WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 5, Issue 2, 373-380. Review Article ISSN 2277– 7105

MI RNA - A BIOMARKER IN ORAL CANCER DETECTION.

[1]Manjari Sonam, [1]Parikshit Sharma, [1]Shaleen Chandra, [2]Nigar Samadi, [2]Naira Khanam and *[1]Fahad M. Samadi

[1]Department of Oral Pathology and Microbiology, KGMU, Lucknow, U.P., India.

[2]Sardar Patel Post Graduate Institute of Dental and Medical Sciences.

Article Received on
22 Nov 2015,

Revised on 14 Dec 2015,
Accepted on 06 Jan 2016

***Correspondence for Author**
Dr. Fahad M. Samadi
Department of Oral Pathology and Microbiology, KGMU, Lucknow, U.P., India.

ABSTRACT

A percent of cancer populations is increasing sharply, the incidence of oral squamous cell carcinoma (OSCC) has also been expected to increase. Cancer prevention is more important than treatment for overcoming increased cancer death in the future. Oral cancer is the most common cancer worldwide which continues to be the most prevalent cancer resulting from the consumption of tobacco and other carcinogenic products. A large part of cancer load in parts of India is formed from Oral cancer. Oral cancer is categorized into precancerous and cancerous stages. Precancerous stage includes Leukoplakia, Erythroplakia and Lichen Planus, while cancerous or malignant stage is Squamous Cell Carcinoma. Oral cancer development is a multistep process which arises from pre- existing malignant lesions. Oral carcinogenesis is a highly complex, multistep process which involves accumulation of genetic alterations that lead to the induction of proteins promoting cell growth (encoded by oncogenes), increased enzymatic (telomerase) activity promoting cell proliferation.

KEYWORDS: Oral cancer, pre-cancer, micro-RNA, salivary biomarker.

INTRODUCTION

90% of oral cancers constitute the malignant form which is Oral squamous cell carcinoma (OSCC). In the United States, it is the sixth most common cancer,.[1] 50% cases shows the average 5-year survival rate for OSCC. As this number has not changed in last three decades.[2] Therefore, there is a need of method for early OSCC detection which can increase patient survival rate. Oral Cancer, is a highly-effected local disease in the oral cavity affecting almost 3 lakh people worldwide annually.[3,4] The American Cancer Society

estimated that more than 30,000 new cases of oral cancer were diagnosed in 2006, representing 3% of all malignancies in men and 2% of all malignancies in women.[5]

Oral precancerous lesions (PCLs), a benign morphologically altered tissue that has a greater than normal risk of malignant transformation, such as leukoplakia etc., is also very common. Precancerous condition is a condition that does not necessarily alter the clinical appearance of local tissue but is associated with a greater than normal risk of precancerous lesion or cancer development in that tissue such as sub mucous fibrosis etc. (Neville et al 2005). Leukoplakia and sub mucous fibrosis are early indicators of damage to the oral mucosa with a transformation rate of 2–12% to frank malignancies.

Oral leukoplakia is a common precancerous lesion, characterized by morphologically altered tissue, in which cancer is more likely to occur than its apparently normal counterpart.

Oral sub mucous fibrosis (OSF) is a pre-cancerous condition characterized by the accumulation of collagen in the lamina propria of oral mucosa. It is characterized by a juxtra-epithelial inflammatory reaction followed by fibroelastic changes in the lamina propria and associated epithelial atrophy.

Patients with OSCC often shows symptoms at the late stage, and there is a high recurrence rate after treatment. The overall 5-year survival rates for oral cancer have remained low and are essentially unchanged during the past few decades.[6,7] Delayed detection of disease is a primary reason for the increasing morbidity rate of oral cancer patients, and this supports the need for sensitive biomarkers to improve early detection of oral cancers.

Biomarker

The term, **biomarker,** refers to measurable and quantifiable biological parameters than can serve as indicators for health and physiology-related assessments, such as pathogenic processes, environmental exposure, disease diagnosis and prognosis or pharmacologic responses to a therapeutic intervention.[8]

A **cancer biomarker** for a specific tumor type can provide vital information needed to successfully treat cancer. The ultimate goal in the discovery of biomarkers is to enhance the survivability of cancer through improved diagnostics and treatment.[9]

Salivary diagnostics is a dynamic and emerging field utilizing nanotechnology and molecular diagnostics to aid in the diagnosis of oral and systemic diseases and using the **salivary biomarkers** for disease detection.[10]

Global profiling of disease-associated molecules, such as proteins, DNA, mRNA, micro RNA, and metabolites is becoming the state-of-the-art method to provide promising disease biomarker candidates.[11]

