REVIEW ARTICLE

Saliva –An imperative medium for Oral Cancer Biomarker Detection - A Brief Review

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ABSTRACT

Oral Cancer is one of the most common epithelial malignancies with significant morbidity and mortality. In spite of diagnostic and therapeutic advances over the decades, the disease still remains a challenge for medical professionals. Early detection followed by appropriate treatment, can increase cure rates to 80 or 90%, and greatly improve the quality of life. The presence of oral cancer markers in saliva has offered renewed interest in the potential use of saliva as a diagnostic fluid. The purpose of the review is to look for the potential oral cancer tumor markers in saliva detected with the modern sophisticated technology which makes saliva an attractive alternative to serum testing for screening and molecular pathology analysis for high risk patients of oral cancer.

Keywords: Saliva, oral squamous cell carcinoma(OSCC), biomarker.

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INTRODUCTION

Malignant neoplasm's are major causes of fear, morbidity and mortality all over the world. Globally 'oral cancer' is the 6th most common cause of cancer related deaths. Oral cancer accounts for approximately 30-40% of cancers in India. Despite recent advances in tumor surgery and multimodal treatment regimes, the prognosis of the oral carcinomas is still relatively poor. This may be attributable to the fact that, symptoms that indicate the presence of carcinoma often appear when the tumor is in advanced stage.[1] Therefore early detection is the most effective means to reduce death from this disease. Currently, diagnosis depends on a thorough oral and histopathological examination by taking a biopsy. Even though a definite diagnosis is based on biopsy, it would be beneficial if could be done through non invasive techniques.[2]

In responding to the call for early detection of OSCC, several diagnostic adjuncts have been developed, or currently are in development, including the use of salivary biomarkers.[3] Biomarker is a biological molecule found in the blood and other body fluids, or tissues that is a sign of normal or abnormal process, or of condition or disease .Tumor markers (biomarker) can be produced directly by the tumor or by non tumor cells as a response to the presence of the tumor. Levels of these markers increase with severity and mortality.[1]The discovery that saliva contains molecular profiles that reflect systemic diseases has opened the doors to a new noninvasive diagnostic methodology- 'salivary diagnostics'. Using saliva in diagnosis is not only practical and non invasive, but at times is also proving to be more accurate than available alternatives.[4]Saliva has the advantages that it contains low background of normal material and inhibitory substances as well as fewer complexes thanblood.[5] It is an informative body fluid containing an array of analytes (Protein, mRNA and DNA) that can be used as biomarkers for translation and clinical applications.[6]

Saliva as the mirror of human health

Saliva is an important body fluid for the detection of physiological and pathological conditions of human body.It is a complex and dynamic biological fluid containing wide range of compounds. In addition, saliva is a good indicator of the plasma/serum levels of various substances such as hormones and drugs. In the last few years, scientific interest has been raised to salivary analysis not only for various compounds present into saliva (e.g., drugs, pollutants, hormones), but also for its well-documented relation with bacterial, viral and systemic diseases. Therefore, it can be used as a non-invasive method for monitoring plasma concentrations of medicines or other substances and for assessment of the severity of an illness. Due to its diverse biological functions, salivary testing is rapidly growing as a practical and reliable means in clinics and research to recognize early signs of systemic illness and exposure to risk factors. Thus components of saliva act as a 'mirror of the body's health'.[7]

Technologies for saliva based diagnosis

The complex nature of saliva consisting of mixture of various components makes it difficult to identify specific constituents. Recently, significant inroads have been made in discovery of a range of technologies which have high specificity and sensitivity in detection of salivary components. For assessment of the diagnostic technologies, a hierarchal model exist which consist of five basic levels of analysis at which the effectiveness of any diagnostic test should be evaluated. These include, analytic (precision and accuracy), diagnostic (sensitivity and specificity), patient outcome efficacy (medical decision making), operational (predictive value and efficiency) and cost / benefit (societal efficacy). Various technologies available for saliva based diagnosis followed by the application aspect in oral cancer includes Proteomics, Transcriptomics, Polymerase chain reaction, Genomics, Hybridization methods, and Flow cytometry.[8]

Saliva collection methods

Whole saliva or gland specific saliva can be collected. Pure parotid saliva can be collected by placing a metal or acrylic cup over the Stenson's duct. Saliva from submandibular glands can be collected by placing the tip of a collection device at the orifice at the Wharton's duct, after placing sterile cotton sponges in the floor of the mouth and over the buccal mucosal areas to occlude the parotid and sublingual ducts. However, usually whole saliva is collected as it is easy to collect and is more representative of the oral milieu. Unstimulated whole saliva can be collected by allowing passive drooling of saliva off the lower lip into a plastic vial. Stimulated whole saliva can be obtained with masticatory movements or with the use of citric acid(gustatory stimulation).It can be collected using absorbent(cotton rolls) and non-absorbent (paraffin, wax, neutral gum base, rubber band) methods.[9]

Till date, many research studies have been published and more than 100 different salivary constituents have been suggested as potential OSCC salivary biomarkers. This review article provides an up-to-date list of potential OSCC salivary biomarkers.

