Researchers develop new, more-sensitive assay for detecting DNA methylation in colon cancer

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A study published in this week's online issue of *Nature Biotechnology*, demonstrates a unique and highly sensitive method for detecting methylation-associated cancers.

Chemical modification of DNA via the addition or deletion of methyl groups has been established as a common biological means of activating or silencing genes. Abnormal levels of DNA methylation, which effectively disrupt the genes responsible for normal cell cycle regulation, has been implicated in a number of different cancers, and has led to the development of novel cancer biomarkers.

However, methylation events are rare and difficult to detect in clinically relevant samples of blood, serum, sputum, urine or feces using currently available methods of analysis. In a joint effort between Case Western Reserve University and John's Hopkins University, researchers have developed a highly sensitive method for detecting methylated DNA.

The authors say the new method, known as Methyl-BEAMing (beads, emulsion, amplification and magnetics) technology, enables absolute quantification of the number of methylated molecules in a sample, and can detect as few as one methylated molecule in approximately 5000 unmethylated molecules in DNA from plasma and fecal samples, an over 60-fold improvement over an alternative commonly used detection method.

The enhanced sensitivity of the test was achieved through the use of PCR amplification of individual DNA fragments covalently attached to specially coated magnetic beads. The process of amplification involved suspending the magnetic beads in tiny water-based nano-compartments immersed in droplets of oil. The beads contained a DNA sequence specific for exon1 of the vimentin gene - a gene known to be hypermethylated in colorectal cancer. If the vimentin gene sequence was present in the sample, subsequent PCR resulted in thousands of copies of the gene attached to each individual magnetic bead. Following amplification, the DNA-coated beads could be hybridized with fluorescent probes specific to the state of methylation, sorted and analyzed using flow cytometry. The researchers say that this method has enabled the accurate detection of a single copy of methylated vimentin sequence in a mixture and improved the technical sensitivity for detecting methylated vimentin exon1 by at least 62 -fold relative to standard methylation-specific PCR.

Using this novel method of digitally detecting methylated DNA, the researchers demonstrated that the test could be used to detect 59 percent of colon cancers in the blood, a 4-fold improvement over CEA, a serum marker commonly used to follow colon cancer patients for recurrence of disease. Additionally, the new method could detect 41 percent of colon cancers in the stool, as well as half of pre-cancerous polyps. The study was led by Bert Vogelstein of John's Hopkins University and Sanford Markowitz, M.D., Ph.D., the Markowitz-Ingalls Professor of Cancer Genetics at the Case Western Reserve University School of Medicine and an oncologist at the Ireland Cancer Center of University Hospitals Case Medical Center.

Colon cancer is the second leading cause of cancer death in the United States. This year 150,000 individuals will develop the disease and 50,000 will die from it. Researchers say that deaths from colon cancer are completely preventable when the disease is detected in its early stages,

before it has spread beyond the colon - yet many individuals do not get screened by colonoscopy.

Although colonoscopy is the most sensitive screening test available, it is time consuming, expensive and uncomfortable for the patient. Dr. Markowitz hopes that a simple blood test will be an additional tool to encourage screening and prevent the disease.

"Methyl-BEAMing provides a new method of screening for colon cancer, and hopefully will reach many of the individuals who are currently not willing or not able to participate in colonoscopy based screening for the disease," explained Markowitz. "Additionally, 10 percent of colon cancers develop in young individuals, age less than 50. These persons are currently largely excluded from colonoscopy based screening. Hopefully, an inexpensive noninvasive screening test such as Methyl-BEAMing will provide a way to offer screening for younger individuals also."

Even though the blood test only catches roughly 60 percent of colon cancers, researchers argue this is comparable to mammograms - a widely accepted screening method that ultimately leads to a more invasive follow-up procedure.

"A positive mammogram leads on to a biopsy. A positive Methyl-BEAMing test would lead on to a colonoscopy," said Dr. Markowitz.

Researchers say that in addition to being used as a screening method in younger individuals or those who are unwilling or unable to have colonoscopies, the blood test can be used in between colonoscopies, and for monitoring of the disease after therapy. Furthermore, Markowitz believes that the discovery of additional colon cancer markers will further improve the value of the test.

"We anticipate that the Methyl-BEAMing test will be improved by testing for a panel of gene DNAs that become methylated in colon cancers. So far we have identified the vimentin gene as a DNA that gets methylated in most, but not all, colon cancers. We are working on discovering additional genes that become methylated in the remaining group of colon cancers that are not detected by the vimentin test," continued Dr. Markowitz.

Future applications of the blood test are not limited to colon cancers. The same technique could be applied to the detection of other cancers, or for pre-natal testing of diseases, like downs syndrome for which altered methylation is a hallmark of the disease.

Source: Case Western Reserve University (news : web)