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"Lymphochip" Genetically Distinguishes Lymphomas

By arraying nearly 18,000 genes on a glass chip about twice the size of a postage stamp and recording the expression patterns of those genes, researchers have obtained detailed molecular portraits of a form of lymphoma.

The gene expression profiling experiments revealed that diffuse large-cell B-cell lymphoma (DLBCL) is actually at least two distinct forms of cancer.

The scientists believe that their achievement which involved making some 1.8 million systematic measurements of gene expression in normal and malignant cells will improve diagnosis and treatment of these particular lymphomas. And they say that their research accomplishment signals the beginning of a new era in which such gene expression profiling will become a powerful clinical tool for revealing the detailed molecular nature of cancers and other diseases.

"We are now able to see the individuality of tumors at the level of gene expression programs that determine much of their behavior and capabilities."

- Patrick O. Brown

"This work shows that the molecular portrait of a tumor that we get from DNA microarray analysis can actually be interpreted as a much clearer, more detailed picture of the tumor's biology and that the new things we can see in this picture really make a difference for the patient," said Patrick O. Brown, a Howard Hughes Medical Institute (HHMI) investigator at Stanford University School of Medicine.

"We showed that gene expression profiling of cancers can uncover new diseases that have different clinical outcomes," said Louis Staudt, a senior investigator at the National Cancer Institute. "In particular, we found that the

most common type of non-Hodgkin's lymphoma, diffuse large B cell lymphoma, is actually two different diseases that have been lumped together using conventional diagnostic methods."

Senior authors Brown and Staudt and a team of 30 scientists published their findings in the February 3, 2000, issue of *Nature*. The lead co-authors of the *Nature* article are former HHMI-NIH research scholar Ash Alizadeh at Stanford University School of Medicine and Michael Eisen, who is now at Lawrence Berkeley National Laboratory.

In their experiments, the scientists sought to create a systematic profile of the genetic script of DLBCL, an aggressive cancer in which B lymphocytes of the immune system proliferate uncontrollably. Although DLBCL has been traditionally classified as a single cancer, patients suffering from the disease showed diverse responses to chemotherapy, leaving a question as to whether the disease might be more than one cancer. While 40 percent of the 25,000 cases that occur each year respond well to treatment, most patients succumb quickly to the cancer.

"It's a disease that has long been recognized as being a real clinical challenge because of such heterogeneity in the clinical behaviors of the tumors," said Brown. "And yet despite a great deal of effort, pathologists have been unable to arrive at a set of diagnostic criteria that would allow them to subdivide this disease into distinct groups that reliably predict clinical outcomes."

To distinguish genetic subtypes of DLBCL, the researchers created a specialized "Lymphochip," a DNA microarray consisting of genes preferentially expressed in lymphoid cells as well as genes known to or suspected to play roles in cancer or normal immune system functions. The Lymphochip included nearly 18,000 of these genes arrayed on a glass chip about twice the size of a postage stamp.

The researchers could see the gene activity in normal and cancerous B cells by collecting and tagging the gene transcripts with a fluorescent dye and bathing the Lymphochip with those fluorescently tagged transcripts. By looking at the color and intensity of fluorescence of each gene, the researchers determined the level of activity of each gene in the microarray.

Mathematical analyses of the huge mass of gene activity data indicated that DLBCL might actually be more than one cancer because DLBCL samples showed wide variation in the expression patterns of hundreds of genes. The researchers noticed that one of the major ways in which the gene expression programs of the tumors varied was in their expression of a large group of genes that are usually associated with a particular stage in development of normal B cells.

"However, statistical analysis of this data also revealed the presence of groups of tumors with similar gene expression patterns. We noticed that one

of these groups of tumors expressed a large group of genes normally expressed at a specific stage in B cell development, while the remaining tumors expressed a set of genes characteristic of a later developmental stage," said Eisen.

This caught the researchers' attention because they knew that one of the most important determinants of the biology of a tumor cell is the normal cell type from which the tumor arose, said Brown. The researchers determined that DLBCL could be categorized into two distinct classes based on the tumor's overall similarity in the pattern of expression of genes involved in B cell development and activation.

The researchers also found that this analytical distinction was reflected in the clinical outcomes of patients with DLBCL. Those patients with cancers of the second genetic subtype tended to have a much more dismal clinical course, dying at a much higher rate. "Patients with these two diseases differed significantly in long term survival following standard chemotherapy," said Staudt. "These results will have practical importance for cancer patients because the molecular profile of a patient's cancer cells could be used to guide patients towards the therapy that is most likely to be successful for them."

Brown added that such new molecular distinctions among cancers would likely allow more targeted treatments and greater insights into basic tumor biology.

"The differences we are seeing are at the level of the molecules and regulatory systems that are the targets of present and future anti-cancer drugs," he said. "So, they not only serve to better classify the tumors, but also suggest ways in which we might tailor specific treatments to each distinct molecular cancer type that we can identify."

Brown emphasized that in the case of DLBCL, gene expression profiling of a larger group of patients may reveal additional distinctive subtypes. As evidence, he cited the researchers' findings that some patients in each group clinically fared either better or worse than others did, hinting at the presence of yet-undiscovered subtypes of the disease.