Scientists develop new, more sensitive nanotechnology test for chemical DNA modifications

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Researchers at The Johns Hopkins University School of Medicine in Baltimore have developed a novel test to screen for chemical modifications to DNA known as methylation. The technology potentially could be used both for early cancer diagnoses and for assessing patients' response to cancer therapies.

During methylation, healthy genes can be switched on or off potentially causing cancer without any changes in the underlying DNA sequence. The current methods for methylation screening, have significant drawbacks, explains lead study author Vasudev Bailey, a biomedical engineering Ph.D. candidate at Hopkins.

Methylation specific PCR, which copies specific DNA sequences millions of times within a few hours, may not be sensitive enough to detect small amounts of methylation, and real time PCR, which allows scientists to view increases in the amount of DNA as it is copied, needs to be run several times and can be expensive, he says.

The Hopkins-developed test makes PCR technology more sensitive and efficient, Bailey said. The work was presented at the American Association for Cancer Research's third International Conference on Molecular Diagnostics in Cancer Therapeutic Development being held September 22-25, 2008, in Philadelphia.

"The impact of detecting DNA methylation is profound, as it has been demonstrated that a larger number of tumor suppressor genes become inactivated through DNA methylation than by mutations," Bailey said. "Our method of methylation screening provides an easy, cost-effective and valuable tool for the early diagnosis of cancer, monitoring tumor behavior and measuring the response of tumors to targeted cancer therapies."

To test the technique, Bailey and colleagues treated segments of DNA with the chemical compound sodium bisulfate. This automatically converted unmethylated cytosines (one of the bases of DNA) to uracils (one of the bases of ribonucleic acid or RNA, which works with DNA to synthesize proteins), while leaving the methylated cytosines untouched.

Then the scientists used PCR with labeled primers to copy and label these DNA segments with the vitamin biotin. Next, they added quantum dots (molecules about a billionth of a meter in size with electrical properties) to the samples that had been coated with the protein streptavidin. Like a magnetic force, the biotin-coated methylated segments of DNA were attracted to the streptavidin coating the quantum dots, highlighting and quantifying DNA methylation.

The new test was sensitive enough to detect as little as 15 picograms of methylated DNA in the presence of a 10,000-fold excess of unmethylated coding sequences, or the equivalent of five cells. In addition, they demonstrated detection capability in as few as eight PCR cycles. In collaboration with his colleague Yi Zhang, also a PhD candidate at Johns Hopkins school of medicine, they were able to see results using very small samples (an average of 800 billionth of a liter per reaction and more than fifty times less sample and reagent as used currently) using a novel lab-on-chip system. This system allows for minimal handing of samples from the researcher, while allowing for simultaneous processing and analysis of multiple samples. Researchers

have a provisional patent on the test.

In additional experiments, the researchers used the technology to accurately detect methylation for the gene ASC/TMS1, which promotes programmed cell death, in low concentrations of DNA from human sputum. This was accomplished with fewer steps and fewer PCR cycles. Scientists also used the test to quantify the amount of methylation reversal in bone marrow fluid samples taken from patients with myelodysplastic syndrome – a disorder in which bone marrow cells don't function normally – before and after they had been treated with medications.

Bailey said the new test allows scientists to detect methylation of multiple genes at the same time, or view methylated and unmethylated DNA at the same time. It also reveals the percentage of methylation at any given time.

Source: American Association for Cancer Research