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Salivary biomarkers for early detection of oral squamous cell carcinoma and oral potentially malignant disorders

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Abstract

Aim: Oral squamous cell carcinoma (OSCC) is one of the most prevalent cancers of epithelial origin associated with high morbidity and death. The tumor markers play an increasingly vital role in the diagnosis and treatment of cancer. The aim of this review was to search for articles in English using the keywords: *Saliva markers, Oral squamous cell carcinoma, and Oral potentially malignant disorder* in PubMed and Google Scholar databases. Article abstracts and entire texts were evaluated.

Background: Recent data suggest that the clinical and histological appearance of oral mucosa may not accurately reflect the underlying genetic alteration. This discrepancy in phenotype and genotype may explain in part for the inability to design effective screening and monitoring strategies based on conventional clinical and microscopic investigation.

Review results: These laboratory-based tests may be helpful for screening for early malignancy, assisting in cancer diagnosis, assessing prognosis, surveillance after curative surgery for cancer, predicting medication response or resistance in advance, and monitoring treatment for advanced illness.

Conclusion: Salivary biomarkers may have a crucial role in the early detection and successful diagnosis of oral cancer and oral potentially malignant disorders.

Clinical Significance: This article may assist in the identification of possible biomarkers for screening and molecular pathology investigation in OSCC patients at high risk.

Introduction

Head and neck squamous cell carcinoma (HNSCC) is one of the leading causes of cancer-related mortality globally, with varied incidence rates across Southeast Asia and Africa. Head and neck squamous cell carcinoma accounts for roughly 8–10% of all malignancies [1]. Oral squamous cell carcinoma (OSCC) mostly affects the buccal mucosa, lip, alveolar ridge, retromolar trigone, hard palate, floor of the mouth, ventral two-thirds of the tongue, and oropharynx [2]. To minimize the mortality and morbidity of this illness, it is essential to develop ways for early identification and detection of OSCC, which will allow for appropriate intervention and treatment. OSCC is currently detected by skilled clinical examination and histological study of suspicious spots. Consequently, sensitive and specific biomarkers for OSCC may be useful for screening people at high risk. The biomarkers should fulfil the following prerequisites [3]:

1. The value attributed to the marker may be measured
2. The marker may be identified in small specimen
3. The altered must be present in high-risk tissue samples, but not in normal oral epithelium
4. The altered must be present early in the development of cancer

OSCC developing in the oral cavity is simpler to monitor, collect specimens for diagnosis, and provide therapy for, due to its superficial localization [4]. Tumor cells either contain or create biochemical compounds known as tumor markers. These may be normal endogenous products generated at a higher rate in cancer cells or the results of newly activated genes in normal cells [5]. Tumor indicators may exist as in the cell as compounds or out of the cell, secreted by it, in bodily fluids such as blood, urine, and cerebrospinal fluid. Examples of bodily fluids used for tumor detection include sputum for the diagnosis of lung cancer, urine for the identification of urologic tumors, saliva for the diagnosis of oral squamous cell carcinoma, breast fluid, and serum or plasma for practically all forms of malignancies [6-9]. With recent developments in diagnostic technology, however, the diagnostic utility of saliva has increased enormously.

Background

Tumor markers have been characterized as specific, new, or structurally changed cellular macromolecules or temporally, geographically, or quantitatively altered normal molecules associated with malignant (and in some circumstances benign) neoplastic cells [10]. Tumor markers may be unique genes, or their products are generated exclusively in tumor cells, or they may be genes or gene products common in normal cells that are aberrantly expressed in distinct sites in tumor cells. Tumor markers are molecules that indicate the presence of malignancy [11]. They are potentially useful in [12]:

1. Cancer screening
2. Aiding diagnosis

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3. Assessing prognosis
4. Predicting in advance a likely response to therapy
5. Monitoring patients with diagnosed disease

A biological marker should possess properties that are universally relevant. The following criteria have been proposed by Kaplan and Pesce for an ideal tumor marker [13]:

1. Be simple and affordable to measure in easily accessible bodily fluids.
2. Be distinct to the investigated tumor and often connected with it.
3. Plasma concentrations of the marker must be proportional to the related tumor mass.
4. Possess an abnormal plasma level, urine level, or both in the presence of micro-metastases, i.e., at a stage when no clinical or current diagnostic procedures detect their existence.
5. Plasma or urine concentrations that are constant and not prone to erratic changes.
6. They should predict a greater or lower risk for subsequent recurrence development.
7. They should be modified as the state of the tumor evolves throughout time.
8. They must precede and anticipate recurrences before they may be detected clinically.

