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Review Article

A Review on Salivary Genomics in Oral Cancer

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Abstract

Oral cancer has emerged as a distressing health problem with increasing incidence and mortality rates all over the world. Early diagnosis and prompt treatment are the need of the hour to reduce the morbidity and mortality rate. The use of tumor biomarkers in disease diagnostics has garnered importance in recent times owing to advancements in technologies used for the identification of such biomarkers. OMICS technologies such as genomics, proteomics, transcriptomics and metabolomics have been used to detect various biomarkers in oral cancer and pre-cancer. The use of saliva for the identification of tumor biomarkers is promising owing to the ease of collection and the close proximity to the oral lesions. The present review highlights the importance of salivary genomic biomarkers in oral carcinogenesis.

Keywords: Oral Cancer; Salivary Diagnostics; Genomics; Oral Pre-Cancer.

Introduction

Oral cancer: An uncontrollable unit

Oral cancer is a major problem in the Indian Subcontinent, where it accounts for 30% of all cancers reported in the country and is quickly being a global health priority [1]. It begins with a small, unfamiliar, unexplained growth or sore in the mouth parts that include lips, cheeks, sinuses, tongue, hard and soft palate, and the base of the mouth extending to the oropharynx [2]**.** Oral squamous cell carcinoma (OSCC) is the most common oral malignancy, representing up to 80-90% of all malignant neoplasms of the oral cavity. It is a disease of adults and the elderly seen between the age group of the 4^{th} to 6^{th} decades of life, with a mean age of 51 years. The numbers of male OSCC patients are significantly higher compared to females, with the ratio of 0.7:0.3 [3]. The common clinical presentation of OSCC is an ulcerated lesion with a necrotic central area surrounded by elevated rolled borders [4]**.** In India, around 77,000 new cases and 52,000 deaths are reported annually, which is approximately one-fourth of global incidences [2]**.** Oral potentially malignant disorders (OPMD) are a group of precursor oral lesions with an increased tendency for malignant transformation, primarily to OSCC [5]**.** OSCC and OPMDs are usually associated with multifactorial etiology, where in tobacco & alcohol are considered to be major risk factors, causing dysregulation in the microenvironment**.** It has been reported that cytokines released through chronic inflammation can initiate cell proliferation activity, which induces irreversible DNA damage, instigating genetic mutation [6].

Genomics: As a dynamic hallmark of cancer

An important hallmark responsible for carcinogenesis is genetic instability and mutation. It includes various molecular alterations, like multiple cancer-driving mutations, gene fusions, amplification, deletion, and post-translational modifications, among others [7]. Apart from these genomic events that are evident during cancer progression, modifications of the nucleotides particularly those of cytosine and post-translational histone modifications, are common in cancer. These modifications, referred to as epigenetic changes, are independent of alterations in the primary DNA sequence and involve changes in DNA methylation and histone modifications. In addition, these changes in cancer constitute the cancer epigenome and play crucial roles in the control of gene activity and nuclear architecture [8]**.**

Genomics in Omics technology

The "Omics" approach is quite different from the conventional technique for studying complex biological systems. In the modern biological era, 'Omics' approach is regarded as a new biomarker sighting tool that focuses on a large set of molecules [9]**.** Rapid advancements in the identification of various molecular targets of cancer cells have led to the revolution of the "omics" group in the field of cancer diagnostics, which categorises these tumour bio-

markers into various techniques. It primarily includes genomics, transcriptomics, proteomics, and metabolomics [10]. Genomics mainly constitutes the detailed study of total DNA of a cell, where genes are analyzed through microarray technologies that measure the altered gene sequences and chromosomes along with thousands of genes analyzed simultaneously [11].

Salivary genomics

Saliva is a clear, slightly acidic, muco-serous exocrine secretion. Whole saliva is a complex mix of fluids from major and minor salivary glands and from gingival crevicular fluid, which contains oral bacteria and food debris. The major salivary glands include the paired parotid, submandibular, and sublingual glands. Minor glands that produce saliva are found in the lower lip, tongue, palate, cheeks, and pharynx. Saliva is composed of a variety of electrolytes, including sodium, potassium, calcium, magnesium, bicarbonate, and phosphates. Also found in saliva are immunoglobulins, proteins, enzymes, mucins, and nitrogenous products, such as urea and ammonia [12].

