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New Test Detects Colon Cancer Gene

Researchers have developed a technique that detects small amounts of a colon-cancer-triggering gene in stool samples. With anticipated improvements, the technique could become a targeted, non-invasive test for finding colorectal cancer early and should greatly improve patients' chances of being cured.

The researchers report the first feasibility trials of the new test for detecting mutations in the cancer-causing *APC* gene in the January 31, 2002, issue of the *New England Journal of Medicine*. They detected gene mutations in about 60 percent of the early-stage colorectal cancer patients tested. There were no false positive tests in patients without cancer.

"Deaths from colon cancer are totally preventable through early detection," said Howard Hughes Medical Institute investigator [Bert Vogelstein](#), senior author of the study. "If colon cancers are detected sufficiently early, before they spread, they are curable through straightforward surgical or colonoscopic methods," he said.

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- Bert Vogelstein

Vogelstein and his colleagues at the Sidney Kimmel Comprehensive Cancer Center at The Johns Hopkins University collaborated with researchers at Exact Sciences Corp. in Maynard, Mass., Uppsala University in Sweden, Lahey Clinic in Burlington, Mass., and the University of Texas M.D. Anderson Cancer Center.

One of the ideas driving the researchers was the need for a specific, non-invasive test that could be broadly applied, and thus increase the number of people taking advantage of early detection. "Current screening tests, such

as colonoscopy and detection of fecal occult blood, have significant problems. Colonoscopies are invasive, and there aren't enough professional colonoscopists in the country to perform the tests, even if people were willing to submit to them. And tests for fecal occult blood, though useful, have several problems which have stimulated the search for more specific non-invasive tests for the early detection of colorectal cancers," said Vogelstein.

The *APC* gene was discovered in 1991 by groups led by Vogelstein and Kenneth W. Kinzler at Johns Hopkins, former HHMI investigator Raymond White at the University of Utah, and Yusuke Nakamura at the University of Tokyo. The *APC* gene was chosen as the target for the new test because it plays a unique role in colon cancer pathogenesis. "Genetically-based tests have advantages over fecal occult blood tests because mutations in these genes are not simply markers of the disease; they drive the disease. And mutations in *APC* initiate the cancer, so they are present in every cancer cell from the very beginning," said Vogelstein. *APC* is a tumor suppressor gene in cells, and when mutation eliminates its function, cells are immediately launched on the pathway toward malignancy.

The researchers faced early problems in detecting *APC* mutations in stool samples. While *APC* mutations are found in nearly all tumors, they are present in as few as one in 250 of the *APC* molecules present in stool; the remainder come from normal cells shed into the feces. Moreover, human DNA represents only about one-billionth of the total DNA found in stool samples, said Vogelstein; the majority of DNA present in feces comes from bacteria. Finally, mutations in *APC* can be of different types and can occur anywhere along a stretch of a thousand or so nucleotides in the gene, making mutations especially difficult to detect in a consistent fashion.

The scientists circumvented these problems by perfecting techniques to isolate human DNA from fecal samples and to isolate and amplify the long stretches of DNA necessary to detect *APC* mutations. The key to detecting the *APC* mutations was the development of a new analytical method that the investigators termed "digital protein truncation."

"Basically, we were looking for a needle in a haystack, but the search was even more complicated because the diversity of mutations meant that we didn't even know the size or shape of the needle," he said. "We found that if we separated the sample into many smaller samples -- like separating a big haystack into tiny haystacks, that made it much more likely that we would detect the 'needles,' or *APC* mutations." The scientists also relied on the knowledge that all mutations stop protein production by the *APC* gene, resulting in truncated proteins that can be detected by their method.

To test the feasibility of the assay, the scientists applied it to stool samples from 28 colon cancer patients, 18 patients with benign adenomas -- tumors that often become malignant -- and 28 healthy people. "We wanted to test the most difficult cases, so we chose patients with early-stage cancers and those

with pre-malignant cancers, both of which could in principle be cured by routine surgery if they were detected early," said Vogelstein.

Using the assay, the researchers detected *APC* gene mutations in 61 percent of the patients with colorectal cancer; in 50 percent of the patients with adenomas; and in none of the healthy people.

"These percentages compare favorably both in sensitivity and specificity with other widely used early detection tests for cancer, including mammography and the Pap smear," said Vogelstein. "We believe that we can increase the sensitivity to over 70 percent simply by analyzing more *APC* molecules from each sample."

The lack of false positives makes the test particularly attractive for screening, he said. "One reason for the relatively poor compliance for other non-invasive tests is a lack of confidence because of false positives those tests yield," said Vogelstein. "But with this test, if a mutation is observed, our results so far suggest that there is very likely to be a cancer or a pre-malignant lesion in the patient's colon or rectum."

Physicians may one day combine the *APC* test with another test designed to detect mutations in a gene called *BAT26* that Vogelstein and his colleagues will report in the February 2, 2002, issue of the British medical journal, *The Lancet*. "This complementary assay could pick up a significant fraction of the cancers that we might miss through the *APC* assays, and together the tests could probably detect over 80 percent of the lesions," he said.

Vogelstein said he sees no insurmountable technical problems in reducing the *APC* assay to a potentially cost-effective clinical screening test. He cautioned that a larger study to replicate the high specificity and sensitivity of the initial results will be required before the test can be considered for potential clinical use. And in the years ahead, Vogelstein hopes that the science of proteomics will provide advances in protein analysis that will make the analysis of the truncated proteins detected in these assays even simpler.