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Researchers Report Early Success Using Saliva to Detect Oral Cancer

Scientists funded by the National Institute of Dental and Craniofacial Research, part of the National Institutes of Health, reported today taking a major step forward in using saliva to detect oral cancer. As published in the current issue of *Clinical Cancer Research*, the scientists found they could measure for elevated levels of four distinct cancer-associated molecules in saliva and distinguish with 91 percent accuracy between healthy people and those diagnosed with oral squamous cell carcinoma.

This so-called "proof-of-principle" study marks the first report in the scientific literature that distinct patterns of "messenger RNA" not only are measurable in saliva but can indicate a developing tumor. Messenger RNA (mRNA) is the molecular intermediate between gene and protein, serving as a chemical record that an individual gene has been expressed.

According to David Wong, D.M.D., D.M.Sc., a scientist at the University of California at Los Angeles (UCLA) School of Dentistry and senior author on the paper, it may be possible with further refinement of the test, possibly by including additional cancer-linked mRNAs, to attain the necessary 99 to 100 percent accuracy of commercial diagnostic tests for oral squamous cell carcinoma, the sixth most common cancer in the United States. Wong noted that currently no biochemical or genetic diagnostic tests are commercially available for oral cancer.

He also noted that the RNA patterns in saliva may be informative for other cancers and common diseases. "Saliva is a mirror of our blood," said Wong. "We're now conducting our initial studies of saliva as a possible diagnostic fluid for other human cancers and system diseases, and we should have our preliminary data in the Spring."

Wong said he and his colleagues never intended to study mRNA patterns in saliva. They had been searching exclusively in the mouth's soft tissues, or mucosa, for proteins that might be associated with oral cancer, when Maie St. John, M.D., Ph.D., a head-and-neck surgery/otolaryngology resident at UCLA, who was then on a training rotation in Wong's lab, posed a simple question: If proteins associated with cancer are present in the oral mucosa, can they also pass from tissue into the saliva?

While looking for cancer-linked proteins in saliva, St. John happened to notice mRNA from the gene encoding one of her proteins of interest. This chance discovery raised two intriguing possibilities that would alter the course of research in Wong's lab: Does saliva contain a wide range of different mRNAs that have value in diagnosing disease? If correct, are there different collective patterns of mRNA in the saliva of a healthy person compared with someone who has a developing cancer?

St. John said pursuing this line of research was potentially important because previous studies had established that mRNA can be as informative of health and disease as changes in protein or DNA. In addition, mRNA has the key advantage over these more traditional analytes in that it can be readily extracted in bulk from a tissue or bodily fluid and processed much faster for a comprehensive picture of which mRNAs are present in a given tissue or bodily fluid and at which levels.

"What really interested us was the idea that mRNA analyses could be performed in a bodily fluid as easily obtained as saliva," said St. John. "If correct, a salivary test in theory would be quick, painless, and most likely less expensive than current diagnostic tests."

But would it be as informative as testing blood? Before they could answer this question, Wong and colleagues first had to define all of the individual mRNAs naturally present in saliva. As published this year in the *Journal of Dental Research*, they found people have about 3,000 chemically distinct mRNAs in their saliva at any one time. Of this total, a "core signature" of about 280 mRNAs are generally present in the saliva of healthy people.

With these baseline data as their scientific anchor, the researchers could begin to test whether saliva contains distinct mRNA patterns. "We obtained saliva and blood from 32 people who had been recently diagnosed with oral squamous cell carcinoma but not treated," said Yang Li, D.D.S., Ph.D., lead author on the study and a researcher in Wong's laboratory. "Because salivary diagnostics with RNA had yet to be tried, we referenced all of our data through blood. That is, whatever we found in saliva, we looked to see if it matched our data in blood."

As presented in their current *Clinical Cancer Research* paper, the scientists extracted the mRNA from the saliva of the cancer patients and soon discovered 1,679 genes were expressed at significantly different levels in the cancer patients compared to healthy individuals. Upon further analysis, they noticed seven mRNAs in particular that were present at a 3.5-fold higher level in the cancer patients. Interestingly, among them was the mRNA for the gene IL-8, whose protein St. John had originally searched for in saliva.

The researchers then whittled down their list of signature mRNAs to four, based on statistical models that indicated the synchronized rise in expression of these four molecules increased the probability that the saliva belonged to a cancer patient. These four mRNAs are from the following genes: Interleukin 1-beta (IL1B), Ornithine decarboxylase antizyme 1 (OAZ1), spermidine/spermine N1-acetyl transferase (SAT), and interleukin 8 (IL-8).

To put this idea to the test, they screened the saliva again to see how often they could correctly identify the samples from the cancer patients. In all instances, they had no foreknowledge of whether a healthy person or a cancer patient provided the saliva sample.

Wong said the group could identify the saliva from cancer patients in nine out of 10 samples. What's more, he said the sensitivity and specificity of their saliva tests were as good or better than their reference work in blood. "This was primarily an exploratory study to validate our initial finding of a unique molecular signature of mRNAs in people with oral squamous cell carcinoma," said Wong. "We will follow up with a larger cohort of about 200 patients in the near future, and this study will hopefully allow us to distinguish in saliva between the various stages of the cancer and ultimately push our accuracy up to as close to 100 percent as possible."

He also noted that these initial results serve to highlight the potential clinical value of saliva as a diagnostic biofluid. "Many have thought of saliva as very difficult to work with in the laboratory, in part because the molecular information contained within it is highly degradable," said Wong. "The truth is oral health researchers have worked on saliva for decades, and they have established defined ways to work with fluids that are consistent, reproducible, and which keep these molecules in a stable state. We have a marvelous way to completely stop the degradation of mRNA in saliva, and it allowed us to gather these data."
