

NOVEMBER 22, 2002

Researchers Identify Cause of Aggressive Childhood Cancer

Researchers have generated a mouse model of a new type of tumor suppressor gene that triggers a rapidly advancing cancer that affects children. The discovery of the fast-onset cancers that result from inactivation of the gene and the technique used to generate the model will likely prove useful in studying genes involved in other forms of cancer.

The research team, which was led by Howard Hughes Medical Institute investigator [Stuart H. Orkin](#), and former HHMI physician postdoctoral fellow Charles Roberts, reported its findings in the November 2002 issue of the journal *Cancer Cell*. Orkin and Roberts are at the Dana-Farber Cancer Institute, Childrens Hospital, Boston, and Harvard Medical School.

The tumor suppressor gene, called *SNF5*, codes for a protein that is a component of a large complex called SWI/SNF that attaches to chromatin to regulate the expression of genes. Chromatin is the complex of DNA and proteins in the nucleus of the cell.

"It will be especially important to link this tumor suppressor with a known pathway of tumorigenesis."

- Stuart H. Orkin

There has been indirect evidence that some types of chromatin remodeling complexes might play a role in cancer, said Roberts. In a key finding reported in 1998, French researchers showed that mutations that inactivated *SNF5* were present in tissue samples from children with malignant rhabdoid tumors. That's what first caught our interest, that we might be dealing with a new type of tumor suppressor, said Roberts. Malignant rhabdoid tumors are rare but highly aggressive cancers that usually appear in infancy. These tumors are resistant to treatment and usually cause death within a year of diagnosis.

With the initial evidence that *SNF5* was involved in such tumors, Roberts, Orkin and their colleagues set out to establish in mice that loss of *SNF5* did

indeed produce cancers. The problem, said Roberts, was that the usual methods for knocking out the gene did not produce a useful model of rhabdoid tumors in the mice.

Mice that are deficient in *SNF5* die very early in embryonic development, and therefore cannot be used to analyze for cancer, he said. And mice that lack only one of the two genes show a relatively low prevalence of tumors, with a median onset of about twelve months. Thus, said Roberts, while these mouse models did demonstrate that *SNF5* was necessary for development, and that its loss caused cancer, such mice could not be used to analyze how *SNF5* loss affected the development of this form of cancer.

To construct a more useful model, the scientists turned to a conditional targeting approach that enabled them to knock out *SNF5* in some mouse cells but not others. This approach involved engineering the mice so that the *SNF5* gene would function normally throughout development, but could later be knocked out in adult mice by the introduction of a triggering chemical. This trigger chemical activates an enzyme that excises the gene under study.

Deletion of *SNF5* in the mice revealed that *SNF5* was required for the survival of adult mice and, in fact, for survival of virtually all normal cells. In order to circumvent the lethality and generate a working cancer model, Roberts and Orkin took the conditional targeting a step further. They engineered the knockout system so that instead of being snipped out, the *SNF5* gene would randomly invert in the process of being knocked out. In some cells, the gene would assume a normal orientation after triggering, and in others it would be inverted, and thus nonfunctional.

This was an adaptation of a technique that researchers Kong-Peng Lam and Klaus Rajewski had used to study lymphoid cells, but it had not been applied to cancer modeling, said Orkin. The trick was to make the gene we wanted to delete, instead of being excised, to flip back and forth and then randomly settle in either the active or inactive orientation.

By employing this technique, Orkin and Roberts created mice whose tissues had a delicate balance of cells with normal and inactivated *SNF5* genes. There were enough cells with normal *SNF5* to allow the mice to live longer, but enough with inactivated *SNF5* genes to give rise to cancers. According to Orkin, the mice engineered to have the reversible, inverting conditional knockout genes showed immediate onset of cancers. Most of the mice developed malignant lymphomas, or cancers of the blood cells, while many also developed rhabdoid tumors.

The fact that the mice showed consistent oncogenesis in a very short time means that we can crossbreed the animals with other genetically altered mice to sort out the cellular pathways that are affected, said Orkin. It will be especially important to link this tumor suppressor with a known pathway of tumorigenesis. Ultimately, if we know what pathway is affected, we can

target therapies to that pathway. Orkin and his colleagues believe that the reversible knockout technique could be applied generally to aid the study of other tumor suppressor genes in which complete deletion of the gene proves lethal.

According to Roberts, understanding the mechanism of *SNF5*-related cancers could have significant clinical impact. There have been many papers showing the role of *SNF5* loss in human cancers, he said. It's clear that the gene is involved in malignant rhabdoid tumors and that it may be involved in certain other aggressive cancers in early childhood. This work has led us to realize the existence of an entirely novel tumor suppressor pathway, the SWI/SNF complex of which *SNF5* is a core member. And, we believe that understanding the basic genetics, biochemistry and molecular biology of SWI/SNF, is likely to generate significant new understanding, and potentially therapies, for many types of human cancer.