Clinical Chemistry

Characterization of Human Salivary Extracellular RNA by Next-generation Sequencing

Article:
Feng Li, et al.
Characterization of Human Salivary Extracellular RNA by Next-generation Sequencing.
Clin Chem 2018;64:1085-95.
http://clinchem.aaccjnls.org/content/64/7/1085

Guest: Dr. David Wong is the Felix and Mildred Yip endowed Distinguished Professor, Associate Dean of Research, and Director of the Oral, Head, and Neck Oncology Research Center at UCLA.

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.

It was once thought that RNA only existed within cells. However, recent discoveries show that RNA is exported from cells and may be functional in cell to cell communication. This extracellular RNA can be abundant and stable and is found in bodily fluids such as saliva. Saliva has long been of interest for biomarker discovery and development, given its noninvasiveness and relative ease of collection.

Despite these advantages, use of saliva for understanding more about extracellular RNA and its role in human biology and disease through RNA sequencing has some challenges. The high bacterial content and low abundance of extracellular RNA in saliva mean that optimization of RNA library construction and isolation protocols are critical to the success of these RNA sequencing experiments.

An original research article in the July 2018 issue of *Clinical Chemistry* compares different RNA isolation methods and library construction kits for long and small RNA sequencing of salivary extracellular RNA. The authors described which protocols provide the best RNA yield and detection by next generation sequencing.

The article's senior author, Dr. David Wong, joins us for this podcast. He is the Felix and Mildred Yip endowed Distinguished Professor, Associate Dean of Research, and Director of the Oral, Head, and Neck Oncology Research Center at UCLA. Dr. Wong is an active and accomplished scientist in oral cancer and saliva diagnostics research.

So Dr. Wong, first question, what were the key and novel findings of this paper?

Dr. David Wong: This paper carries with it that discovery, or insight, into a landscape of constituents within body fluid saliva which previously very little is known. The saliva proteome has been in the scientific community for, perhaps, the last

decade due to an NIDCR activity. But the presence of other -omics constituents has not been developed or identified.

So this initiative here is an inroad into the existence of a landscape of extracellular RNA, where RNA is conventionally thought of to be within cells, in nucleus, being transcribed and translated in the cytoplasm, into protein use. And this consortium, which is funded by the NIH Common Fund, is to elucidate and foundation the existence of RNA outside the cell and their presence, their activities, which present the new paradigm in biological communication.

So in this manuscript here, it represents a body fluid, saliva and its constituent composition, presence of extracellular RNA, its landscaping using a high throughput technology, and with that foundation, it begins to pose a number of biological insights and perhaps clinical utilities as well of the existence of these extracellular RNA.

Bob Barrett: Could you please explain the scientific and clinical significance of salivary RNA?

Dr. David Wong: Indeed. So scientifically and clinically, the existence of extracellular RNA in saliva presents opportunities that haven't been available and debut scientifically while RNA is traditionally thought to be existent within cells transcribed in the nucleus, translated into cytoplasm. Now, the existence of these molecules outside the cell either in free form, or encapsulate it vesicular entities, and present those questions that really prompted investigations. While most people think of saliva as being spitted out, but as we know they are being swallowed into the stomach down the GI tract where in the distal ileum, 70%, seven-zero, of a mucosal immunity lies, the Peyer's patches that get associated with lymphoid tissues.

The original discovery of these vesicles are communicated between the TMB cells. So it really is very intriguing to, perhaps, project the possibilities that these vesicular entities that are holding these extracellular RNA cargo from various systemic origins coming into the salivary gland into saliva being swallowed and delivered to the distal ileum and could they serve as a immunological source to elicit a new response into a mucosa immunity. Those are really intriguing questions that now allow to be tested that we didn't have those insights before.

Clinically, the existence of extracellular RNA truly presents a landscape of translation a way in clinical research where extracellular RNA can present a biomarker source for disease association, early predictiveness of cancer onset, and monitoring of clinical efficacy, truly echoing the current

theme of precision and medicines, so there lies these clinical and translation opportunities.

But perhaps, last and certainly not least is that in the translational diagnostic in the landscape, noninvasiveness is of utmost importance and saliva, it holds that capability. And on a daily basis, each one of us produce a liter worth of saliva. Noninvasively, non-painfully, and non embarrassingly, we can obtain it continuously, repetitively, and it carries these -omics targets in this scenario, the extra cellular RNA that we can harness, we can train, and we can develop them into discriminatory biomarkers for early detection of diseases or monitoring of therapeutic efficacies.

Bob Barrett: So how does salivary extracellular RNA fit into the extracellular RNA atlas and the NIH's Extracellular RNA Communication Consortium?

