, but are unable to provide direct experimental evidence showing that such genes contribute to tumor progression.

Therefore, although we know that stromal fibroblasts are critical for tumor progression, the genes and signaling pathways that are relevant to human tumor progression have remained largely elusive until recently.

Pten Tumor Suppressor Function in Mammary Stromal Fibroblasts

Our group set out to develop mouse models that more accurately recapitulated the human breast cancer microenvironment, at both histopathologic and molecular levels. We developed a transgenic tool, *FSP-cre*, on the basis of the promoter for *Fibroblast-specific protein 1/S100A4* (9) to delete genes in fibroblasts. We chose to interrogate the *Pten* tumor suppressor pathway in mouse breast cancer models as a first approach. Although somatic mutations of tumor suppressors like *PTEN* in human stromal fibroblasts remain controversial (10, 11), the conserved function of the *PTEN* pathway in maintaining homeostasis in many cell types is well established. In addition, Cowden syndrome patients contain germline mutations in *PTEN* that predispose them to breast cancer, suggesting the possibility that loss of *PTEN*

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both in stromal cells and epithelial cells can affect tumor development.

Pten-loxP alleles were combined with *FSP-cre* in the well characterized *MMTV-Erbb2* breast cancer model. The *RosaA-stop-loxP-lacZ* allele (12) was also at hand, allowing the fibroblasts to be genetically marked. A mammary fat pad transplantation model was adapted to ensure the effects observed on tumor progression were due to *Pten* deletion in fibroblasts located in the mammary gland, and not indirectly by deletion in other tissues or organs. Several noteworthy outcomes emerged from the analysis of this model (13):

1) Tumor incidence and tumor load in mice with fibroblast-specific deletion of *Pten* were significantly increased compared with controls. Notably, although *Pten* deletion in fibroblasts promoted tumor growth, epithelial transformation was not observed in the absence of oncogene, showing requisite interaction between *Pten* signaling in stromal fibroblasts and *ErbB2* signaling in epithelial cells in the development of the observed phenotype;
2) LacZ staining and PTEN immunohistochemistry showed that the fibroblasts present in the transplant were derived from the host and were present during the initial transplant, 16 weeks before tumors were first detected in the Pten-fibroblast deleted model. Thus, resident fibroblasts, and not cells recruited from bone marrow or other tissues, were sufficient to elicit the observed phenotype during tumor progression;
3) Tumors with *Pten* deletion in fibroblasts had extensive expansion of the ECM, including increased collagen levels, and the tumor histology remarkably resembled human breast tumors;
4) Gene expression profiling of the Pten-null fibroblasts compared with controls revealed deregulation of genes involved in inflammation, angiogenesis, and ECM remodeling. Strikingly, a dramatic increase in macrophages, but not other innate or adaptive immune cell types, was observed even in the absence of epithelial oncogene expression. These results indicate that tumor fibroblasts have a central role in promoting inflammation in the breast tumor microenvironment. A subsequent study by Erez and colleagues has confirmed the important function of fibroblasts in tumor inflammation (14). In contrast to the findings of this group, the NFκB pathway is not activated in our *Pten*-null tumor fibroblasts.
5) Instead, expression profiling also revealed that transcription factor *Ets2* was upregulated upon *Pten* deletion. Ets2 is a target of extracellular signal regulated kinase (Erk) and c-Jun NH, kinase (JNK) mitogen-activated kinase pathways, both of which are activated in the *Pten*-null fibroblasts. We showed that deletion of *Ets2* in stromal fibroblasts in the *MMTV-PyMT* model caused significantly reduced tumor growth through decreased matrix metalloproteinase 9 (MMP-9) activity in the ECM and reduced VEGF signaling in endothelial cells. Using a double knockout strategy, we were able to show that *Ets2* deletion in *Pten*-null fibroblasts restricted tumor growth when compared with *Pten* knockout alone in an orthotopic injection model. Recruitment of both macrophages and endothelial cells was diminished in these double knockout mammary glands.
6) Immunohistochemistry of a breast tumor tissue microarray showed that PTEN expression was diminished in the stroma of approximately 50% of the samples surveyed, and a significant inverse correlation was observed between PTEN expression and the expression of activated ETS2 and AKT.
7) Comparison of the gene expression signature derived from *Pten*-null fibroblasts to gene expression data obtained from human cancer stroma and matched normal stroma (15) revealed that a 70-gene *Pten*-dependent signature was able to completely separate normal stroma from breast cancer stroma and to predict patient outcome.

