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Can Stroma Reaction Predict Cancer Lethality?

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Stromal features in carcinomas may provide a relatively consistent means to stratify patients afflicted with solid tumors. Stroma-derived transcriptome signatures can now be used to make predictions about patient survival, suggesting the potential for their clinical application in precision medicine to predict disease progression and emergence of therapeutic resistance. *Clin Cancer Res; 19(18); 4905–7.* ©*2013 AACR.*

Stroma–cancer interactions recapitulate highly conserved molecular programs that are active during embryonic development. In response to cancer growth and progression, stromal reactions are believed to be specific and nonrandom and initiate a series of reciprocal chain reactions that modulate cancer progression. In this issue of *Clinical Cancer Research*, Chen and colleagues have taken a computational approach to determine whether cancer stroma gene expression profiles are able to predict survival of ovarian, lung, colon, and prostate cancer patients. Unlike other groups attempting to define stromal responses to cancer epithelium using less well-defined stromal signatures from cancer cases (1), the group led by Dr. Robert B. West pioneered an approach that involves characterizing gene expression patterns of two relatively pure populations of stromal cells isolated from soft-tissue tumors, the desmoid-type fibromatosis (DTF), and the solitary fibrous tumor (SFT). They tested the hypothesis that DTF and STF molecular signatures offer prognostic insight in differentiating the survival of breast cancer patients. The West group published two earlier articles (2, 3) about their work using datasets from different institutions in which they concluded that patients with breast carcinomas expressing the DTF fibroblastic signature had significantly better prognosis than patients expressing the SFT signature. The current publication is an extension of the previous work in which the authors set out to test another hypothesis, i.e., whether the usefulness of the DTF fibroblastic signature is specific to breast cancer or can be applied to other solid tumors. They analyzed gene expression profiles of five ovary, five lung, two colon, and three prostate cancer microarray datasets, and conducted immunohistochemical (IHC) analyses of two selected stromal biomarkers, osteonectin (SPARC), and versican (CSPG2), with a focus on whether the DTF signature can predict survival in these malignancies. They concluded that, in contrast with breast cancer, the DTF signature predicts worse outcome in ovarian cancer (rather then better outcome in the case of breast carcinoma). In this study, the authors noted that significant variations exist in the publicly available datasets subjected to their statistical analyses. Within five ovarian cancer datasets, one with a smaller number of patients failed to show statistical significance in predicting survival outcomes. Colon cancer datasets were discrepant. One DTF signature dataset showed a correlation with better patient survival. The other showed a correlation with worse survival. Further investigation of a new set of colon cancer tissues using a different technical approach revealed that positive IHC staining for SPARC and CSPG2 as surrogates for the DTF signature predicted worse colon cancer survival.

Overall, the idea of harnessing stromal fibroblastic signatures, rather than epithelial signatures, to predict the progression and survival of cancer patients has received substantial endorsement in the published literature (4, 5). The main reason stromal signatures may elicit greater predictive power in comparison with cancer epithelium is that cancer-associated stroma presumably contains a normal genotype. Stromal reactions in response to cancer epithelium most likely only affect gene expression, not drastic and stochastic disruptions of the genome. Because cancer and stroma coevolve during cancer progression (6, 7), many genes expressed by stroma are also expressed by cancer epithelium (7). A promising opportunity for continuing progress in understanding the clinical significance of stromal signatures is represented in attempts to use this type of information to identify and quantify soluble or cellular blood-based biomarkers. Nevertheless, although Chen and colleagues provide new approaches toward clinical translation of stromal analyses in ovarian cancer using the more homogenous DTF signature, the authors' conclusion that the "DTF fibroblast signature is a common tumor stroma signature in different types of cancer including ovarian, lung

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and colon carcinomas" may not be fully warranted based on the findings presented.

The concept that tumor–stroma interactions play key roles in dictating cancer growth and differentiation originated from the rich developmental biology literature, which has shown that local organ development is profoundly programmed by embryonic connective stromal tissues (8). In the context of cancer, these interactions are reciprocal and in tissue recombinant model systems their consequences can be either an acceleration (9) or inhibition (10) of tumor growth, depending on the composition and receptor subtypes in the chimeras. Therefore, it is not surprising that some stromal molecular signatures correlate positively with survival in one type of cancer but correlate inversely with that of another tumor type, or are not correlated at all. The stromal and epithelial composition of tumors is heterogeneous, comprising a large number of cell types represented in different proportions and with different lineage relationships. Their reciprocal interactions and intrinsic plasticity when interacting with growth factors and extracellular matrices (Fig. 1) are yet another source of variability in transcriptomics analyses. It is likely that future studies will define the molecular features of this heterogeneity and apply accordingly the knowledge of mathematical modeling learned from the analysis of clinical specimens on an individualized basis for improved prediction of cancer- or progression-free survival (11).

