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The Human Salivary RNA Transcriptome Revealed by Massively Parallel Sequencing

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Guest:
Dr. Wong is the Felix and Mildred Yip Professor, and Associate Dean of Research at the UCLA School of Dentistry.

Bob Barrett: This is the podcast from *Clinical Chemistry*. I am Bob Barrett.

Evaluation of RNA in oral fluid is an emerging diagnostic technology with great potential for disease detection. In the September 2012 issue of *Clinical Chemistry*, Dr. David Wong and his colleagues at UCLA explored massively parallel sequencing for providing nucleotide-level sequence information for RNA in saliva. Dr. Wong is the Felix and Mildred Yip Professor, an Associate Dean of Research at the UCLA School of Dentistry, and he is our guest today in this podcast.

Dr. Wong, you and your team have published many studies that investigated different aspects of saliva. What are the key findings of this present study?

Dr. David Wong: This paper represent a significant milestone in the advancement of the science and the capability of utilizing the transcriptomic constituents in saliva for clinical as well as for fundamental science understanding.

From the clinical perspective, it really represents a frontier, a new frontier intoward the harnessing and development of biomarkers in saliva. The concept of extracellular RNA in cellular communication and biomarker development now is an accepted concept and the saliva is no different than other bodily fluid. So that really represents an advancement in terms of its resolution, of its boundary, and the level that we can harness all-in-one run so to speak, both the mammalian content and the RNA, microRNA, non-coding RNA, as well as the microbiome content of it.

And from the fundamental science perspective, it represents a credentialing, if that's a good word for it, of the credibility of the existence of the salivary transcriptome from a scientific perspective and how we can sort of begin to

project the biological functionality of how these constituents may be served in a biological and physiological perspective.

So, it really opens up a new frontier whereby the clinical utilities as well as the fundamental science of how these extracellular RNA in saliva could work in a cellular communicative venue.

Bob Barrett: Where exactly does the RNA come from in saliva and what's its function in the oral cavity?

Dr. David Wong: These are two very, very important questions where currently, there may not be any obvious answers. But, the landscape is changing very rapidly from a number of perspectives.

This paper, what it sort of allowed us to conclude, is the credentialing. The salivary transcriptome is now a credible entity within saliva. The sources of it is very interesting, and we have approached this and others as well, that basically every fluid that comes into the oral cavity either from the gingival crevicular fluid, that little gap between the tooth and gum, or from the salivary gland which on a daily basis, the three major pairs of salivary gland that surround the oral cavity, produce collectively a liter to a liter-and-a-half of whole saliva. That's equivalent to two to three bottle water that we consume on a daily basis. Imagine that two to three volumes of that, constantly real-time, come into our oral cavity sort of non-painfully and non-invasively and carries with it this sort of informative biological -omics information that we can harness.

So, these are the sources, but perhaps what is really more telling is a recent discovery that in our bodily function, all of these vesicular entities where organs and cells sources, shed these vesicular entities that is generally under term exosomes, that harbors cellular constituents including RNA, microRNA, and they were disseminated through the vasculature to different parts of the body including the salivary gland and we have shown that, and they come into the salivary gland, and then present themselves in saliva. Thereby, it really represents a novel signaling mechanism, if you will, how information and constituents from a part of the body could be disseminated through the vasculature to different parts of body including salivary gland.

The interesting thing about salivary gland is where does it go, and where does it exert its biological effect, which is not systemic or circulatory, but it goes down the oral mucosa tract, the GI system.

So the source is multiple, it's from the salivary gland itself and a defined mechanism is now in place which are these

vesicular entities that's sort of a signaling information that's shed from different parts of the body and they come to different parts including the salivary gland as well.

Bob Barrett: Doctor, unlike DNA, RNA is very sensitive and easily degraded. So how is it possible to detect intact RNA in saliva?

Dr. David Wong: It's a very, very insightful question and we asked that very same question when we approached this work. But, what is really perhaps is novel and impactful in this field is that the RNA in saliva as well as in other bodily fluid, in blood, in cerebrospinal fluid, in tears and urine, they are not in free form.

So as we learn more about these extracellular RNA, they don't exist in freeform and they're encapsulated within these vesicular entities which serve as a protective vesicle that shuttles these cellular constituents from a source, for example, a tumor. We recently saw that in a series of paper that shuttle melanoma and the vesicular entity was seeded in bone marrow and facilitate, interestingly, a tumor-metastatic phenotype.

So the body has a mechanistic basis in shedding this information and there is a protective moiety that harbors this information and through this mechanism, it confers its stability and thereby answer the question that at the very beginning we discovered it in 2004, where the salivary transcriptome was first reported and its stability has since then continued to be demonstrated, and a mechanistic underpinning to it was unraveled in recent years, which would pinpoint to this vesicular entity which now constitute a central working mechanism, how the extracellular RNA could exert an effect not only in saliva but in blood, in urine, and other body fluid that shuttles information from a part of the body to another.

Bob Barrett: In the study published in *Clinical Chemistry*, you not only describe the transcriptome from the human subjects but also the microbiome. Is there an interaction between these signatures and why does it make sense to identify both signatures at once?

Dr. David Wong: It's a very, very exciting question. It also paints a very exciting landscape as why these emerging technology has value from a scientific and clinical perspective. So from a technological scientific point of view, that all these information emerge at the nucleotide level of both a mammalian transcriptome, which includes the RNA and the microRNA, and the non-coding RNA, where that interplay itself is currently emerging. We've seen it in other body

organ and in fluid, that story is unfolding itself, that these constituents are not there by accident.

