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Salivary Biomarkers in Cancer Detection

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Abstract

Cancer is the second most common cause of death in the United States. Its symptoms are often not specific and absent, until the tumors have already metastasized. Therefore, there is an urgent demand for developing rapid, highly accurate and non-invasive tools for cancer screening, early detection, diagnostics, staging and prognostics. Saliva as a multi-constituent oral fluid, comprises secretions from the major and minor salivary glands, extensively supplied by blood. Molecules such as DNAs, RNAs, proteins, metabolites, and microbiota, present in blood, could be also found in saliva. Recently, salivary diagnostics has drawn significant attention for the detection of specific biomarkers, since the sample collection and processing are simple, cost-effective, precise and do not cause patient discomfort. Here, we review recent salivary candidate biomarkers for systemic cancers by dividing them according to their origin into: genomic, transcriptomic, proteomic, metabolomic and microbial types.

Keywords

Biomarkers; Cancer; Exosomes; Saliva

INTRODUCTION

Cancer is the second most common cause of death in the United States, trailing only behind the incidence of heart disease. A total of 1,658,370 new cancer cases and 589,430 cancer deaths were projected to occur in the United States in 2015 [1]. Early cancer detection is the hallmark of successful treatment. With recent advances in diagnostic technologies, including computed tomography and magnetic resonance imaging, sufficient sensitivity has been achieved. However, high costs and radiation exposure restrict their use for the purpose of

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CONFLICTS OF INTEREST

The other authors declare no conflict of interest.

INFORMED CONSENT:

Informed consent was obtained from all individual participants included in the study.

screening [2]. Therefore, more reliable, relatively inexpensive, and noninvasive methods are required for early diagnosis of cancer.

Saliva is a biological fluid composed of more than 99% water and less than 1% proteins, electrolytes and other low-molecular weight components [3]. It originates mainly from three pairs of major salivary glands (the parotid, submandibular, and sublingual glands) as well as from 300 to 400 minor salivary glands present in the oral cavity [4]. The gingival crevicular fluid containing bacteria, epithelial cells, erythrocytes, leukocytes and food debris contributes only in small part to the formation of oral fluids. Thus, saliva plays a key role in the lubrication, mastication, swallowing and digestion. It protects the integrity of the oral tissues, but also provides clues for local and systemic diseases and conditions [5]. Molecules such as DNAs, RNAs, proteins, metabolites, and microbiota, present in blood, could be also present in saliva. Thus, their concentration changes can be used as biomarkers to detect early-stage cancer or to monitor the response to therapeutic management [6]. Salivary diagnostics is a non-invasive, easy to use tool for patient specimen collection. Saliva testing potentially allows the patient to gather their own saliva samples, even at home, thus savings healthcare costs, enabling convenient and multiple sampling as well as having a positive impact on patient compliance [7].

The aim of this paper was to provide a review on the potential mechanisms by which the distal tumors mediate changes in salivary biomarker profiles as well as to describe recent advances in salivary biomarkers used for systemic cancer detection.

Biomarkers in Saliva

“Salivaomics” is a broad collection of technologies used to explore different types of molecules contained in saliva. This term includes genome and epigenome (the study of genes and their methylation), transcriptomics (the study of mRNA within cells or organisms), metabolomics (the study of global metabolite profiles in a system), proteomics (the study of proteins) and microbiota (the study of microbiology).

Genome and Epigenome

The salivary genome consists of both human and microbial DNA [8]. Both, the quantity and the quality of salivary DNA are quite good: the mean total DNA in saliva is approximately 24 μg, ranging from 0.2 to 52 μg. Although it is approximately 10 times lower than in blood (mean 210 μg, range 58–577 μg), genotyping requires as low as 5 ng/mL of DNA to work effectively. Saliva samples also yield sufficient DNA for sequencing arrays and polymerase chain reaction (PCR) assays [9]. The ratios of absorbance at 260 nm and 280 nm (A260/A280) can be measured to evaluate the purity of DNA. The mean value of A260/A280 is 1.56 for saliva and 1.71 for plasma, which indicates that the quality of salivary DNA is comparable to that in blood [9]. Tumorigenesis is a multistep process, involving both genetic and epigenetic changes in its pathology [10]. Aberrant DNA methylation was the first epigenetic mark to be associated with cancer as a consequence of the alteration it causes in normal gene regulation [11]. Salivary genome and epigenome are assayable by a diverse collection of biomolecular techniques, including methylation arrays, PCR and quantitative

PCR (qPCR)-based genotyping. Some innovative methods are also developed to detect gene mutation related to lung cancer [12].

