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Gene Screen Nabs New Colon-Cancer Genes

Howard Hughes Medical Institute researchers have combed through a catalog of all known tyrosine kinase enzymes to identify new gene mutations that occur in a significant fraction of colon cancers.

Researchers have known for years that abnormal activity of tyrosine kinases can hasten the development of certain forms of cancer. In this first-of-a-kind survey, researchers used a forward-looking approach to screen the genes that produce tyrosine kinases—reasoning that somewhere in this genomic landscape they might encounter novel mutations that spur colon cancer and other types of cancer.

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The researchers—including Howard Hughes Medical Institute investigators Bert Vogelstein and Sanford Markowitz—published their findings in the May 9, 2003, issue of the journal *Science*. Vogelstein is at the Sidney Kimmel Comprehensive Cancer Center at The Johns Hopkins University, and Markowitz is at Case Western Reserve University and University Hospitals of Cleveland.

The starting point for the studies was a detailed catalog of all kinases in the cell, similar to one published in December 2002 in the journal *Science*.

Kinases are important enzymes that activate other proteins by adding a phosphate group to them. According to Vogelstein, this catalog, which its developers named the “kinome,” constitutes an ideal collection of targets for cancer researchers seeking to stop the uncontrolled proliferation of cancer cells.

“The good news is that many of the genes responsible for common cancers, like colon cancer, have been identified,” said Vogelstein. “The bad news is that most of these genes are tumor suppressor genes, the metabolic brakes on cells. These suppressor genes are inactivated in tumors, and since cancer drugs work by reducing the activity of enzymes, they won't work on such suppressors because a drug can't inhibit a gene that is already inactivated.”

According to Vogelstein, genetic mutations can produce a tyrosine kinase that is essentially “turned on” in the absence of a normal activation signal, which is called constitutive activation. This represents an ideal drug target, said Vogelstein, and is exemplified by the success of the drug Gleevec in treating chronic myeloid leukemia.

“In the past, the search for drug targets has been guided mechanistically - by asking what karyotypic abnormalities are found in a cancer cell or what's responsible for a hereditary cancer predisposition,” said Vogelstein. “But with the completion of the human genome sequence and the availability of the kinome, one can begin to think about how to do this in a much wider and unbiased sense.”

The approach taken in this work actually represents a marriage of two technologies. “The availability of the human genome sequence allows scientists to scan sequences to identify kinases, and the increased speed with which DNA can be sequenced enables us to rapidly search for mutations in those kinases in human cancers,” said Markowitz.

In beginning their survey of the kinome for kinases activated in colon cancers, the group first explored tyrosine kinases and related enzymes. To reduce the amount of gene sequencing required, they focused their search on mutations in the “kinase domain” of these enzymes, which is the region that is principally responsible for enzymatic activity.

“The kinase motifs are fairly stereotypical,” said Markowitz. “They always include an adenosine triphosphate binding site and a set of conserved amino acid residues. That motif makes it possible to identify kinases using computer-based information and to screen genome sequences for all of the possibilities.”

“We figured that if there were going to be mutations that constitutively activated these enzymes, and that would thus be targetable by drugs, the kinase domains would be the ones to go for,” said Vogelstein.

The researchers first identified the kinase domains of 138 tyrosine kinases and similar enzymes from the kinome database. They next extracted these same domains from 35 colorectal cancer cell lines, most of which had been generated by Markowitz and his colleagues. They then sequenced those domains for comparison.

Their studies revealed mutations within the kinase domains in 14 genes. The researchers then analyzed another 147 colorectal cancer cell lines in the same way for kinase-domain mutations in these genes, and then sequenced the

entire coding region of all kinases that were mutated, in all discovering 46 new mutations.

The major difficulty, said Vogelstein, was distinguishing the “signal from the noise,” in this case, the mutations that would trigger cancer from the huge number of harmless variants in kinase genes. “We saw hundreds of changes in genes in cancer cells that had not been identified before, and for each of these, HHMI research associate Alberto Bardelli had to perform comparisons with the normal tissues of the same patient, to see if the mutation was specific to the cancer,” he said.

Analyses of mutations unique to the cancer cells strongly indicated that the mutations affect the function of the tyrosine kinases in the cells, constitutively activating them, said Vogelstein. “Most importantly, they were in positions of the kinase domains predicted to alter function, based on analogous mutations that have been seen before in other kinases,” he said.

According to Vogelstein, these studies of the mutations indicate that about 30 percent of colorectal cancers have mutations in at least one kinase, meaning that these cancers will theoretically be vulnerable to drugs that can block the action of the kinases.

The survey method demonstrated by the researchers could have a profound impact on colon cancer treatment strategies, said Vogelstein. “We envision that in the future, there could be tailored cancer therapies. For example, each patient with colon cancer could have a diagnostic analysis to determine which kinases are activated by mutation—an easy task once you know which ones to look for. Then, that patient could be treated with a drug that specifically targets that kinase.”

More broadly, he said, the approach could impact treatment of other types of cancer. “I think it's a perfectly reasonable speculation to suggest that ultimately a great majority of cancers will be found to have at least one drug-targetable mutation, and this could lead to new avenues for individualized therapy,” he said.

“Two of the major questions to be addressed by our laboratories are the functional consequences of the mutations we are finding, and whether these kinases are targets for drugs,” said Markowitz. “Discovering a new oncogene in a tumor now allows one to ask the next big questions, what happens when you turn it off? How big an impact will it have on the behavior of the tumor? Gleevec works so well in chronic myeloid leukemia because it turns off a tyrosine kinase that dramatically reverses the cancer process.”