Saliva as a Biomarker

Human saliva is a slightly acidic (pH = 6.0–7.0) biologic fluid containing a mixture of secretions from multiple salivary glands (parotid, submandibular, sublingual) and other minor glands beneath the oral mucosa as well as gingival crevice fluid. This complex mixture of oral fluid serves as the execution of multiple physiologic functions such as oral digestion, food swallowing and tasting, antibacterial and antiviral protection.[12] In addition to maintaining the homeostasis of the oral cavity system, the oral fluid is a perfect medium to be explored for health and disease surveillance. Similar to the case of blood, saliva is a complex fluid containing a variety of enzymes, hormones, antibodies, antimicrobial constituents, and cytokines.[13,14] Many of these enter saliva from the blood by passing through cells by transcellular, passive intracellular diffusion and active transport, or paracellular routes by extracellular ultra filtration within the salivary glands or through the gingival sulcus.[15,16,17] So, most of the compounds found in blood are also present in saliva. Accordingly saliva can reflect the physiologic state of the body, including emotional, endocrinal, nutritional and metabolic variations. This fluid also provides a source for the monitoring of oral and also systemic health. This is the basis of our vision to develop disease diagnostics and promote human health surveillance by analysis of saliva.

Saliva is used as new a diagnostic tool for oral cancer detection, and some salivary analytes such as proteins and DNA are also used to detect Oral cancer.[18,19] The mRNAs from saliva enters the oral cavity through various sources like saliva glands, gingival crevice fluid, and desquamated oral epithelial cells.[20] The majority of salivary mRNAs is found in partially degraded forms[21] which maintain their stability in saliva through their association with unidentified macromolecules.

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. Moreover, saliva is readily available and easily collected without specialized equipment or personnel. For the past two decades, saliva has been increasingly evaluated as a diagnostic fluid for detecting caries risk, periodontitis, oral cancer, breast cancer, salivary gland diseases and 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. It may reflect levels of therapeutic, hormonal, and immunologic molecules and can yield diagnostic markers for infectious and neoplastic diseases. With a salivary specimen, one can collect multiple specimens from the same individual at the optimum times for diagnostic information.[22]

The first report of saliva as a diagnostic tool for oral cancer detection was published in 2000.The authors claimed that exon 4, codon 63 of the p53gene was mutated in salivary DNA from five of eight(62.5%) oral cancers patients.[23]

Micro-rna Biomarker

MicroRNAs (miRNA) lin-4 and let-7 were firstly discovered in C. elegans . miRNAs are transcribed by RNA polymerase II or RNA polymerase III as a part of an intron of mRNA or as an independent gene unit.[24,25] Initially transcribed miRNAs are several hundred to thousands of nucleotides long and have a distinct stem-loop structure, which is then cleaved into a <100 nucleotide stem loop structure by a type III RNase named Drosha . These pre-miRNAs are then exported from the nucleus to the cytoplasm with the help of exportin 5 protien. In the cytoplasm, these pre-miRNAs then undergo another round of endonucleolytic cleavage by Dicer, another type III RNase. Fully processed miRNAs are 18 to 24 nucleotides in length. These mature miRNAs are bound by a protein complex called the RNA-induced silencing complex (RISC), which is composed of four argonaute family proteins Ago1-4.[25] This active miRNA-RISC complex binds to target mRNAs based on complementary base pairing between the miRNA and the mRNA. The miRNA blocks the translation of mRNA and leads to mRNA degradation. Because miRNAs bind with slightly imperfect complementary to target mRNAs, it is estimated that one miRNA is capable of binding >100 different mRNAs with different binding efficiencies.

With about 1,000 miRNAs expected to be present in the human genome, it is postulated that 30% of all mRNAs are post transcriptionally regulated by miRNAs.[26,27] The recent discovery of hundreds of miRNAs, from various organisms, and functional assays have

determined that miRNAs serve important functions in cell growth, differentiation, apoptosis, stress response, immune response, and glucose secretion.[28,29,30]

miRNAs are also differentially expressed in various cancer cells compared with normal cells, and it seems that miRNAs more accurately cluster different types of solid tumors than mRNA, suggesting that miRNAs can be used to detect cancer.[28]

Currently, oral cancer diagnosis depends on a thorough oral examination, usually by a dentist. Elevated levels of salivary soluble CD44 in the majority of OSCC distinguishes the cancer from benign stage with malignant.[31] In the saliva of OSCC patients, the level of three tumor markers, (cytokeratin 19 fragment Cyfra21- 1, tissue polypeptide antigen, and cancer antigen 125), were elevated and combined use of these markers resulted in similar diagnostic value to those obtained when measuring them in the sera of OSCC patients.[32] The level of p53 autoantibody in saliva was also found correlated with its serum levels in OSCC and analysis of p53 antibody in saliva may offer a specific method for detection of a subset of OSCC with p53 aberrations.[33] Considering that these candidate biomarkers were discovered at an individual basis, their predicting power for OSCC detection is limited.

