Molecular Pharmacogenomics Exposes Leukemia's Weaknesses

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The Education Session on Molecular Pharmacogenomics yesterday (which will be presented again this morning at 7:30 a.m.) focused on new technologies that will play an integral part in cancer therapy in the future. As Dr. Stella Davies from Cincinnati Children's Hospital emphasized, we must understand and identify variability of drug response for individual patients in order to optimize therapy. Genome-wide experiments are envisioned to identify candidate genes for further study.

One such genomic study was described by Dr. James Downing from St. Jude's Children's Research Hospital, whereby single nucleotide polymorphism (SNP) array assays were used to identify both genotype and copy number of genomic DNA from pediatric acute lymphoblastic leukemia (ALL) cells.

The technique is elegant — genomic DNA was digested with a restriction enzyme, and the fragments were amplified by polymerase chain reaction (PCR). Purified PCR products were further fragmented and then labeled with fluorescent dyes, generating glowing genomic probes that were used to hybridize to high-resolution SNP oligonucleotide arrays, allowing the SNP genotype and copy number to be determined.

DNA purified from 249 pediatric ALL samples at presentation and most of their corresponding remission DNA were analyzed using the SNP array. Interestingly, the transcription factor PAX5 was found to be altered in copy number in approximately 30 percent of B-progenitor ALLs, through deletions, mutations, or epigenetic silencing. PAX5 is required for B-cell differentiation beyond the pro-B stage; thus, haploinsufficiency of PAX5 is thought to lead to a block of differentiation of leukemic progenitors at the pro-B stage.

Many perturbations in the signaling latticework remain unidentifiable because traditional biochemical techniques are too crude to elucidate details of signaling networks of a single cell. Dr. Garry Nolan's group at Stanford University did just that, using multiple-channel, multi-color flow cytometry. This key technology simultaneously detects multiple wavelengths of fluorescence when a laser beam excites a single cell. To do this, different fluorescent dyes were used to label various antibodies that could recognize phosphorylated proteins specifically. Primary cells were stimulated with various agents such as G-CSF, and then were fixed, permeabilized, and stained with the phospho-specific antibodies. The resulting readout from the flow cytometer showed the phosphorylation status to a given stimulus, thus generating a "physiologic profile."

In correlation with clinical outcome, Nolan's group found that although there was marked heterogeneity among AML samples, there were patterns noted. For example, G-CSF-stimulated phosphorylation of Stat3 and Stat5 correlated with poor response to chemotherapy. Also discovered was that in follicular lymphomas and colon cancer, there was an underlying signaling alteration of infiltrating T cells and regulatory T cells, respectively. Furthermore, Dr. Nolan demonstrated that the multi-color flow cytometry technique may be able to diagnose JMML in a matter of hours instead of weeks.

Both of these technologies shed light on the mechanism of oncologic disease, which is markedly heterogeneous. It remains to be seen if cancer stem cells differ in their genomics than their daughter tumor cells. Nevertheless, it is clear that the emerging patterns of abnormalities may yield future targets of molecular therapies, and individualized profiling of a particular patient's cancer will allow clinicians to select and combine molecularly targeted therapies rationally.