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Noninvasive Detection of Bladder Cancer by Shallow-Depth Genome-Wide Bisulfite Sequencing of Urinary Cell-Free DNA for Methylation and Copy Number Profiling

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Guest: Dr. Timothy Cheng is a Resident and Honorary Tutor in the Department of Chemical Pathology at the Chinese University of Hong Kong.

Bob Barrett: This is a podcast from *Clinical Chemistry,* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I am Bob Barrett.

Bladder cancer is the ninth most common cancer worldwide. The current diagnosis and monitoring of bladder cancer is still heavily reliant on cystoscopy, an invasive procedure where the tumor is directly visualized. However, a paper appearing in the July 2019 issue of *Clinical Chemistry* from Dr. Dennis Lo's laboratory in Hong Kong reports that bladder cancer can be detected noninvasively in urinary cell-free DNA by methylomic and copy number analysis. Such analyses could be used as a liquid biopsy to aid in the diagnosis and monitoring of bladder cancer.

We are pleased to have the first author of that study, Dr. Timothy Cheng, as our guest in this podcast. He is a Resident and Honorary Tutor in the Department of Chemical Pathology at the Chinese University of Hong Kong.

So Dr. Cheng, let's start off by just telling us a little bit about urine testing for bladder cancer.

Dr. Timothy Cheng: Sure. So, bladder cancer testing is very important for diagnosis. For example, for those who are passing blood in the urine and it's also very important for cases of bladder cancer, because for some cases, even after complete excision, you get recurrence rates as high as 70 to 80%. And so, this really necessitates that these patients go in for regular cystoscopies in up to about three-monthly intervals. And I think I speak for most if not all of us that we would all prefer a noninvasive urine test as compared to undergoing cystoscopy or an endoscopy once every three months.

So currently, in the state of things, urine cytology where they collect urine and look at the cells, is the only urine-based test which is widely used, and it's actually known to have quite a low sensitivity and this is particularly true for low-grade tumors. So, it struggles to pick up low-grade tumors.

So therefore, a more sensitive and more specific urine-based test would be very helpful in the diagnosis and monitoring of bladder cancer. And for example, if it's a really good test, you could actually do it in shorter intervals and that would aid the prioritization of the high-risk cases and those cases, you really concentrate on for the cystoscopy and also further treatment.

And so basically, even before, there have been multiple attempts at targeted tests, where a single genomic aberration or a single target or several targets looked at, at a single time, and this is in hopes of improving the urine testing over urine cytology.

Bob Barrett: So how is this cell-free DNA methylation and copy number profiling approach different from previous molecular tests?

Dr. Timothy Cheng: So actually when you think about it, for all targeted tests, it's really predicated on the tumor expressing what you are targeting, so the targeted mutation. For example, some of the commonest bladder cancer mutations for example, occurring in the TERT or FGFR3 loci, they are only found in about less than about half of the bladder tumors.

And so, for example, if the tumor does not express the mutation that you are looking for, then of course, that test will test negative. So, based on how heterogeneous bladder tumors are, we thought that we could use a genome-wide methylation and copy number scan instead, and so we have no preconception or no previous knowledge of where the mutations are occurring or might be and we would just look at the whole genome, and our approach is actually a combination of three approaches.

So, we used a deconvolution process, where we deduce the tissue of origin based on methylation pattern. So, it's basically like looking at the pattern on small pieces of DNA and then, matching up with the methylation pattern in the tissues that it could have come from and then, deducing how much of the DNA actually came from tumor.

On the second approach, we use is something called global hypomethylation. So, we know that this is really a hallmark of many different cancers, and so, it's associated for example, with the loss of regulation. So, we looked to see if there was this global hypomethylation pattern.

The third approach that we used is called looking at copy number gains and losses. This also is seen in many different cancers of different tissues. And we were able to have ROC curves with the area under curve of 0.9 to 0.97 using these three approaches. And I think what's special about this is that, in a single analytical run, each approach

is actually looking at a slightly different aspect of the bladder tumor and thus, when we can combine all three approaches, we can improve the sensitivity to 94% and the specificity to 96%.

Bob Barrett: Dr. Cheng, could you talk about some of the challenges for detecting bladder cancer in the urine, particularly noninvasive low-grade disease?

