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What is the Importance of Analyzing Tumor Tissues and Blood Cells in the Study of Circulating Tumor-Derived DNA?

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Guest: Dr. K.C. Allen Chan, Professor 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'm Bob Barrett.

Circulating tumor DNAs are promising biomarkers for early tumor detection and assessment of residual disease in early-stage cancer. However, quantifying minute amounts of circulating tumor DNA is challenging and well-designed studies with early-stage cancers are still lacking.

The December 2022 issue of *Clinical Chemistry* published a paper where the investigators adapted a sensitive next-generation sequencing technology and performed parallel analysis of pre- and postoperative circulating tumor DNA and matched tumor tissues in a prospective cohort of patients with resectable pancreatic ductal adenocarcinoma.

The same issue of the journal had an accompanying editorial that asked the question, What is the Importance of Analyzing Tumor Tissues and Blood Cells in the Study of Circulating Tumor-Derived DNA?

The authors of that editorial are Stephanie CY Yu and KC Allen Chan from the Chinese University of Hong Kong. We're pleased to have Dr. Chan as our guest in this podcast. He is currently Professor of Chemical Pathology at the Chinese University of Hong Kong, where his research focuses on the development of clinical applications of circulating cell-free DNA analysis. He is a co-inventor, with Dr. Dennis Lo, of the non-invasive prenatal test for Down Syndrome, which has been adopted in over 70 countries with millions of tests performed each year.

So first of all Dr. Chan, what are the major challenges in the early detection of cancer using circulating tumor-derived DNA?

Allen Chan: Actually detecting cancer at an early stage using circulating tumor-derived DNA, ctDNA, is challenging. First, in cancer patients, only a small proportion of the circulating DNA is derived from tumor cells, and most of the other DNA is

derived from normal tissues. The fraction of the DNA coming from tumor cells is positively correlated with the size of the tumor.

So, in patients with early cancers, the proportion of DNA deriving from tumor cells is very low and typically less than 1%, and detecting mutations at such low fractional concentration is technically challenging because the detection methods can generate errors.

For example, the sequencing errors using the common next-generation sequencing platforms is in the range of 0.1%. Hence, it is challenging to differentiate sequencing errors from real mutations present at very low levels.

Moreover, the mutational profiles of different cancer patients vary widely even if they suffer from the same type of cancer. Therefore, we need to search for a large genomic region to find these mutations. The larger the genomic region we search would generate more sequencing errors.

Bob Barrett: What strategies did Lee and colleagues use in their study to overcome such challenges?

Allen Chan: As sequencing errors is one key challenge, Lee and colleagues used two strategies. One is molecular barcoding and the other one is in silico filtering of background artifacts; that is the sequencing errors.

In molecular barcoding, each original DNA would be given a barcode using DNA tags. So each original DNA molecule would be given one unique barcode and after sequencing, because the sequencing involve PCR amplifications and all the subsequent daughter molecules amplified from the same original molecule would bear the same barcode so that after sequencing, all the molecules sharing the same barcodes can be analyzed together.

And the other one is they use bioinformatics algorithms to filter out the background artifacts associated with these mutations. And these two strategies allow them to eliminate a lot of sequencing errors so that they would be able to reliably achieve a detection of mutation at a variable early fraction of 0.1%, so they can achieve a very low detection limit for the mutations.

Bob Barrett: And how satisfactory was the performance of these strategies in the detection of early-stage pancreatic cancer?

Allen Chan: With these strategies, they can detect mutations present at very low concentration. Unfortunately, even with these strategies, because they are detecting oncogenic mutations, mutations were only detected in less than 40% of patients

before surgical resection of the tumor. So in another 50% of patients, oncogenic mutations were detected in the tumor tissues but not in plasma.

So the observations suggest that even if we have a method that has a very low detection limit, oncogenic mutations could only be detected in less than half of the patients with early cancers because the concentrations of tumor-derived DNA in plasma is very low in patients with early-stage cancer.

Bob Barret: So, how can we further improve the performance of liquid biopsy for detecting early cancers and minimal residual disease after treatment?

Allen Chan: In the study by Lee et al., they search for driver oncogenic mutations in circulating DNA of cancer patients. In patients with detectable mutations in plasma, only one mutation could be detected. So one possible way to improve the clinical sensitivity is to look for more cancer-associated targets.

For example, in a previous study, it was shown that the detection of Epstein-Barr Virus DNA, the EBV DNA, is useful for the screening of early asymptomatic nasopharyngeal cancer. In the study, the assay targets a repeat region in the EBV genome. Each EBV genome has 10 repeats of that target.

Furthermore, each tumor cell contains approximately 50 EBV genomes. As a result each tumor cell would contain approximately 500 targets. As the number of targets increase, the sensitivity would accumulate. So this has greatly enhanced the clinical sensitivity for detecting the EBV DNA in the plasma. So, for the detection of early cancers or minimal residual disease, we can increase the number of targets to be detected so as to improve the clinical sensitivity.

Bob Barrett: Well finally, Dr. Chan, in the study by Lee and colleagues, matched tumor tissues and blood cells were analyzed in addition to the plasma samples. What are the advantages of analyzing the matched tumor tissues and blood cells?

Allen Chan: For the analysis of matched tumor tissues, so one key application for the detection of oncogenic mutations in cancer patients is to guide the use of target therapy. As demonstrated by Lee et al., less than half of patients with oncogenic mutations in tumor tissues had the corresponding mutation detected in plasma.

In other words, the analysis of plasma for mutations can give false negative results if we target these oncogenic mutations. Therefore, the testing of tumor tissues for mutations is necessary in patients with undetectable mutations in plasma

to reflect the true mutational profile so as to more accurately guide the use of target therapy.

In addition to false negative results, analysis for mutation in plasma can also have false positive results. Clonal hematopoiesis is one important source of such false positive results. Mutations can be observed in certain clones of hematopoietic cells. These mutations can be mistaken as mutations derived from cancer cells. The chance of having clonal hematopoiesis increases with age. The analysis of blood cells is useful for differentiating clonal hematopoiesis from the presence of cancer-associated mutations and hence reduce the false positive detection of cancer-associated mutations.

Bob Barrett: That was Dr. Allen Chan, Professor of Chemical Pathology at the Chinese University of Hong Kong. He has been our guest in this podcast on circulating tumor DNA. His editorial, as well as an original scientific paper on circulating tumor DNA and matched tumor tissues in pancreatic adenocarcinoma, appear in the December 2022 issue of *Clinical Chemistry*.

I'm Bob Barrett. Thanks for listening.