

MARCH 24, 2005

Trio of Leukemias Shares a Single Mutation

Three leukemias that affect as many as 100,000 people in the United States are all caused by acquired mutations that alter a specific enzyme controlling blood cell proliferation, according to new studies by Howard Hughes Medical Institute (HHMI) researchers.

Identifying the genetic malfunction that causes these disorders raises the hope that researchers may be able to devise a targeted therapy, just as they have done for chronic myelogenous leukemia (CML), which is presently treated with Gleevec. The three leukemias that share a common genetic cause are polycythemia vera (PV), essential thrombocythemia (ET) and myeloid metaplasia with myelofibrosis (MMM).

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— **D. Gary Gilliland**

The research team, which was led by Gary Gilliland, an HHMI investigator at Brigham and Women's Hospital and Harvard Medical School, published its findings on March 24, 2005, in an immediate early publication in the journal *Cancer Cell*. Gilliland and Stephanie Lee of the Dana-Farber Cancer Institute, and co-first authors Ross Levine and Martha Wadleigh, collaborated with researchers from the University of Leuven - Flanders Interuniversity Institute for Biotechnology in Belgium, University Hospital of Ulm in Germany, the Broad Institute, and the Mayo Clinic.

According to Gilliland, the researchers analyzed the blood of patients with the three leukemias for a defect that permanently activated a particular type of enzyme called a tyrosine kinase. Tyrosine kinases are cellular switches that control an array of cellular processes. The researchers concentrated on the

enzymes because activated tyrosine kinases had been shown to cause other similar myeloproliferative diseases such as CML, he said. Also, in earlier work, Gilliland and his colleagues had isolated the mutated gene that produces the activated tyrosine kinase responsible for the myeloproliferative disease hypereosinophilic syndrome.

“The three disorders that we studied were the last of these diseases whose causes had not yet been discovered,” said Gilliland. “We thought it was a good bet that the cause would be a constitutively activated tyrosine kinase.” According to Gilliland, while PV, ET and MMM are rare, their prevalence is about five times higher than CML, with about 100,000 cases in the U.S. each year.

The researchers performed high-throughput DNA sequence analysis of blood and mouth-swab samples from 164 PV patients, 115 ET patients and 46 MMM patients. The patients were recruited via a notice posted on the web site of an advocacy group for people with myeloproliferative disease. The researchers used a sequencing technique developed in collaboration with co-authors William Sellers and Matthew Myerson of Dana-Farber. Specifically, they sequenced regions of tyrosine kinases that were likely to be mutated in the leukemias.

Their sequencing analysis revealed that about 75 percent of the PV patients, 32 percent of the ET patients and 35 percent of the MMM patients showed the same defect in the gene for a tyrosine kinase known as JAK2.

“There are some similarities among these three different diseases, and some overlap in the diagnostic criteria, but it was a surprise to us that the same mutation appears to account for at least a fraction of cases for all three,” said Gilliland

By comparing the DNA sequences from the blood with those from the mouth swabs, the researchers could determine which mutations the blood cell progenitors had acquired—since the mouth-swab DNA represents inherited germline DNA that had not undergone mutation. Their comparisons revealed that the characteristic mutation in *JAK2* was acquired, not inherited. And since the researchers did not find the mutation in a large number of normal blood samples, they were able to conclude that the mutation was characteristic of a large fraction of the three leukemias.

JAK2 normally functions as a molecular switch to trigger proliferation of red blood cells in response to events such as blood loss, said Gilliland. Also, mice in which the gene has been knocked out exhibit defective red blood cell production. Thus, he said, abnormal activation of the gene for JAK2 would be expected to lead to myeloproliferative disease.

In test tube studies, the researchers found that a drug that inhibits the JAK2 tyrosine kinase did inhibit growth of cells with the mutant *JAK2* gene. “While this is a very preliminary finding, and there is much developmental work to be done, it does suggest that inhibition of this kinase—just as Gleevec inhibits the kinase that causes CML—might be an effective therapeutic

approach for these diseases,” said Gilliland. He noted that the discovery of the characteristic mutation will have diagnostic value in distinguishing the myeloproliferative diseases from secondary causes of similar blood cell pathologies, such as smoking or cardiac disorders.

Further studies are needed to understand why only a fraction of the three leukemias show the mutation, said Gilliland. For example, there may be other genes whose mutations are also involved in causing the diseases, he said. Furthermore, it is still not understood how the mutation causes permanent activation of the JAK2 enzyme.

The researchers' approach to finding patients using the internet could be of particular value for studying rare genetic diseases, Gilliland emphasized. To recruit patients with the disorders, the researchers posted a notice on the web site of an advocacy group for patients with myeloproliferative diseases—MPDInfo (www.mpdinfo.org).

“These are relatively rare diseases, and because it is important for these high-throughput genome sequencing strategies to have large numbers of patients, we needed to recruit about a hundred patients for each disease,” said Gilliland. “It would be hopeless to do that at even a large medical center. So, we presented this problem to the patient advocacy group, and there was a huge outpouring of interest. We developed a clinical protocol for internet-based recruitment, posted a questionnaire on their web site, and we had six hundred responses within just a couple of weeks.”

Once candidates for the study had been identified and consent forms obtained, the researchers sent the patients kits that would enable collection of blood and mouth-swab samples by mail, said Gilliland. The patients were instructed to have their blood drawn at their local clinic, and the researchers obtained clinical records from the patients' physicians.

Gilliland concluded that the internet-based clinical protocol is “a very valuable strategy, especially for studying relatively rare genetic diseases. It has been used by epidemiologists for gathering data using questionnaires about clinical problems. But as far as I know, this is the first time that a research group has used it as a protocol for collecting blood samples to identify the cause of a disease.”