The notion of well, saliva carries with it, allow me to say, it used to be thought of like a trashcan, you know, things just end up there. You drink your Coca-Cola and you drink your coffee and you sneeze and you somehow end up with certain composition, but the more we know about the physiology and biology of the organ is that these are highly regulated and now we know that these entities are conveyed and then transfer into the salivary gland into saliva in a very defined way.

So the interplay of the transcriptomic content as well as the other constituents as well, proteins and DNA and the metabolites, which is not the subject of our topic today, but they are encapsulated in the same way. So the dynamic interplay of this transcriptome as well as the potential interplay between the microbiome and the host in this milieu of the oral cavity is very much a landscape, very fertile for scientific investigation.

So a new chapter of cellular communication, a new chapter of signaling and communication that is conveyed, perhaps, through another bodily source and bio-fluid, that is salivary gland and saliva, is this landscape that we are very excited about in terms of how we may convey both a clinical perspective for a biomarker development, which obviously is very fertile ground as well and a noninvasive fluid that comes in continuously on a daily basis that we can harness using technology like we report in this paper that can identify discriminatory marker for life-threatening diseases to a fundamental understanding that we have a segment of cellular biological communication that is outside of a circulatory system but is companion bio-fluid.

And if we remember that where the saliva go, so when they sort of end up in oral cavity, most people would think that it got spitted out, but biologically and physiologically, it goes down the esophagus, into our GI tract where 60% of our mucosal immune system is there and where, it's assumed being discovered in the first place, all within the immune system.

So there lies these exciting testable hypotheses that we are excited about, where this paper and its outcomes poise us to begin to explore these scientific and mechanistic interactions.

Bob Barrett: Were you surprised to find small nuclear RNAs in saliva and why do you think they're so highly abundant there?

Dr. David Wong: It's a very insightful question and we are just as surprised as you were. Again, that sort of points to the uniqueness and perhaps the discovery of the utilization of deep sequencing. RNA-seeking exosomes is really just beginning to happen. I would tend to think that as more and more groups are sort of applying this level of technologies as to the resolution to their exosomal content in blood and urine, perhaps a similar outcome will emerge.

But then again, you know, it's sort of having this level of presence of noncoding RNA in the salivary, exosomes is indeed surprising and perhaps it sort of echoes the thought that these are noncoding, new information that are regulatory in nature and so there lies the excitement of this new chapter of biological interplay that we would like to sort of unravel in saliva, utilizing the extracellular RNA.

Bob Barrett: As you said, the oral cavities exposed to many influences, such as foods consumed and saliva flow rates, circadian salivary secretion, and other factors. How do these aspects come into play?

Dr. David Wong: They all do and in particular that there is this dynamic open, communication between the exogenous environment and the oral cavity. However, saliva could turn itself into a scientific entity whereby in our clinic here, in our lab here, that we ask patients to come in with two hours of fasting. When they come in, they would rinse with distilled water three times and then there is a SOP in place for them to produce saliva. So that exogenous environment is there. It is part of the reality, but we could obtain saliva and obtain its biological constituents in a very scientific manner.

And on the topic of diurnal variation amongst other things, this is not unique to saliva. This is present in blood, in every other bodily fluid. This is just part of our body.

So what we need to do and what we've done is to define the scientific parameters whereby we can control these confounding factors and minimize it or utilize it to the best possible way for both translation and basic understanding.

So while saliva, sort of, has historically, have this notion and reputation of not a, perhaps, a very scientific entity, that landscape has changed. The National Institute of Dental and Craniofacial Research in the past 10 years has invested into this area here to turn saliva into a credible scientific entity where translational and scientific work can be performed.

And I think one of our essence today is what do these RNA do in saliva, it really synergized with this recent landscape of extracellular RNA communication program.

So it's a very exciting time where what this paper reports a technology we can really, you know, finally survey the transcriptomic content from both a mammalian and microbiome origin and begin to look at their interplay and also look at their eventual capability for both the translational and for mechanistic understanding as well.

Bob Barrett: Well, finally Dr. Wong, why do you believe that saliva is such an important bio-fluid?

Dr. David Wong: Well, perhaps it's a… another way to, sort of, pose this question is, while my efficacy is looking into saliva, the capability of defining the scientific foundation in saliva and from what we have known so far and I think in part, that's the NIH investment is, is this -omics information, the genomic, the proteomic, the transcriptomic of being faster and developed in the multitude of personalized medicine sort of application.

These very constituents are present in saliva, as I call them, as diagnostic alphabets for clinical applications. The point here is that the awareness of the presence of this -omics information that are clinically, translationally discriminatory and yet at the same time could open up these new chapters in cellular communication, presents a body of information that we otherwise would not have been aware of.

Now with that being said, when the scientific foundation is credentialed. That really is the key. Is there any value to harnessing the salivary transcriptome for biomarker development, say, for example? I mean when that is credentialed, then these secondary level, what I call second level of advantages kicks in. It's noninvasive, it's non-painful, and it's non-embarrassing.

And as a consumer, at the end of the day, if I need to do a spinal tap to get a diagnostic result and have no other choice, sure, that has to be done. But if there is a choice of performing the same procedure noninvasively, non-painfully, non-embarrassingly, the answer is clear. The Holy Grail of diagnostics is noninvasiveness. Yes, blood is minimally invasive, but give that time to be credential is endowed within saliva, it's the Holy Grail.

Bob Barrett: Dr. Wong is the Felix and Mildred Yip Professor, and Associate Dean of Research at the UCLA School of Dentistry. He has been our guest in this podcast from *Clinical Chemistry.*

I'm Bob Barrett. Thanks for listening.

