Saliva is a complex body fluid that comprises secretions from the major and minor salivary glands, which are extensively supplied by blood. Therefore, molecules such as proteins, DNA, RNA, etc., present in plasma could be also present in saliva. Many studies have reported that saliva body fluid can be useful for discriminating several oral diseases, but also systemic diseases including cancer. Most of these studies revealed messenger RNA (mRNA) and proteomic biomarker signatures rather than specific non-coding RNA (ncRNA) profiles. NcRNAs are emerging as new regulators of diverse biological functions, playing an important role in oncogenesis and tumor progression. Indeed, the small size of these molecules makes them very stable in different body fluids and not as susceptible as mRNAs to degradation by ribonucleases (RNases). Therefore, the development of a non-invasive salivary test, based on ncRNAs profiles, could have a significant applicability to clinical practice, not only by reducing the cost of the health system, but also by benefitting the patient. Here, we summarize the current status and clinical implications of the ncRNAs present in human saliva as a source of biological information.
Saliva is a complex body fluid that comprises secretions from the major and minor salivary glands, nourished by body's vasculature. Although many circulatory molecules (DNA, RNA, and proteins) can also be present in saliva, saliva harbors unique molecular constituents that can be discriminatory for oral and systemic disease screening and detection. Many studies have reported that salivary constituents can discriminate oral diseases (oral cancer and Sjögren's syndrome) and also systemic diseases (lung cancer, breast cancer, pancreatic cancer, and ovarian cancer). Noncoding RNAs (ncRNAs) are emerging new regulators of diverse biological functions, playing important roles in oncogenesis and tumor progression. Indeed, the short size of these molecules makes them stable in different body fluids such as urine, blood, and saliva, being not as susceptible as mRNAs to degradation by RNases. Here, the current status and clinical implications of the ncRNAs present in human saliva are reviewed for translational applications and basic biological research. The development of noninvasive salivary test (based on ncRNAs profiles) for disease detection could have effective applications into the clinical context with a translational significance as emerging molecular biomarkers for non-invasively disease detection, not only by reducing the cost to the health care system but also by benefitting patients.

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KEYWORDS: biomarkers; body fluid; cancer; diagnostics; noninvasiveness; saliva

cells derived mediators, such as exosomes, in the development of saliva-based mRNA biomarkers. To further our understanding of the origins of disease-induced salivary biomarkers, we here evaluated the hypothesis that tumor-shed secretory lipidic vesicles called exosome-like microvesicles (ELMs) that serve as protective carriers of tissue-specific information, mRNAs, and proteins, throughout the vasculature and bodily fluids. RNA content was analyzed in cell free-saliva and ELM-enriched fractions of saliva. Our data confirmed that the majority of extracellular RNAs (exRNAs) in saliva were encapsulated within ELMs. Nude mice implanted with human lung cancer H460 cells expressing hCD63-GFP were used to follow the circulation of tumor cell specific protein and mRNA in the form of ELMs in vivo. We were able to identify human GAPDH mRNA in ELMs of blood and saliva of tumor bearing mice using nested RT-qPCR. ELMs positive for hCD63-GFP were detected in the saliva and blood of tumor bearing mice as well as using electric field-induced release and measurement (EFIRM). Altogether, our results demonstrate that ELMs carry tumor cell-specific mRNA and protein from blood to saliva in a xenografted mouse model of human lung cancer. These results therefore strengthen the link between distal tumor progression and the biomarker discovery of saliva through the ELMs.


Publication Types, Secondary Source ID, Grant Support

4. **Saliva diagnostics: utilizing oral fluids to determine health status.**
Schafer CA¹, Schafer JJ, Yakob M, Lima P, Camargo P, Wong DT.

