

OCTOBER 15, 2008

Genetic Analysis Predicts Whether Liver Cancer Likely to Recur

Researchers are poised to unlock the genetic secrets stored in hundreds of thousands of cancer biopsy samples that are in long-term storage and previously thought to be useless for modern genetic research. With the aid of a new technique developed by Howard Hughes Medical Institute researchers, scientists can now reconstruct thousands of genes that are shredded into tiny pieces when tissue samples are treated with a chemical fixative and stored in wax - a protocol commonly used to preserve tissue samples indefinitely.

The scientists tested their new technique on liver tissue samples from 307 patients enrolled in clinical studies in four different countries. The scientists used sophisticated microarray technology to examine RNA from stored liver tissue samples. Their studies turned up a tell-tale genetic profile that indicated whether liver cancer will recur.

"The fact that the predictive information comes not from the tumor but from surrounding tissue could offer important insights into the mechanism of liver cancer."

— **Todd R. Golub**

Since the testing was done on tissue samples of patients whose clinical outcomes were known, the researchers were able to associate specific gene expression signatures with a likelihood of tumor recurrence.

The researchers are optimistic that oncologists will be able to use this information to determine which liver cancer patients would likely develop recurrence and treat them to help prevent it.

It is now possible to scan the entire genome for gene expression profiles in tissues that have been fixed for a very long time—in our study as long as twenty-four years, said Howard Hughes Medical Institute investigator Todd R. Golub, who led the study. There are lots of those tissues available compared with frozen ones, and tissue availability has been a real bottleneck in cancer genomic research.

The findings were published October 15, 2008, in an advance online article in the *New England Journal of Medicine* by an international team including collaborators from Japan, Spain, Norway and Italy.

Liver cancer is the third leading cause of cancer deaths worldwide. Most primary liver cancers begin in liver cells called hepatocytes. This type of cancer is called hepatocellular carcinoma or malignant hepatoma. Although liver cancer is often detected at an early stage, it can recur despite early diagnosis and treatment. If the cancer recurs, it often proves fatal.

For decades, hospitals have stored tissue samples from biopsies and surgeries by soaking them in a combination of formaldehyde and water (called formalin) and then embedding them in wax. However, this type of storage breaks the genetic material into pieces that are so small that they are essentially useless for genetic studies, explained Golub, a researcher at the Dana-Farber Cancer Institute and the Broad Institute of the Massachusetts Institute of Technology and Harvard University.

Golub's research is based on the premise that extraordinary insights into the molecular basis of cancer can be obtained by taking global views of the genomes of tumor samples. To broaden the view of cancer genomes, Golub and his colleagues use DNA microarrays (DNA chips) to monitor the gene expression (gene activity) of thousands of genes simultaneously across the human genome. This technique, pioneered by HHMI investigator Patrick O. Brown at Stanford University, involves extracting messenger RNA (mRNA) from tumor samples, fluorescently labeling the mRNA and hybridizing it to an array of DNA probes in the DNA chip. By measuring the mRNA levels from each gene, researchers can determine the activity of the genes in the tumor.

On average, mRNA samples in fresh tissue are around two thousand bases long, said Golub said. But when a tissue sample is fixed in formalin, the mRNA gets cleaved into little bits between fifty and one hundred bases long. So, these samples are not amenable to conventional microarray technology.

However, scientists at Illumina, Inc., in San Diego, had recently developed a way to perform gene expression analysis on these degraded samples. The company had successfully used their technique to study the expression levels of several hundred genes in formalin-fixed samples. We reasoned that it might work for analyzing the entire genome, Golub said. Their goal was to measure or infer the activity of roughly 6,000 genes in the samples - a large improvement over the previous method.

To find out if that was feasible, Golub's team looked at tissue samples from 307 patients enrolled in liver cancer studies in Tokyo, Milan, New York and Barcelona. They first analyzed samples of the tumors themselves, looking for gene expression patterns that might be predictive of cancer recurrence. You would think that if you wanted to learn about a tumor's probability of coming back, you would look at the genomic profile of the tumor, he said. But we found that the genetic profile of the tumor wasn't predictive of outcome, survival, or late recurrence.

However, the paper's first author, Yujin Hoshida, suggested that they also analyze gene expression in what appeared to be normal liver tissue adjacent to the tumor. Hoshida knew that liver cancer researchers have been debating a hypothesis that a field defect of the whole liver may predispose a person to liver cancer. That theory posits that liver tissue that appears normal might in fact harbor detectable genetic abnormalities that would give rise to new tumors after the main tumor was removed. Analysis of this adjacent tissue revealed a characteristic gene expression signature in 186 genes that reliably correlated with a high frequency of tumor recurrence.

These findings indicate that we might be able to identify patients at risk of recurrence and target those patients with interventions to help prevent it, he said. The fact that the predictive information comes not from the tumor but from surrounding tissue could offer important insights into the mechanism of liver cancer.

More broadly, said Golub, this analytical technique could be applied to any type of cancer. We don't know whether there will be a recurrence signature in the non-tumor tissue, of, for example, breast cancer, he said. But we can now explore that possibility. Golub noted that the technique also opens the way for genomic study of fixed tissue samples in diseases, such as multiple sclerosis.

In an accompanying editorial in the *NEJM*, Morris Sherman of the University of Toronto wrote that the findings bring the possibility of individualized therapy for hepatocellular carcinoma one step closer. He wrote that the new research has opened the door to identifying the relevant gene expression in the pathogenesis of hepatocellular carcinoma as it evolves from non-tumorous liver, as well as possibly initiating research into a molecular method for determining more precisely who is at risk for the development of hepatocellular carcinoma.

Golub said the next step is to analyze samples from more patients to confirm findings in liver cancer. He said he sees no major barriers to translating the findings into a clinical diagnostic technique, but some technical issues remain to be resolved.

Golub and his colleagues at the Broad Institute and Dana-Farber Cancer Institute collaborated on the studies with researchers from the Mount Sinai School of Medicine in New York; Toranomon Hospital in Tokyo, Japan; the National Cancer Institute in Milan, Italy; the University of Bergen in Norway; the Institut d'Investigacions Biomèdiques August Pi i Sunyer Centro de Investigaciones en Red de Enfermedades Hepáticas y Digestivas Hospital Clínic in Barcelona, Spain; and the Institut Català de Recerca Avançada in Barcelona.