Scientists use Pap test fluid to detect ovarian, endometrial cancers

January 9 2013

Using cervical fluid obtained during routine Pap tests, scientists at the Johns Hopkins Kimmel Cancer Center have developed a test to detect ovarian and endometrial cancers. In a pilot study, the "PapGene" test, which relies on genomic sequencing of cancer-specific mutations, accurately detected all 24 (100 percent) endometrial cancers and nine of 22 (41 percent) ovarian cancers. Results of the experiments are published in the January 9 issue of the journal, *Science Translational Medicine*.

The investigators note that larger scale studies are needed before clinical implementation can begin, but they believe the test has the potential to pioneer genomic-based cancer screening tests.

The Papanicolaou (Pap) test, during which cells collected from the cervix are examined for microscopic signs of cancer, is widely and successfully used to screen for cervical cancers. However, no routine screening method is available for ovarian or endometrial cancers.

Since the Pap test occasionally contains cells shed from the ovaries or endometrium, cancer cells arising from these organs could be present in the fluid as well, says Luis Diaz, M.D., associate professor of oncology at Johns Hopkins and director of the Swim Across America Laboratory. The Laboratory is sponsored by a volunteer organization that raises funds for cancer research. "Our genomic sequencing approach may offer the potential to detect these cancer cells in a scalable and cost effective way," adds Diaz.

Cervical fluid of patients with gynecologic cancer carries normal cellular DNA mixed together with DNA from cancer cells, according to the investigators. Their task was to use genomic sequencing to distinguish cancerous from normal DNA.

The scientists had to determine the most common genetic changes in ovarian and endometrial cancers in order to prioritize which genomic regions to include in their test. They searched publically-available genome-wide studies of ovarian cancer, including those done by other Johns Hopkins investigators, to find ovarian-cancer specific mutations. Such genome-wide studies were not available for the most common type of endometrial cancer, so they conducted genome-wide sequencing studies on 22 of these endometrial cancers.

From the ovarian and endometrial cancer genome data, the Johns Hopkins-led team identified 12 of the most frequently mutated genes in both cancers and developed the PapGene test with this insight in mind.

The investigators then applied PapGene on Pap test samples from ovarian and endometrial cancer patients at The Johns Hopkins Hospital, Memorial Sloan-Kettering Cancer Center, the University of Sao Paulo in Brazil and ILSBio, a tissue bank. The new test detected both early and late stage disease in the endometrial and ovarian cancers tested. No healthy women in the control group were misclassified as having cancer.

The investigators' next steps include applying PapGene on more samples and working to increase the test's sensitivity in detecting ovarian cancer. "Performing the test at different times during the menstrual cycle, inserting the cervical brush deeper into the cervical canal, and assessing more regions of the genome may boost the sensitivity," says Chetan Bettegowda, M.D., Ph.D., assistant professor of neurosurgery at Johns Hopkins.

Together, ovarian and endometrial cancers are diagnosed in nearly 70,000 women in the United States each year, and about one-third of them will die from it.

"Genomic-based tests could help detect ovarian and endometrial cancers early enough to cure more of them," says graduate student Yuxuan Wang, who notes that the cost of the test could be similar to current cervical fluid HPV testing, which is less than $100.

PapGene is a high-sensitivity approach for the detection of cancer-specific DNA mutations, according to the investigators; however, false mutations can be erroneously created during the many steps—including amplification, sequencing, and analysis—required to prepare the DNA collected from a Pap test specimen for sequencing. The investigators needed to build a safeguard into PapGene's sequencing method, designed to weed out artifacts that could lead to misleading test results.

"If unaccounted for, artifacts could lead to a false positive test result and incorrectly indicate that a healthy person has cancer," says graduate student Isaac Kinde.

Kinde added a unique genetic barcode—a random set of 14 DNA base pairs—to each DNA fragment at an initial stage of the sample preparation process. Although each DNA fragment is copied many times before eventually being sequenced, all of the newly-copied DNA can be traced back to one original DNA molecule through their genetic barcodes. If the copies originating from the same DNA molecule do not all contain the same mutation, then an artifact is suspected and the mutation is disregarded. However, bonafide mutations, which exist in the sample before the initial barcoding step, will be present in all of the copies originating from the original DNA molecule.