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Cell-Free DNA Fragmentomics: The New "Omics" on the Block

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Guest: Dr. Rossa Chiu, a Professor of Chemical Pathology and Associate Dean for Development 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.

Many may have the impression that with the explosive growth of nucleic acid-based technologies in the last three decades, that the idea of the genome and genomics are relatively new concepts, but the year 2020 marks the 100th anniversary of the first use of the word "genome" by Winkler in a 1920 textbook. "Proteomics," the large-scale study of proteins, was coined in 1994, opening the floodgates to literally hundreds of other so-called "-omics."

A Q&A feature appearing in the December 2020 issue of *Clinical Chemistry* examined one of the new -omics on the block, namely cell-free DNA "fragmentomics." Four experts with different roles in this field discussed recent advances and ongoing challenges in this area. The moderator for that feature is Dr. Rossa Chiu, a Professor of Chemical Pathology and Associate Dean for Development at The Chinese University of Hong Kong. She is a clinician-scientist specializing in the research of the diagnostic potential of cell-free nucleic acids, and we're pleased to have her as a guest in this podcast.

So, Dr. Chiu, we know there are many -omics, but fragmentomics, that's a new one on me. What are they and how can fragments be an "omics"?

Dr. Rossa Chiu: All right. So, the name fragmentomics, as it suggests, it means nucleic acid fragments across the genome. So, where do we get fragmented nucleic acids? Well, what we refer to is actually the cell-free DNA or RNA molecules that naturally exists as fragments in bodily fluids, for example plasma, and when we talk about fragments, it's because they are highly degraded DNA or RNA molecules compared to what we normally find in an intact cell. And as for why the studying of these nucleic acid fragments has -- we refer it to as an -omics, I guess there are two levels to this definition. Firstly, it's because initially when we started off studying the fragments, because there are typically very few of them, we had to pull the fragments across the genome in order to get enough signal to make any meaningful

interpretation. So, the analysis itself is an -omics type of analysis. But now, after we have found interesting findings, we found that these findings are actually scattered across the genome. So, what do we mean by fragmentomic findings? That is for example, the length of the DNA molecules at different parts of the genome, which part of the genome has more DNA molecules of particular sizes, or which part of the genome has a fragment that entered with a particular sequence. So, to make it easy to describe that complex web of features, we call it fragmentomics.

Bob Barrett: And how have studies regarding DNA fragments in human bodily fluids become a branch or field of study on its own?

Dr. Rossa Chiu: Well, initially, the main motivation for studying a cell-free DNA analysis is to look for species of DNA that might have been released from various organs that might be useful for doing diagnostics on them. So, for example, we have analyzed DNA that came from the placenta for prenatal diagnosis. We've analyzed DNA release from tumors for cancer assessment, but we need to somehow distinguish these molecules of interest with the molecules that are just floating around in the background that might not be from a diseased organ or the organ of interest. So previously, when the field first started, we tried to use genetic differences. For example, for tumor, we might be looking for mutations that were present in the tumor genome and use those features to distinguish the tumor DNA from the non-tumor DNA. But then, as time went on, we realized that those DNA of interest, they typically are shorter. For example, the DNA from placenta or tumor cells, they are shorter than the background DNA that we are not as much interested in. Then, when we looked into why these DNA molecules are shorter, then we found out that the fragmentation process is actually non-random. From this point onwards, people started as, why is it not random and how does the cell control which part of genome becomes fragmented and how can we make use of these fragmented features? And very soon, I mean, it became a field and the studies -- the number of studies just exploded.

Bob Barrett: Well, besides learning about the fascinating biological basis of cell-free DNA generation, what else can fragmentomics offer?

Dr. Rossa Chiu: Interestingly, there are already a number of clinical applications that can be based on fragmentomics. The first one that has been used is to measure fetal DNA fraction. So, when we take a plasma sample from a pregnant woman to do prenatal analysis, we will be interested to know, in her sample, how much of a DNA came from the baby and we call this proportional DNA fetal fraction. Researchers have found out that the DNA from the placenta are typically a

little bit shorter than the DNA coming from the mother. So, by measuring the amounts of short DNA in the sample, one can actually work out the approximate amount of baby's DNA in the mother's blood sample. So, this is one application that has been used in the field. And recently, there are other interesting applications. Remember I said that people are interested in analyzing tumor DNA molecules in a person's circulation, as in a liquid biopsy. But now, we found out that some of the mutations are not from the cancerous growth because some of our background non-cancer cells with aging, it may accumulate some mutations or some changes, let's say. And this typically occurs with the blood cells and we call this clonal hematopoiesis. But researchers in the field have taken advantage of the fact that tumor DNA are generally shorter in nature, so if they see a mutation in a shorter DNA molecule, then that signal is more likely to be coming from cancer cells. So, this is another utility. Then, another area of utility that is really interesting is that now we realize that the fragmentation pattern of DNA coming from cells of different tissues or organs are different because the fragmentation pattern is influenced by the gene expression or the DNA methylation of those cells. And so, by analyzing the fragmentation profile, one may be able to guess which organ is having a pathology and releasing excess amounts of DNA and so researchers are trying to make use of fragmentomic features to do what we call as "tissue origin analysis" of DNA molecules present in the circulation.

Bob Barrett: Well finally, Dr. Chiu, we have been watching the emergence of various new diagnostics based on cell-free DNA or RNA analysis. Why are researchers now so particularly excited about fragmentomics of cell-free DNA?

Dr. Rossa Chiu: Well number one is that I believe now, we have the tools that are powerful enough for us to study the fragmentomic features because we have sequences that could provide a deep enough signal for us to analyze enough DNA molecules to look for those subtle fragmentomic features. The mathematic skill sets have improved greatly in the field and also, we have digital PCR platforms that allow us to physically count the number of molecules that are present in the sample. So, this is one reason, I guess. And another reason is, besides the potential value fragmentomic features may add to improving diagnostics which I have just alluded to in the other question, by studying fragmentomics, we're beginning to understand the biology that governs cell-free DNA production, that is how do they become fragmented in the way that they are? So for example, some recent studies even have gone into studying what nuclease enzymes are responsible for cutting off these DNA molecules. So, I guess this is a new field that we are learning things that we have

not learned before and this is why I believe researchers are so excited about this.

Bob Barrett: That was Professor Rossa Chiu from the Department of Chemical Pathology and Li Ka Shing Institute of Health Sciences at The Chinese University of Hong Kong. She served as the moderator of the Q&A feature appearing in the December 2020 issue of *Clinical Chemistry* titled "Cell-free DNA Fragmentomics: The New "Omics" on the Block." I'm Bob Barrett. Thanks for listening.