These observations show the importance of the PTEN-Ets2 axis in stromal fibroblasts in the *MMTV-ErbB2* model in suppressing breast cancer growth and indicate the stromal pathway contributes to the complexity of human breast cancer stroma.

Pten Collaboration Is Oncogene Specific: Modeling Coevolution in the Tumor Microenvironment?

In companion studies, the effect of *Pten* fibroblast deletion in the *MMTV-myc* and *MMTV-ras* models yielded unexpected and surprising results (G. Leone and M.C. Ostrowski, unpublished results). Deleting *Pten* in fibroblasts with epithelial *c-myc* overexpression provided an even more dramatic effect than the *ErbB2* results, as more than 90% of the transplanted mammary glands had developed large tumors by 26 weeks compared with less than 10% of the *c-myc*–alone controls. In contrast, *Pten* deletion in the *ras* model had no effect: few tumors arose in either the *Pten*-null or *Pten*–wild-type genetic groups after 26 weeks. Intriguingly, the histopathology of the stroma with *Pten* deletion in the 3 models was very similar, with expanded ECM, increased macrophage infiltration, and increased angiogenesis. So how do we interpret these results?

Our working hypothesis is that the defined genetic changes engineered into both epithelial and stromal cell compartments in our models reflect the coevolution of tumor and stroma that occurs in a spontaneous manner in human breast cancer. For example, an ERBB2-overexpressing human tumor would be more aggressive if it coevolved with PTEN loss in the stroma. One tenet of this model is that the final breast tumor subtype is composed of both epithelial and stromal components (Fig. 1). The fact that both tumor epithelium and stromal components contribute to the biological diversity of breast cancer is well established (16, 17).

An important implication of the working hypothesis is that multiple stromal subtypes exist in human breast tumors. For example, an epithelial tumor cell with ERBB2 amplification