Transcriptome

Salivary transcriptome researches mainly focus on mRNA and miRNA, which can be found in oral cavity that are distant to original cells [13]. The salivary transcriptome was first profiled in our UCLA lab [14]. We also developed the simple methods to stabilize the salivary mRNA at a room temperature and its analysis without further processing [15]. Noncoding RNAs (ncRNAs) are emerging as new regulators of diverse biological functions, playing an important role in oncogenesis and tumor progression. Because of the small size of these molecules, they are very stable in different body fluids and not as susceptible as messenger RNAs (mRNAs) to degradation by ribonucleases (RNases) [16]. Based on gene microarray and quantitative real-time PCR (qRT-PCR) technology, several mRNA and micro-RNA (miRNA) candidates were discovered in lung cancer [17], pancreatic cancer [18, 19], and breast cancer [20] with good sensitivity and specificity.

Proteome

The salivary proteome comprises the entire protein content of the oral cavity. Saliva contains greater than 2000 proteins and peptides, that are involved in a multitude of different biological functions in the oral cavity [21]. Approximately one fourth of the whole-saliva proteins are found in plasma. Proteomic analysis in saliva have distinct advantages over blood, especially for low-abundance proteins, due to the more even distribution of distinct salivary peptides [22].

Currently, mass spectrometry (MS) is the core technology for salivary protein identification. Surface-Enhanced Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (SELDI-TOF-MS) can get stable profile of salivary proteome of healthy controls [23]. It could be also used in detecting the differences between pre- and post-orthodontic treatment [24] or in high-throughput breast cancer biomarker discovery [25]. Two-dimensional gel electrophoresis (2DE) combined with MS performed well in lung cancer [26] and breast cancer [20] biomarker detection with high sensitivity and specificity.

Raman spectroscopy (RS) has been regarded as a promising optical technique for the comprehensive investigation of cancer diagnosis over the last 2 decades. Feng et al. demonstrate that saliva protein Surface Enhanced Raman Spectroscopy (SERS) analysis combined with partial least squares– discriminant analysis diagnostic algorithms has great potential for the noninvasive and label-free detection of breast cancer [27].

Metabolome

Metabolome, a global comprehensive overview of the metabolic status provides a new insight into pathophysiologic mechanisms of various diseases. It allows measuring the levels of endogenous metabolites, thus enabling biomarker discovery [28]. The endogenous metabolites, including nucleic acids, lipids, amino acids, peptides, vitamins, organic acids, thiols, and carbohydrates, represent a valuable tool for detection of biomarkers for various diseases and monitoring disease progression [29, 30].

In 2010, Sugimoto et al. identified that cancer-specific signatures are embedded in saliva metabolites by capillary electrophoresis time-of-flight mass spectrometry (CE-TOF-MS). They conducted a comprehensive metabolite analysis of saliva samples obtained from oral, pancreatic, breast cancer, periodontal disease patients and healthy controls. Fifty-seven principal metabolites were found to accurately predict the probability of being affected by a specific disease, yielding large area under the receiver operating characteristic curves (AUCs) [31]. Other salivary metabolites were established to differentiate oral squamous cell carcinoma [32] and neurodegenerative dementia patients [33] from controls based on MS.

Microbiota

The recent advances in next generation sequencing allowed the identification of about 19,000 phylotypes in the oral cavity [34]. Evidences shows that bacteria and microorganisms can lead to oral diseases, such as caries [35], periodontitis [36] as well as systemic diseases including cancer [37, 38]. Based on microarray and qPCR, Farrell et al. demonstrated that the combination of *N.elongata* and *S.mitis* in saliva can distinguish pancreatic cancer patients from healthy subjects [39]. Torres et al. got the similar results by using high-throughput sequencing of bacterial small subunit ribosomal RNA (16S rRNA) gene [40]. *Helicobacter pylori (H.pylori)*, which is known to cause inflammation of the stomach lining, can also lead to gastric cancer. Two metabolites of *H.pylori* can be detected in saliva with good sensitivity [41].

How the Distal Tumor Relate to Saliva Biomarkers?

Previous studies have confirmed that many discriminatory salivary biomarkers can be detected upon the development of systemic cancers such as pancreatic cancer [18], breast cancer [42], lung cancer [26] or ovarian cancer. However, none of them clearly explains why a cancer located far from the oral cavity could affect biomarker profiles in saliva.

Lau et al. used a breast cancer cell model to demonstrate that breast cancer-derived exosome-like microvesicles are capable of interacting with salivary gland cells, altering the composition of their secreted exosome-like microvesicles [43]. They found that the salivary gland cells secreted exosome-like microvesicles, thus encapsulating both proteins and mRNAs.

Exosomes are small vesicles (diameters of 30–120 nm) that contain lipids [44], mRNA, microRNA [45], DNA [46] and proteins [47]. They are perceived to carry these contents from distant locations to the whole body. Exosomes exist in almost all cell types around the body and in most bodily fluids including saliva [48–50]. Studies demonstrate that exosomes can be involved in RNA processing and degradation [51], spreading pathogen [52], tumor promotion [53, 54] and immune function [55].

Later, Lau et al. found that the constructed pancreatic cancer mouse model yielded discriminatory salivary biomarkers by implanting the pancreatic cancer cell line into the pancreas of host [56]. These studies confirm that exosomes provide a mechanism for altered salivary cancer specific biomarkers.

