Clinical Chemistry

Maternal Plasma RNA Sequencing for Genome-Wide Transcriptomic Profiling and Identification of Pregnancy-Associated Transcripts

Article:
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Maternal Plasma RNA Sequencing for Genome-Wide Transcriptomic Profiling and Identification of Pregnancy-Associated Transcripts
Clin Chem 2014;60:954-962.
http://www.clinchem.org/content/60/7/954.abstract

Guest:
Dr. Yuen Fei Wong is a researcher at the Li Ka Shing Institute of Health Sciences at the Chinese University of Hong Kong.

Bob Barrett: This is a podcast from C*linical Chemistry* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I am Bob Barrett.

Analysis of circulating RNA in the plasma of pregnant women can be a powerful tool for noninvasive prenatal testing and research. However, unbiased and high throughput detection of circulating RNA in plasma is a technical challenge.

The July 2014 Issue of *Clinical Chemistry* published a paper, describing massively parallel sequencing for plasma transcriptome profiling in first, second and third trimester pregnant women.

Those studies were carried out at the Chinese University of Hong Kong led by Dennis Lo.

One of the authors from this team Dr. Yuen Fei Wong joins us in this podcast, and Dr. Wong, can you tell us a bit about the background that's led to this work that you recently published in *Clinical Chemistry.*

Dr. Yuen Fei Wong: Sure. We have been studying cell-free nucleic acids that is circulating the blood plasma for 17 years. In 1997, our group first reported that during pregnancy, a small amount of cell-free DNA originated from the fetus, could be detected in the blood plasma of the pregnant mother.

The fetal DNA comes predominantly from the placenta, so that means one could actually analyze the fetal DNA by examining the maternal plasma rather than taking a sample of the fetus or the placenta through invasive methods such as chorionic villus sampling or analytic synthesis that are associated with a small percentage risk of miscarriage.

This advance has led our group to the development of a noninvasive method to determine fetal chromosomal aneuploidy prenatally by massively parallel sequencing of DNA in maternal plasma.

This method approach has already been used by over 700,000 pregnant women in over 50 countries. And interestingly, we discovered in year 2000 the fetal derived RNA could be detected in the maternal plasma too, and just like fetal DNA, fetal RNA is also largely derived from the placenta and we were able to detect many placental RNA in the maternal plasma.

Along the way, many interesting research questions have come up, for example what are the spectrum of placental RNA that could be detected in the maternal plasma and what are the proportions of these placental RNA into total plasma RNA pool.

These are some of the questions we wish to address, which have led to this work.

Bob Barrett: Why is it important to study pregnancy associated RNA transcripts and what could be the main clinical application of this work?

Dr. Yuen Fei Wong: Well, we know that differences in gene expression level, not only in the placenta, but also in other tissues, is associated with abnormal fetal development and many pregnancy related diseases, but the question is whether we can detect and quantify such differences in the maternal plasma.

This question could be addressed through examination of the plasma RNA, particularly the RNA transcripts that are either up or down regulated during pregnancy.

The clinical application of this work is far reaching and would broaden the horizon of noninvasive prenatal testing. For example we may be able to apply this technology on many pregnancy related disorders such as preterm labor, intrauterine growth retardation and preeclampsia.

Currently, the diagnosis of these prenatal disorders is largely based on clinical assessment. It would be ideal if one could supplement such assessment with objective molecular tests.

We postulate that changes in RNA expression which may or may not be confined to the placenta would take place during the early stage of such disorders.

Detection of these changes noninvasively from the maternal plasma, would allow an early diagnosis to be made and a suitable medical care plan to be formulated in a timely manner.

Bob Barrett: Doctor, what are the key findings in this study and what do you believe to be to be the most important advance reported here over other papers from your group?

Dr. Yuen Fei Wong: As I have mentioned earlier, we have been examining circulating RNA for many years, well before the era of massively parallel sequencing. Until then we have predominantly relied on the reverse transcriptase quantitative polymerase chain reaction technology which is no doubt an analytically sensitive method, but with a low throughput as it allows us to examine only a very limited number of transcripts in a given sample each time.

But in this work, we have adopted massively parallel sequencing which has allowed us to interrogate the maternal plasma transcriptome in a high throughput manner.

Using this technology we can have a bird's eye view of the proportion of transcripts contributed by the fetus in the maternal plasma transcriptome.

We have also discovered a number of novel circulating RNA transcripts that are associated with pregnancy and more importantly the abundance of these transcripts in the maternal plasma was positively correlated with the abundance in the placental tissue.

This means that if there were any gene expression alterations in the placenta during disease progression, we would be able to detect these changes by examining the maternal plasma.

Bob Barrett: What were the major difficulties or challenges you encountered in studying the plasma RNA, compared to studying the plasma DNA?

Dr. Yuen Fei Wong: We have indeed encountered a lot of difficulties studying the plasma RNA when we first started. Now to begin with the transcriptome is far more complex than the genome and in fact scientists have yet to fully decide that understand such complexity.

A highly intricate activity that involves multiple levels of synchronization and coordination is in place to determine whether a gene is to be switched on or switched off.

So not all genes are switched on at all times and when they do get switched on, their levels of expression vary amongst different tissues in the body and even within the same tissue of the same individual the gene expression levels could vary at different time points.

On another note, of all those RNA molecules that are expressed, the bulk of them are actually the ribosomal RNA that often do not provide much biological insight.

And coming back to our very own work, it is even more challenging to study the plasma transcriptome. Firstly, the amount of RNA that you could get from the plasma is substantially lower than what you could get from tissues or culture cells.

And obviously fetal RNA constitutes only a minor proportion of the total RNA pool and secondly plasma RNA molecules are often not as intact as tissue or cellular RNA.

Our challenging goal was to capture as much information of the plasma RNA transcripts as possible, despite their low quantity and the low quality.

And this is where massively parallel sequencing technology comes in. But unlike DNA sequencing which already is a rather mature application as far as the technology is concerned, RNA sequencing remains a huge challenge.

The complexity of the transcriptome and low quantity and quality of plasma RNA has caused the sample preparations that the sequence matching accuracy as well as the normalization algorithm for gene expression quantification to be far less forward than DNA sequencing.

We have worked very hard to optimize quite a number of experimental steps and parameters at both the wet lab and dry lab stages and we are very glad that the group's effort has paid off as we finally managed nail down the protocol and bioinformatics pipeline for plasma RNA sequencing for the purpose of our study.

Bob Barrett: Finally Doctor, what's next? What's the follow-up to this work?

Dr. Yuen Fei Wong: Since this work is a proof of concept study, we are now planning to apply this technology on a bigger cohort of pregnant women to include those with pregnancy related disorders that I have mentioned earlier.

We would also continue to refine our protocols and bioinformatics pipeline in order to reap the maximum benefit from the sequencing data.

And hopefully, our follow-up work will lead us to discovering many more novel biomarkers related to prenatal diseases in a very near future, which could contribute toward noninvasive prenatal monitoring.

Bob Barrett: Dr. Yuen Fei Wong is a researcher at the Li Ka Shing Institute of Health Sciences at the Chinese University of Hong Kong. She has been our guest in this podcast from *Clinical Chemistry* on circulating RNA. I am Bob Barrett, thanks for listening!