Review results

Saliva as diagnostic medium

Blood and saliva are the bodily fluids, most intensively studied, due to the fact that they may contain cancer markers. The saliva is a bodily fluid that contains proteins, mRNA and DNA that are used as biomarkers [14]. Saliva offers significant benefits over blood and tissues as a clinical tool, including ease of collection, storage, and transport, cost-effectiveness, readily available large sample volumes for analysis, and recurrent sampling for monitoring [15]. Thus, saliva-based analysis, a noninvasive alternative to serum analysis, is a useful method for cancer diagnosis, prognosis, and monitoring the therapeutic response of patients' post-treatment. Consequently, the development of salivary diagnostic tools is of the utmost significance, particularly for identifying high-risk groups, individuals with premalignant lesions, and patients with a history of cancer [16]. Oral cancer is one of the cases for which fluid examination provides the highest advantage due to its direct interaction with the cancerous lesions. The most crucial factor in picking saliva as a diagnostic tool is that it includes fallen cells from the mouth cavity, making it the optimal option for screening and identifying possible biomarkers in oral cancer [4].

The genomic identifiers in saliva

In light of the fact that the beginning and development of malignant tumors are driven by the accumulation of unique genetic abnormalities, salivary tumor-specific genomic markers comprising of DNA and RNA markers are investigated for the diagnosis of oral cancer. DNA exhibits cancer related features [17,18]:

Microsatellite alteration

Unregulated methylation of the promoter

Mitochondrial DNA alteration

Mutation in tumor suppressor genes such as p53

Presence of tumor-related viral DNA

Loss of heterozygosity (LOH) is the absence of genomic material from one of the chromosomal pairs. Detection of LOH related to tumor suppressor gene is an early indicator of the malignant transformation of a potentially malignant lesion [19]. Studies have shown that frequent LOH on chromosomes 3p, 9q, 13q, and 17p represents an early event in the development of oral cancer [20-23]. Mitochondrial DNA alterations have also been used to identify OSCC cells in exfoliated saliva. Direct sequencing has detected these mutations in 67% of saliva samples from OSCC patients [24].

In response to DNA damage, the P53 gene located on chromosome 17p is responsible for cell cycle arrest and onset of apoptosis. A mutation of p53 in the DNA collected from the saliva of OSCC patients was identified, indicating its potential application as a biomarker for the identification of oral cancer [25]. The research focused on p53 exon 4 codon 63 mutations, which were much more prevalent [25]. Other genes associated to p53, such as p16, p27, p63, and p73, and the cell cycle are changed to variable degrees in oral cancer. Multiple genes have been shown to have hypermethylated promoters in head and neck cancer. In the OSCC, the abnormal methylation of DAP-K, p16 and MGMT was observed [26]. Zhong, *et al.* identified telomerase activity in 75% of oral squamous cell carcinoma (OSCC) saliva samples, indicating that telomerase detection may be employed as an adjunct marker in OSCC [27]. Amplification of the Cyclin D1 gene is linked with a bad prognosis in OSCC [28]. Microsatellite DNA changes were also detected in the saliva of small cell lung cancer patients [29].

Markers of the salivary transcriptome

Diagnostics based on the salivary transcriptome provide a unique clinical method in which a vast panel of human RNAs may be conveniently identified in the saliva. It is hypothesized that salivary mRNA resides in apoptotic bodies or is actively released by exosomes or microvesicles. Recently, 18–24 molecule-long microRNAs and short RNA molecules that seem to influence transcription were found in existing saliva samples [30]. Using microarray study of the salivary transcriptome, Li, *et al.* determined that seven markers were significantly elevated in the saliva of OSCC patients [31].The seven validated genes were classified into three groups based on the magnitude of mRNA upregulation: highly upregulated mRNA: interleukin-8 (IL-8); moderately upregulated mRNA: H3F3A (H3 histone, family 3A); IL-1-; and low upregulated mRNA: DUSP1 (dual specificity phosphatase 1); OAZ1 (ornithine decarboxylase antizyme 1); and SAT (spermidine/spermine N1-acetyltransferase) [31].