Saliva Biomarkers of Cancer Detection

Lung Cancer

Lung cancer is the leading cause of cancer-related deaths in both men (28%) and women (27%) in the United States. The American Cancer Society estimated that over 220,000 patients were diagnosed with lung cancer and over 150,000 deaths were caused by this disease in 2015 [1]. The majority of lung cancers are diagnosed at an advanced stage, thus resulting in much lower 5-year survival rate (17%) compared to breast (89%), prostate (99%) and colon carcinomas (65%) [1]. Despite advances in the management of lung cancer, this disease still remains a significant global health burden with survival rates that have not significantly improved in last decades. Reduced mortality with low-dose helical CT (LDCT) screening of high-risk patients is challenged by the high false positive rate and the potential morbidity associated with follow-up diagnostic evaluation in patients at high risk for iatrogenic complications. The diagnostic dilemma of the indeterminate nodule incidentally identified on diagnostic or screening CT has created a need for searching for reliable biomarkers capable of distinguishing benign from malignant disease.

Wei et al. developed a novel core technology, electric field–induced release and measurement (EFIRM), which can detect the epidermal growth factor receptor (EGFR) mutations directly in bodily fluids, including saliva. This approach is an electrochemical method based on immobilized nucleic acid probes for capturing mutated sequences and applying electric fields to facilitate the hybridization process. Because of the speed and simplicity of the method, EFIRM has the potential to be a suitable tool for oncogenic mutation monitoring in clinics. A blinded test was performed on saliva samples from 40 patients with non-small cell lung carcinoma (NSCLC). The receiver operating characteristic (ROC) analysis indicated that EFIRM detected the exon 19 deletion with the AUC of 0.94 and the L858R mutation with an AUC of 0.96 [12]. Pu et al. used EFIRM to detect exon 19 deletion and L858R mutations in saliva and plasma samples of lung cancer patients [57]. This study resulted in the detection of exon 19 deletion with the AUC of 1.0 in both saliva and plasma samples in lung cancer patients. For L858R mutation detection, the AUC of saliva was 1.0, while the AUC of plasma was 0.98. Strong correlations were also found between pre- and post-surgical samples for both saliva (0.86 for exon 19 deletion and 0.98 for L858R mutation) and plasma (0.73 and 0.94, respectively).

A salivary transcriptome was analyzed in a cohort of 42 lung cancer patients and 74 healthy controls by gene microarray. Seven mRNA transcripts [BRAF (v-raf murine sarcoma viral oncogene homolog B1), CCNI (cyclin I), EGFR, FGF19 (fibroblast growth factor 19), FRS2 (fibroblast growth factor receptor substrate 2), GREB1 (growth regulation by estrogen in breast cancer 1), and LZTS1 (leucine zipper, putative tumor suppressor 1)] expressed in saliva were identified and prevalidated. The logistic regression model with the combination of five mRNA biomarkers (CCNI, EGFR, FGF19, FRS2 and GREB1) could differentiate lung cancer patients from control subjects, yielding the AUC value of 0.925 with 93.75% sensitivity and 82.81% specificity [17]. Li et al. introduced SERS to identify lung cancer biomarkers in saliva. There were nine significant peaks between patients and controls, most

of them assigned to amino acids and nucleic acid bases. The accuracy, sensitivity, and specificity of the measurement were 80%, 78% and 83%, respectively [26].

Xiao et al. investigated the proteomic biomarkers in saliva by 2-DE combined with MS, 16 candidate protein biomarkers were discovered. Three proteins (haptoglobin, zinc-a-2-glycoprotein and calprotectin) were further verified with the discriminatory power of 88.5% sensitivity and 92.3% specificity in lung cancer patients compared to healthy controls (AUC=0.90) [58].

Pancreatic Cancer

Pancreatic cancer is the fourth leading cause of cancer-related deaths in males and females of all ages with a 5-year survival rate of 3%–5%. It has been estimated that this disease causes over 40,000 deaths per year in the USA [1]. Close to 100% of patients with pancreatic cancer develop metastases and die because of the late-stage presentation, lack of effective therapy protocols, biomarkers and early detection tools [59, 60].

Zhang et al. profiled the transcriptomes of saliva samples from 42 pancreatic cancer patients, including 30 chronic pancreatitis patients and 42 healthy control individuals by using the Affymetrix HG U133 Plus 2.0 Array [18]. Their results showed that the combination of 4 mRNA biomarkers (*KRAS, MBD3L2, ACRV1* and *DPM1*) could differentiate pancreatic cancer patients from cancer-free subjects with high sensitivity of 90.0% and specificity of 95.0% (AUC=0.971).