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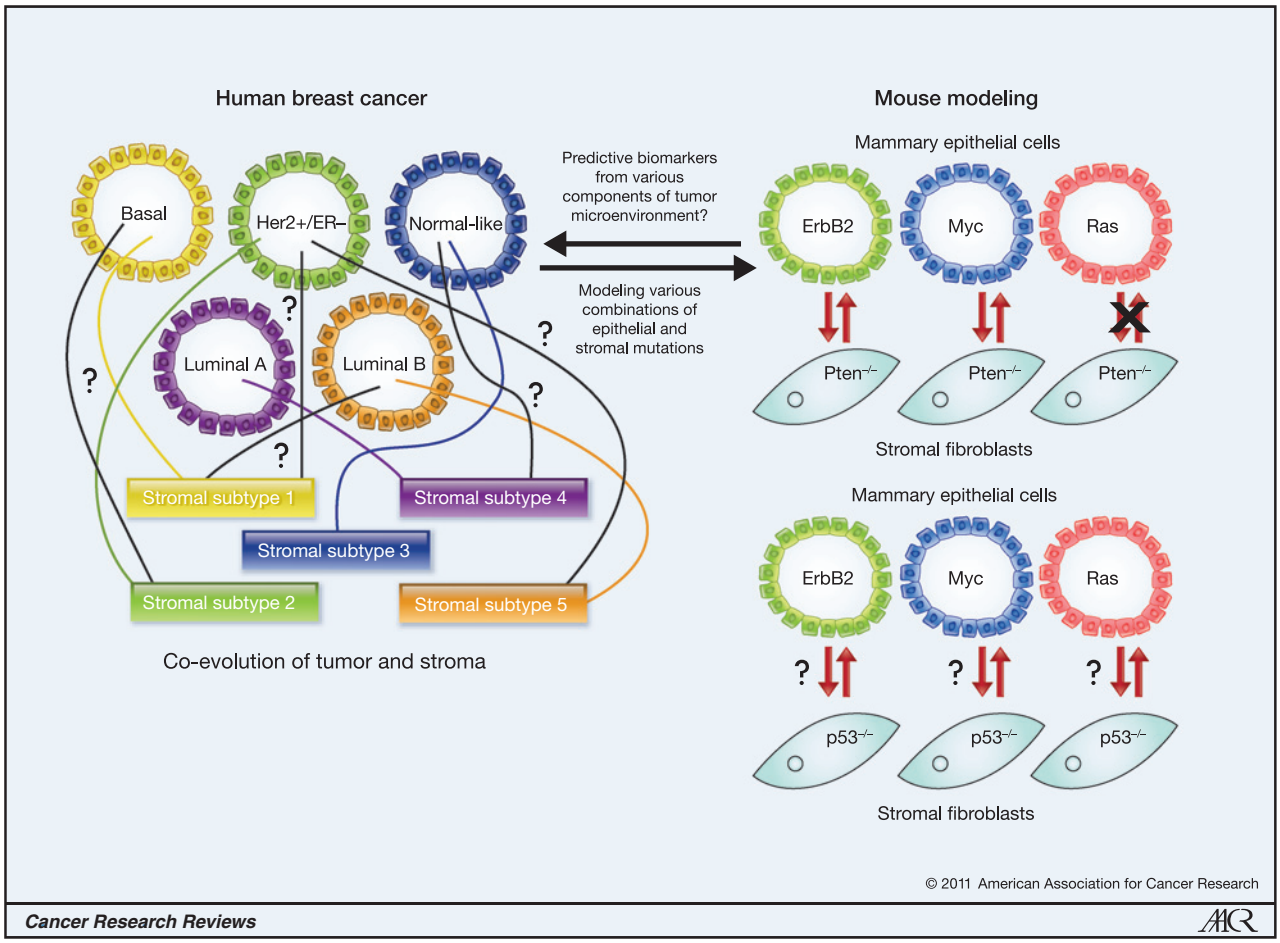

Figure 1. Modeling tumor–stroma coevolution: spontaneous development of tumor subtypes and stromal subtypes in human breast cancer. Collaboration of both the epithelial and stromal compartments during tumor development contributes to the biological diversity in breast cancer and could be associated with various clinical parameters and patient outcomes; that is, a basal-like tumor developing in combination with stromal subtype 1 may be more aggressive or more resistant to treatment than the same basal-like tumor developing simultaneously with stromal subtype 4. Conversely, the presence of a particular subtype of stroma might adversely affect growth of a particular tumor subtype, thereby predicting a better patient outcome. By examining the effects of stromal *Pten* signaling in several models of breast cancer in mice, we were able to show cooperation between *Pten* and both *ErbB2* and *c-myc*, however, not between *Pten* and *Ras*. Similar experiments are underway to examine the collaboration of *p53* in stromal fibroblasts with various oncogenes in mammary epithelial cells. Using gene signatures from various cell types in these and other models currently being developed, we will have a powerful tool in predictive biomarkers for both human tumor and stromal samples.

could coevolve with different stromal subtypes, leading to distinct phenotypes and patient outcomes (Fig. 1). The heterogeneity in stromal subtype most likely reflects the genetic heterogeneity that occurs in tumor epithelial cells, for example, other genetic and epigenetic events likely occur in epithelial cells harboring ERBB2 amplification. In support of this notion, gene expression profiling of laser-captured human tumor stroma showed that multiple stromal subtypes exist and that these stromal subtypes are independent predictors of patient outcome (15). Patient response to specific therapies and final outcomes likely depends on this coevolution of tumor and stroma.