Challenges of Salivary biomarkers in other field

Saliva, a multiconstituent oral fluid, has high potential for the surveillance of general health and disease. Particularly, it represents a promising diagnostic fluid for the screening of various oral diseases. For the past two decades, saliva has beenincreasingly evaluated as a diagnostic fluidfor detecting caries risk, periodontitis, salivary gland diseases, hereditary diseases, autoimmune diseases, systemic disorders such as hepatitis and the presence of human immunodeficiency virus (HIV) or hepatitis C virus.

The simple and non-invasive nature of saliva collection and its high-sensitivity assay development has led to an emphasis on the promise of salivary biomarkers.

Table:Potential biomarkers in Oral cancer :

Potential OSCC Salivary biomarkers	Authors
Defensin-1	Mizukawa et al.[10]
Proteins P53 autoantibody	Warnakulasuriya et al.[11]
α-amylase	Chen et al.[12]
Inter Leukin-8	St. John et al.[13] Rhodus et al.[14] Arellano-Garcia et al.[15] Brinkmann et al.[16] Elashoff et al.[17]
Tumor Necrosis Factor-α	Rhodus et al.[14]
Inter Leukin-1	Rhodus et al.[14] Cheng et al.[18]
Inter Leukin-6	Katakura et al.[19] Saheb-Jamee et al[20] Sato et al.[21]
Basic fibroblast growth factor	Vucicevic et al.[22] Gorugantula et al.[23]
Statherin	Contucci et al.[24]

Cyfra 21.1	Nagler et al.[25]
Tissue polypeptide antigen (TPA)	Nagler et al[.25]
Cancer antigen 125 (CA125)	Nagler et al.[25] Balan et al.[26]
Endothelin-1	Pickering et al.[27] Cheng et al.[28]
Inter Leukin-1β	Katakura et al.[19] Brinkmann et al.[16] Elashoff et al[.17]
CD44	Franzmann et al.[29]
Total salivary protein	Shpitzer et al.[30]
Insulin growth factor 1 (IGF-1)	Shpitzer et al.[30]
Matrix Metallo Proteins-2, 9	Shpitzer et al.[30]
CD59 Catalase Profilin S100A9/MRP14	Hu et al.[31]

M2BP	Hu et al.[31] Brinkmann et al.[16]
Carcinoembryonic antigen (CEA) Carcinoma associated antigen CA-50	He et al.[32]
Salivary carbonyls,Cyclin D1,Maspin, Phosphorylated-Src,Ki-67	Shipitzer et al.[33]
Lactate dehydrogenase	Shipitzer et al.[33] Shety et al.[34]
Transferrin	Jou et al.[35]
Zinc finger protein 501 peptide	Jou et al.[36]
Hemopexin Haptoglobin Complement C3 Transthyretin α1-antitrypsin	Jessie et al[37]
P53 gene codon 63	Liao et al.[38]
Loss of heterozygosity in the combination of markers D3S1234, D9S156, and D17S799	El-Naggar et al.[39]
Mitochondrial DNAs (cytochrome c oxidaseI and cytochrome c oxidase II)	Jiang et al.[40]

Hypermethylation of promoters in tumor uppressor genes: DAPK, DCC, MINT-31, TIMP-31, TIMP-3, p16, MGMT, CCNA1	Carvalho et al.[41]
Inter Leukin-8	Li et al.[42] Brinkmann et al.[16] Elashoff et al.[17]
Inter Leukin-1β	Li et al.[42]
DUSP1 (dual specificity phosphatase 1)	Li et al.[42] Elashoff et al.[17] Cheng et al.[43]
H3F3A (H3 histone family 3A)	Li et al.[42] Elashoff et al.[17]
AZ1 (ornithin decarboxylase antizyme 1)	Li et al.[42] Elashoff et al.[17] Cheng et al.[43]
S100P (S100 calcium binding protein P) S100P (S100 calcium binding protein P)	Li et al.[42] Brinkmann et al.[16] Elashoff et al.[17] Cheng et al.[43]
SAT (spermidine/spermine N1-acetyltransferase EST)	Li et al.[42] Brinkmann et al.[16] Elashoff et al.[17]
miR-125a miR-200a	Park et al.[44]
miR-31	Liu et al.[45]