Dr. Timothy Cheng: If I may just to start off with, I think there are some very good points or actually some positives about detecting bladder cancer in the urine. The urinary system is like a unidirectional drainage system, where you have basically a constant flow of the urine and the bladder tumor can shed this kind of tumor DNA and it's doing so quite close to the exit where we're collecting the urine.

On the flip side though, cell-free DNA in the urine is actually quite a bit shorter than the cell-free DNA fragments that we find in the plasma and thery're generally less than about a 100 base pairs in length. In our previous work, we demonstrated that the urine cell-free DNA is actually fragmented in a time-dependent manner. So, it means that, as it travels down the urinary tract, it is actually getting shorter and shorter, and in some sense that it means, it's getting degraded.

Dr. Timothy Cheng: And the other challenge really is that bladder tumors are quite genetically heterogeneous, and so, this really makes it very tricky for the previous targeted assays that I talked about. So, although some genes are commonly mutated, the exact combination or constellation of genetic mutations can vary a lot from tumor to tumor.

So, I think all of these things make it more suited in a sense to a genome-wide approach that we're doing. For your non-muscle invasive low-grade disease, it can be more challenging, because the tumor load is relatively smaller and the genomic aberrations are less widespread.

However, in our case series, we had cases of low-grade noninvasive bladder cancer and we were still able to achieve a sensitivity of 84%. And it was quite encouraging that we had a single case, which was a case with the noninvasive disease and also the lowest possible histological grade and this is called a papillary urothelial neoplasm of low malignant potential. And so, basically, it was normal in terms of two of our three approaches that we were able to detect copy number gain in chromosome 10.

And so, I think, this goes to show how using diversified approaches based on a single assay, we are able to increase the chances of detecting a low-grade tumor.

Bob Barrett: All right. What else did this study reveal?

Dr. Timothy Cheng: So, we did several things as well. For example, we looked at pre- and postoperative urine samples and we were able to detect residual disease, and this really opens up the chances of using this for longitudinal monitoring for example.

And also, we realized that our test is more than a binary test. So, in the three approaches that we are using, we are able to see a positive correlation between the degree of the aberrations, and there is a correlation between that and the tumor size, the muscle invasiveness, and the tumor grade.

And so, for example, you can imagine, because of this positive correlation, we are less likely to miss late phase disease, and also we may be able to track tumor burden longer term. I think the other thing that was very interesting in terms of biological characteristics is that we looked at the size of cancer DNA compared to the DNA that originated from normal tissue.

Dr. Timothy Cheng: We noticed in some cases that, in bladder cancer patients, the cell-free DNA in the urine was slightly longer compared to non-cancer controls. And then, we delved a bit deeper and we looked at the copy number variation. So basically, in the genomic regions where you get copy number gain, you could imagine that's actually enriching for bladder cancer DNA whereas, regions of copy number loss are actually enriching for normal DNA.

And so, when we compared the size of cell-free DNA fragments between the gains and the loss regions, we found that tumor DNA was indeed slightly longer than a normal DNA. This is actually quite a fascinating phenomenon and this is because this is the exact opposite of what we observed in plasma and although it just seems like a slight size difference, something like this can be exploited potentially, as a strategy to further enrich for tumor DNA and to improve the detection.

Bob Barrett: Okay. Well, finally doctor, look ahead, what do you think the next steps will be?

Dr. Timothy Cheng: I think ,there are really exciting opportunities ahead and of course, it would be very important to validate this on a large number of cases to see how it's helpful for diagnosis and monitoring and the other thing is, because this is a genome-wide test, basically, each test is like a genome-wide scan. And with this, we can actually build a larger database to understand and potentially predict disease recurrence and progression.

And I think lastly, one opportunity may be upper tract urothelial cancers, so these are cancers that arise in the upper urinary tract and these are actually quite difficult to diagnose in general. If we were able to detect that in the urine based on a noninvasive test, I think, that would be very promising.

Bob Barrett: That was Dr. Timothy Cheng from the Department of Chemical Pathology at the Chinese University of Hong Kong. He's been our guest in this podcast on noninvasive detection of bladder cancer by sequencing of urinary cell-free DNA. That paper appears in the July 2019 issue of *Clinical Chemistry*. I am Bob Barrett. Thanks for listening.