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(REVIEW ARTICLE)

A review on salivary biomarkers in carcinoma diagnosis

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Abstract

Carcinoma is the 2nd leading mortality in the U.S. Signs & symptoms include typically unspecific until the tumours metastasize. Hence, an urgency is there for quick, precise, and non-invasive carcinoma diagnosis, rapid detection, diagnosis, stage surveys, & forecasts. Saliva is a multi-structural fluid, found in the oral region, containing secretions from primary and minor salivary glands. Species can even be found in blood-present molecules including Deoxyribo Nucleic Acids, RNAs, hormones, metabolites, and microbiota. Recently, saliva testing received considerable interest in identifying specific biomarkers as sample collection and processing is quick, cost-effective, accurate and doesn't put any distress on the patient. We examine recent salivary biomarkers of systemic carcinoma by separating them into genomically, transcriptomically, proteomically, metabolomic and microbially dependent forms.

Keywords: Carcinoma, Deoxyribo Nucleic Acids, RNAs, hormones, metabolites, and microbiota

1. Introduction

Carcinoma is the 2nd leading mortality in the U.S., second only to cardiac failure. In 2015, the U.S. reported around 1.66M new onco cases and about 0.6 carcinoma mortality [1]. Rapid carcinoma diagnosis is typical of good treatment. Latest advances in medical science have provided ample sensitivity, including CT-scan and MRI. High prices and radiation exposure, however, hinder their screening use [2]. Therefore, early carcinoma detection involves more reliable, relatively affordable and non-invasive procedures.

Saliva is an organic mixture consisting of approximately 99% water and below 1% protein, electrolyte and other components of small molecular weight [3]. It comes primarily from three broad glands (parotid, submandibular, and sublingual) and even through 3-400 small oral glands [4]. Bacterium, epithelial cells, erythrocytes, leucocytes, and gingival crevicular liquid food waste only contribute small quantities to oral fluid output. Saliva thus conducts essential lubrication for chewing, swallowing & digestive functions. This also preserves oral tissues integrity though it is often indicative of local and systemic conditions [5]. Species can include molecules such as blood-borne Deoxyribo Nucleic Acid sequence, RNA sequence, proteins, metabolites and microbes. Their variations in concentration can also be used to identify early carcinoma or detect clinical infection as biomarkers [6]. Salivary diagnostics are simple to use, since medical specimens are collected. Saliva testing will enable patients also at home to gather their samples, thereby reducing health care expenses, allowing rapid and multiple samples and major effects on the conformity of patients [7].

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The current study explores possible pathways by which distal tumours intervene in saliva's composition, oncological biomarker profiling and clarifies recent progress in the detection of systemic carcinoma by saliva oncological biomarkers.

2. Salivary Onco-biomarkers

"Salivaomics" is a vast variety of techniques employed in investigating different saliva components. This classification includes genomes (all genetic material of an organism) and epigenomes (record of the chemical changes to the Deoxyribo Nucleic Acid), transcriptomics (set of all RNA transcripts), metabolomics (chemical processes involving metabolites), and proteomics (protein study).

2.1. Genome

Genomes of humans & microbes coexist in the saline in a good condition [8]. Total salivary Deoxyribo Nucleic Acid is 24 g, ranging from 0.02 to 52 g. Amount & accuracy of oral Deoxyribo Nucleic Acid are both high. While the genome amount is ten folds smaller compared to blood (mean 210 g, scale 58–577 g), the genotyping procedure needs 5 ng/mL of the genetic material. Salivary sampling often provides enough genetic material for PCR & DNA sequencing [9]. Deoxyribo Nucleic Acid purity values were measured through colorimetric methods at 260 nm and 280 nm. The A260/A280 ratio of saliva and plasma is 1.56 and 1.71 respectively, indicating that saliva Deoxyribo Nucleic Acid is equivalent to blood Deoxyribo Nucleic Acid [9]. Tumorigenesis is a multi-stage mechanism that includes genetic and epigenetic changes [10]. Because of the altered modulation of natural genes, aberrant Deoxyribo Nucleic Acid methylation being the first carcinoma-related epigenetic marker [11]. A number of biomolecular instruments, such as a methylation collection, PCR, and quantitative PCR (qPCR), may be used to study the genome and epigenome of saliva. Innovative methods for identifying lung carcinoma-related gene mutations have also been developed [12].

