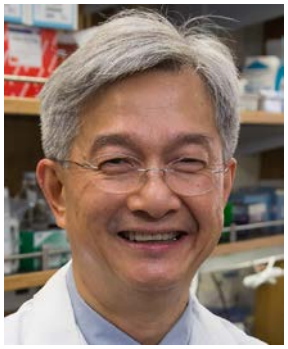
Clinical Chemistry

The Landscape of MicroRNA, Piwi-Interacting RNA, and Circular RNA in Human Saliva



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Guest:
Dr. David Wong is Professor and Associate Dean of Research and also the Director of the Oral/Head & 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.

Human saliva has been increasingly used for biomarker development to allow noninvasive detection of diseases. Extracellular RNA was discovered in saliva about 10 years ago, and since then the nature, origin, and characterization of salivary RNA have been actively pursued.

In the January 2015 issue of *Clinical Chemistry,* a special issue devoted to molecular diagnostics, a group of researchers from UCLA described the landscape of RNAs in human saliva that will facilitate further biomarker discoveries.

In this podcast, we're joined by one of the authors of that paper, Dr. David Wong. He is Professor and Associate Dean of Research and also the Director of the Oral/Head & Neck Oncology Research Center at UCLA.

Dr. Wong, what prompted you to explore the presence of non-coding RNA in saliva?

Dr. David Wong: So the existence of RNA in this oral fluid in extracellularly has been on our research program for almost a decade. In 2004 we reported the presence of extracellular RNA in saliva. It was an initiative from the Dental and Craniofacial Institute at NIH and we would define that definition, that resolution, and for translation of a clinical application as well.

And interestingly, of interest is that two years ago the NIH Common Fund has initiated a consortium to look at extracellular RNA for cellular communication and one of the programs within this umbrella program is to evaluate extracellular RNA for translational and clinical application. We responded to it, and we now have a project to evaluate

the clinical utility of extracellular RNA. And as technology advanced we were engaging in high super micro array a decade ago and deep sequencing is the technology of choice, and utilizing this technology and optimizing it for applications deciphering the extracellular RNA in saliva, that's what generated the excitement is the unexpected presence of these extracellular RNA, in particular the non-coding RNA have virtually 95% of the RNA component that are present in saliva are of a non-coding nature, so to speak of the microRNA and the Piwi-RNA and the circular RNA alike.

Bob Barrett: Doctor, what are the novel findings of his paper?

Dr. David Wong: There are a number of unexpected and truly novel findings or discoveries and largely due to the engagement of an emerging tool, a technology that allows us to look at any bodily tissues of fluid with unprecedented resolution and capability, and that's deep sequencing.

And in utilizing this technology, optimizing it for oral fluid application, which we have been engaging in the area for the past decade, allow us to begin to have these revelations, the presence of the great abundancy, number one of non-coding RNA in saliva, and that in itself is impactful, and then further delineating into the composition of these non-coding RNA. That's where we have found the presence of the microRNA which hasn't been defined before.

And then the discovery of the presence of at least two other major classes of non-coding RNA, the Piwi-associated RNA and then this circular RNA. This circular RNA has never been detected in bodily fluids. So the finding, the detection, and the validation of their presence in saliva is novel, and importantly, while the finding is one thing, the question that keeps emerging obviously is, why are they there? Why are they in saliva and as we know of the nature and function of non-coding RNA are predominantly regulatory in nature, that really is a most intriguing outcome in addition to the finding itself.

Bob Barrett: So how does the landscaping of microRNA, Piwi-interacting RNA, and circular RNA advance salivary diagnostics?

Dr. David Wong: In perhaps -- in a most unexpected and perhaps impactful ways--in the sense that while saliva has not yet been in the mainstream of clinical diagnostic or utilities, the emergence of the genomic component, in particular the extracellular component, has always been in our experience and others that has been the top ranking discriminatory constituents.

Amongst all the -omics constituents that's in decipher in saliva--the proteomics, the metabolomics and now the

genomics and the transcriptomics--the RNA markers as we develop them for disease detection has always been top ranking in their performance in classifying a disease from a non-disease individuals and their alike.

Now with this resolution that we're looking deeper into the transcriptome and the emergence of these non-coding RNA, these circular and the Piwi and the microRNA, it gives us additional handle, and while the eventual translational value remain to be seen, we already have data that indicate that the most discriminatory fraction within the non-coding RNA are the Piwi-RNA, which again, had this effort not been made we would not even have known their presence in saliva. And so we're really poised, in a very exciting way, as we look forward of a new landscape of how the translational utility of saliva constituents then can present the constituents for clinical applications, at the same time the biological questions are equally exciting if not more is, is why these Piwi-RNA that are restricted to its stem cells and germ cells are so unusually abundant in saliva, unlike their cousin biofluid in serum or in cerebrospinal fluid, which is dramatically reduced.