Recently, miRNAs show their importance in salivary diagnostics. A miRNA PCR array (miRBase version 18, containing 384 miRNAs; Qiagen) was used to detect the miRNA fraction in salivary supernatant of 30 patients diagnosed with pancreatic cancer and 32 healthy controls. The top 5 miRNA candidates (miR-17, miR-21, miR-181a, miR-181b and miR-196a) were differentially expressed in the saliva samples of pancreatic cancer patients compared to controls and validated by qRT-PCR [19]. Humeau et al. screened 94 salivary candidate miRNAs by qRT-PCR in patients with pancreatic cancer, pancreatitis, intraductal papillary mucinous neoplasia and healthy controls [61]. They identified hsa-miR-21, hsa-miR-23a, hsa-miR-23b and miR-29c significantly upregulated in saliva of pancreatic cancer patients compared to controls, showing perfect specificity (100%), but with relative low sensitivities of 71.4%, 85.7%, 85.7% and 57%, respectively. In other study, an Agilent microarray was used to profile the salivary miRNA of patients with resectable pancreatic cancer, followed by validation by means of qPCR. Logistic regression model combining miR-3679-5p and miR-3679-5p was able to distinguish resectable pancreatic cancer within the three categories, showing sensitivities of 72.5%, 62.5%, 70.0% and specificities of 70.0%, 80.0%, 70.0%, respectively [62].

Lau et al. provide the mechanistic and biological rationales, why the biomarkers of pancreatic cancer can be appearing in saliva [56]. They investigated the role of pancreatic cancer-derived exosomes in salivary biomarker development by constructing a pancreatic cancer mouse model. Their results showed that the salivary biomarker development was disrupted by inhibiting the biogenesis of pancreatic cancer-derived exosomes.

Sugimoto et al. identified eight pancreatic cancer-specific metabolites (leucine with isoleucine, tryptophan, valine, glutamic acid, phenylalanine, glutamine and aspartic acid) using CE-TOF-MS. The AUC value to discriminate healthy controls from pancreatic cancer subjects was 0.993 [31].

Farrell et al. observed significant variation in salivary microbiota between 10 pancreatic cancer and 10 control subjects by using Human Oral Microbe Identification Microarray (HOMIM), later validated by means of qPCR in an independent cohort [39]. The combination of *N.elongata* and *S.mitis* provided the AUC of 0.90 with 96.4% sensitivity and 82.1% specificity in distinguishing patients with pancreatic cancer from healthy subjects. Torres et al. characterized the salivary microbiota of pancreatic cancer, healthy and subjects diagnosed with other diseases by using high-throughput sequencing of 16S rRNA gene [40]. Similar to Farrell's research, the decreased abundances of *N.elongata* as well as significantly higher ratio of *Leptotrichia* to *Porphyromonas* were detected in the saliva of patients diagnosed with pancreatic cancer compared to healthy controls or those with other diseases. These reports open a new venue for salivary microbiota to serve as an informative source for discovering non-invasive biomarkers for systemic diseases.

Breast Cancer

Breast cancer is the most common form of cancer and the second leading cause of cancer deaths in women in the USA [1]. Despite advances in treatment, more than 40,290 people died of this disease in USA in 2015 [1]. Most breast cancers are diagnosed at a late stage, resulting in high mortality rates. Traditional screening mammography is considered to be the gold standard for breast cancer diagnosis, but the sensitivity is not ideal depending on the type of mammography [63].

Affymetrix HG-U133-Plus-2.0 Array and 2-DE were used to profile salivary transcriptomes and proteomes of 10 breast cancer patients and 10 matched controls. Preclinical validations were performed by qRT-PCR (transcriptomic biomarkers) and quantitative protein immunoblot (proteomic biomarkers) in an independent cohort. Eight mRNA biomarkers and one protein biomarker were prevalidated, yielding an accuracy of 92% (83% sensitivity, 97% specificity) [20].

Salivary and serum level of concentrations of protein CA15-3 could be positively correlated when comparing breast cancer patients to controls [42, 64]. CA15-3 now is a proteomic biomarker approved by US Food and Drug Administration (FDA) for monitoring the metastasis of breast cancer [65]. SERS was applied to explore the protein biomarkers of saliva samples obtained from 33 healthy subjects, 33 patients with benign breast tumors and 31 with malignant breast tumors, followed by analysis using regularized multinomial regression techniques. The diagnostic accuracy of 92.78%, 95.87% and 88.66% were acquired in those three groups, respectively [27].

The expression of lung resistance protein (LRP) in saliva was measured in 16 healthy women and 16 women with confirmed breast cancer stage I using gel electrophoresis and Western blot technology. The levels of LRP were reported at significantly higher concentrations among breast cancer subjects as compared to healthy women [66].

Jinno et al. conducted a metabolite analysis of samples obtained from 60 breast cancer patients and 20 healthy controls by capillary electrophoresis time-of-flight mass spectrometry. Five potential biomarkers (Choline, Isethionate, Cadavarine, N1-Acetylspermidine and Spermine) demonstrated significantly higher concentrations in breast cancer patients comparing with healthy individuals ($p < 0.05$), showing high AUC values of 0.850, 0.819, 0.809, 0.765 and 0.716, respectively [67].