The salivary marker proteins

Proteomics is the study of the proteins that are encoded by the genome. While a genome is relatively stable, the protein levels in a cell may fluctuate significantly when genes are switched on and off in response to the cell's environment [32]. To help in the early diagnosis of oral cancer and the implementation of an appropriate therapy regime, the protein biomarkers in the saliva are evaluated both individually and as a panel of indicators. The human salivary proteome project (HSP project) has identified over 1100 nonredundant proteins in human parotid and submandibular/sublingual secretions [33]. Using a comprehensive investigation of the human salivary proteome, Hu, *et al.* identified many salivary proteins (such as Mac-2 binding protein, myeloid associated protein 14, CD59, profilin 1, and catalase) with varying concentrations in oral cancer patients [34]. Several salivary protein indicators in OSCC

have been studied in several studies and have shown rather modest sensitivity and specificity values for prognostication. Defensins, for instance, are peptides with antibacterial and cytotoxic activities [35]. Higher levels of defensin-1 in the saliva are indicative of the presence of oral mucosal diseases [36]. In a separate investigation, soluble CD44 was shown to be raised in the majority of OSCC patients and discriminated cancer from benign illness with excellent specificity [37].

The study of IL-6 and IL-8 in, regarding OSCC, showed that an increase in IL-6 promotes immunological unresponsiveness and induces wasting, cachexia, and hypercalcemia, all of which are present in OSCC patients with a poor prognosis [38]. IL-8 has a crucial role in the activation of angiogenesis and chemotaxis and is correlated to increased numbers of granulocytes and macrophages, which are significant elements of OSCC stroma [38]. In their investigation, IL-6 levels in serum and IL-8 levels in saliva of patients with OSCC were all over the threshold limit. IL-6 and IL-8 levels may be elevated, in general, in inflammatory disorders, however, the findings were statistically significant for IL-8 in saliva but not for IL-6, indicating that the OSCC's contribution to the increase of IL-8 in saliva surpasses any possible contribution from the host's inflammatory conditions [38]. Numerous proteins with increased levels in the saliva of OSCC patients have been previously linked to human malignancies (e.g., squamous cell carcinoma antigen 2 [SCC-Ag 2], calcyclin, Rho GDP dissociation inhibitor, heat shock 70-kDa protein 1, Annexin I, cathepsin G, peroxiredoxin II, thioredoxin, short palate, and lung and nasal epithelium carcinoma-associated protein). In addition to their potential therapeutic benefits, these target proteins may aid in the knowledge of the disease's molecular process [34].

In addition, a number of salivary proteins are underexpressed in OSCC. Clusterin, for example, is present in normal controls but lacking in OSCC, as shown by subtractive proteomic analysis. Clusterin is involved in apoptosis and its downregulation was in esophageal squamous cell carcinoma and prostate cancer [34]. Other biomarkers, altered in OSCC, compared to normal oral epithelium, are the following [39]:

Inhibitors of apoptosis

SCC-Ag

Carcino-embryonic antigen

Carcinoantigens CA19-9, CA128 and CA125

Intermediate filament protein (Cyfra 21-1)

Tissue polypeptide-specific antigen,

Reactive nitrogen species and 8-OHdG DNA damage marker

Lactate dehydrogenase and immunoglobulin

S-IgA

Insulin growth factor,

Metalloproteinase MMP-2 and MMP-11

Discussion

Since the process for collecting saliva is simple and inexpensive, it may be the best option as the main screening test for high-risk patients of oral squamous cell carcinoma (OSCC). In addition, the sample contain less background chemicals than blood and is, thus, less complicated than blood. In addition to proteins, saliva also includes cells that may have shed from the OSCC and the potentially malignant disorders. It is now necessary to do more research, however dissecting the extraordinarily complicated genomic or proteomic expression profile and identifying the "real" biomarker, remains a formidable obstacle. Since more and more bioinformatic computing platforms are being developed, systematic analysis will aid in the development of sensitive and specific biomarkers for OSCC and other malignancies.

Clinical significance

This article may assist in the identification of possible biomarkers for screening and molecular pathology investigation in OSCC patients at high risk.

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