Team effort in discovery of blood disorder's missing 'LNK'

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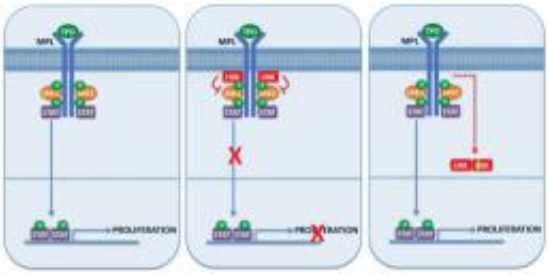

LEFT: Scientists have known that in a typical cell, the Jak-Stat pathway cues it to proliferate in response to external chemical signals. Mutations in the Jak2 protein that leave the pathway stuck in the “on” position are associated with some blood disorders. CENTER: New research has focused on how another protein, LNK, inhibits the Jak-Stat pathway. RIGHT: A new study has found some patients with these disorders whose Jak2 genes are normal but have mutations in LNK.

(PhysOrg.com) -- It might seem, watching a cell dividing languidly under a microscope, that nature has a straight-forward game plan: an orderly progression of "first this, then that." But backstage, it's much more messy.

In the end, a cell’s actions are the sum of a bewildering array of internal signals, including ”start,” “stop,” and sometimes even “reverse.” Upset the balance in any direction and you’re likely to cause a catastrophe in the form of cellular suicide, or the uncontrolled cell division that can lead to cancer.

That's what happens in a particular class of blood cell disorders called myeloproliferative neoplasms, or MPNs. Over 150,000 people in this country live with one of three forms of the disorders, which are characterized by overproduction of red blood cells, white blood cells, and/or platelets. Patients can develop life-threatening blood clots and, in some cases, their condition progresses to acute leukemia. Some people respond well to currently available treatment, but many do not. Jason Gotlib, MD, would like to know why.

In 1999, Gotlib, now an assistant professor of medicine and a member of the Stanford Cancer Center, became the first fellow in the Division of Hematology to pursue what was then a newly created career track: that of clinical investigator. The program was developed to allow young physicians who have completed their patient-care training to focus their attention on clinical research and the design and implementation of clinical trials. By choosing the clinical investigator pathway, rather than a more-traditional route that spends time in a basic research laboratory, Gotlib ensured that he would remain in close contact with patients throughout his training period.

Since then, Gotlib has balanced the care of patients who suffer from a variety of blood disorders with translational research into the causes of their conditions. He has recruited patients for numerous phase-1 to phase-3 clinical trials of novel therapies for acute and chronic myeloid leukemias, with a focus on myelodysplastic syndrome and MPNs. To figure out what exactly is going wrong in patients with MPNs, Gotlib has joined forces with hematology fellow Stephen Oh, MD, PhD; and professor of microbiology and immunology Garry Nolan, PhD; and Jim Zehnder, MD, professor of pathology and of medicine.

Previous research has shown that many, but not all, MPN cases exhibit abnormal activation of a protein pathway called JAK-STAT that is critical for translating "grow" signals from outside the cells. Specifically,

a recurring mutation in the gene for the JAK2 protein causes the pathway to be locked into the on position, even in the absence of external signals to divide. Although this mutation is identified in greater than 95 percent of patients with one form of the disorder, it is found in only about 50-60 percent of the patients with the other two types of the disorder.

Gotlib and Oh were intrigued by studies of mice that involved another molecule, called LNK. Although originally identified as an important player in B-cell development, it was subsequently found that LNK can block JAK-STAT signaling, and mice missing the protein have some interesting symptoms.

“These mice have the same large spleens and an increase in white blood cell and platelet counts observed in patients with MPNs,” said Gotlib. “We looked at this data and said, ‘Wow, this seems like a gene we should investigate in patients who don’t have a mutation in the gene for JAK2.’”

Fortunately, Gotlib had been collecting blood and bone marrow samples from many MPN patients and clinical trial participants.

Gotlib and Oh hit on the idea of sequencing portions of the gene for the LNK protein in these individuals to see if anything was amiss. They found that two of 33 patients whose JAK2 gene was un-mutated instead had mutations in the LNK protein in their blood cells. Although that’s only about 6 percent, the finding pinpoints a novel genetic abnormality for these diseases, and provides new molecular insights into the mechanism underlying the condition.

“This is an important paradigm change in our understanding of these disorders,” said Gotlib. “We’re learning that LNK acts like a brake on cell division, and other proteins in the pathway, like JAK2, act like a gas pedal.

“When the LNK gene is mutated,” he added, “it’s as if the cell’s brakes are defective.” As a result, the cells begin dividing abnormally.

The finding marks the first time that mutation of the LNK gene has been implicated in human disease, and it may lead to diagnostic and therapeutic avenues for people with the condition, say the researchers.

The research was published on April 19 in the journal *Blood*.

Since the earlier discovery of the JAK2 mutation’s role in MPNs, many companies have pursued the development of inhibitors of the pathway as a treatment option for these patients. Although Gotlib and Oh showed that cells engineered to express a mutant LNK protein can respond to a JAK inhibitor, they speculate that the degree or duration of the response may differ between patients depending on what mutations they exhibit.

“Our identification of mutations in LNK provides an alternative biologic basis for activation of the JAK-STAT pathway in these patients,” said Oh, a postdoctoral scholar in Nolan’s lab and first author of the recent study. “These data should generate not only substantial interest in the feedback controls governing this pathway, but have therapeutic implications in the current era of clinical trials with biologically targeted agents.”

The research represents the benefits of an academic medical center focused on translational medicine, the scientists said. Gotlib provided the patient samples and the clinical expertise, while Oh, who also trained as a clinical fellow, conducted the follow-up experiments in Nolan’s laboratory.

“We’re straddling both sides,” said Oh. “I got involved because of my desire to be working at the lab bench, while still staying as close to the patient as possible.

“It’s very rewarding to be able to elucidate novel mechanisms of pathogenesis while working directly with patient samples,” Oh added.

Oh’s work confirmed that LNK mutations can cause blood cells grown in the laboratory to respond inappropriately to external growth signals. Using a technique refined in the Nolan laboratory called phospho-flow, Oh was also able to show that these cells also keep their JAK-STAT pathway active longer than in normal cells.

The researchers are continuing to sequence the LNK gene in more patients to learn how frequently they occur and what types of mutations might contribute to the initiation and progression of the disorder.

“We’re beginning to acquire an understanding of the genetic basis for these myeloproliferative neoplasms,” said Gotlib, “and it’s more complex than what we had first thought. Although there are likely to be many cooperating mutations, people can now begin to look for LNK mutations in early or late-phase disease and assess their biologic effects and impact on patient outcomes.”

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