So why is this so uniquely present in this oral fluid, are the exciting biological questions that need to be explored, and perhaps it also puts additional light on what saliva could do functionally, physiologically impacting our health or diseases as this fluid is traversed through vasculature coming through a salivary gland.

And while most people think saliva is being spitted out, they are more often swallowed down the esophagus through the stomach and into our gastrointestinal tract, whereas 70% of our mucosal immunity in the distal ileum, the gut-associated lymphoid tissues, the Peyer's patches are there in our G.I. tract. The initial discovery of these extracellular RNA, they are contained within these vesicular entities called exosomes and these exosomes are communicating entities between the T and B cells within the immune system.

So there lies a host of questions or hypothesis that could be evaluated how, in addition to the fluid itself, how these constituents, how these targets extracellular RNA, how they could be influencing our health and perhaps in situations where disease develop as well. So it's a very exciting landscape as we look forward in this new horizon how the presence of these extracellular RNA could poise these bio fluids in health and in diseases as well.

Bob Barrett: How can RNA be stable extracellularly in saliva where it's known to have ribonuclease?

Dr. David Wong: Absolutely! That's the very, very insightful question, and saliva is known to have presence of ribonucleases. So there lies perhaps the initiative from a common front is the embracement or the realization that RNA no longer are residing predominantly within cells, they have been found outside of cellular entities, in biological fluid, and perhaps the most important realization is the finding that these, these extracellular RNA are not in free form, they are encapsulated in the lipid vesicles, predominantly in exosomes, and also in microvesicles.

So they are not in free form, they are encapsulated in lipid bilayers and serve as communicative or shuttling vehicles that could shuttle from its cell of origin to a distal target either through vasculature, lymphatics, cerebrospinal fluid, or in our case here through saliva. And they actually have been also found to be able to mediate biological sequelae in the target cells like hormones, like simply molecules.

So this is I think one of the exciting realizations that RNA can have functional capabilities outside cells and their stability or the resistance to “degradation” and largely is due to their encapsulation and these vesicular entities are called exosomes or microvesicles.

Bob Barrett: Well, finally Dr. Wong, what could be the biological functions of these non-coding RNA in saliva?

Dr. David Wong: So while the paper is really a delineation or landscaping, but the presence and the abundance and the unusual distribution of Piwi-RNA and circular RNA in saliva really sort of begs the question as to why is there?

And knowing why the non-coding RNA are predominantly regulatory in nature really poise these questions in a most exciting biological manner.

So some of the thoughts that have emerged and that we're currently exploring is, there are physiological processes that in the oral cavities are very, very different than other parts of the body, and knowing the Piwi-RNA are restricted to germ cell and stem cell begin to clue us into, are there regenerative capabilities in the oral cavity that perhaps is different from other parts of the body?

One thing comes to mind and a good example is, if you were to have a skin wound, a two-inch skin wound on the skin, and then have the same sized wound in your oral cavity, you may be surprised to know that that wound in your oral cavity would heal five to 10 times faster, and also interestingly they don’t scar.

So whether these biological constituents that are present in oral fluid that constantly surrounds this oral wound could have the effect as they are being restricted primary to germ cells and stem cells, contribute to this process. So these are very testable hypotheses amongst others, circular RNA certainly have biological function that can serve as docking stations to microRNA and provide additional debilities of these extracellular RNA in saliva, amongst other biological regulatory functions.

So I think perhaps the one thing that really summarizes for us is the presence of these non-coding RNA as we know now is in such tender stage of biological evaluation and their unusual abundance, unique presence in saliva, getting it really begin to shed light and consolidate.

There is a horizon or a landscape of saliva biology that we may not have been paying attention to, that I think the finding begin to paint the landscape and allow us to address these questions and sort of relating a fluid that is in oral cavity that maybe integrating and interacting and exerting a systemic effect that we may not have been paying attention to before.

So it's a very exciting future that we look forward to based on these findings that we can continue to explore and evaluate.

Bob Barrett: Dr. David Wong is Professor and Associate Dean of Research and also the Director of the Oral/Head & Neck Oncology Research Center at UCLA. He has been our guest in this podcast from *Clinical Chemistry* on Human Salivary RNAs. His paper appeared in the January 2015 issue of *Clinical Chemistry*, a special issue devoted to molecular diagnostics.

I am Bob Barrett. Thanks for listening.