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A Study of Hypermethylated Circulating Tumor DNA as a Universal Colorectal Cancer Biomarker

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Guest: Dr. Valerie Taly leads the Translational Research and Microfluidics Group at the Université Paris Descartes.

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.

Noninvasive dynamic monitoring of tumors is an exciting area of development in cancer diagnosis and care. Recent advances are based on the quantification of circulating tumor DNA in body fluids. Despite its promise, there are a number of challenges with so-called liquid biopsies and several technical approaches have been used.

The August 2016 issue of *Clinical Chemistry* published details of a method for a colon cancer follow-up that combines the simplicity of picoliter droplet-based digital PCR with pertinent hypermethylated sequence monitoring.

The article senior author, Dr. Valerie Taly, joins us for this podcast. Dr. Taly leads the Translational Research and the Microfluidics Group at the Université Paris Descartes. Her research is dedicated to the development of new tools, procedures and strategies for cancer diagnosis and care.

So, Dr. Taly, what are the principal findings of your work?

Dr. Valerie Taly: Okay. So, many works, including ours have shown that you could detect circulating DNA in body fluids, droplet-based digital PCR is a perfect tool to do such analysis because it could give both high sensitivity and accuracy on the measurement leading to highly quantitative measurement. If you think about classic strategies that are used to detect circulating tumor DNA, the body can rely on the identification of the mutations, specific of the tumor of the patient generally identified by next-generation sequencing, for example, and then what you do is that you follow the specific mutation with a specific assay within the DNA coming from the body fluids. Basically, this assay could be either commercially available or designed specifically for this patient, and this requires the development of a very high quantity of assay. Sometimes they are specific for the patient and sometimes you just manage to design an assay because it's a difficult target, for example.

So, in the case of circulating DNA, we have been calculating that if we wanted to, we have a big cohort of a high number of patients and if we wanted to -- if we would be using 50 assays, we would just be able to follow basically half of our patients in the circulating DNA. So with regard to these results, we were trying to find some more universal markers that will allow us to detect much more patients with a limited number of assays.

In this study, it's exactly what we did. We basically demonstrated that we could use just two methylation markers, DNA methylation markers to screen all the patients that we have in our cohort. We just look in the tumor DNA, the DNA coming from the tumor and from adjacent tissue and demonstrated that these two markers that has been previously highlighted by Professor Iradj Sobhani, with whom we collaborate, could be detected in all the tumor and not in the adjacent tissue, and this is independently of the state of the tumor and then we demonstrated that within circulating tumor DNAs, a follow-up of the circulating DNA by mutation analysis or by methylation analysis was correlated, and that we could basically track in the circulating tumor DNA this methylated marker to really be able to see the tumor DNA within the body fluids.

Also, for a limited number of patients, let's say nine patients, we demonstrated that we could basically monitor treatment efficiency for a person with metastatic colorectal cancer using this methylation marker and also that we could detect for localized cancer earlier occurrence, but this is very preliminary and we need to analyze much more patients to really have the conclusion here.

Bob Barrett: Do you think that digital PCR is the best option to detect and monitor circulating tumor DNA?

Dr. Valerie Taly: As we mentioned earlier, droplet-based digital PCR presents a high sensitivity of (00:03:51) experiments of the marker. Multiplex analysis is visible, but limited to few markers (00:03:59) ten here, five to ten. Next-generation sequencing in parallel could also detect high quantity of marker, but presents lower sensitivity and accuracy. Of course optimized procedure has been developed, but they always present lower sensitivity than digital PCR.

In our work, by targeting methylation marker, as we said, just with two markers, detect all patients leading to both low occurrence and higher sensitivity than other procedure and thus we could use droplet-based digital PCR in this context.

Bob Barrett: What's the difference between your approach and the ones classically used for circulating DNA follow-up based on the detection of specific mutations?

Dr. Valerie Taly: As we mentioned earlier, the use of tumor-specific mutation requires the development of a very high number of assays to be able to follow all of our patients from a specific cohort; sometime we need to develop one assay for one patient. This process is very expensive and often inefficient.

In our case, we demonstrated that two methylation markers could be used to detect all colorectal cancer patients independent of the stage of the illness, and in addition since the marker got present in all the colorectal tumors that we've been testing -- actually you don't have to test the colorectal tumors before to look for circulating DNA. So you could go directly for circulating tumor DNA without having to rely on tissues that sometimes are difficult to obtain.

Bob Barrett: Why did you decide to choose these markers in your study?

Dr. Valerie Taly: So, it could be that the calculation that's been described as early in cancer evolution and we still decided to -- we still, with Professor Pierre Laurent-Puig with whom we collaborated with the group to use these markers for the follow-up of circulating DNA. We have an ongoing collaboration with Professor Iradj Sobhani from APHP and he has presently shown that the use of three different methylation marker using a classic conventional PCR approach allowed to detect colorectal cancer tumor DNA. However, this procedure was not using digital PCR. We combined droplet-based digital PCR and these markers and demonstrated that two of them could be used to track the circulating tumor DNA very efficiently.

We have tested and validated this marker and demonstrated that the use actually of one of this marker, which is WIF1 allowed to detect 94% of the tumor sample and that's hiding the NPY marker also detect 100% of the tumor DNAs that we've been testing.

Bob Barrett: Dr. Taly, in your opinion, do you think that hypermethylated circulated tumor DNA markers can replace, for example, KRAS mutated circulating tumor DNAs for a circulating DNA detection and monitoring?

Dr. Valerie Taly: So I believe that follow-up of hypermethylated marker in case of colorectal cancer could replace the detection of specific mutations to highlight this circulating tumor DNA. However, this can't replace the detection of specific mutation when these mutations are associated to a specific treatment. For example, KRAS mutations are predicted to resist to sensitivity to targeted treatment, targeting EGFR

and this can't be replaced by the use of a methylated marker.

Bob Barrett: Finally, doctor, what do you think is the biggest challenge with development of methods for detecting circulating tumor DNA and cancer?

Dr. Valerie Taly: There are many challenges for the detection of circulating DNA cancer, and if we can fight some of them, I would say that the first one is actually to have an assay that will allow to discriminate circulating DNA coming from the tumor, from the one coming from normal cells and you have a very amazing -- well, you have a high quantity of DNA coming from the death of normal cells. Second, you need to have an assay that is highly specific. You need to have a procedure that is highly specific and quantitative to be able to track (00:07:55) modification in the circulating tumor DNA. It should allow very precise measurement of quantity of tumor DNA present in the sample, but also to perform real time analysis of its evolution. In our study, I believe answers both of these challenges.

In addition, you also need to be able to get the (00:08:12) amount of DNA as possible to be able to reach a very high sensitivity, and this will depend on our capacity to get enough blood, but also probably on the development of new accurate procedures for tumor DNA extraction from serum or plasma.

Bob Barrett: Dr. Valerie Taly leads the Translational Research and Microfluidics Group at the Université Paris Descartes. She's been our guest in this podcast from *Clinical Chemistry*.

I'm Bob Barrett. Thanks for listening!