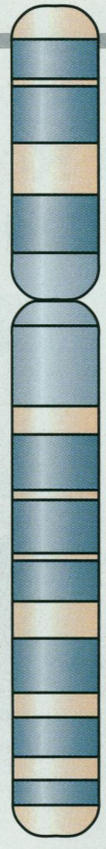
CANCER RESEARCH

A Challenge to *p16* Gene as A Major Tumor Suppressor



Hotspot. Chromosome 9p21 contains an important cancer gene. But is it *p16*?

Barely 2 months ago, a potential major new star appeared, blazing in the firmament of cancer genes. Two research teams, one in Salt Lake City and the other in San Diego, produced evidence indicating that the gene for a newly identified inhibitor of the cycle that underlies cell division, a protein that goes by the name p16, might be an important tumor suppressor. And that was big news, since tumor suppressors have become key players in cancer research because their loss or inactivation contributes to the uncontrolled growth of many types of cancer. Indeed, the work suggested that *p16* might be as important as the *p53* gene, which is thought to be involved in as many as 50% of all human cancers.

Now, new data, some presented earlier this month at Cold Spring Harbor Laboratory's annual symposium on quantitative biology,* are raising questions about whether *p16* is a sparkling new star or just a flash in the pan. Researchers who have looked at primary tumor samples rather than the cultured cell lines chiefly used in the original work have so far found that few tumors show the *p16* mutations that would be expected if inactivation of this gene is a major step in cancer causation. Molecular geneticist Curt Harris of the National Cancer Institute said in his presentation of the data at the Cold Spring Harbor meeting: "I believe that *p16* is a tumor suppressor, but it's uncommonly involved in human cancers."

But the researchers who pointed to *p16*'s possible involvement in cancer, including Alexander Kamb of Myriad Genetics Inc. in Salt Lake City and Mark Skolnick of Myriad and the University of Utah Medical Center, are standing firm in their view that it is a major tumor suppressor. "I think when all has been sorted out, our observations will be confirmed," Skolnick asserts. Finding out whether he's right is of more than theoretical significance, because if the work is confirmed, it could provide new leads to cancer therapies. It might be possible, for example, to inhibit cancer growth either by using *p16* for gene therapy or by mimicking its growth-suppressive abilities with drugs.

Kamb, Skolnick, and their colleagues' identification of *p16* as a possible tumor suppressor was an outgrowth of the Skolnick group's earlier finding that segment p21 of human chromosome 9 is the site of a gene whose alteration can result in susceptibility to the dangerous skin cancer melanoma. That work didn't identify the gene itself, but by early spring the Utah workers zeroed in on *p16*, which David Beach's group at Cold Spring Harbor had already identified as an inhibitor of one of the key enzymes that drives the cell division cycle (*Science*, 15 April, pp. 344 and 436).

Adding another link to the growing number of connections between the cell cycle machinery and cancer was intriguing, but there was a special reason for heightened interest in *p16*: It appeared that mutations in that gene might contribute to a wide range of cancers. Researchers have found abnormalities, including the deletions characteristic of tumor suppressors, at 9p21 in many kinds of tumors. The region is "commonly deleted in human cancers," says David Sidransky of the Johns Hopkins University School of Medicine in Baltimore, one of the researchers doing that work. Among the tumors in which abnormalities have been found, he notes, are such frequently occurring cancers as lung, bladder, brain, head and neck, and breast cancer, and also leukemias.

The excitement mounted when Kamb and his colleagues found that *p16* was in fact deleted in cell lines derived from a wide variety of cancers. It was lost from about 50% of the 290 cell lines they looked at, and some of the cell lines that didn't have deletions had smaller mutations that would change one or a few amino acids in the p16 protein. Meanwhile, Tsutomu Nobori, Dennis Carson, and their colleagues at the University of California, San Diego, had also found that the *p16* gene was frequently deleted in cancer cell lines. (Their results appeared in the 21 April issue of *Nature*.)

But finding losses of *p16* or mutations in cultured cell lines from human tumors doesn't prove that those changes were involved in the development of the cancers, since the alterations might have arisen in the lab as artifacts of cell culture. And that's just what some researchers are now suggesting has happened with *p16*. Harris's group, in collaboration with Beach's, has now looked at 200 primary tumors, including cancers of the lung, breast, esophagus, and colon. They find that "*p16* mutations and homozygous deletions [loss of both gene copies] are relatively frequent in cell lines and relatively infrequent in primary tumors," says Harris. Sidransky confirms that his group has had similar results, although he declined to discuss them because the data have not yet been published.

The Utah workers, however, are in no way ready to agree that these results mean that *p16* isn't a major tumor suppressor. Kamb does readily concede one point: The smaller, more subtle *p16* mutations occur infrequently both in primary tumors and cell lines. His group has confirmed that, he says.

But Kamb maintains the homozygous deletions, which are much more common in the cell lines, may be obscured in the primary tumors because of an inherent problem with their analysis. While it's easy to detect the loss of both copies of a gene in cell lines, which have only one cell type, it's much more difficult, he explains, in primary tumors, which are likely to be contaminated with normal, noncancerous cells. Since the normal cells retain at least one copy of the target gene, their presence may obscure the gene's total absence in the cancer cells. When Kamb brought this point up at Cold Spring Harbor, Harris maintained that such contamination should not have been a problem with their primary tumors, which had been carefully microdissected to remove normal cells.

Still, at least one group has preliminary evidence that Kamb may be right about the high rate of homozygous deletions in specific tumor types. Jin Jen, a postdoc in the lab of Johns Hopkins cancer gene expert Bert Vogelstein, has found that many brain tumors have homozygous deletions of *p16*. "The problem," Vogelstein says, "is that we don't know it [*p16*] is the target [of the deletions]. There may be something else out there, too." He means by this that other genes may have been deleted along with *p16*, and these may be the important ones for cancer.

As matters now stand, the fate of *p16* is up in the air. That state of affairs probably won't last long, though, since one thing everyone agrees on is that 9p21 does contain an important tumor suppressor gene—whether it's *p16* or not. That conviction has made that region an object of intense interest. "Within 6 months you'll see these issues addressed much more carefully by all the groups, and the answer will be much more clear than now," predicts Vogelstein. And then cancer researchers will know whether *p16* is just a meteor streaking across the sky or a glowing permanent addition in the constellation of cancer.

–Jean Marx

**The symposium, on the "Molecular Genetics of Cancer," was held from 1 to 8 June.*