Dr. David Wong: The NIH Common Fund five years ago has implemented and supported an initiative under the title of "Extracellular RNA Communication Consortium," or ERCC for short, that presents a new paradigm in cellular communication in clinical translational research by foundationing the scientific and clinical credibility of the existence of RNA that are outside of cells.

As I mentioned that RNA is within the nucleus, transcribed, translated into cytoplasm, the new paradigm is that RNA now is known, and found in foundation to be existent with outside cells either in free form or within the vesicular energy and which confer to them stability, number one. And secondarily, a vehicle for transfer or trafficking or delivery to a distal attracted cell which upon entry could deliver and be translated and influence the physiology or functionality of the recipients cells, truly a new paradigm in cellular communication in signaling and also in the ability for therapeutic implication is immense.

So the ERCC through the Common Fund has been in existence for almost five years now, where different body fluids have been looked into extensively, using massive parallel NGS sequencing, and they're very informative and innovative bow informatic analyses in terms of how this extracellular RNA could be a value of this scientific function, the biogenesis of these vesicles to the transfer of this extracellular RNA hosting package within these vesicular entities to the translation of utility of extracellular RNA for disease detection and for therapeutic applications.

There also exists a very resourceful consortium in cataloging all of the body fluids, and also their existence of extracellular RNA, of coding as well as non-coding origin,

into this entity known as the extracellular RNA outlets in a wonderfully resourceful entity that is now in the public domain and is searchable, and everyone could look into this entity and seek out the biological presence of the fluid of interest or whether a certain RNA of interest is present in a biological fluid of interest to a particular study.

So the existence of the establishment of this activity through the NIH Common Fund, and obviously being supported by various ICs has presented a new paradigm. And in the past five years, we also begin to see specific initiatives, either RSA's or SOA's are coming into the community in terms of seeking out the basic evaluation and clinical evaluations of extracellular RNA of vesicular entities for early detection of cancer or in the therapeutic applications for a host of biological impactful clinical questions. So these are all present in the public domain.

And saliva comes in as a facet, as a facet, as a part of the whole body constituents on terms of biological fluid, and how it echo's into this essential foundationing of extracellular RNA in this communicative entity.

Bob Barret: So finally, Dr. Wong, does salivary extracellular RNA have any functional roles?

Dr. David Wong: That truly is the most intriguing question of the discovery of extracellular RNA, in any body fluid, present these provocative questions as to what biological functions are they conveying?

Are they sort of carried out? In particular, when one thinks of a sample type such as saliva, one perhaps begins to think that this is a bystander or simply a byproduct of a certain scenario that extracellular RNA end up into the saliva. But as I mentioned earlier, the saliva is also not spitted out and it maybe should not be spitted out. They are swallowed. It goes down into the stomach, into the GI tract. And these vesicular entities which are known to be communicative between the immune cells and in the distal ileum of the GI tract lies 70% of a mucous immunity. This is one of the sort of projected scenarios here.

The question that keeps coming back to all of us is that the non-coding RNA in particular are regulatory in nature. And their presence is ubiquitous in all body fluids and in saliva is no different. They are being packaged as a cargo in these vesicular entities which goes down the GI tract which could influence and elicit an immunoresponse in the distal ileum. Amongst other properties what could they be eliciting? What biological function could maybe be carried out in a systemic nature?

In other aspects, we have been looking into what are they carrying out in terms of regulatory function in the oral cavity. So a manuscript has recently been published that is demonstrating a very unique interkingdom interaction with hosts extracellular RNA is influencing the microbiome activities of pathogenic strains in the oral cavity. And that truly represents a very novel and yet timely insight into extracellular RNA and how it could interact with the 700 different microbial species that co-inhabit in our oral cavity.

And the other scenario we're looking into is biological functions that we know have very unique features and functions in the oral cavity, that's different than in other parts of the body. How extracellular RNA can impact on those.

So it really presents a very provocative and intriguing and perhaps it would be a source for landscape of biology that we haven't noticed before, but it opens up this new door and opportunities that we can have a better understanding of this contribution in terms of local phenotype as well as a systemic capability of eliciting an immunoresponse to the host to what's infection disease or perhaps towards neoblastic development. So those are the very intriguing and perhaps functional opportunities that open up for us that we have not been realizing in this biofluid of saliva.

Bob Barrett: Dr. David Wong is the Felix and Mildred Yip Endowed Distinguished Professor, Associate Dean of Research and Director of the Oral Head and Neck Oncology Research Center at UCLA. He has been our guest in this podcast from *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.