2.2. Transcriptome

Experiments on salivary transcriptomes focus on mRNA and microRNA located in the oral cavity, away from the initial cells [13]. The UCLA laboratory first profiled the salivary transcriptome [14]. They also developed basic methods for monitoring and testing salivary mRNA at room temperature [15]. As novel regulators with diverse biological functions, non-coding RNAs (ncRNAs) play a major role in tumorigenesis. Since these molecules are minute, they are extremely stable in a variety of body fluids and are less prone to ribonuclease degradation than messenger RNAs [16]. Several candidates of messenger RNA and miRNA got identified in lung carcinoma [17], pancreatic carcinoma [18, 19] and breast carcinoma [20] dependent on microarray & PCR technologies (qRT-PCR).

2.3. Proteome

The proteome content in saliva contains whole cavity protein structure. Saliva comprises a multitude of oral cavity biochemical roles [21] comprising over 2,000 proteins and peptides. Around 25% of all saliva protein is in plasma. Because of the fair distribution of various salivary peptides [22], proteomic analysis of saliva offers distinct benefits, especially for low-abundance proteins.

Mass spectrometry (MS) is these days an essential in detecting salivary proteins. Enhanced Laser Desorption/Ionization Time-of-Flight MS (SELDI-TOF-MS) can provide a reliable profile of healthy control salivary proteins [23]. It may also be used to classify differences in pre- and post-orthodontic therapy [24] or biomarkers for high-throughput breast carcinoma [25]. 2-D gel electrophoresis (2DE) coupled with MS was effective in identifying high sensitivity and specific biomarkers in lung carcinoma [26] and breast carcinoma [20].

Raman spectroscopy (RS) has been deemed to be a successful optical technique for systemic carcinoma detection during the past two decades. Feng and his colleagues demonstrated potential for non-invasive and mark-free breast carcinoma diagnosis in combination with partial least squaresalivary protein analysis and Surface Enhanced Raman Spectroscopy (SERS) [27].

2.4. Metabolome

A global metabolism analysis provides a new snapshot of various diseases' pathophysiological pathways. It enables endogenous metabolite numbers to be measured and enables biomarkers to be discovered [28]. An important mechanism for detecting biomarkers and monitoring disease progression via endogenous metabolites such as nucleic acids, lipids, amino acids, peptides, antioxidants, organic acids, thiols, & carbs [29, 30].

Sugimoto and his colleagues. used mass spectrometry and capillary electrophoresis to classify carcinoma-specific signatures in salivary metabolites in 2010. Oral, pancreatic, breast carcinoma, periodontal, and healthy controls were collected for a thorough metabolite examination of salivary samples. The risk of diseases impacting the receptor's operating curves (AUCs) were estimated and perfectly speculate fifty-seven primary metabolites [31]. Other salivary metabolites have been reported to separate patients from neurodegenerative patients [33] between orally squamous cell carcinoma [32] and neurodegenerative patients [33].

2.5. Microbiota

In the oral cavity, some 19k phylotypes have been discovered thanks to recent developments in next-generation sequencing [34]. Oral diseases like caria [35], periodontitis [36], and structural issues like carcinoma [37, 38] are assumed to be caused by bacteria and other microbes. Patients with microarray- and qPCR-based pancreatic carcinoma are separated from healthy subjects by N.elongata and S.mitis, according to Farrell and his colleagues [39]. Torres and his colleagues [40] found similar results using a high-performance sequence of the tiny bacterial rRNA subunit (16*S*) gene. *Helicobacter pylori* (H.pylori), a bacteria that induces inflammation in the stomach lining, can also play a role in the production of gastric carcinoma. There are two H.pylori metabolites associated with safe diagnosis [41].

