Researchers find RNA molecules in urine and tissue that detect prostate cancer

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Researchers at Sanford-Burnham Medical Research Institute have identified a set of RNA molecules that are detectable in tissue samples and urine of prostate cancer patients but not in normal healthy individuals. The study sets the stage for the development of more sensitive and specific noninvasive tests for prostate cancer than those currently available, which could result in fewer unnecessary prostate biopsies with less treatment-related morbidity, according to a new study in The *Journal of Molecular Diagnostics*.

According to the American Cancer Society, prostate cancer is the second most common type of cancer in American men (after skin cancer), and the second-leading cause of cancer-related death in men (after lung cancer). In 2014, more than 230,000 new cases of prostate cancer will be diagnosed. One in seven American men will get prostate cancer during his lifetime, and one in 36 will die from it. Since most men with prostate cancer have indolent (nonaggressive) disease for which conservative therapy or surveillance is appropriate, the clinical challenge is not only how to identify those with prostate cancer, but also how to distinguish between those who would benefit from surgical or other aggressive treatment from those who would not.

Prostate cancer is primarily detected and monitored by testing for high concentrations of prostate-specific antigen (PSA) in blood samples. High PSA levels are often followed by a biopsy to confirm the presence of cancer and whether it is slow growing or aggressive.

"While elevated PSA can be an alert to a lethal cancer, it can also detect less aggressive cancers that may never do any harm," said Vipul Patel, MD, medical director of the Global Robotics Institute at Florida Hospital in Orlando. "Moreover, only 25 percent of men with raised PSA levels that have a biopsy actually have prostate cancer. Prostate cancer needs to be screened for; we just need to find a better marker."

The researchers believe that they have identified a group of RNA molecules – known as long noncoding RNAs (lncRNAs) – that hold the potential for serving as better prognostic markers for prostate cancer. lncRNAs were dismissed until recently by scientists as non-functional noise in the genome. However, they are now thought to regulate normal cellular development and are increasingly reported as contributing to a range of diseases, including cancer.

"We have identified a set of lncRNAs that appear to have an important role in prostate cancer diagnostics," commented Ranjan J. Perera, PhD, associate professor and scientific director of Analytical Genomics and Bioinformatics at Sanford-Burnham's Lake Nona campus in Orlando. "The findings advance our understanding of the role of lncRNAs in cancer biology and, importantly, broaden the opportunity to use lncRNAs as biomarkers to detect prostate cancer."

The study profiled the lncRNAs in three distinct groups: (1) human prostate cancer cell lines and normal prostate epithelial cells; (2) prostate adenocarcinoma tissue samples and matched normal tissue samples; and (3) urine samples from patients with prostate cancer or benign prostate hyperplasia, and normal healthy individuals. In each case, the lncRNAs were elevated in prostate cancer patient samples, but not in patients with benign prostate hyperplasia or normal healthy individuals.

One advantage of lncRNAs is that the molecules can be detected in urine samples, which are more easily available than blood tests. One lncRNA,

PCA3, was recently commercialized in a urine test to identify which men suspected of having prostate cancer should undergo repeat prostate biopsy. However, discrepancies exist between PCA3 levels and clinicopathologic features, noted Dr. Perera. In the current study, PCA3 was detected in some, but not all of the study samples, suggesting that reliance on a single biomarker may be insufficient for prostate cancer detection, whereas combining additional markers may increase the specificity and sensitivity of the test.

"There is a tremendous unmet clinical need for better non-invasive screening tools for early detection of prostate cancer to reduce the overtreatment and morbidity of this disease," added Dr. Patel. "Our findings represent a promising approach to meet this demand."

Technical details of the study

The goal of the first experiment was to see whether lncRNAs are differentially expressed in prostate cancer by measuring total RNA from prostate cancer cell lines and normal epithelial prostatic cells using NCode human ncRNA array and SurePrint G3 human lncRNA microarrays. Hierarchical clustering revealed distinguishable lncRNA expression profiles. Thirty lncRNAs were up-regulated and the expression levels of three top-ranking candidates [XLOC_007697, LOC100287482, and AK024556 (also known as SPRY4-IT1)] were confirmed in prostate cancer cell lines by quantitative real-time polymerase chain reaction (qPCR) analysis. The SPRY4-IT1 was found to be up-regulated more than 100-fold in PC3 cells compared with prostatic epithelial cells.

In a second experiment, lncRNA expression was compared in pooled prostate cancer tissue samples and matched normal tissues from 10 frozen biopsy specimens. Hierarchical clustering of the differentially expressed lncRNAs was observed and 10 up-regulated lncRNAs were

detected using microarrays. An additional set of 18 prostate cancer tissue samples was analyzed by qPCR and five lncRNAs were found to be significantly higher in prostate tumor tissues compared with matched normal tissues.

Researchers used qPCR to analyze total RNA isolated from urine in another experiment. Urine was collected from 13 prostate cancer patients and 14 healthy controls. All six lncRNAs were found to be significantly up-regulated in the urine samples from the prostate cancer patients compared with normal patient controls, whereas there were no differences between normal and benign prostatic hyperplasia patient samples.

In other studies focused particularly on SPRY4-IT1, using both qPCR and highly sensitive droplet digital PCR, expression of SPRY-IT1 was found to be increased in 16 of 18 (89 percent) tissue samples from patients with prostatic adenocarcinoma, compared to normal tissue samples. The researchers developed chromogenic in situ hybridization (CISH) techniques to visualize SPRY4-IT1 expression in cancerous and matched normal tissue. Intense staining was seen in all adenocarcinoma samples, but not in normal prostatic tissue. Finally, the investigators showed that reduction of SPRY4-IT1 in prostate cancer cells through the use of small interfering RNA (siRNA) leads to decreased cell viability and cellular invasion as well as increased apoptosis, similar to what is seen in melanoma cells.

More information: "Long Noncoding RNAs as Putative Biomarkers for Prostate Cancer Detection," by Bongyong Lee, Joseph Mazar, Muhammed Nauman Aftab, Feng Qi, John Shelley, Jian-Liang Li, Subramaniam Govindarajan, Felipe Valerio, Inoel Rivera, Tadzia Thurn, Tien Anh Tran, Darian Kameh, Vipul Patel, and Ranjan J. Perera, DOI: dx.doi.org/10.1016/j.jmoldx.2014.06.009. Published online ahead of The *Journal of Molecular Diagnostics*, Volume 16, Issue 6 (November

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