Zhong et al. declared that they successfully established metabonomics analysis in human saliva for identifying biomarkers to diagnose and to determine the stage of breast cancer. Hydrophilic interaction chromatography and reversed-phase liquid chromatography separations, operated in both positive and negative ionization modes, were used to analyze the global saliva metabonome. Among the potential 18 biomarkers which disclosed significant differences, LysoPC (18:1), LysoPC (22:6) and MG (0:0/14:0/0:0) displayed the AUC values of 0.920, 0.920 and 0.929, respectively [68].

Gastric Cancer

Although the incidence and mortality have dramatically decreased over the past several decades, gastric cancer is still a major public health issue as the fifth most common malignancy in the world and the third leading cause of cancer-related death worldwide [69, 70]. The symptoms of gastric cancer tend to emerge late in the development of the disease and thus treatment options are often limited [26]. Moreover, gastric cancer occurs in juvenescence, and is usually diagnosed at advanced stage [70]. The quantitative proteomic approach, tandem mass tag (TMT) technology, was applied to develop discriminatory salivary protein biomarkers for the detection of gastric cancer. More than 500 proteins were identified and quantified in this study, among which 48 showed significant differential expression profile between controls and gastric cancer patients. Cystatin B, triosephosphate isomerase, and malignant brain tumors 1 protein were successfully verified by ELISA. The combination of these three biomarkers could reach 85% sensitivity and 80% specificity with accuracy of 0.93 [71].

H.pylori, causing the stomach lining inflammation, can also lead to gastric cancer. Zilberman et al. detected clinically relevant levels of two metabolites of H.pylori, NH_3 and CO_2, in saliva, which provides a platform for cross-reactive sensitivity and allows detection of salivary CO_2 and NH3 at ppm levels [41].

CONCLUSIONS

The goal of cancer screening is to detect tumor at an early stage, when treatment is most likely to be successful. Screening methodologies, exhibiting the combined features of high sensitivity and high specificity, are greatly needed. Moreover, the screening tools should be sufficiently noninvasive and inexpensive to allow widespread applicability. Salivary diagnostics has all the advantages mentioned above over a blood test, and different salivary biomarkers could be applied to detect systematic cancers, other than used for diagnosis of oral local diseases.

The understanding of exosome secretion and liquid biopsy provides the information about the origin of salivary biomarkers and the mechanism responsible for the development of discriminatory biomarkers in saliva and distal systemic diseases. As detailed above, the recent approaches of salivary biomarker development have elucidated great progress towards clinical application. Several biomarkers for systematic cancer detection have been identified and validated at the preclinical level. We truly believe that integration of better understanding of salivary and emerging novel, accurate detection technology will open a new era for salivary diagnostics.

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David Wong is co-founder of RNAmeTRIX Inc., a molecular diagnostic company. He holds equity in RNAmeTRIX, and serves as a company Director and Scientific Advisor. The University of California also holds equity in RNAmeTRIX. Intellectual property that David Wong invented and which was patented by the University of California has been licensed to RNAmeTRIX.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin. 2015; 65:5–29. [PubMed: 25559415]
2. Huang MY, Tsai HL, Huang JJ, Wang JY. Clinical Implications and Future Perspectives of Circulating Tumor Cells and Biomarkers in Clinical Outcomes of Colorectal Cancer. Transl Oncol. 2016; 9:340–347. [PubMed: 27567958]
3. Soini HA, Klouckova I, Wiesler D, Oberzaucher E, Grammer K, Dixon SJ, et al. Analysis of volatile organic compounds in human saliva by a static sorptive extraction method and gas chromatography-mass spectrometry. J Chem Ecol. 2010; 36:1035–1042. [PubMed: 20809147]
4. Aps JK, Martens LC. Review: the physiology of saliva and transfer of drugs into saliva. Forensic Sci Int. 2005; 150:119–131. [PubMed: 15944052]
5. Slavkin HC. Toward molecularly based diagnostics for the oral cavity. J Am Dent Assoc. 1998; 129:1138–1143. [PubMed: 9715016]
6. Lee JM, Garon E, Wong DT. Salivary diagnostics. Orthod Craniofac Res. 2009; 12:206–211. [PubMed: 19627522]
7. Lawrence HP. Salivary markers of systemic disease: noninvasive diagnosis of disease and monitoring of general health. J Can Dent Assoc. 2002; 68:170–175. [PubMed: 11911813]
8. Rylander-Rudqvist T, Håkansson N, Tybring G, Wolk A. Quality and quantity of saliva DNA obtained from the self-administrated oragene method—a pilot study on the cohort of Swedish men. Cancer Epidemiol Biomarkers Prev. 2006; 15:1742–1745. [PubMed: 16985039]
9. Abraham JE, Maranian MJ, Spiteri I, Russell R, Ingle S, Luccarini C, et al. Saliva samples are a viable alternative to blood samples as a source of DNA for high throughput genotyping. BMC Med Genomics. 2012; 5:19. [PubMed: 22647440]
10. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. Cell. 1996; 87:159–170. [PubMed: 8861899]
11. Feinberg AP, Vogelstein B. Hypomethylation distinguishes genes of some human cancers from their normal counterparts. Nature. 1983; 301:89–92. [PubMed: 6185846]
12. Wei F, Lin CC, Joon A, Feng Z, Troche G, Lira ME, et al. Noninvasive saliva-based EGFR gene mutation detection in patients with lung cancer. Am J Respir Crit Care Med. 2014; 190:1117–1126. [PubMed: 25317990]
13. Park NJ, Zhou H, Elashoff D, Henson BS, Kastratovic DA, Abemayor E, et al. Salivary microRNA: discovery, characterization, and clinical utility for oral cancer detection. Clin Cancer Res. 2009; 15:5473–5477. [PubMed: 19706812]
14. Li Y, Zhou X, St John MA, Wong DT. RNA profiling of cell-free saliva using microarray technology. J Dent Res. 2004; 83:199–203. [PubMed: 14981119]

