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Molecular Portrait of Metastasis-Competent Circulating Tumor Cells in Colon Cancer Reveals the Crucial Role of Genes Regulating Energy Metabolism and DNA Repair

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Guest: Dr. Catherine Alix-Panabières is the Director of the Laboratory of Rare Human Circulating Cells and an Associate Professor at the University Medical Center of Montpellier in France.

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 cells are fundamental to the concept of a liquid biopsy for cancer. These cells are shed from the primary tumor and are carried in the blood to remote sites, potentially leading to metastasis formation. Understanding more about this process is critical as tumor metastasis is a leading cause for cancer death. Yet this is challenging as the number of circulating tumor cells in an individual is very small, making them difficult to study in detail.

Cell lines derived from circulating tumor cells can be created to overcome this problem. This has been done in colon cancer, for example, and enables researchers to examine molecular and functional differences between the circulating tumor cells and those from the primary tumor. In this way, researchers may further our ability to identify and characterize cells that initiate metastasis and to ultimately develop new therapies to stop them.

An original report in the January 2017 issue of *Clinical Chemistry* describes the differential gene expression between colon cancer cell lines derived from circulating tumor cells and the primary tumor. Dr. Catherine Alix-Panabières is the primary author of this article and joins us for this podcast. Dr. Alix-Panabières is the Director of the Laboratory of Rare Human Circulating Cells and an Associate Professor at the University Medical Center of Montpellier in France.

So doctor, what is the current status of research work on circulating tumor cells? Are there challenges to translating this to clinical care?

Dr. Alix-Panabières: Circulating tumor cells, or CTCs, in blood are considered as the real-time liquid biopsy of cancer. They're optimizing new biomarkers potentially useful for prognostic prediction and monitoring of therapies in patients. We study tumors including colon cancer. You know that Professor Pantel from Hamburg and I, we coined this phrase, "liquid biopsy" for

the first time in 2010. It is now used for all circulating biomarkers in cancer all over the world in the cancer field.

The application of CTCs for the early detection of cancer is of high public interest, but it faces serious challenges regarding specificity and sensitivity of the current cell. Prediction of prognosis in patients with curable disease can already be achieved in several tumor entities, particularly in breast cancer. And monitoring the success or failure of specific therapies, for example chemotherapy, hormonal therapy or all the targeted therapies by sequential measurement of CTCs is also feasible. Interventional studies on treatment stratification based on the analysis of CTCs are needed to implement liquid biopsy into personalized medicine.

We are looking forward to getting the results of the STIC METABREAST study to get the clinical utility of CTCs. This is a French interventional phase three clinical trial on the enumeration of CTCs to guide the clinicians for giving hormonal therapy for less than five CTCs. This is chemotherapy for at least five or more CTCs detected to metastatic breast cancer patient. The clinical validity has been already demonstrated with the robust meta-analysis on almost 2,000 patients with metastatic breast cancer. Only CTCs could give prognostic information at baseline, three to five weeks or six to eight weeks after the initiation of treatments whereas the standard serum tumor markers in breast cancer did not.

CTC research opens a new revenue for understanding the biology of metastasis in cancer patients. However, an in-depth investigation of CTCs is impaired by a very low number of these cells especially in the blood of colorectal cancer patients. First, the establishment of cell cultures and permanent cells lines from CTCs has become the most challenging task over the past years.

Bob Barrett: Doctor, you and your colleagues established the first cell line for circulating tumor cells in cancer, why is this such an important breakthrough and how did it factor into this study?

Dr. Alix-Panabières: In 2013, our group established the first stem cell line named CTC-MCC-41, derived from metastasis competent CTCs of a patient with a metastatic colon cancer. Unraveling the molecular mechanisms that regulate the biology of metastasis compete in CTCs are urgently needed to understand metastasis formation and tumor relapse. The current CTC line is in culture for more than three years now and cells have been characterized at the genome, transcriptome, proteome and secretome levels.

These data have been published in *Cancer Research* in 2015. The thorough analysis show that CTC-MCC-41 cells resembled characteristics of the original tumor cells in the colon cancer patient, and display a stable phenotype characterized by an intermediate endothelial-mesenchymal phenotype stem cell-like properties and in those two (00:05:29) signature including a bone marrow origin.

Functional studies show that CTC-MCC-41 cells induced rapidly in vitro endothelial cell tube formation to mimic angiogenesis in vitro and in vivo tumors after the xenografting in immunodeficient mice. The establishment of these first colon CTC line eludes now a wealth of functional studies on the biology of CTCs as well as in vitro and in vivo direct testing.

Bob Barrett: Doctor, please tell us more about the key findings in the differential gene expression between these two types of cells lines.

Dr. Alix-Panabières: Comparison of the transcriptome data of metastasis complete in CTC-MCC-41 cells and of HT-29 cells derived from a primary colon cancer highlights the differential expression of genes that regulates energy metabolisms and stemness. The differential expression of 20 genes has been validated by quantitative RT-PCR. But then we also tested many other cancer cell lines established from primary tumors and from metastatic sites.

Hierarchical clustering analyses using Affymetrix transcriptome analysis controlled software mapped the samples into two main major clusters. We observed a clear segregation between the CTC-MCC-41 cell line on one side and the other colon cancer cell lines on the other side. The HT-29 samples self-cluster into another branch and are situated in the vicinity of the primary and metastatic colon cancer cell line. These results are very interesting and highlights clearly that CTC-MCC-41 line displays a very specific transcription program.

Interestingly, among the 1,624 transcripts exclusively upregulated in CTC-MCC-41 samples, key genes related to energy metabolisms, DNA repair, and stemness genes were observed.

Bob Barrett: What do these results mean for our understanding of colon cancer progression or metastasis?

Dr. Alix-Panabières: What is important to understand is that these metastasis complete in CTCs are very rare in the blood stream of colon cancer patients. We could establish CTC lines only from one unique patient with a metastatic colon cancer out of 165. It was really a challenge and only a few groups. less than five

in the world, could achieve establishing in vitro permanent CTC lines in breast, prostate, and lung cancer. These subsets of CTCs needs to have specific properties allowing them to generate new tumors at different sites.

Based on our very recent results, they have clearly stem cell properties, they can repair their DNA differently than the other tumor cells, and they show a special metabolism producing a lot of energy making them super tumor cells. For us, it's very important to understand these new pathways allowing the CTCs to disseminate, to survive in the bloodstream, and to extravasate in different organs to form metastasis causing relapses in cancer patients and subsequently their death.

Bob Barrett: Well, finally doctor, let's look ahead. What are the next steps in this area of work?

Dr. Alix-Panabières: We have established eight other colon CTC lines from the same patients during these treatments and after two subsequent relapses. It will give us precious information on the cancer progression and their therapy with special clonal selection and their chemotherapy and targeted therapy. Such data may supply insights from the discovery of new biomarkers to identify the most aggressive CTC subpopulation and for the development of new drugs to inhibit metastasis initiators, CTCs in colon cancer. At the end, we must find a way to eradicate specifically these rare, aggressive CTCs with new targeted therapies.

These new original data just published in *Clinical Chemistry* may ultimately help developing new personalized treatments for patients with colon cancer.

Bob Barrett: Dr. Catherine Alix-Panabières is the Director of the Laboratory of Rare Human Circulating Cells and an Associate Professor at the University Medical Center of Montpellier in France. She's been our guest in this podcast from *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.