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Circular RNAs as Urinary Biomarkers

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Guest: Dr. Jacky Lam is a clinical lecturer at the Department of Chemical Pathology 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 am Bob Barrett.

Circular RNAs have the unique topological feature of circularity among the RNAs species. They are single-stranded, covalently closed, circular RNA molecules and are hypothesized to be generated largely through back-splicing of exons from precursor messenger RNAs. Circular RNAs were once considered as aberrant splicing by-products, which were only present in minute amounts in cells. The potential of this RNAs species as a disease biomarker is to be explored, but there are biological properties of circular RNAs which make them suitable for biomarker development.

An Editorial appearing in the October 2019 issue of *Clinical Chemistry* by Drs. Jacky Lam and Dennis Lo discussed circular RNAs as potential urinary biomarkers. Dr. Jacky Lam is a clinical lecturer at the Department of Chemical Pathology of the Chinese University of Hong Kong and he is our guest in this podcast. So, Dr. Lam, first, what is circular RNA?

Dr. Jacky Lam: Okay, so circular RNA belongs to the group of non-coding RNA, which is comprised of all RNA species with no protein coding potential. They were once considered as artifacts of aberrant RNA splicing. But with more knowledge, it is now believed that they possess the biological function as sponge in micro RNA and have indirect involvement in gene regulation. So, in the broadest term, circular RNA refers to the RNA species which bears the unique topological feature of circularity as you can see from its name. But most of the circular RNA reported today originate from the exons of the coding (00:01:48) messenger RNAs. So, for these circular RNAs, they are hypothesized to be generated through back splicing of exons from precursor messenger RNAs. Therefore, some researchers may directly refer to circular RNA as circular RNA specifically with exonic sequences only. There are some other circular RNAs which are originated from intronic sequence so they could be made a circular intronic RNAs and there are also some circular RNA species

which we think have both exons and also introns. And they are termed as exon new intron circular RNAs. Therefore, I think it is important for all of us to pay attention to the terminology and understand which apply when the researchers referred to the various types of circular RNAs.

Bob Barrett: And what are some of the recent technical advances for circular RNA detection and analysis?

Dr. Jacky Lam: So, there are two fundamental molecular characteristics of circular RNA, that is the circularity and also the presence of the back-splice junction that has been used for circular RNA detection. Here, the back-splicing junction refers to the ordering of the exons in a sequence being reversed relative to the annotated template. So, there are several technical advances which facilitate circular RNA detection. First, circular RNA in a sample could be enriched through exonuclease digestion. Here, I refer to when the sample has been treated with exonucleases and they would digest/degrade most of the linear RNAs while leaving the circular RNA intact. So, second, with the advancement in the high throughput sequencing, so it allows genome-wide profiling of the RNA species. And also there are now full-bodied informatics algorithms which are specially designed for identification of these back-splicing junctions, while early RNA sequencing matching algorithms will filter out such sequences.

Bob Barrett: Doctor, what are the advantages of using circular RNA as a disease biomarker?

Dr. Jacky Lam: So, there are a few biological properties of circular RNA which make them suitable for biomarker development. So, first, it is their unique circular topology, so there are the lack of the open ends of the circular RNA. This particular molecular characteristic we convert some of the resistance through the exoribonucleaic-mediated degradation and convert extra stability to the circular RNA compared to the linear counterpart. Second, there is an abundance of the circular RNA in the various human cell types. And third, the circular RNA expression patterns are diverse among different cell types and could exhibit tissue specificity. And also it has been reported cell free circular RNA has been shown to be present in human plasma, so including urine, saliva samples. So, all these things suggest the potential of circular RNA as biomarkers for noninvasive disease detection and also monitoring.

Bob Barrett: Doctor, I'd like you to talk about the major finding of the study by Kölling et al., on using circular RNA for monitoring acute graft rejection in renal transplant patients.

Dr. Jacky Lam: Okay, in this issue of *Clinical Chemistry*, Kölling and his colleagues have explored the diagnostic potential of urinary cell free circular RNA for monitoring acute graft rejection in renal transplant patients. Several patients with renal transplant, they may suffer from subsequent clinical rejection, so for the clinical rejection it means that the condition may be left unrecognized, because there is actually no change in the conventional blood test or serum creatinine concentration. This may delay the recognition of the rejection and it is very important because it may delay the timely administration of immunosuppressive therapy. Currently, the gold standard for detection of acute rejection is graft biopsy, and all of us know it is invasive in nature. So, Kölling and his colleagues have aimed to identify the urinary cell free circular RNA biomarker for the non-invasive detection of acute graft rejection. So, what they do is that they first analyze the circular RNA expression profile in the urine samples of nine patients with acute rejection, and in another nine transplant recipients, it's control. They found that there were distinct urinary cell free circular RNA profiles between the rejection group and also the control group.