Peroxidase	Bahar et al.[46]
Reactive nitrogen species (RNS) such as nitricoxide (NO), nitrites (NO2) and nitrates (NO3	Bahar et al.[46]
Glutathione S-transferase (GST)	Bahar et al.[46]
Superoxide dismutase (SOD) 8-hydroxy-2-deoxyguanosine (8-OHdG)	Agha-Hosseini et al.[47]
Glutathione	Almadori et al.[48]
Malondialdehyde (MDA)	Agha-Hosseini et al.[47]
adaverine, alpha-aminobutyric acid, anine,C5H14N5, piperidine, taurine perideine,pipercolic acid, C4H9N, C8H9N, pyrrolinehydroxycarboxylic acid, betaine, C6H6N2O2, leucine+isoleucine, tyrosine, histidine,tryptophan, beta-alanine, glutamic acid,threonine, serine, glutamine, choline, carnitine, C4H5N2O11P	Sugimoto et al.[49]
Phenylalanine Valine	Wei et al. [50] Sugimoto et al.[49]

Lactic acid	Wei et al.[50]
Sialic acid α-L-fucosidase	Vajaria et al.[51]

It may reflect levels of therapeutic, hormonal, and immunologic molecules and can yield diagnostic markers for infectious and neoplastic diseases. Various mediators of chronic inflammation and tissue destruction have been detected in whole saliva of patient with oral diseases. Moreover, its local inflammatory process may promote early diagnosis and aid in the monitoring of treatment.[52]Significant milestone in salivary diagnostics has also been reached for diagnosis of breast cancer. pancreatic cancer, lung cancer, ovarian cancer and gastric cancer.[4]

DISCUSSION

Till date, most of the markers have been identified from various body fluids. Among whichsaliva and blood are the most extensively studied body fluids. Both basic and clinical research on the development of methods to assay salivary biomarkers are increasing. Reviews states that oral carcinoma is the first carcinoma to have its biomarker mapped using salivary diagnostics. Studies state that over expression of the mutant p53 protein can induce a specific humoral response in cancer patients and the detection of p53 antibodies can offer a specific and non-invasive method for the detection of a subset of tumours with p53 aberrations.[11]St John MA et al using laser-capture microdissection, identified the expression of 2 cellular genes that are uniquely associated with OSCC: interleukin (IL) 6 and IL-8 and correlated that cytokines may contribute to the pathogenesis of this disease, and have been linked with increased tumor growth and metastasis. Cheng Y S et al in their study used seven salivary mRNAs-IL-8; IL-1β; dual specificity phosphatase 1 (DUSP1); H3 histone family 3A (H3F3A); ornithin decarboxylase antizyme 1 (OAZ1); S100 calcium-binding protein P (S100P); and spermidine/spermine N1-acetyltransferase 1 (SAT1)-for detecting development of oral squamous cell carcinoma (OSCC) and OSCC patients whose disease was in remission.They concluded that salivary OAZ1, S100P, and DUSP1 mRNAs are candidate biomarkers for detecting OSCC development in OSCC patients in remission.[43] Hu S et al explored the presence of informative protein biomarkers in the human saliva proteome for detection of oral squamous cell carcinoma (OSCC). Patient-based saliva proteomics is a promising approach for OSCC biomarkers detection. The discovery of these new targets may lead to a simple clinical tool for the noninvasive diagnosis of oral cancer. [31]

CONCLUSION

Currently saliva matrix is an upcoming area of research for basic and clinical application purposes. The future for saliva diagnostics relies on combinations of biomarker panels used as screening tools to improve on diagnostic accuracy and specificity. Biopsy remains the gold standard for definitive diagnosis of oral squamous cell carcinoma but it would be highly desirable and beneficial if salivary biomarkers may be made as a diagnostic tool, especially when a concurrant analysis for significantly increased markers is done. That is because salivary harvesting is non invasive which may make it an attractive, effective alternative to serum testing. Further, enormous efforts from researchers and clinicians are essential to turn salivary diagnostics into clinical and commercial reality to combat oral cancer.

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