**Author information**

**Abstract**
Imagine a time where your health status could be available to you without the pain, discomfort and inconvenience of a physical examination. Distant vision of an inconceivable future or impending reality with potentially immeasurable impact? Recent advancements in the field of molecular diagnostics indicate this is not only possible, but closer than we think. Novel discoveries and substantial advancements have revealed that saliva may contain real-time information describing our overall physiological condition. Researchers are now reporting that, like blood and tissue biopsies, oral fluids could be a source of biochemical data capable of detecting certain diseases. What is even more intriguing is that this phenomenon not only applies to local disorders like oral cancer and Sjögren's syndrome, but distant pathologies like autoimmune, cardiovascular and metabolic diseases as well as
viral/bacterial infections and even some cancers. These revelations have provided a foundation for the burgeoning field of salivary diagnostics and hence spurred the onset of investigations poised at deciphering the salivary milieu. This paper overviews salivary diagnostics from biomarker development to the multitude of techniques utilized in identifying saliva-based molecular indicators of disease. In doing so, we present oral fluids as an easily accessible noninvasive alternative to traditional diagnostic avenues and not just an essential component of the digestive process. Determining saliva as a credible means of evaluating health status represents a considerable leap forward in health care, one that could lead to enormous translational advantages and significant clinical opportunities.

PMID: 24862597 [PubMed - indexed for MEDLINE]

5. Salivary biomarkers for detection of oral squamous cell carcinoma - current state and recent advances.

Abstract
Oral squamous cell carcinoma (OSCC) is the most common malignant neoplasm of the oral cavity. Detection of OSCC is currently based on thorough clinical oral examination combined with biopsy for histological analysis. Most cases of OSCC are not detected until the cancer has developed into advanced stages; thus, a reliable early stage diagnostic marker is needed. This literature review presents an overview of the status of current advances in salivary diagnostics for OSCC. Though many protein and mRNA salivary biomarkers have been identified that can detect OSCC with high sensitivity and specificity, the most discernable findings occur with the use of multiple markers. Studies that incorporate proteomic, transcriptomic, and potentially additional "omics", including methylicos, need to be initiated to bring technology to clinical applications and allow the best use of saliva in diagnosing OSCC.

KEYWORDS: exosomes; metabolomics; methylomics; microbiomics; oral cancer; oral fluid diagnostics; oral squamous cell carcinoma; proteomics; salivaomics; salivary biomarker; salivary diagnostics; transcriptomics
Salivary microRNAs and oral cancer detection.

Yoshizawa JM, Wong DT.

Abstract
MicroRNAs (miRNAs) in human saliva have recently become an emerging field in saliva research for diagnostics applications and its potential role in biological implications. miRNAs are short noncoding RNA molecules that play important roles in regulating a variety of cellular processes. Dysregulation of miRNAs are known to be associated with many diseases. miRNAs were found present in the saliva of OSCC patients and could serve as potential biomarkers for oral cancer detection. Understanding the biological function of miRNAs in association with diseases is important towards utilizing miRNAs as diagnostic markers. There are currently a variety of profiling methods available for detecting miRNA expression levels. In this chapter, we overview the Applied Biosystem Stem-loop RT based Taqman MicroRNA Assay for salivary miRNA profiling. Using this highly sensitive and specific assay, miRNAs in saliva are profiled with only a few nanograms of starting RNA. This method is also applicable for studying biomarkers in other body fluids or clinical samples that contain small amounts of RNA.

Salivary diagnostics: moving to the next level.
Brinkmann O, Spielmann N, Wong DT.

Abstract
The evolution of salivary diagnostics has reached a new level toward the goal of using saliva
as a powerful fluid for early detection and the first line of diagnosis for life-threatening diseases such as cancer, metabolic disorders, infections, and inflammatory diseases. Newly developed tools such as the novel saliva-based POCT and the SKB are helping to realize the goal of making salivary diagnostics available to clinicians worldwide. This is a unique moment where dentistry may be paving a new path for primary healthcare.

