Liver cancer marker could yield blood test for early detection

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In the face of an emerging liver cancer crisis in Asia, researchers at the Chinese University of Hong Kong have developed a test that could help millions. Due to widespread hepatitis B virus (HBV) infection, nearly 10 percent of China's population is at high risk for hepatocellular carcinoma (HCC), a liver cancer with low survival rates if not detected and treated early. Researchers report on a new blood screening technique that could make it possible to detect early-stage liver cancer and predict how well a patient will do following treatment.

They present their data today at the American Association for Cancer Research's Second International Conference on Molecular Diagnostics in Cancer Therapeutic Development, in Atlanta, Georgia.

According to their report, the Chinese team has detected an altered version of RASSF1A, a tumor suppressing gene, in the blood of HCC patients and in 58 percent of HBV-infected test subjects. Healthy subjects showed no signs of the altered gene. They also found that patients treated for HCC with high blood levels of the gene were more likely to have a relapse of the disease.

"A large portion of the population throughout Hong Kong and China are carriers of hepatitis B, so many people are at risk for hepatocellular carcinoma," said K.C. Allen Chan, MBBS a professor at the Chinese University of Hong Kong. "And we hope that this will form the basis of an effective clinical test for early detection of hepatocellular carcinoma."

Hepatocellular carcinoma is one of the deadliest forms of cancer in China and throughout Asia, according to the researchers. In the West, liver cancer is usually a secondary cancer, caused by the spread of tumor cells from elsewhere in the body. In China, however, liver cancer mainly manifests as HCC, a primary cancer, which has been linked to hepatitis B and C infection and cirrhosis. Noticeable symptoms do not usually appear until the cancer has progressed, so it is rarely caught early, when intervention would be most effective, and survival rates are typically low, said Chan.

Currently, ultrasound and CT scans are the gold standard for detecting HCC. However, they are too expensive to be an effective mass screening tool, the researchers said. About 70 percent of patients exhibit a detectable increase in bloodstream amounts of alphafetoprotein, but a screen for this protein would miss many potential patients. “We need a new biomarker for hepatocellular carcinoma, something that can be used to screen large populations of at-risk people for follow-up studies,” Chan said.

RASSF1A is a good candidate, according to Chan. Researchers have known that the DNA of HCC tumor cells lack a functioning copy of RASSF1A. In these cells, RASSF1A is “hypermethylated,” meaning the RASSF1A gene has been physically altered by cancer-related processes that added clusters of carbon and hydrogen atoms, called methyl groups, to portions of the DNA within the gene. Hypermethylation is epigenetic – the gene is altered by environmental circumstances and is not inherited. Since the cell’s protein making system can’t access the gene, hypermethylation effectively knocks out the tumor-suppressing RASSF1A gene, which is then unable to stop cells from becoming cancerous.

While hypermethylated RASSF1A would make a useful biomarker for HCC, methylation-specific PCR – the polymerase chain reaction used to

specifically amplify and detect methylated DNA – destroys about 85 to 93 percent of the DNA in a blood sample. Together with the fact that tumoral DNA is only present at very low concentrations in blood during early stages of HCC, this method has not been sensitive enough to detected altered RASSF1A in blood for the purpose of early cancer detection, Chan said.

To compensate, Chan and his colleagues invented a new technique that they call “methylation-sensitive enzyme-mediated real-time PCR,” which combines real-time PCR, a technique that enables researchers to simultaneously detect and amplify a given gene, with an enzyme that breaks unmethylated DNA apart. With this new technique, Chan’s team was able to separate out the altered methylated DNA, thus developing a more sensitive technique for detecting and quantifying hypermethylated RASSF1A derived from cancer cells in blood.

To test the relationship between altered RASSF1A and HCC – as well as test the new detection technique -- Chan and his colleagues conducted two studies involving HCC patients. In the first, they matched 63 pairs of patients, one with HCC and the other a chronic HBV carrier by age and sex, along with 30 healthy volunteers. They detected hypermethylated RASSF1A in 93 percent of the HCC patients, 58 percent of the HBV carriers and none of the healthy patients. The median RASSF1A levels for the HCC patients were 770 copies per milliliter and 118 copies per milliliter for HBV carriers.

“The respective levels of the gene for HCC patients and HBV carriers, is consistent with what we already know about the progression of the disease,” Chan said. “The gene is altered very early in the procession of malignant transformation, and so we can see that the levels of the altered gene increase as the cancer process progresses.”

In the second study, the researchers looked at 22 pairs of sex- and age-

matched patients who had been enrolled in a HCC surveillance program involving 1018 HBV carriers. For the 22 HBV carriers who subsequently developed HCC, there was a significant increase in circulating RASSF1A levels from the time of enrollment to the time of cancer diagnosis. On the contrary, there was no significant change in RASSF1A levels over the same period for the 22 matched subjects enrolled in the same program who didn't develop HCC.

"As we refine the process of detecting hypermethylated RASSF1A, we hope to have a functioning test for hepatocellular carcinoma," Chan said. "A significant number of people will develop this cancer and it is only through early screening and detection that we can hope to help them."

Source: American Association for Cancer Research