Saliva is a clinically informative biological fluid (biofluid) that is useful for novel approaches to prognosis, laboratory or clinical diagnosis, and monitoring and management of patients [13]. It is also considered a potential alternative to blood serum and urine to provide a mirror of the body's health. It is easily collected and stored and ideal for early detection of disease as it contains specific soluble biological markers (biomarkers) [14].

Salivary DNAs represent the genetic information (genome) of the hosting human body, oral microbes present in the mouth (oral microbiota) and infecting DNA-viruses. By a general analysis of the value of total DNA content in human whole saliva, it was found that it ranges between 1.8 – 128.4 µg/mL with a mean value of 21.6 µg/mL. The quality of salivary DNA yield was found to be good, 72% to 96% of samples could be genotyped, 84% could be amplified, and 67% could be sequenced [15]. Hence, it can aid in identifying various tumour-specific circulating DNA markers in oral cancer, by utilizing nanotechnology and molecular techniques. Hence, DNA has the ability to show tumour-specific features such as p53 and other tumour suppressor genes [16].

Genomic biomarkers in oral cancer

According to the extensive literature survey in genomic studies, numerous articles related to OSCC were found in saliva. The use of selective genetic markers, including loss of heterozygosity (LOH), in combination with other biomarkers such as D3S1234, D17S79, and D9S156 can be beneficial in the early detection of head and neck squamous cell carcinoma, especially in high-risk population [17]. Mitochondrial DNAs such as cytochrome cooxidase I and I can be used to check for any damage to DNA, which may help in the detection of oral cancer [18].

Nagata et al. investigated aberrant methylation of 13 genes and found that aberrant methylation of ECAD, TMEFF2, RARb, MGMT, FHIT, WIF-1, p16, and DAPK marker genes present in oral rinse samples has great potential for the non-invasive detection of OSCC [19]. In a few studies *EDNRB* promoter methylation has been associated with the presence of dysplasia or invasive cancer. It has been proven as a salivary biomarker particularly useful in oral premalignancy and malignancy screening [20]. In a study by Preston RG, six out of the eight genes *EDNRB, HOXA9, GATA4, NID2, KIF1A and DCC* show differential promoter methylation status in OSCC. Among these, *HOXA9 and* NID2*, can be used as efficient biomarkers for oral cancer detection [21].*

Ferlazzo N., *et al*. investigated the influence of the MTHFR C677T and A1298C gene polymorphisms on DNA methylation and site-specific methylation on *p16* and O^6-methylguanine-DNA methyltransferase *(MGMT)* gene promoters in patients with OSCC by isolating genomic DNA from saliva samples. These genes were suggested to have a major role in oral cancer development and as an early event during carcinogenesis, thus representing a powerful diagnostic approach for cancer at its early stage [22].

Liyangage., *et al*. have explored a few promoter methylation markers like $p16^{INK4a}$*, RASSF1A, TIMP3,* and *PCQAP/MED15* TSGs, in DNA derived from saliva which can serve as a diagnostic marker panel in the early detection of oral precancer and oral cancer [23]. TP53 is a tumour suppressor protein playing an important role in the control of cell division. A study by D Cruz., *et al*. has shown the superiority of P53 in saliva as an alternative to invasive tissue biopsy for the detection of genetic changes [24]. According to a study by Mewara., *et al*. the detection of this gene at codon 63 can provide a fast, accurate, and sensitive diagnosis [25]. Sun W validated a panel of methylation-based salivary rinse biomarkers and found that the detection of hypermethylation of CCNA1, MGMT, and MINT31 was significantly associated with poor overall survival and TIMP3 was associated with local recurrence-free survival and disease-free survival [26].