2.6. Relation of distal tumour & salivary onco-biomarkers

Previous study has found that discriminatory biomarkers may be used to treat chronic carcinomas such as pancreatic carcinoma [18], brain carcinoma [42], lung carcinoma [26], and ovarian carcinoma. Not a single one of them explain how carcinoma outside of the oral cavity may have an effect on saliva biomarker profiles.

Lau and his colleagues. employed a breast carcinoma oncocell model to present that exosome-like breast carcinoma microvesicles interact with saliva gland cells and alter the exosome-like microvesicles that are secreted [44]. Salivary cells secreted microvesicles that looked like exosomes and formed of both mRNAs and proteins, according to the researchers.

Minute (30-120 nm) vesicles of lipid [44], mRNA, microRNA [45], Deoxyribo Nucleic Acid [46], and protein [47] are all found in exosomes. These compounds have been assumed to carry and travel across the body. Exosomes can be present in nearly any cell in the body, as well as the bulk of body fluids, including saliva [48–50]. Exosomes have been shown in studies to be capable of RNA treatment, degradation [51], pathogenic dislocation [52], tumour promotion [53,54], and immune function [55].

By injecting the carcinoma cell line in hosts, the pancreas model of the mice was able to create salivary markers [56]. Exosomes seem to be a pathway to altered salivary carcinoma biomarkers, according to his research.

3. Lung Carcinoma

In the United States, lung carcinoma is the leading cause of carcinoma-related death in both men (28%) and women (27%). Lung carcinoma was identified in over 22Lakhs individuals in 2015, resulting in over 15Lakhs deaths, according to the American Cancer Society [1]. Many severe lung carcinoma patients have a 5-year survival rate (17percent ofthe cases), equivalent to (89 percent of the cases) for breast carcinoma, (99 percent of the cases) for prostate carcinoma, and (65 percent of the cases) for colon carcinoma [1]. Although lung carcinoma continues to be a significant public health concern, survival rates have remained relatively unchanged over the last few decades. In iatrogenic challenging patients, low-dose helical CT (LDCT) screening reduced mortality through increased false positive rates and possible prevalence correlated by follow-ups. The need to find the correct biomarker to distinguish between benign and malignant diseases has arisen as a result of the diagnostic issue of the undetermined CT nodule.

Wei and his colleagues. have developed a cutting-edge technology (EFIRM) for detecting epidermal growth factor mutations (EGFR). It's an electrochemical approach for detecting mutant sequences from nucleic acid and utilising electrical domains for hybridization with the probes. EFIRM may be a good tool for detecting oncogenic mutations in clinics because of the pace and simplicity of the technique. A blind examination done on salivary samples of 40 individuals suffering from non-small cell lung carcinoma. The ROC analysis concluded that EFIRM found deletion of exon19 with the AUC being 0.94 and the L858R mutation AUC being 0.96 [12].This study discovered AUC 1.0 exon 19 deletion in saliva and plasma samples from lung carcinoma patients. Positive connections were also seen in pre- & post-chemical plasma (0.86 in exon-19 deletion & 0.98 in L858R mutation) (0.73 & 0.94, respectively).

42 individuals with lung carcinoma and 74 healthy microarray controls were studied for the salivary transcriptome. There were seven saliva transcripts [BRAF (v-raf murine oncogene homolog B1), CCNI (Cyclin I), EGFR, FGF19

(Fibroblast Growth Factor 19), GREB1 (Breast Cancer Growth Control 1), LZTS1(Leucine Zipper Tumor Suppressor 1) and FRS2 (Fibroblast Growth Receptor Substrate 2). The Regression Model with separate lung carcinoma cases from controls, and 5 mRNA pooling (CCNI, EGFR, FGF19, FRS2, and GREB1), yielded an AUC = 0.925, with 93.75 percent sensitivity, and 82.81 percent specificity [17]. SERS are established by Li and his colleagues. to identify biomarkers in saliva lung carcinoma. Amino acids and nucleic acid bases are represented by nine main peaks. The numbers of consistency, (80%) quality (78%), and precision (83) were recorded [26].

