Teaming up against cancer

Finding cancer's genetic drivers

Genetically speaking, cancer cells are a mess. Their DNA is ravaged by mutations, some of which spawn cancer by driving the cells to grow and divide abnormally. Other mutations pile up as cells bypass built-in restraints and error-checking mechanisms and careen into chaos. Cells in human pancreatic and colorectal tumors, for example, contain an average of 60 altered genes.

In 2006, a massive multi-institutional project got under way in the United States to analyze hundreds of samples from patients with different tumor types. The object was to compile an atlas of the cancer genome. At around the same time, a coalition of scientists at Cold Spring Harbor Laboratory began tackling cancer's murky genetics with a different approach.

"Cataloging every mutation in a tumor will help construct a detailed genetic fingerprint of each patient's cancer," says Scott Lowe, the architect of the CSHL strategy. "But we also need to annotate this list with functional information."

Lowe and the other CSHL scientists are, in other words, finding out which of the mutated genes actually cause cancer ("drivers") as opposed to those that have no effect on cancer ("passengers"). These investigators are charting how mutated genes work in tandem to let tumors thrive and develop resistance to drugs. Importantly, they are also searching for mutations that, if targeted by drugs, could halt cancer.

Not only might this provide leads for more effective therapies. It could also help doctors predict the course of a patient's disease and anticipate drug resistance. And it has great potential for helping them choose better options among existing treatments.

The CSHL approach, dubbed "integrative oncogenomics," is essentially a rapid, large-scale dragnet for genes that are deleted in human cancers. These genes are suspected of functioning as tumor suppressors — a class of genes that inhibit the activity of tumor-promoting oncogenes, which are multiplied in cancer. Suspect genes are then evaluated for their ability to trigger cancer in mice.

In a pilot experiment last year, Lowe and members of four other CSHL labs whittled down a candidate list of 360 genes that are frequently missing — deleted — in samples of human liver cancer. They confirmed 13 of them as tumor suppressors. In understanding the genetic causes of liver cancer, the fifth most common type of cancer worldwide and one of the deadliest, these findings are a treasure trove.


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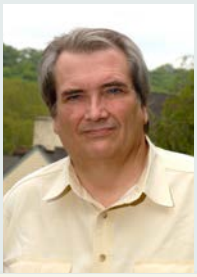
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> "Each group in this collaboration brought something unique and critical to the table."
>
> Scott Lowe

Fine-tuning a cancer mouse model

The CSHL approach has coalesced and evolved around Lowe's push to establish useful animal models for cancer. "Tumors that grow in animals are much more realistic than cancer cells growing in a Petri dish, and are a better system for ferreting out cancer-driving genetic changes," explains Lars Zender, formerly a Clinical Fellow and mouse expert in Lowe's lab, and who is currently on the front lines as a practicing oncologist in Germany.

1 Scott Lowe
2 Scott Powers
3 Mike Wigler
4 Greg Hannon
5 Richard McCombie

Lowe's group first shortened the amount of time it took to induce cancer in mice from the year or more taken by standard techniques to two months, a record. Their approach was to introduce two cancer-causing mutations — one that switched on an oncogene and another that switched off a tumor suppressor — into liver stem cells harvested from mouse embryos.

"The effect is like jamming on a car's accelerator while cutting off its brakes," explains Lowe. When transplanted into an adult mouse, the mutant cells embed themselves in the liver to create a "mosaic" animal, and quickly produce tumors similar to those seen in humans. This innovation set the scene for a multi-group collaboration.

Piecing together a unique gene screen

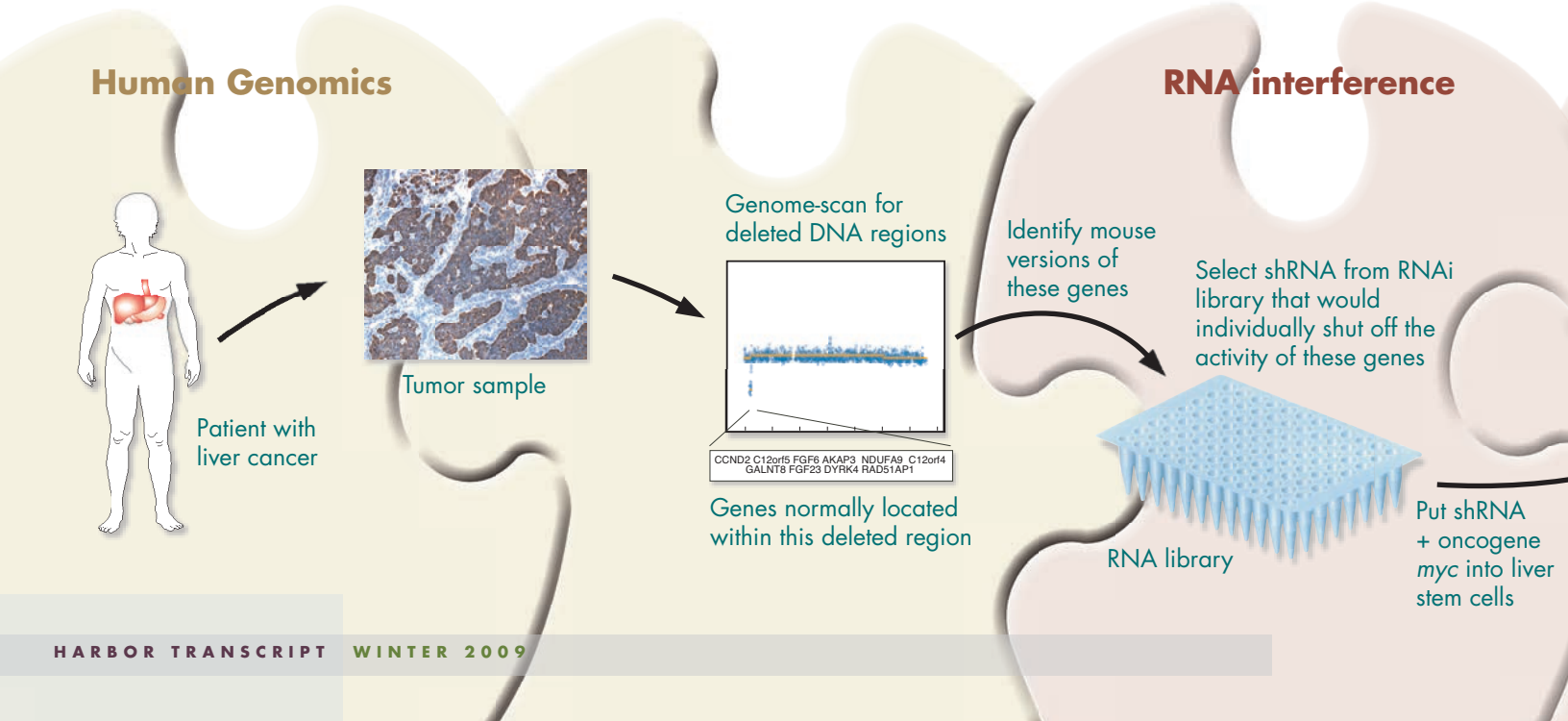
To take a closer look at the genetic landscape of the mouse tumors, Lowe's team enlisted help from CSHL's Mike Wigler and Rob Lucito, co-inventors of a genome-scanning technique called ROMA. This method enables researchers to identify segments of DNA deleted or abnormally multiplied in cancer cells.

