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Role of Salivary Biomarkers in Oral Cancer Detection- A Systematic Review

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ABSTRACT

Introduction: Saliva is an informative biological fluid that has gained a lot of interest because of its physiologic diagnostic medium. The salivary secretions are under control of sympathetic and parasympathetic stimulation. It contains enzymes, proteins, biochemicals. It has been suggested to be ultrafiltrate of blood. The recent findings suggest that saliva could be used as a biomarker in diagnosing oral cancer. The study of saliva as a biological matrix has been identified as a new landmark initiative in search of a useful biomarker to diagnose oral cancer through and transcriptomes. Most oral cancers are oral squamous cell carcinoma using saliva for early oral cancer detection in the search for new clinical markers is a promising approach because of its noninvasive sampling and easy collection method. Identification of this salivary biomarker could help to screen patients at risk, predict disease outcome and effectively contribute to planning treatment strategies. Biomarkers in saliva can be used as for diagnostic & response to treatment in oral squamous cell carcinoma.in this review, we explored their application in this increasingly common disease. A systematic review of the literature was performed based on the English titles listed in the PubMed, EBSCO, Cochrane, Science Direct, ISI web Science, and SciELO databases using the keywords. This article may help to identify the potential biomarkers for screening and the molecular pathology analysis in the high-risk patients with the OSCC.

Keywords: squamous cell carcinoma, transcriptome, biomarker

Introduction:

Oral Squamous Cell Carcinoma usually arises from a pre-existing potentially malignant disorder. It includes a variety of lesions and conditions like leukoplakia erythroplakia, erosive oral lichen planus, and oral submucous fibrosis. Early detection of OSCC can not only significantly reduce mortality and morbidity but also enable effective intervention and therapy.

Saliva sampling is a noninvasive, simple and low-cost method which does not bother the patient.

Besides, saliva is an exceedingly desirable body fluid for clinical applications.[1] It contains numerous biomarkers used for detecting and monitoring of oral and systemic health and determining disease stages such as IL-6 and IL-8. At the molecular level, the quantity of transcriptome (the full range of mRNA) change in relation to health and disease. There are about 3,000 mRNAs in cell-free saliva, and only 180 of them are common among healthy subjects. It is worthy to mention that salivary transcriptomic profile reflects those originated in distant diseased tissues as well as transcripts that originated in salivary

glands. In a pioneer study that concern the associated of salivary RNAs and OSCC, authors registered that IL-8, SAT, IL-1β and OAZ1 as potential biomarkers for OSCC detection. [1]

During the past decades, salivary diagnostics have received increasing attention. The saliva-based analysis is a noninvasive alternative compare to serum analysis. The development of salivary diagnostic tools is essential, especially in the identification of a high-risk group, patients with premalignant lesions and patients with a previous history of cancer. Among all the malignancies, oral cancer is one such malignancy, where the saliva examination for detection shows the most significant benefit because of its direct contact with oral cancer lesions. The most critical point for selecting saliva as a diagnostic tool is that it also contains the fallen cells in the oral cavity which allow saliva to be the first choice of screening and identification of potential biomarkers in the OSCC.[2,3]

These biomarkers are going to be important indicators of physiological or pathological conditions and provide information for the detection of early and differential markers for disease. Based on the mentioned above, this study mainly highlights the role of saliva as a diagnostic tool in OSCC and its uses for the early diagnosis of OSCC.[2,3]

SUMMARY OF THE TUMOR MARKERS IN THE DIAGNOSIS OF ORAL CARCINOMA

Salivary genomic markers	Salivary transcriptome markers	Salivary protein markers	Salivary microbiota
Somatic mutations in tumor suppressor genes (p53)	IL-8	Elevated levels of defensin-1	Significant increase in the levels of *Porphyromonas gingivalis, Tannerella Forsythia* and *Candida albicans*
Loss of heterozygosity in chromosome 3p, 9q, 13q and 17p	H3F3A	Elevated CD44	Significantly elevated levels of *Bacteroides melaninogenica* and *Streptococcus mitis*
Promoter hypermethylation of genes (p16, MGMT, or DAP-K)	IL1β	Elevated IL-8	Presence of HPV and EBV
Cyclin D1 gene amplification	S100P	SCC-Ag	
Decrease in 8-oxoguanine DNA glycosylase, phosphorylated-Src and mammary serine protease inhibitor (Maspin)	DUSP1	Calcyclin, Rho GDP dissociation inhibitor	
Microsatellite alterations of DNA	OAZ1	CEA, carcinoantigen (CA19-9), CA128	
	SAT (spermidine/ spermine N1-acetyltransferase)	Intermediate filament protein (Cyfra 21-1)	
		RNS	
		8-OHdG DNA damage marker	
		LDH)	