PMID: 22746063 [PubMed - indexed for MEDLINE]

**MeSH Terms, Substances**

**Bioinformatics advances in saliva diagnostics.**
Ai JY<sup>1</sup>, Smith B, Wong DT.

**Author information**

**Abstract**
There is a need recognized by the National Institute of Dental & Craniofacial Research and the National Cancer Institute to advance basic, translational and clinical saliva research. The goal of the Salivaomics Knowledge Base (SKB) is to create a data management system and web resource constructed to support human salivaomics research. To maximize the utility of the SKB for retrieval, integration and analysis of data, we have developed the Saliva Ontology and SDxMart. This article reviews the informatics advances in saliva diagnostics made possible by the Saliva Ontology and SDxMart.

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**Publication Types, MeSH Terms, Substances, Grant Support**

**Prevalidation of salivary biomarkers for oral cancer detection.**

**Author information**
Abstract

BACKGROUND: Oral cancer is the sixth most common cancer with a 5-year survival rate of approximately 60%. Presently, there are no scientifically credible early detection techniques beyond conventional clinical oral examination. The goal of this study is to validate whether the seven mRNAs and three proteins previously reported as biomarkers are capable of discriminating patients with oral squamous cell carcinomas (OSCC) from healthy subjects in independent cohorts and by a National Cancer Institute (NCI)-Early Detection Research Network (EDRN)-Biomarker Reference Laboratory (BRL).

METHODS: Three hundred and ninety-five subjects from five independent cohorts based on case controlled design were investigated by two independent laboratories, University of California, Los Angeles (Los Angeles, CA) discovery laboratory and NCI-EDRN-BRL.

RESULTS: Expression of all seven mRNA and three protein markers was increased in OSCC versus controls in all five cohorts. With respect to individual marker performance across the five cohorts, the increase in interleukin (IL)-8 and subcutaneous adipose tissue (SAT) was statistically significant and they remained top performers across different cohorts in terms of sensitivity and specificity. A previously identified multiple marker model showed an area under the receiver operating characteristic (ROC) curve for prediction of OSCC status ranging from 0.74 to 0.86 across the cohorts.

CONCLUSIONS: The validation of these biomarkers showed their feasibility in the discrimination of OSCCs from healthy controls. Established assay technologies are robust enough to perform independently. Individual cutoff values for each of these markers and for the combined predictive model need to be further defined in large clinical studies.

IMPACT: Salivary proteomic and transcriptomic biomarkers can discriminate oral cancer from control subjects.

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Publication Types, MeSH Terms, Substances, Grant Support


Salivary transcriptome biomarkers in oral squamous cell cancer detection.

Brinkmann O1, Wong DT.

Author information
Abstract

In all, 350,000 new cases of oral cancer are reported annually worldwide, 35,000 of these occur in the United States. For decades, the 5-year survival rate has remained low at only 60%, which is mainly due to cancer diagnosis at late and progressed stage. Using saliva as a diagnostic medium could be the key for early detection and thus improved survival rates. Among all salivary constituents, the transcriptome has turned out to be a highly promising biomarker source. So far, seven mRNA and two microRNA markers were found to be discriminatory in saliva of oral cancer patients. This review will give an overview on the field of salivary transcriptome research with focus on oral cancer detection as well as the translation of salivary diagnostics into clinical reality.

PMID: 22126022 [PubMed - indexed for MEDLINE]

Publication Types, MeSH Terms, Substances

Scientific frontiers: emerging technologies for salivary diagnostics.

Baum BJ, Yates JR 3rd, Srivastava S, Wong DT, Melvin JE.

Author information

Abstract

Saliva, a biofluid historically well-studied biochemically and physiologically, has entered the post-genomic 'omics' era, where its proteomic, genomic, and microbiome constituents have been comprehensively deciphered. The translational path of these salivary constituents has begun toward a variety of personalized individual medical applications, including early detection of cancer. Salivary diagnostics is a late-comer, but it is catching up where dedicated resources, like the Salivaomics Knowledge Base (SKB), now have taken center stage in the dissemination of the diagnostic potentials of salivary biomarkers and other translational and clinical utilities.

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