Among the hundreds of circular RNA with high differential expression between the two group, they selected two particular circular RNA cut candidates, and in the validation cohort, they saw that one particular circular RNA was significantly higher in the rejection group compared to control group, while there was no difference in the concentration in the other circular RNA marker.

So, the authors especially highlighted that the majority of, around 80 percent of, the urine samples in the rejection group were actually collected from patients with the clinical rejection, that is at the time of measurement that serum creatinine were actually within normal range. And the diagnostic performance of this special urinary circular RNA marker was re-evaluated with receiving operator for curve analysis. The area under the curve value was 0.85. Moreover, this concentration of this circular RNA were normalized in 10 patients with acute rejection after the successful immunosuppressant therapy. Also, this urinary circular RNA marker predicted the loss of renal function at one year after transplantation. Overall, Kölling and his colleagues have demonstrated clinical potential of this urinary circular RNA as biomarkers for acute kidney rejection as I have mentioned just now.

Bob Barrett: OK, finally doctor, in the review article, you talked about the study of the different topologic forms of DNA and RNA and also the term plasma DNA topologics, could you please elaborate on that a bit more?

Dr. Jacky Lam: Sure, so in this study by Kölling, so the study of circular RNA could actually represent one form of analysis, that is the analysis of one topologic form of the RNA species. I'm sure that it is of biological interest that if we could simultaneously analyze a different topologic form of RNA, that is the circular form and also the linear isoforms from the same gene locus. But as I have mentioned just earlier, the conventional method of global circular RNA profiling involves the enrichment step. In the sequence (00:08:58) that is we have to deplete the linear isoforms and that detection step would preclude such simultaneous analysis. In one recently published large-scale study involving circular RNA profiling of over 800 tumor samples of 17 different types of cancer and that research group has developed a capture sequencing base assay, which would target the gene body for enrichment of both the linear form and also the circular isoforms of the RNA. This specially designed assay therefore would allow to quantification of the circular RNA in relationship to the corresponding linear isoform. So, in that study I have just mentioned, it has been reported that the circular RNA expression was independent of the basal expression pattern, and the others has partially different factors that may contribute to the level of circular RNA in that cell types. So, this study, as it has highlighted the simultaneous analysis of the different topologic forms of the RNA species, may actually provide a novel biological insight in the disease mechanism.

So, you have just mentioned our group has recently proposed the study of the plasma DNA topologies and actually we have reported the analysis of the different topologic forms of plasma by the control of DNA as an example. The detail is because previously it was only known that the short linear forms of the mitochondrial DNA fragments exist in the plasma. We have actually designed a new assay which allows the analysis of both the linear and also the circular forms of the mitochondrial DNA molecules. So, in this new assay, we incorporate one additional step, that is we use the restriction enzyme digestion step before the sequencing library preparation. So, this restriction enzyme would cut both the linear and also the circular forms of the mitochondrial DNA. This step would linearize the circular mitochondrial DNA. So, circular mitochondrial DNA which previously, because of the circular topology, they could not sequence because the free ends were absent on both sides. Therefore, adaptor ligation step could not be performed and they hence could not be subject to sequencing analysis. So, this restriction enzyme would cut the circular forms of the mitochondrial DNA. So, we use the cleavage signature at both ends to infer whether these sequence mitochondrial DNA segments were actually derived from the linear or from the circular mitochondrial DNA. In detailed studies, if the sequence mitochondrial DNA

carries a cleavage signature at one end, we believe that it should be originated from linear mitochondrial DNA. So, if the mitochondrial DNA carries the cleavage signature at both ends, we believe that it should be originated from the circular mitochondrial DNA. We actually showed that both topologic forms, that is, a linear form and also the circular complete forms of the mitochondrial DNA, coexisted in the plasma. With that in mind, we have shown that these linear and also circular mitochondrial DNA molecules in plasma might actually have different tissue origins. So, we believe that these plasma mitochondrial DNA topology studies would illustrate the potential of the analysis of the different topological forms of the cell free DNA in plasma and we may expand to the analysis of different body fluids as well in future studies.

Bob Barrett: That was Dr. Jacky Lam, a clinical lecturer at the Department of Chemical Pathology of the Chinese University of Hong Kong. He has been our guest in this podcast on circular RNAs. His Editorial with Dennis Lo on this interesting topic appears in the October 2019 issue of *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.