H3F3A: H3 histone, family 3A, DUSP1: Dual specificity phosphatase 1, SCC-Ag: Squamous cell carcinoma antigen 2, IL: Interleukin, OAZ1: Ornithine decarboxylase antizyme 1, CEA: Carcino-embryonic antigen, RNS: Reactive nitrogen species, LDH: Lactate dehydrogenase, HPV: Human papilloma virus, EBV: Epstein–Barr Virus, CA: Cancer antigen

TRANSCRIPTOMICS

Salivary transcriptome diagnostics constitutes a novel clinical approach where a large panel of human RNAs is readily detected in the saliva. A speculation is that salivary mRNA is contained in apoptotic bodies or actively released in exosomes or macrovesicles. Recently, microRNAs and small RNA molecules, 18–24 molecules in length, that seem to regulate transcription were also discovered in the existing saliva samples.[4]

Transcriptomics studies the full global complement of mRNA molecules expressed in cells and tissues. Salivary transcriptomics is based on the analysis of the oral transcriptome, the set of all mRNA molecules that can be found in the salivary milieu. In order to analyze the transcriptome of a patient, saliva is collected and then RNA extraction is performed. Following the extraction, the RNA is amplified for analysis. After quantification of the nucleic acid content present in the saliva, comparisons can be made with known transcriptomic information.[5,6]

Saliva contains an assortment of extracellular RNA species, including mRNA, miRNA, and other small non-coding RNAs. The human salivary transcriptome was initially described using microarray technology. In various cancers, such as oral, esophageal, lung, pancreatic, breast, and ovarian cancers, certain RNA biomarkers have been discovered in saliva and proposed as possible biomarkers. MicroRNA (miRNA) is small noncoding RNA sequences with strong regulatory potential for normal biological processes ranging from cell growth, and differentiation to death. It has been demonstrated that genes for miRNAs genes are dysregulated in various types of cancer and that miRNAs themselves can act as both oncogenes and as tumor suppressors. Many research groups have shown that miRNAs are differentially expressed in various cancer cells compared with normal cells, and it seems that miRNAs can more accurately classify different types of solid tumors than can mRNA, suggesting that miRNAs can be used to detect cancer.[5,6]

Li et al. by using the microarray analysis of the salivary transcriptome showed that seven markers displayed a significant elevation in the saliva of the OSCC patients. The validated seven genes could be classified in three ranks by the magnitude of increase into highly upregulated mRNA: interleukin-8 (IL-8), moderately upregulated mRNA: H3F3A (H3 histone, family 3A), IL-1-β, S100P (S100 calcium binding protein P) and low upregulated mRNA: DUSP1 (dual specificity phosphatase 1), OAZ1 (ornithine decarboxylase antizyme 1), and SAT (spermidine/spermine N1-acetyltransferase).[7]

THE SALIVARY GENOMIC MARKERS

Tumor-specific genomic markers consisting of DNA and RNA markers which are identified in the saliva for the detection of oral cancer considering that the initiation and progression of malignant tumors is driven by the accumulation of specific genetic alterations. DNA shows tumor-specific characteristics such as somatic mutations in tumor suppressor genes and p53, microsatellite alteration, abnormal promoter methylation, mitochondrial DNA mutations, and presence of the tumor-related viral DNA.

Studies have shown that LOH in regions that contain a known human suppressor gene is an early predictor of malignant transformation of precancerous lesion.[26] Studies have demonstrated frequent LOH in chromosomes 3p, 9q, 13q, and 17p as an early event in oral carcinogenesis. [27,28,29,30] Mitochondrial DNA mutations have also been useful to detect the exfoliated OSCC cells in saliva. Such mutations have been identified in 67% of the saliva samples from the OSCC patients by direct sequencing.[8]

Liao et al. have observed the mutation of p53 in the DNA extracted from the saliva of the OSCC patients, suggesting a potential use as biomarker for the oral cancer detection. The study concentrated on p53 exon 4 codon 63 mutations which was significantly higher.[9] Other genes such as p16, p27, p63, p73

related to p53, and cell cycle are altered in varying degrees in oral cancer.[10] Main functional activity of p53 is cell cycle arrest and initiation of apoptosis in response to the DNA damage.