MS proteomic 2-DE biomarkers were tested by Xiao and his colleagues. and sixteen biomarker candidates were discovered. In addition, differentiation was tested in 3 different proteins (haptoglobin, zinc-a-2-glycoprotein, & calprotectin) against stable controls (AUC) in patients with lung carcinoma, with 88.5 percent sensitivity and 92.3 percent precision [58].

3.1. Pancreatic Carcinoma

Pancreatic carcinoma is the 4th leading reason of carcinoma-related mortality in men & women of age matched population, with a 5-year survival rate varying from three to five percent. In the United States, the disease is estimated to kill over 40,000 people a year [1]. Nearly all carcinoma patients develop metastases and are subjected to late-stage therapies, as well as a shortage of effective surgical methods, biomarkers, and early-stage testing techniques [59, 60].

Zhang and his colleagues employed the Affymetrix HG U133 Plus 2.0 Array [18] in 42 pancreatic carcinoma cases, namely 30 cases with chronic pancreatitis and 42 healthy controls. According to his results, a combination of four messenger RNA biomarkers (KRAS, MBD3L2, ACVR1, and DPM1) are able to discriminate between 90 and 95 percent of cases of pancreatic carcinoma (AUC = 0.971).

MiRNAs have recently been seen to play a part in the detection of salivary carcinomas. MiRNA PCR was acquired (miRBase, version 18, including 384 miRNAs, Qiagen) and used in the salivary-surnament of 30 individuals diagnosed with pancreatic carcinoma and 32 healthy controls. In saliva samples from pancreas carcinoma patients, the top 5 miRNA candidates (miR-17, miR-21, miR-181a, miR-181b, and miR-196a) were found to be distinct from controls and were tested using qRT-PCR [19]. Humeau and his colleagues. used qRT-PCR to test 94 Salivar candidates' miRNAs in individuals suffering from pancreatic carcinoma, pancreatitis, and intraductal Papillary Neoplasm, as well as stable controls [61]. In pancreatic carcinoma saliva, hsa-miR-21, hsa-miR-23a, hsa-miR-23b, and miR-29c were notably high relative to controls, while another analysis used an Agilent Microarray to profile and validated salivary miRNA in individuals suffering from resectable pancreatic carcinoma who had qPCR. The logistic regression model, when used in accordance with miR-3679-5p and miR-3679-5p, were successful to discriminate sensitivity and specificity between the three forms of pancreas resectable carcinoma, with 72.5 %, 62.5 % & 70.0 %, [53] indicating 70.0 %, 80.0 %, 70.0 %, respectively.

The role of pancreatic biomarkers in saliva has been explained mechanically and biologically by Lau and his colleagues. [56]. The study used the pancreatic mice model to investigate the function of exosomes isolated from pancreatic carcinoma in the production of salivary biomarkers. Exosome biogenesis from pancreatic carcinoma was shown to inhibit the synthesis of salivary biomarkers, according to their findings.

Sugimoto and his colleagues. used CE-TOF-MS to classify eight carcinoma-specific metabolites, including isoleucine, tryptophan, valine, glutamic acid, phenylalanine, glutamine, leucine, and aspartic acid. AUC was found to be 0.993 [31] essential for successful pancreatic carcinoma care.

Farrell and his colleagues discovered significant variations in salivary microbial composition using the Human Macrobiotic Detection Microarray (HOMIM) in 10 pancreatic carcinomas and 10 control subjects, as well as an independent cohortusing qPCR [39]. In safe individuals and in pancreas carcinoma patients, N.elongata and S.mitis provided 96.4 percent sensitivity and 82.1 percent precision, while differentiating them. Through the use of 16S rRNA sequencing, Torres and his colleagues were able to differentiate the salivary microbiota from pancreatic carcinoma, healthy, & other diseases [40]. Low level of N.elongata and a significantly higher leptotrichia-porphyromonas ratio were found in individuals diagnosed with pancreatic carcinoma, similar to Farrell's research in individuals diagnosed with pancreatic carcinoma. These findings open up the possibility of using salivary microbiota to find noninvasive biomarkers for systemic diseases.

