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Study Finds Lung Cancer Subtype Test from UNC, Startup GeneCentric to be Accurate, Reproducible

May 30, 2013

By Ben Butkus

A team led by clinical researchers from the University of North Carolina, Chapel Hill, has published a peer-reviewed study describing the development and validation of a gene expression-based predictor of lung cancer histology for formalin-fixed, paraffin-embedded tissue samples.

The 57-gene, RT-qPCR-based assay was able to distinguish between four different lung cancer histological subtypes – adenocarcinoma, squamous cell carcinoma, carcinoid, and small cell carcinoma – with a mean accuracy of 84 percent in cross-validation studies, making it at least as accurate as diagnosis by pathologists but with much higher reproducibility and reduced workflow, according to the researchers.

In addition, the intellectual property surrounding the test has been exclusively licensed to two-year-old startup GeneCentric Diagnostics. GeneCentric in turn licensed the first application of the test – the ability to distinguish between adenocarcinoma and squamous cell carcinoma – to Laboratory Corporation of America, which has independently validated the assay and currently offers it as a laboratory-developed test called HistoPlus: Lung Cancer.

Classification of lung cancer subtypes is critical to optimizing patient care, but consensus histologic diagnoses as measured by pathologists has proven difficult. However, recent studies have shown that different histological subtypes differ not only in their morphology, but also in genetic features such as gene expression, DNA copy number, and sequence mutations.

As such, gene expression assays could be a boon for accurately diagnosing various histological subtypes. However, DNA microarrays are not the most ideal platform for such assays due to their relative incompatibility with widely used FFPE tissue samples.

To tackle this problem, the researchers from UNC-Chapel Hill's Lineberger Comprehensive Cancer Center developed an RT-qPCR-based assay on the Roche LightCycler platform – work they described in a paper published last week in the *Journal of Molecular Diagnostics*.

To select their gene signature, they sifted through published lung cancer cohorts assayed by Affymetrix gene expression microarrays. Specifically, they identified six genes that differentiated each of the following pairs of histological types: carcinoid versus other, small cell versus other, and stromal lung versus other, for a total of 18 genes. They also selected 16 genes that differentiated squamous cell carcinoma versus adenocarcinoma; 18 genes that differentiated among the three adenocarcinoma expression subtypes; and five housekeeping genes for normalization, resulting in an overall 57-gene signature.

The researchers collected 442 FFPE specimens from patients with lung cancer receiving curative-intent surgery at UNC-Chapel Hill and the University of Utah Health Sciences Center, and used the RT-qPCR test to diagnose different lung cancer subtypes, comparing the results to pathologist diagnoses on the same tumor block specimens.

They found that their histology expression predictor, or HEP, yielded similar accuracy and precision as pathologist diagnoses, and in cross-validation studies yielded a mean accuracy of 84 percent. The HEP also exhibited good performance in specimens with low tumor cellularity.

"One of the really challenging things in [diagnosing] lung cancer is that there is no gold standard," Neil Hayes, associate professor of clinical research at the UNC Lineberger Comprehensive Cancer Center, and corresponding author on the study, told *PCR Insider* this week.

"You may quote an accuracy of 84 percent and ask is that good or not good … and we had these same slides read by up to seven pathologists … and really in almost no case did every pathologist agree on the slides," Hayes added. "So it becomes very hard to comment on what the right answer is if you can't get the pathologists to agree on it. So that was a big part of developing a reproducible and reliable test."

Further, Hayes said, when the researchers took the same samples and assayed them twice, then took a paraffin slide immediately adjacent to the tissue used for the assay and had them assessed twice by pathologists, the reproducibility was around 50 percent. "So the pathologists disagree with themselves about half the time," Hayes said.

However, the PCR assay was "rock solid," Hayes said. "There were two cases where there is a discrepancy compared to over half the cases with the pathologist. You have a test that's at least as good as a pathologist at getting the diagnosis correct, but much more reproducible."