The group found a genetic alteration in the mouse tumors that was identical to an alteration that Scott Powers, another CSHL researcher, had previously identified in human liver cancer. Lowe and Powers immediately began to collaborate. Their initial idea for a side-by-side comparison study between human and mouse tumors eventually stalled, Powers recalls. But he got over his disappointment when Lowe pitched him a broader and much more exciting idea when they met one evening at the coffee bar in Blackford Hall, a favorite hangout on the CSHL campus.

Instead of comparing genetic alterations in mouse and human tumors, Lowe proposed using ROMA to first gather data on genomic alterations that occur in human cancers and then mimicking these alterations in mice to see which of them caused cancer. "Of course, in retrospect, it is clear that this is a much more straightforward and widely applicable approach to testing which altered genes are cancerous," Powers remarks.

The final piece of technology needed to make this idea work came from another CSHL researcher, Greg Hannon. Hannon had created a collection of short, hairpin-shaped RNA (shRNA) molecules, which, via a cellular mechanism called RNA interference (RNAi), suppress the activity of specific genes. Because each shRNA molecule is tagged with a unique molecular "barcode," researchers using Hannon's shRNA "library" to screen the activity of a multitude of genes at once can still keep track of shRNA molecules that trigger a particular change.

The CSHL team first used shRNA to switch off one suspected tumor suppressor and induce liver cancer in mice. This proved that shRNA, which had thus far only been tested on cells grown in lab dishes, could also work in mice. With this proof-of-principle in hand, the team was ready to ramp up the scale of their experiment and screen hundreds of genes for tumor-suppressing activity.



Stimulus for collaboration

The importance of CSHL's innovative team approach to cancer genetics has been recognized by the federal government. The American Recovery and Reinvestment Act (ARRA) of 2009 awarded CSHL $4.75 million to set up a facility to analyze the wealth of data generated by human cancer genome projects. The aim is to discover cancer pathways and establish a new set of cancer biomarkers. Scientists will use both RNAi-based tools and another strategy developed by Scott Powers that uses molecules called cDNAs to validate candidate genes in mouse models for various types of cancer. Another aim is to discover and validate a new generation of cancer drugs with which doctors will be able to target a patient's specific constellation of mutations.

Powers used his expertise in analyzing cancer genomes to first identify DNA regions that were recurrently deleted in more than 100 human liver cancer samples. With software developed by Alex Krasnitz, a computational scientist in Wigler's group, Powers then picked out the regions likeliest to be the locations of deleted tumor suppressor genes, and compiled a list of the 360 genes that normally reside at these sites.

Selecting shRNAs from Hannon's library, Zender and his labmates Wen Xue and Johannes Zuber then systematically knocked out each of the 360 genes in mice that had also been engineered to overproduce a protein called Myc encoded by an oncogene. This painstaking work produced results within a month. Liver tumors appeared in mice in which a tumor suppressor gene had been turned off by a shRNA.

A rich pay-off

The researchers extracted DNA from the tumors and analyzed it with help from another CSHL scientist, Richard McCombie. The exciting result: the identification of 13 tumor suppressor genes, most of which had yet to even be linked to cancer. "We wouldn't have guessed their relationship to cancer if we hadn't followed this approach," says Lowe. Published in *Cell*, a leading journal, the paper describing this first "RNAi-based screen" in animals quickly rose to the top of the scientific community's must-read list.

CSHL's oncogenomics approach is a major step forward in the international effort to understand cancer. Now, researchers can rapidly filter genomic information to pick up only those genes that affect cancer development in living animals, and focus follow-up studies on those that might be most useful clinically.

"Each group in this collaboration brought something unique and critical to the table," says Lowe. CSHL President Bruce Stillman concurs. "On their own, each of the five labs involved in this work is doing groundbreaking research. Their various areas of expertise, brought to bear on a single problem, demonstrates the power of team science." **Hema Bashyam**