

JUNE 29, 2006

Technique Reveals Genes that Drive Growth of Liver Cancer

Researchers have greatly shortened the time it takes to create a mouse model of human liver cancer—going from about a year with standard techniques down to about one month with the new approach. Using this technology, Howard Hughes Medical Institute scientists have already identified two genes that play a key role in driving the establishment of liver cancer.

The researchers say their approach will help scientists distinguish driver genes, which are mutated genes that cause cancer, from bystander genes, which are mutated but do not cause cancer. The new mouse model is also likely to facilitate development and testing of new drugs to target liver cancer, which is the third leading cause of cancer deaths worldwide.

"This is a nice demonstration that shows us that not every gene that contributes to cancer does so all the time. A gene must be in combination with the right other genes to produce cancer, and our mouse model enables us to precisely control and explore that context."

— **Scott W. Lowe**

More broadly, the researchers believe their approach will aid the national human cancer genome project, which aims to use genomic approaches to identify all of the genes that contribute to human cancer. Our study suggests an approach that can complement this project, said senior author Scott Lowe, a Howard Hughes Medical Institute investigator at the Cold Spring Harbor Laboratory. Mouse models that develop cancers that accurately recapitulate the human disease may be useful to help more rapidly identify the relevant genetic changes and speed up the pace of discovery to focus on those that will have the biggest impact on diagnosing and treating the disease.

Lowe and his colleagues described the new mouse model in a paper published in the June 29, 2006, issue of the journal *Cell*. Lowe's co-authors are at Hannover Medical School in Germany, Memorial Sloan-Kettering Cancer Center, The Walter and Eliza Hall Institute of Medical Research in

Australia and General Hospital Celle in Germany. A related article by Lynda Chin and colleagues at the Dana-Farber Cancer Institute was published in the same issue of *Cell*.

The new technique for producing liver cancer in mice is unlike most other approaches used to create mouse models of specific tumors. Those involve laboriously creating mutations in the whole mouse and then cross-breeding strains of mice to combine mutations.

In contrast, the *ex vivo* technique created by Lowe and his colleagues begins with the isolation of liver progenitor cells from fetal mice. These cells can develop into adult liver cells when transplanted into adult mice. The researchers can then easily induce precise cancer-causing mutations in the fetal cells and transplant them into adult mice, where the fetal cells colonize the liver and produce cancers.

It's much faster than methods of inducing individual mutations in mice and then crossing mice to combine those mutations, Lowe said. That approach takes can take years, whereas we can produce mice with multiple mutations in a few months.

Lowe and his colleagues also used a genome-wide scanning technique called ROMA (representational oligonucleotide microarray analysis) to scan the tumors arising in the mice and in human liver tumor samples.

One advantage of ROMA is its precision, said Lowe. It is well known that human tumors have a lot of genetic instability, with a lot of 'noise,' he said. There are a great many mutations in such tumors, and one of the big challenges has been to identify the critical mutations that, if targeted by drugs, would lead to a reversal of the cancer process. This technique enables us to pinpoint and validate *in vivo* such 'driver genes' with great precision.

In a test of their approach, the researchers produced three sets of mice that combined one of three known cancer gene mutations with a knockout of the *p53* gene. The researchers tested mutations in the genes *c-myc*, *Akt* and *Ras*. The *p53* gene—which is functionally compromised in about half of all human cancers—is a protective 'sentinel' gene that normally triggers cell death when cells have damaging mutations.

When the researchers transplanted the altered liver cell progenitors bearing mutations in *c-myc*, *Akt*, and *Ras* into mice, they found that, the *c-myc* mutation, in particular, produced liver cancers with chromosomal abnormalities resembling human liver cancer. Genetic analysis revealed that the *c-myc*-derived tumors showed an increased number of copies of a chromosomal region. Further analysis revealed that chromosomal region identified in the mice corresponds to a copy-number abnormality also seen in the counterpart chromosome in human liver cancers.

This comparative finding in mice and humans was important, because it showed us that what we had found in our mouse model quite likely had relevance in human cancers, said Lowe.

Subsequent genetic studies of this amplified region of DNA revealed two important genes that could be potential drivers of the cancer. These genes were *cIAP1*, which prevents cell death, and *Yap*, which promotes cell proliferation.

In the next round of experiments, the researchers discovered that the two genes were, indeed, cancer drivers. When they overexpressed either gene in mice with liver cancer, they saw accelerated tumor growth. But what really surprised us was that when we combined the two genes, they cooperated to accelerate tumor growth more than either of the two alone, said Lowe. And the effect was much more than additive.

Lowe said the identification of this synergistic effect offers insight into how amplification of one chromosomal region can drive cancers in such a powerful way. In this case, the individual genes in the amplified region cooperate in the cancer-causing process. Lowe said this type of knowledge could aid the search for other, still unknown, cancer genes.

For example, the chromosomal region that the researchers showed to be amplified in the mouse model of liver cancer is also amplified in some cancers of the lung, ovary and esophagus, said Lowe. And while it is only found in about five percent of these cancers, given that these are major cancers, the number of patients affected is quite large. Thus, these genes encode important drug targets, he said.

Lowe also noted that neither *Akt* nor *Ras* produced the same amplification of the cancer-causing genes, *cIAP1* or *Yap*, when combined with *p53* deficiency. This is a nice demonstration that shows us that not every gene that contributes to cancer does so all the time, said Lowe. A gene must be in combination with the right other genes to produce cancer, and our mouse model enables us to precisely control and explore that context, he said.

Lowe said that the research team's findings demonstrate that the new mouse model will offer a well-defined experimental system in which to develop and test liver cancer drugs. We know that the response to cancer drugs can vary from patient to patient, and that may be because the underlying genetics of their cancers are different, he said. So, we need to understand the relationship between this genetic variability and therapeutic response, and models such as ours enable us to get a handle on that relationship.

The new mouse model should enable researchers involved in the cancer genome project to prioritize their targets for further study. In human cancers, there is a lot of genetic noise, a lot of variability among cancers, Lowe said. Mouse models produce tumors that, by definition, are more defined than human tumors. By studying these cancers one learns more about the specific genetic context in which a putative cancer-causing gene might contribute to the disease. This makes follow-up validation studies much more efficient than if we only had information from human tumors alone, where such information is not available.