A prediction of the hypothesis is that the mouse models showing synergy between stromal and epithelial changes correspond to distinct human tumor subtypes. If this idea is accurate, gene expression signatures from the mouse models can be compared in parallel to human stroma data to determine if this is the case. Our initial result with the Pten fibroblast signature suggests that this approach is tenable. We are expanding these studies to include expression profiles of the major cell types affected by *Pten* deletion in fibroblasts, for example, macrophages, endothelial cells, and epithelial tumor cells. Preliminary studies indicate that *Pten* deletion in fibroblasts affects gene expression in these compartments as well. The combined profiles of these microenvironment cell types should be more powerful for testing the idea of concordance with distinct human breast cancer subtypes. This approach may lead to the development of biomarkers for predicting treatment options that consist of markers expressed in several different stromal compartments.

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Another approach to test the hypotheses is to determine whether deletion of a different tumor suppressor in the stroma exhibits an altered spectrum of interactions with specific oncogenes present in the epithelial cell. The *p53* gene would be a good candidate because there is evidence for loss of expression of this gene in the tumor stroma during prostate cancer progression (3, 18). Deletion of *p53* in tumor fibroblasts should affect a distinct gene pathway in the stromal compartment, which should also correspond to different human gene expression patterns than in the *Pten* model.

Studying the Mechanisms That Control Stromal Interactions with the Tumor

These models and approaches also provide a means to determine the mechanisms underlying the communication between the various cellular components of the tumor microenvironment. By manipulating signaling in one cell type, it is possible to examine responding signaling pathways in other cell types. For example, we observed activated AKT, JNK, and ERK pathway signaling not only in fibroblasts with *Pten* deletion, but also in adjacent mammary epithelial cells even in the absence of oncogene (13). Whether these activated signaling pathways contribute to the oncogenic transformation of epithelial cells remains to be determined. In the future, a more detailed genomic, molecular, and biochemical characterization of different cell types in the microenvironment of mammary tumors will provide insights into how these cells interact. These types of studies will uncover the network of interactions that ultimately control tumor growth and spread. This research provides a significant technical challenge to the field, both experimentally and at the level of bioinformatics. Tools to connect different types of data obtained from mRNA and microRNA expression profiling, ChIP-seq, proteomics, DNA mutation and copy number profiling, imaging studies, and a multitude of other high-throughput data platforms rapidly becoming available, remain at an early stage of development, but will be required to create the robust network analysis needed for the task. Nevertheless, understanding these networks will identify the crucial molecular targets that can be experimentally verified and then used for the development of therapies aimed at blocking tumor–stroma interactions.

Among the many challenges facing the microenvironment field, perhaps the most daunting is identifying additional stromal-dependent pathways involved, especially pathways that are unique to the stroma, unlike the tumor suppressor pathway we identified initially. Functional genomic studies of human tumors may provide some clues, but such studies also yield a bewildering number of possibilities that makes attempts to model them in mice difficult. Taking a cue from the early studies of oncogene action, model systems including *Caenorhabditis elegans* and *Drosophila* may be useful in identifying pathways in one cell type that confer a phenotype in adjacent cells.

In conclusion, we have successfully modeled the breast tumor and its microenvironment and showed the relevance of the mouse genetic model to human breast cancers. The mechanism of tumor-stromal cross-talk and how it evolves depending on specific changes in the epithelial and stromal compartments can be revealed by detailed analysis of this and similar models. This research holds open the possibility of better classification of human tumors based on stromal properties in combination with those of the tumor cell, which will improve decisions about what current therapies may be useful for individual patients. Ultimately, these types of studies will lead to new therapeutic strategies that target the pathways through which stroma and tumor communicate.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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