The disparity in results with the use of different public datasets could be viewed as a potential barrier for hypothesis testing. The number of patients in each database, the quality of specimens collected, the experimental protocols used to create such databases, including sample processing, instrumentation used for gene expression read-out, and bioinformatics methodology all contribute to variations in the quality of databases derived from patient material. In the study by Chen and colleagues, despite their use of multiple large datasets across several cancer types, significant disparities were noted in the predictive models developed, suggesting a lack of uniformity and unknown biases in the datasets. Hence, future studies must establish rigid criteria for database selection, incorporating or rejecting datasets using objective criteria that will increase the chances for prognostic success in a single patient. This type of study can be further strengthened by integrating transcriptome data with analyses of other molecular targets, such as quantitative protein data obtained using multiplex technologies. Methods are emerging allowing expression of proteins to be assessed quantitatively at the single-cell level using antibodies linked to quantum-dot nanoparticles with light emission at specific wavelengths. Because multiplex quantum-dot labeling technology has been validated by quantitative reverse-transcription PCR and Western blot (12), we suggest such technology may be applied together with

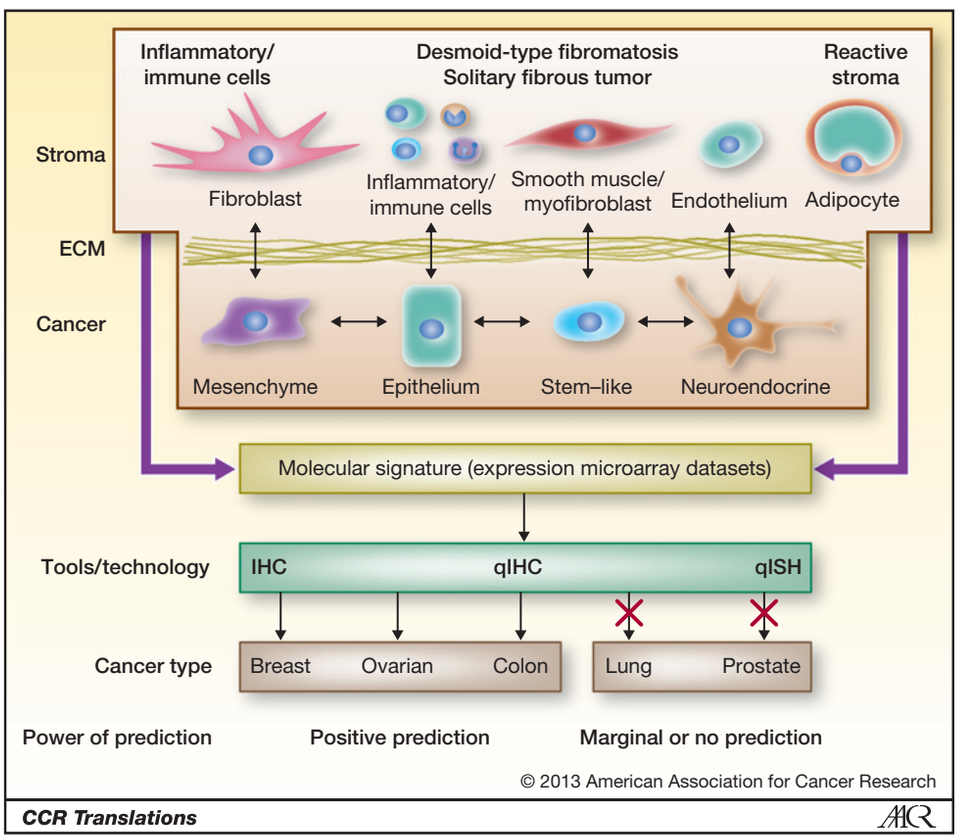

Figure 1. Reciprocal tumor–stroma interactions: the question of cellular heterogeneity and the potential for prediction of cancer progression and survival. Although DTF and SFT represent homogeneous, fibroblastic tumor stroma, other cell types, including inflammatory/immune cells, smooth muscle/myofybroblast, endothelium, and adipocytes, reside in the cancer-associated stromal compartment. This stromal element is known to interact in a reciprocal manner with mesenchyme, epithelium, and stem-like, and neuroendocrine cells in the cancer epithelial compartment. Novel technologies including advanced quantitative immunohistochemistry (qIHC) and *in situ* hybridization (qISH) methods could enhance the power of prediction of cancer progression and lethality.

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microarray data for improved power of prediction of cancer patients' survival. Mass spectrometry–based proteomics technologies are also rapidly advancing and are becoming relevant to clinical medicine. In addition to exon-capture microarray transcriptomics, technologies to record miRNA and long noncoding RNAs are also now available and are being applied to clinical specimens. These additional targets, multiple RNA species representing a majority of the expressed genome as well as new quantitative protein-based methods, will likely increase the power of large datasets to predict more faithfully what lies in store for individual patients with cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: L.W.K. Chung
Development of methodology: L.W.K. Chung
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): L.W.K. Chung
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M.R. Freeman, L.W.K. Chung
Writing, review, and/or revision of the manuscript: M.R. Freeman, L.W. K. Chung, Q. Li
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): L.W.K. Chung
Study supervision: L.W.K. Chung

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