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Toward a functional taxonomy of cancer

Interrogating the genomes of tumor cells with genomic and proteomic methods is becoming a mainstay of modern cancer classification efforts. This notion is brought to a new level by a paper in the July 23 issue of *Cell*, in which the dynamic responses of leukemia cells to perturbation are cataloged by flow cytometry, and the leukemias classified in terms of their functional responses. This study paves the way for more systematic attempts to bring functional genomics to the study of human cancer.

The beginning of the 21st century has brought the hope and expectation that an emerging understanding of the human genome will bring about a transformation of the practice of medicine. In the field of cancer, it now becomes likely that over the next decade or two, it will become possible to classify all cancers on the basis of their underlying genetics and physiology. These goals are being largely addressed through the use of DNA microarrays for monitoring the RNA profiles of tumor specimens. Significant progress has been made in many areas including breast cancer (van de Vijver et al., 2002), lymphoma (Rosenwald et al., 2002), and most recently, acute myeloid leukemia (AML) (Bullinger et al., 2004; Valk et al., 2004), among others. A molecular taxonomy of cancer thus appears feasible.

Yet, RNA profiles of tumor biopsies or resected specimens cannot possibly capture all of the relevant molecular detail of a given cancer. For one, much of cellular behavior is governed by translational and posttranslational control mechanisms that are not reflected in RNA profiles. This has led some to argue that a definitive molecular classification of cancer would require proteomic analysis. While this may be correct in principle, the ability to perform high-throughput, detailed proteomic analysis of tumors is at least several years off. As such, RNA profiling still represents the most tractable, high information content, high-throughput classification platform.

Far more important than the RNA versus protein debate is the fact that molecular analysis of tumors creates a snapshot of the biological state of the tissue at the time of biopsy. Differences in dynamic response to environmental conditions (e.g., growth factor stimulation, microenvironmental effects) are not exposed. That is, the resting profiles of two tumors could be the same, yet their response to provocation entirely different—and highly relevant to understanding the clinical behavior of human cancers.

This notion of classifying cancers according to their dynamic response to perturbation is explored for the first time in an important paper by Garry Nolan and colleagues in the July 23 issue of *Cell* (Irish et al., 2004). In this work, the authors use flow cytometry of leukemic cells to assess the phosphorylation state of 6 signaling proteins (Stat1, Stat3, Stat5, Stat6, p38, and Erk1/2) in response to 5 cytokine perturbations (FLT3 ligand, GM-CSF, G-CSF, IL-3, and interferon γ). The experiments were first conducted in leukemic cell lines, and then extended to primary blasts from patients with AML. The studies show quite convincingly that the phosphorylation status of signaling proteins at baseline is not predictive of their response to cytokine stimulation. For example, Stat5, known to be a downstream effector of the receptor tyrosine kinase FLT3, exhibited equivalent phosphorylation in FLT3 wild-type versus mutant (resulting in constitutive FLT3 activity) AMLs. However, Stat5 phosphorylation in response to cytokine stimulation differed significantly between FLT3 wild-type and mutant leukemias. In fact, FLT3 status could be predicted based on Stat5 (and other Stat proteins) response to cytokine treatment. Along those same lines, clustering of these dynamic responses led to successful prediction of response to chemotherapy.

This paper is noteworthy for several reasons. First and foremost, it demonstrates the feasibility of classifying tumors (or in fact any cell) on the basis of their response to cellular perturbation, thereby exposing a new dimension of cellular activity not otherwise accessible. Second, the study demonstrates the power of phospho-proteomic analysis, where single cell phosphorylation status is examined. Standard flow cytometry is reinvented as “single cell profiling” in

keeping with current enthusiasm around proteomics technologies. But perhaps this new spin will reawaken flow cytometry's proven record as a robust means of quantitatively assessing protein expression at the individual cell level. Third, the authors demonstrate that this approach can be taken not only with established cell lines, but also with primary leukemic blasts. Indeed, a significant amount of heterogeneity in cellular response was observed within individual patient samples. Whether this heterogeneity will prove to be clinically important remains to be determined.

To be sure, the study is limited by the small numbers of analytes (e.g., Stat proteins) and small number of perturbations (cytokine stimulations). But, the proof of principle is established that classification based on dynamic response to perturbation is feasible and informative. As higher complexity proteomic profiling methods are established, they should be able to be utilized within this same conceptual framework. Less clear is whether or not specific new insights into signal transduction in leukemia were garnered by this study. Similarly, the sparseness of the data makes it difficult to form a true network understanding of signaling in these cells.

Nevertheless, the study does raise the provocative notion that a functional taxonomy of cancer—that is, a taxonomy built on functional response (however measured) to a diverse set of cellular perturbations—could be highly informative. Of course there are at present numerous technical limitations to the widespread application of this approach to solid tumors, but the principle is indeed established, and the study will hopefully prompt others to use the tools of genomics and high dimensionality data analysis and bring them to bear on studying the functional consequences of perturbation of cancer cells. Such efforts will at last put the *functional* in functional genomics.

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SRCircumventing imatinib resistance

The ABL inhibitor imatinib is a highly effective therapy for patients with chronic myeloid leukemia. Relapses after an initial response have been observed in some patients, and mutations of the *BCR-ABL* gene are the most common mechanism driving these relapses. Alternative ABL inhibitors have been identified that inhibit most of the common BCR-ABL mutations, and one has entered clinical trials. The structural basis for these results has yielded significant insights into the mechanism of action of these compounds, mechanisms of resistance, and their ability to inhibit the BCR-ABL mutants. These studies demonstrate the importance and impact of conducting scientific studies as part of clinical trials.

Imatinib, a selective inhibitor of the ABL tyrosine kinase, is a highly effective treatment for chronic myeloid leukemia (CML), a disease driven by the activated BCR-ABL tyrosine kinase. Relapses after an initial response have occurred in a small percentage of chronic phase patients, but are quite common in patients with advanced disease. In the majority of cases, resistance is caused by reactivation of BCR-ABL kinase activity, indicating that resistance could be overcome if inhibition of BCR-ABL was restored. After the original report of a threonine to isoleucine substitution at amino acid 315 (T315I) (Gorre et al., 2001), it has become clear that mutations in the kinase domain of BCR-ABL are the predominant mechanism underlying acquired drug resistance, although some patients have amplification of BCR-ABL (Hochhaus et al., 2002; Shah et al., 2002). Mutations have now been observed in at least 17 different amino acids scattered throughout the ABL kinase domain and render the kinase variably less sensitive to imatinib (Shah et al., 2002; Corbin et al., 2003).

Once it became clear that resistance to imatinib was frequently due to mutations of BCR-ABL, alternative inhibitors that could inhibit these ABL mutants were sought. The first compound identified with this capability, PD180970, a pyridopyrimidine derivative, had originally been developed as a SRC kinase inhibitor, but was subsequently shown to inhibit wild-type ABL at nanomolar concentrations (Dorsey et al., 2000). Based on structural data discussed later, we reasoned that SRC/ABL inhibitors would likely inhibit the kinase domain mutants detected in patients and showed, with the notable exception of T315I, that PD180970 inhibited all imatinib-resistant BCR-ABL kinase domain mutants tested in vitro (La Rosee et al., 2002). Although the unfavorable pharmacokinetic profile of the pyridopyrimidine derivatives precluded their clinical development, these studies provided proof of principle for the