In addition, typical pathologist diagnosis involves examining the specimen under a microscope, then performing two or three special immunohistochemical stains, "and then in addition you frequently have to do DNA assays as well, including mutational analysis," Hayes added. "This is going to require 10 or 15 paraffin sections. But with this assay, you don't have to do that anymore. You just need one 5-micron section, and you can get all of that."

Last year, Hayes, along with UNC-Chapel Hill colleague Charles Perou, co-founded GeneCentric Diagnostics as a vehicle to commercialize the assay, which they had dubbed the Lung Subtype Platform. Other company co-founders included Hatteras Venture Partners and Myla Lai-Goldman, former CMO and CSO of LabCorp and a venture partner at Hatteras Venture Partners.

As the UNC-Chapel Hill researchers were finishing their study on the Lung Subtype Platform, GeneCentric, which has an exclusive license to discoveries from the Hayes and Perou laboratories, licensed the lung cancer test to LabCorp for the purposes of distinguishing solely between adenocarcinoma and squamous cell carcinoma.

According to LabCorp's website, the HistoPlus: Lung Cancer test should be used in conjunction with other clinical and laboratory findings, such as histopathology, to help select appropriate therapies for patients.

"The [*JMD*] paper is a bit different than the first test that LabCorp has launched in that the paper shows the ability to do all of the subtypes of lung cancer – all of the small cell subtypes, so adenocarcinoma and squamous, as well as carcinoid and small cell," Lai-Goldman told *PCR Insider* this week.

"LabCorp independently validated the non-small-cell subtypes, the adeno and squamous," she added. "They initially focused solely on that area, because there is utility today in being able to provide that test … for several drugs that are on the market. There is opportunity in the future to expand that utility, and that is what we plan. But we thought it was wise to start with something that could be useful for patient care today."

Underscoring the importance of distinguishing between adenocarcinoma and squamous cell carcinoma, Hayes noted that one current therapy, pemetrexed (sold as Alimta by Eli Lilly) has been shown in a Phase III clinical trial to be ineffective for squamous cell carcinoma.

In addition, a second drug, bevacizumab (sold as Avastin by Genentech/Roche) "is specifically dangerous for patients with squamous cell carcinoma, because it tends to cause tumor necrosis and life threatening bleeding in patients when they're treated with it."

Lai-Goldman also noted that LabCorp validated its test using a different RT-qPCR platform than the LightCycler platform used by the UNC researchers, though she declined to disclose which platform the company used due to confidentiality concerns.

"The paper uses a LightCycler methodology, and LabCorp uses a different platform," she said. "But it shows the strength of our gene set that it doesn't hinge upon any particular platform or instrument in the lab. The strength is the gene set, and that's what our IP is based on."

GeneCentric and the UNC researchers have also developed a second platform technology called the Hypoxia Signature, which has the potential to identify patients that respond to anti-angiogenesis therapies. This test, Lai-Goldman said, is currently in clinical trials.

"GeneCentric is very much focused on overcoming barriers [to test adoption] by building a strong evidence base for how these tests can benefit patients," she said. "Our plan is to commercialize through partnerships, and the partnership we developed with LabCorp is an example of executing our business model. It gave us the ability to independently validate the technology."

As for the Lung Subtype Platform, Hayes would like to see it become a candidate for a gold standard diagnostics, but noted that his team still needs to conduct more work demonstrating its usefulness.

"We want to show that if we make the correct diagnosis with the molecular assay, compared to the standard diagnosis, that we actually see an improved outcome," Hayes said. "The second thing is, we would really like to see this offered in the small volume biopsy realm."

And finally, Hayes said, the researchers would like to push the technology further for other uses, much in the same way that Genomic Health has demonstrated multiple applications for its Oncotype Dx.

"Not just making existing diagnoses better – we'd like to make new diagnoses," Hayes said. "We have the content, the gene set for that. In fact, it's actually embedded in the same content as this assay. But in order to deliver that you have to provide the evidence that you're doing what you say you're doing, and that's another thing we're working on."

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