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# Jagged Ends on Multinucleosomal Cell-Free DNA Serve as a Biomarker for Nuclease Activity and Systemic Lupus Erythematosus

**Article:**
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**Guest:** Dr. Spencer Chen Ding from the Centre for Novostics in Hong Kong Science Park and Li Ka Shing Institute of Health Science of 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.

Jagged ends of plasma DNA are a recently recognized class of fragmentomic markers for cell-free DNA, reflecting the activity of nucleases. A number of recent studies have also highlighted the importance of jagged ends in the context of pregnancy and oncology. However, our knowledge regarding the generation of these jagged ends is incomplete.

To address this issue, a paper from Dr. Dennis Lo's Laboratory in Hong Kong, appearing in the July 2022 issue of *Clinical Chemistry,* examined jagged ends on multinucleosomal cell-free DNA and how they serve as biomarkers for nuclease activity and systemic lupus.

The lead author for that study is Dr. Spencer Chen Ding. She joined Dennis Lo's group at the Centre for Novostics in Hong Kong Science Park and Li Ka Shing Institute of Health Science of the Chinese University of Hong Kong in 2018. Her work is mainly focused on investigating genetic and epigenetic markers for cell-free based liquid biopsy, and she is our guest in this podcast.

So, Doctor, from your work, we know that not all cell-free DNA molecules are completely double-stranded in plasma. There are some double-stranded molecules carrying single-stranded ends, and you call this kind of end the "jagged end." Would you please briefly introduce the jagged ends and tell us how you measure them?

Spencer C. Ding: Yes, I'm happy to answer the question. So actually, the jagged end is emerging characteristic in the fragmentomics. So, our first study related to this topic could be dated back to our publication in *Genome Research* in 2020.

So, if you ask me what is the jagged end, I will try my best to explain that in an easier way. We all know that the cells in our body would goes through the apoptosis and these cells will release DNA into the blood circulation.

So, we know that there are a lot of nucleases in our blood circulation and those nucleases may cut the DNA in a flush manner or not. So, we can imagine that those resultant circulating DNA molecules could carry blunt ends or they might carry the single-stranded overhang, and that is the jagged [end].

So, in our normal procedure, if we want to sequence the plasma DNA molecules, we would need to process the extracted DNA for subsequent library preparation. And if we want to ligate the adapters to the fragment, we have to repair the ends first.

So, now you can imagine that this kind of repairing procedure will convert all the jagged ends into the blunt ends and this would make the jagged ends untreatable with our conventional data process.

So, that's why people have overlooked this question for a long time. They are not sure whether the original DNA molecules carry the jagged ends or they are carrying the blunt ends. We know that we can recognize the jagged ends if we introduce differential signals during the end repair process.

So, for example, we can incorporate the methylated cytosines, so that the measured methylation level will increase if the jagged ends are present. So, you know the cytosines are the CH sites, which is C-A, C-C, and the C-T. The cytosines there are generally unmethylated. So, the increased methylation signals can be clearly detected, basically. So, we are trying to introduce some noticeable markers into the jagged ends which do not exist in the original DNA strand, so that we can recognize them, and that is how we measure the jagged ends.

Bob Barrett: Well Doctor, let's back up just a bit because you have mentioned that the jagged end is an emerging new characteristic in fragmentomics. What exactly is fragmentomics and why do you think jagged ends have an advantage over other fragmentomic properties?

Spencer C. Ding: Yeah, so fragmentomics actually includes several unique properties related with fragmentation patterns. So, the difference in these properties would enable us distinguishing patients in virus physiological or pathological states.

So, for example, the fetal cell-free DNA molecules are normally shorter than the maternal cell-free DNA in the

plasma of the pregnant women and the patients with hepatocellular carcinoma tend to have some lower frequency of the fragments ended with the signature CCCA when compared with some healthy human subjects, etc.

So, one reason to explain such different pattern is that nucleases may play important roles in generation, or we say, the clearance of those DNA molecules. So, the DNA nucleus activities would shape the fragmentation patterns of those cell-free DNA molecules like the *Dffb*, which is *DNA fragmentation factor subunit beta*. It might cut the DNA into some high weight molecules in the nucleus *Dnase 1 like 3*. It may generate some molecules ended with the C.

So, all of those properties could be utilized for deducing the fragments origins in the plasma DNA flow. Of course, they will give some different levels in the analytical resolutions, but they are informative enough.

As for the advantage of the jagged ends over other fragmentomic properties, I would say that the jagged ends may potentially reflect the intrinsic cleavage patterns caused by those nucleases, the DNA molecules, and as such the original cutting states are supposed to be well-preserved in the jagged ends.

Bob Barrett: Can the jagged ends also be observed in other materials, for example, urine or saliva?

Spencer C. Ding: Yes. So, our group did look into the jagged ends in the urine, and since the urinary cell-free DNA are kind of naked and the *Dnase1* expression level is really, really high in the urine. We could easily observe the drastic decrease of jagged ends in the urine cell-free DNA of those *Dnase1* knockout mice. And we also apply the jagged end analysis of the urinary cell-free DNA to distinguish those patients with bladder cancer or kidney cancer from the healthy human subjects.

The results turned out to be pretty promising. Patients with increased degrees of bladder cancers would present progressively decreased jagged ends. Hopefully, we could translate those findings into some practical clinical utilities.

Bob Barrett: So jagged ends do sound like they're of practical utility but sequencing is quite an expensive technique. Is there any more cost-effective ways to detect the jagged ends?

Spencer C. Ding: Actually, it's not that expensive as we imagined. So, the jagged ends can be deduced from any bisulfite sequencing data which are commonly used for the cancer detection.

So, I may think that such measurement can be obtained for free, so when one patient used the bisulfite sequencing for

the cancer detection. But indeed, I think we can play some other tricks to lower the cost. So, for example, we can try to incorporate some fluorescent nucleotide to the jagged ends and then we can detect for the fluorescence intensity by those machines such as a fluorescence microplate reader, which would cost less than like $10 per sample, I guess.

Bob Barrett: Well finally Doctor, what brought you to this field of study?

Spencer C. Ding: I have to admit that the finding of the jagged ends was kind of incidental. At that time, our team was trying to study the methylation signals across the fragments and we consistently noticed a drop of the methylation level at the downstream end of the sequencing reads. And at first, we thought it might be an artificial thing, but we then noticed that these kind of decrease of methylation level only happened in every single plasma DNA sample, and we couldn't find the similar pattern in our sonicated genomic DNA samples, even though those samples were prepared following the same protocol. So, we know that there must be something important behind this.

So, we then look back to my protocols step by step, and noticed that we would manually edit some dNTPs to generate the blunt ends for the library preparation and that artificial incorporated and methylated cytosines were the cause of the dropped optimization level.

So naturally, we hypothesized that this kind of single-stranded part could reflect the cutting features of the nucleases. So, we used the mouse models to verify that and I would say that every weird phenomenon always has its own reason and for science, we need to find out that reason.

Bob Barrett: That was Dr. Spencer Chen Ding from the Centre for Novostics in Hong Kong Science Park and Li Ka Shing Institute of Health Science of the Chinese University of Hong Kong. She has been our guest in this podcast on Jagged Ends in Multinucleosomal Cell-Free DNA. She is a co-author of a paper on that topic that appears in the July 2022 issue of *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.