15. Lee YH, Zhou H, Reiss JK, Yan X, Zhang L, Chia D, et al. Direct saliva transcriptome analysis. Clin Chem. 2011; 57:1295–1302. [PubMed: 21791578]
16. Majem B, Rigau M, Reventós J, Wong DT. Non-coding RNAs in saliva: emerging biomarkers for molecular diagnostics. Int J Mol Sci. 2015; 16:8676–8698. [PubMed: 25898412]
17. Zhang L, Xiao H, Zhou H, Santiago S, Lee JM, Garon EB, et al. Development of transcriptomic biomarker signature in human saliva to detect lung cancer. Cell Mol Life Sci. 2012; 69:3341–3350. [PubMed: 22689099]
18. Zhang L, Farrell JJ, Zhou H, Elashoff D, Akin D, Park NH, et al. Salivary transcriptomic biomarkers for detection of resectable pancreatic cancer. Gastroenterology. 2010; 138:949–957. [PubMed: 19931263]
19. Gao S, Chen LY, Wang P, Liu LM, Chen Z. MicroRNA expression in salivary supernatant of patients with pancreatic cancer and its relationship with ZHENG. Biomed Res Int. 2014; 2014:756347. [PubMed: 25126577]
20. Zhang L, Xiao H, Karlan S, Zhou H, Gross J, Elashoff D, et al. Discovery and preclinical validation of salivary transcriptomic and proteomic biomarkers for the non-invasive detection of breast cancer. PLoS One. 2010; 5:e15573. [PubMed: 21217834]
21. Bassim CW, Ambatipudi KS, Mays JW, Edwards DA, Swatkoski S, Fassil H, et al. Quantitative salivary proteomic differences in oral chronic graft-versus-host disease. J Clin Immunol. 2012; 32:1390–1399. [PubMed: 22806177]
22. Loo J, Yan W, Ramachandran P, Wong DT. Comparative human salivary and plasma proteomes. J Dent Res. 2010; 89:1016–1023. [PubMed: 20739693]
23. Papale M, Pedicillo MC, Di Paolo S, Thatcher BJ, Lo Muzio L, Bufo P, et al. Saliva analysis by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF/MS): from sample collection to data analysis. Clin Chem Lab Med. 2008; 46:89–99. [PubMed: 18020972]
24. Ciavarella D, Mastrovincenzo M, D'Onofrio V, Chimenti C, Parziale V, Barbato E, et al. Saliva analysis by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS) in orthodontic treatment: first pilot study. Prog Orthod. 2011; 12:126–131. [PubMed: 22074837]
25. Streckfus CF, Bigler LR, Zwick M. The use of surface-enhanced laser desorption/ionization time-of-flight mass spectrometry to detect putative breast cancer markers in saliva: a feasibility study. J Oral Pathol Med. 2006; 35:292–300. [PubMed: 16630293]
26. Li X, Yang T, Lin J. Spectral analysis of human saliva for detection of lung cancer using surface-enhanced Raman spectroscopy. J Biomed Opt. 2012; 17:037003. [PubMed: 22502575]
27. Wu, W., Gong, H., Liu, M., Chen, G., Chen, R. 2015 8th International Conference on Biomedical Engineering and Informatics (BMEI): 2015. IEEE; 2015. Noninvasive breast tumors detection based on saliva protein surface enhanced Raman spectroscopy and regularized multinomial regression; p. 214-218.
28. Nicholson JK, Lindon JC. Systems biology: metabonomics. Nature. 2008; 455:1054–1056. [PubMed: 18948945]
29. Arakaki AK, Skolnick J, McDonald JF. Marker metabolites can be therapeutic targets as well. Nature. 2008; 456:443.
30. Park C, Yun S, Lee SY, Park K, Lee J. Metabolic profiling of Klebsiella oxytoca: evaluation of methods for extraction of intracellular metabolites using UPLC/Q-TOF-MS. Appl Biochem Biotechnol. 2012; 167:425–438. [PubMed: 22555499]
31. Sugimoto M, Wong DT, Hirayama A, Soga T, Tomita M. Capillary electrophoresis mass spectrometry-based saliva metabolomics identified oral, breast and pancreatic cancer-specific profiles. Metabolomics. 2010; 6:78–95. [PubMed: 20300169]
32. Wei J, Xie G, Zhou Z, Shi P, Qiu Y, Zheng X, et al. Salivary metabolite signatures of oral cancer and leukoplakia. Int J Cancer. 2011; 129:2207–2217. [PubMed: 21190195]
33. Tsuruoka M, Hara J, Hirayama A, Sugimoto M, Soga T, Shankle WR, et al. Capillary electrophoresis-mass spectrometry-based metabolome analysis of serum and saliva from neurodegenerative dementia patients. Electrophoresis. 2013; 34:2865–2872. [PubMed: 23857558]