4. Breast Carcinoma

Breast carcinoma is the most common carcinoma in women in the US and the 2nd leading cause of carcinoma mortality [1]. More than 40,290 people died in the United States from this disease in 2015, despite better treatment [1]. Breast carcinoma is often diagnosed late, resulting in an increased mortality rate. Routine mammography testing is regarded as the gold standard for breast carcinoma detection, but the accuracy of mammography is not optimal [63].

The Affymetrix HG-U133-Plus-2.0 Array along with 2-DE were used to profile 10 patients with salivary transcriptome and 10 safe controls. In a separate cohort, the qRT-PCR and quantitative protein immunoblot were statistically evaluated. 8 messenger RNA biomarkers & 1 protein biomarker have been pre-substantiated, resulting in a precision of 92 percent (83 percent sensitivity, 97 percent specificity) [20].

In comparison to controls, the salivary and serum protein levels of CA15-3 may be positively correlated in patients with breast carcinoma [42, 64]. CA15-3 is now an FDA-certified proteomic biomarker for breast carcinoma monitoring [65]. Protein biomarker samples from 33 healthy people, 33 individuals with benign breast tumours, and 31 individuals with malignant tumours were analysed using SERS followed by multinomial regression analysis. The 3 classes had diagnostic reliability of 92.78 percent, 95.87 percent, and 88.66 percent, respectively [27].

Gel electrophoresis & Western blot technology were used to examine the expression of the pulmonary resistance protein in 16 healthy women & 16 women who had stage-I breast carcinoma. Pulmonary resistance protein levels are significantly higher in women with breast carcinoma issues than in healthy women [66].

Jinno and his colleagues. conducted a metabolite analysis and 20 healthy samples & 60 patients with breast carcinoma through CE-TOF-MS. A marginally high amount 5 potential biomarkers (choline, isethionate, cadaverine, N1-acetylspermidine, and spermine) were observed in carcinoma patients with AUC being 0.850, 0.819, 0.809, 0.765, and 0.716.

5. Gastric Carcinoma

Despite the fact that the prevalence and mortality of gastric carcinoma have declined dramatically in recent decades, it remains the world's fifth most prevalent carcinoma-related malignancy and the 3rd leading reason of carcinoma-related mortality [69, 70]. Since gastric carcinoma symptoms occur later as the disease advances, therapeutic choices are scarce [26]. Furthermore, gastric carcinoma may occur in children & is considered common in advanced stages of diagnosis [70]. Tandem Mass Tag (TMT) technology has been used in creating differentiating biomarkers of oral fluid protein for the diagnosis of gastric carcinoma. Above five hundred proteins were discovered and quantified in this research, with forty-eight demonstrating substantial differences in expression profiles among controls & gastric carcinoma patients. ELISA successfully reported the existence of cystatin B, triosephosphate isomerase, & malignant brain tumour-1 protein. The mixture of these three biomarkers has a sensitivity of 85 percent and a specificity of 80 percent (0.93).

H. pylori, which causes inflammation in the stomach lining, can play a role in the development of stomach carcinoma. Zilberman and his colleagues. scientifically defined the two metabolites associated with H. Pylori, NH3, and CO2 saliva in order to establish a cross-reaction susceptibility platformen to detect CO2 and NH3 in ppm [41].

6. Conclusion

Cancer screening aids in the early detection of tumours, and increases the likelihood of a successful procedure. Screening techniques with a combination of high specificity and high accuracy are crucial. Furthermore, screening tests should be non-invasive and cost-effective such that they can be used widely. Salivary diagnosis has many benefits over blood tests, and numerous salivary biomarkers may be used to diagnose systemic carcinomas rather than local oral diseases.

Understanding the roots of salivary biomarkers and the pathway responsible for expression of discriminatory biomarkers in saliva & distal systemic diseases is made possible by understanding exosome seclusion and fluid biopsy. As previously reported, recent approaches to salivary biomarker discovery have produced positive clinical outcomes. At the preclinical stage, a number of biomarkers for the diagnosis of systemic carcinoma were discovered and validated. The new age for salivary diagnosis is characterised by expanded salivary knowledge and the development of specialised and detailed detection methods.

Compliance with ethical standards

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Disclosure of conflict of interest

None.

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