Cyclin D1 gene amplification is associated with poor prognosis in the OSCC.[11] In another study, Ki67 marker was increased, while 8-oxoguanine DNA glycosylase, phosphorylated-Src, and mammary serine protease inhibitor (Maspin) were decreased in the saliva of patients with the OSCC.[12]

THE SALIVARY PROTEIN MARKERS

The proteome represents the complete set of proteins encoded by genome and proteomics which is the study of the proteome that investigates the cellular levels of all the isoforms and post-translational modifications of proteins that are encoded by the genome of the cell under a given set of circumstances. While a genome is more or less static, the protein levels in a cell can change dramatically as genes get turned on and off during the cell's response to its environment.[13]

The protein biomarkers in the saliva are being analyzed both individually and as a panel of markers to aid in the early detection of the oral cancer and in implementing appropriate therapeutic regime.

Hu et al. by using the in-depth analysis of the human salivary proteome revealed several salivary proteins (such as Mac-2 binding protein, myeloid related protein 14, CD59, profilin 1, and catalase) at differential levels between the oral cancer patients.[14] Several salivary protein markers in the OSCC have been investigated in various studies and have shown relatively moderate sensitivity and specificity values relative to prognosis prediction.

St John et al. investigated whether IL-6 and/or IL-8 could serve as informative biomarkers for the OSCC in the saliva. Elevation of IL-6 has been shown to promote immune unresponsiveness and induction of wasting, cachexia, and hypercalcemia, all of which are observed in patients with OSCC who have a poor prognosis. IL-8 plays an important role in the stimulation of angiogenesis, proliferation, and chemotaxis of granulocytes and macrophages, which are prominent constituents in the stroma of OSCCs.[15]

THE SALIVARY MICROBIOTA

Certain alterations in the diet, medications, habits, and host immune status may lead to the overgrowth of minor components of the oral microflora which predispose the site to disease. Kang et al. demonstrated a significant increase in the levels of Porphyromonas gingivalis, Tannerella forsythia, and Candida albicans in the cancer group than in the normal controls.Mager et al. found significantly elevated levels of P. gingivalis, P. melaninogenica, and Streptococcus mitis in the saliva of OSCC patients, thereby suggesting the role of salivary microbiota as a diagnostic indicator in OSCC.[16]

Studies have found increased candidal carriage in the salivary samples of the OSCC group than in the normal controls.[17] This indicates that the salivary analysis of candida species might be useful as a diagnostic and prognostic indicator of the oral precancer and cancer.

The presence of Human Papilloma virus and Epstein–Barr virus genomic sequences has been identified.[18]

APPEARANCE OF TUMOR MARKERS IN SALIVA

Although it is inevitable that the markers of oral cancer will be reflected in the saliva, the relationship between systemic disease/ cancer and appearance of biomarkers in saliva is yet unclear. Several intracellular and extracellular pathways enable the biomolecules to move from blood capillaries to the saliva**.** Many of the biomolecules enter into saliva from the blood by passing through the spaces between cells by transcellular (passive intracellular diffusion and active transport) or paracellular routes (extracellular ultrafiltration). These markers may be carried by the local capillaries of the salivary glands to the oral cavity through the flow of gingival crevicular fluid (GCF).[19] In a mouse model of melanoma and lung cancer conducted to check for the altered salivary biomarkers in systemic cancer revealed the production of growth factors at the tumor tissue site representing the Egr-1 signaling pathway, a mechanism in which nerve growth factor (NGF) produced by the tumor tissue alters the transcriptome of the salivary glands leading to altered gene expression reflecting in the salivary protein profiles. This study using mouse models of melanoma and non-small cell lung cancer has revealed that biomarkers specific for the lung cancer tumor were detectable in saliva of the mouse. This is suggestive that the salivary glands may be regulated by mediators released from remote tumors. The altered salivary protein pattern in the tumor bearing mice, confirmed the speculation of the researchers that the tumors secrete mediators which may affect the activity of transcription factor in salivary glands, thereby inducing either an up or down-regulation of protein transcript levels in saliva.[20]

CONCLUSION

Salivary screening can be the best choice as the primary screening test for the high-risk cases of OSCC, since the collection procedure is noninvasive and low cost. In addition, the specimen is with low background and inhibitory substances and less complex than blood. Not only the proteins, but also the saliva contains cell which may fall from the cancerous tissue in the oral cavity.

It can be concluded that saliva would serve as a very good diagnostic tool to improve the quality of life of the cancer patients. This review suggests that pursuing of saliva as a tool to detect the different types of cancer can pave the way for improved outcome of future noninvasive investigation in the same field giving the patient a chance for a better quality of life. With the advances in nanotechnologies applied in proteomics and genomics, many biomarkers for the OSCC have been identified extensively. It is now required further studies to confirm its specifi city in a large sample size, although to dissect the extraordinary complex genetic or proteomic expression profile and to find the “true” biomarker remain a challenge.

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