34. Keijser BJ, Zaura E, Huse SM, van der Vossen JM, Schuren FH, Montijn RC, et al. Pyrosequencing analysis of the oral microflora of healthy adults. J Dent Res. 2008; 87:1016–1020. [PubMed: 18946007]
35. Burne RA, Zeng L, Ahn SJ, Palmer SR, Liu Y, Lefebure T, et al. Progress dissecting the oral microbiome in caries and health. Adv Dent Res. 2012; 24:77–80. [PubMed: 22899685]
36. Ge X, Rodriguez R, Trinh M, Gunsolley J, Xu P. Oral microbiome of deep and shallow dental pockets in chronic periodontitis. PLoS One. 2013; 8:e65520. [PubMed: 23762384]
37. Cox MJ, Cookson WO, Moffatt MF. Sequencing the human microbiome in health and disease. Hum Mol Genet. 2013; 22:R88–R94. [PubMed: 23943792]
38. Schwabe RF, Jobin C. The microbiome and cancer. Nat Rev Cancer. 2013; 13:800–812. [PubMed: 24132111]
39. Farrell JJ, Zhang L, Zhou H, Chia D, Elashoff D, Akin D, et al. Variations of oral microbiota are associated with pancreatic diseases including pancreatic cancer. Gut. 2012; 61:582–588. [PubMed: 21994333]
40. Torres PJ, Fletcher EM, Gibbons SM, Bouvet M, Doran KS, Kelley ST. Characterization of the salivary microbiome in patients with pancreatic cancer. PeerJ. 2015; 3:e1373. [PubMed: 26587342]
41. Zilberman Y, Sonkusale SR. Microfluidic optoelectronic sensor for salivary diagnostics of stomach cancer. Biosens Bioelectron. 2015; 67:465–471. [PubMed: 25223554]
42. Agha-Hosseini F, Mirzaii-Dizgah I, Rahimi A. Correlation of serum and salivary CA15-3 levels in patients with breast cancer. Med Oral Patol Oral Cir Bucal. 2009; 14:e521–524. [PubMed: 19680209]
43. Lau CS, Wong DT. Breast cancer exosome-like microvesicles and salivary gland cells interplay alters salivary gland cell-derived exosome-like microvesicles in vitro. PLoS One. 2012; 7:e33037. [PubMed: 22448232]
44. Subra C, Grand D, Laulagnier K, Stella A, Lambeau G, Paillasse M, et al. Exosomes account for vesicle-mediated transcellular transport of activatable phospholipases and prostaglandins. J Lipid Res. 2010; 51:2105–2120. [PubMed: 20424270]
45. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007; 9:654–659. [PubMed: 17486113]
46. Pisetsky DS, Gauley J, Ullal AJ. Microparticles as a source of extracellular DNA. Immunol Res. 2011; 49:227–234. [PubMed: 21132466]
47. Théry C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. Nat Rev Immunol. 2002; 2:569–579. [PubMed: 12154376]
48. Yamada T, Inoshima Y, Matsuda T, Ishiguro N. Comparison of methods for isolating exosomes from bovine milk. J Vet Med Sci. 2012; 74:1523–1525. [PubMed: 22785357]
49. Gonzalez-Begne M, Lu B, Han X, Hagen FK, Hand AR, Melvin JE, et al. Proteomic analysis of human parotid gland exosomes by multidimensional protein identification technology (MudPIT). J Proteome Res. 2009; 8:1304–1314. [PubMed: 19199708]
50. Ogawa Y, Kanai-Azuma M, Akimoto Y, Kawakami H, Yanoshita R. Exosome-like vesicles with dipeptidyl peptidase IV in human saliva. Biol Pharm Bull. 2008; 31:1059–1062. [PubMed: 18520029]
51. Mahmoodzadeh Hosseini H, Imani Fooladi AA, Soleimanirad J, Nourani MR, Davaran S, Mahdavi M. Staphylococcal entorotoxin B anchored exosome induces apoptosis in negative esterogen receptor breast cancer cells. Tumour Biol. 2014; 35:3699–3707. [PubMed: 24399649]
52. Ritchie AJ, Crawford DM, Ferguson DJ, Burthem J, Roberts DJ. Normal prion protein is expressed on exosomes isolated from human plasma. Br J Haematol. 2013; 163:678–680. [PubMed: 24117007]
53. Yamashita T, Kamada H, Kanasaki S, Maeda Y, Nagano K, Abe Y, et al. Epidermal growth factor receptor localized to exosome membranes as a possible biomarker for lung cancer diagnosis. Pharmazie. 2013; 68:969–973. [PubMed: 24400444]
54. Beninson LA, Fleshner M. Exosomes: an emerging factor in stress-induced immunomodulation. Semin Immunol. 2014; 26:394–401. [PubMed: 24405946]