Conclusion

Salivary diagnostics in oral carcinogenesis have advanced exponentially in the recent past owing to the advent of newer and more sensitive techniques that help in identifying various tumour biomarkers, even when present in small quantities. Numerous research studies are ongoing under omics technology to identify the potential candidate biomarkers that have high diagnostic, prognostic and therapeutic utility. Salivary genomics can serve as a potential biomarker, which can be used to predict the disease at the genomic level before it gets expressed phenotypically. It is hence imperative to identify, characterize, and quantify genomic

Sr. No.	Author and Year	Genomic Biomarkers	Techniques	Significance
1	Cui Y, 2021[29]	ctDNA	PCR followed by Next-Generation Sequencing	Determine early tumor recurrence through genetic analysis
2	D'cruz., *et al*. 2021 [24]	TP53 gene	PCR	Detection of genetic changes in oral cancer
3	El-Naggar AK., *et al*. 2001 [17]	Loss of heterozygosity (LOH) in combination of other biomarkers D3S1234, D17S79, and D9S156	PCR	An early indicator of the precancerous lesion that is most likely to be changed in malignancy
4	Fendt L., *et al*. 2020 [28]	Mitochondrial DNA detected high-level heteroplasmies (9868R, 4196Y, 6978R, 11682R)	PCR followed by Next-Generation Sequencing	Potentially deleterious high heteroplasmic level mutations in protein-coding regions are significantly associated with shorter patient survival.
5	Ferlazzo N., *et al*. 2017 [22]	Influence of methylenetetrahydrofolate reductase (MTHFR) enzyme on DNA methylation: p16 and O^6-methylguanine-DNA methyltransferase (MGMT) gene	PCR	MTHFR polymorphisms may have an important role in OSCC carcinogenesis, probably due to their influence on gene-specific methylation processes
6	Guerrero-Preston., *et al*. 2011 [21]	Promoter methylation: KIF1A, HOXA9 , NID2 and EDNRB	PCR	Early detection and cancer prevention studies.
7	Jiang W., *et al*. 2005 [18]	Mitochondrial DNAs such as cytochrome cooxidase I and I	PCR	DNA mutations allows checking DNA damage, which is essential for OSCC detection at its all stages
8	Liyanage C., *et al*. 2019 [23]	Promoter hypermethylation of tumor-suppressor genes: $p16^{INK4a}$, RASSF1A, TIMP3, and PCQAP/MED15	Methylation-specific PCR coupled with densitometry analysis	Excellent diagnostic accuracy in the early detection of oral cancer
9	Mewara A., *et al*. 2010 [25]	P53 gene codon 63	PCR and microarrays followed by qPCR	Detection of this gene at codon 63 gives fast, accurate, and sensitive diagnosis of OSCC
10	Nagata S., *et al*. 2012 [19]	Aberrant DNA methylation of tumor-related genes ECAD, TMEFF2, RARb, MGMT, FHIT, WIF-1, p16, and DAPK	PCR	Useful tool for the diagnosis and detection of oral cancer
11	Pattani KM., *et al*. 2010 [20]	Tumor suppressor genes: Endothelin receptor type B (EDNRB) and kinesin family member 1A (KIF1A)	PCR	Potentially identify patients with premalignant and malignant lesions
12	Rosas., *et al*.2001 [31]	Promoter hypermethylation of p16, O-methylguanine-DNA-methyltransferase (MGMT) and death associated protein kinase (DAP-K)	Methylation-specific PCR	
13	Righini CA., *et al*. 2007 [32]	Tissue inhibitors of metalloproteinase, ECAD, MGMT, p16, DAPK (27%) & RASSF1A.	Methylation-specific PCR	TIMP3 encodes for metalloproteinase inhibitor that suppresses tumor growth, angiogenesis, invasion and metastasis
14	Schussel J., *et al*. 2013 [27]	Promoter methylation: Endothelin receptor type B (EDNRB) and deleted in colorectal cancer (DCC)	Quantitative methylation-specific PCR	Oral premalignancy and malignancy screening
15	Sun W., *et al*. 2012 [26]	Tumor suppressor genes i.e., CCNA1, MGMT, MINT31, TIMP3	Quantitative methylation-specific PCR	Detection of OSCC with accuracy
16	Varun C., *et al*. 2015 [30]	Amplification and protein over expression: Her2/neu gene	ELISA	Distinguishing premalignant and malignant condition

Table 1: Candidate genomic biomarkers according to their significance in oral cancer.

biomarkers that can aid in the implementation of newer screening and early diagnostic approaches for the early detection of oral squamous cell carcinoma, as well as to predict the behaviour of oral potentially malignant disorders.

Conflict of Interest

No conflict of interest.

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