55. O'Loughlin AJ, Woffindale CA, Wood MJ. Exosomes and the emerging field of exosome-based gene therapy. Curr Gene Ther. 2012; 12:262–274. [PubMed: 22856601]
56. Lau C, Kim Y, Chia D, Spielmann N, Eibl G, Elashoff D, et al. Role of pancreatic cancer-derived exosomes in salivary biomarker development. J Biol Chem. 2013; 288:26888–26897. [PubMed: 23880764]
57. Pu D, Liang H, Wei F, Akin D, Feng Z, Yan Q, et al. Evaluation of a novel saliva-based epidermal growth factor receptor mutation detection for lung cancer: A pilot study. Thorac Cancer. 2016; 7:428–436. [PubMed: 27385985]
58. Xiao H, Zhang L, Zhou H, Lee JM, Garon EB, Wong DT. Proteomic analysis of human saliva from lung cancer patients using two-dimensional difference gel electrophoresis and mass spectrometry. Mol Cell Proteomics. 2012; 11 M111. 012112.
59. Jenkinson C, Earl J, Ghaneh P, Halloran C, Carrato A, Greenhalf W, et al. Biomarkers for early diagnosis of pancreatic cancer. Expert Rev Gastroenterol Hepatol. 2015; 9:305–315. [PubMed: 25373768]
60. Li D, Xie K, Wolff R, Abbruzzese JL. Pancreatic cancer. Lancet. 2004; 363:1049–1057. [PubMed: 15051286]
61. Humeau M, Vignolle-Vidoni A, Sicard F, Martins F, Bournet B, Buscail L, et al. Salivary microRNA in pancreatic cancer patients. PLoS One. 2015; 10:e0130996. [PubMed: 26121640]
62. Xie Z, Yin X, Gong B, Nie W, Wu B, Zhang X, et al. Salivary microRNAs show potential as a noninvasive biomarker for detecting resectable pancreatic cancer. Cancer Prev Res (Phila). 2015; 8:165–173. [PubMed: 25538087]
63. Skaane P. Studies comparing screen-film mammography and full-field digital mammography in breast cancer screening: updated review. Acta Radiol. 2009; 50:3–14. [PubMed: 19037825]
64. Laidi F, Bouziane A, Lakhdar A, Khabouze S, Amrani M, Rhrab B, et al. Significant correlation between salivary and serum Ca 15-3 in healthy women and breast cancer patients. Asian Pac J Cancer Prev. 2014; 15:4659–4662. [PubMed: 24969900]
65. Füzéry AK, Levin J, Chan MM, Chan DW. Translation of proteomic biomarkers into FDA approved cancer diagnostics: issues and challenges. Clin Proteomics. 2013; 10:13. [PubMed: 24088261]
66. Wood N, Streckfus CF. The Expression of Lung Resistance Protein in Saliva: A Novel Prognostic Indicator Protein for Carcinoma of the Breast. Cancer Invest. 2015; 33:510–515. [PubMed: 26506284]
67. Jinno, H., Murata, T., Sunamura, M., Sugimoto, M. Investigation of potential salivary biomarkers for the diagnosis of breast cancer; ASCO Annual Meeting Proceedings: 2015; 2015. p. 145
68. Zhong L, Cheng F, Lu X, Duan Y, Wang X. Untargeted saliva metabonomics study of breast cancer based on ultra performance liquid chromatography coupled to mass spectrometry with HILIC and RPLC separations. Talanta. 2016; 158:351–360. [PubMed: 27343615]
69. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015; 65:87–108. [PubMed: 25651787]
70. Wu ZZ, Wang JG, Zhang XL. Diagnostic model of saliva protein finger print analysis of patients with gastric cancer. World J Gastroenterol. 2009; 15:865–870. [PubMed: 19230049]
71. Xiao H, Zhang Y, Kim Y, Kim S, Kim JJ, Kim KM, et al. Differential proteomic analysis of human saliva using tandem mass tags quantification for gastric cancer detection. Sci Rep. 2016; 6:22165. [PubMed: 26911362]