CONCLUSION

In the past several years, the importance of microRNA (miRNA) in cancer cells has been recognized. Proper control of miRNA expression is essential for maintaining a steady state of the cellular machinery. Recently, it was discovered that extracellular miRNAs circulate in the blood of both healthy and diseased patients, although ribonuclease is present in both plasma and serum. Most of the circulating miRNAs are included in lipid or lipoprotein complexes, such as apoptotic bodies, microvesicles, or exosomes, and are, therefore, highly stable. The existence of circulating miRNAs in the blood of cancer patients has raised the possibility that miRNAs may serve as a novel diagnostic marker. However, the secretory mechanism and biological function, as well as the meaning of the existence of extracellular miRNAs, remain largely unclear. In this review, we summarize the usefulness of circulating miRNA for cancer diagnosis, prognosis, and therapeutics. Furthermore, we propose a mechanism for the secretion and incorporation of miRNA into the cells.

REFERENCES

1. Brinkman BM, Wong DT. Disease mechanism and biomarkers of oral squamous cell carcinoma. Curr Opin Oncol 2006; 18: 228–33.

2. Funk GF, Karnell LH, Robinson RA, Zhen WK, Trask DK, Hoffman HT. Presentation, treatment, and outcome of oral cavity cancer: a National Cancer Data Base report. Head Neck 2002; 24: 165–80.
3. Greenlee RT, Murray T, Bolden S, et al. Cancer tatistics, 2000. CA CancerJClin 2000; 50: 7^33.
4. Parkin DM, Bray F, Ferlay J, et al. Global cancer statistics, 2002. CA CancerJClin 2005; 55: 74^108.
5. Cancer facts and figures 2006. Atlanta: American Cancer Society; 2006.
6. Lippman SM, Hong WK. Molecular markers of the risk of oral cancer. NEngl JMed 2001; 344: 1323^6.
7. Vokes EE,WeichselbaumRR, Lippman SM, et al.Head and neck cancer. NEngl JMed 1993; 328: 184^94.
8. Nadine Spielmann and David T. Wong, Saliva:diagnostics and therapeutic perspectives, School of Dentistry and Dental Research Institute, University of California–Los Angeles:Published in final edited form as:Oral Dis. 2011 May ; 17(4): 345–354.
9. Author : Emily Humphreys, Cancer Biomarker in Human Saliva Proteomics, Proteomics-January 7, 2013.
10. Daniel Malamud, PhDa,b and Isaac R. Rodriguez-Chavez, PhDc, Saliva as a Diagnostic Fluid, Published in final edited form as: Dent Clin North Am. 2011 January ; 55(1): 159–178.
11. Prevalidation of Salivary Biomarkers for Oral Cancer Detection Cancer Epidemiol Biomarkers: Prev; 21(4); 664–72. _2012 AACR
12. Mandel ID (1987). The functions of saliva. J Dent Res 66 Spec No: 623–627.
13. Rehak NN, Cecco SA, Csako G. Biochemical composition and electrolyte balance of ‘‘unstimulated’’ whole human saliva. Clin Chem Lab Med 2000; 38: 335–343.
14. Zelles T, Purushotham KR, Macauley SP, Oxford GE, Humphreys-Beher MG. Saliva and growth factors: the fountain of youth resides in us all. J Dent Res., 1995; 74: 1826– 1832.
15. Drobitch RK, Svensson CK. Therapeutic drug monitoring in saliva. An update. Clin Pharmacokinet 1992; 23: 365–379.
16. Jusko WJ, Milsap RL. Pharmacokinetic principles of drug distribution in saliva. Ann N Y Acad Sci., 1993; 694: 36–47.
17. Haeckel R, Hanecke P. The application of saliva, sweat and tear fluid for diagnostic purposes. Ann Biol Clin (Paris) 1993; 51: 903–910.

18. Chai RL, Grandis JR. Advances inmolecular diagnostics and therapeutics in head and neck cancer. Curr TreatO ptions Oncol 2006; 7: 3–11.
19. Hu S, Arellano M, Boontheung P, et al. Salivary proteomics for oral cancer biomarker discovery. Clin Cancer Res 2008; 14: 6246–52.
20. Park NJ, Li Y, Yu T, Brinkman BM, Wong DT. Characterization of RNA in saliva. Clin Chem 2006; 52: 988–94.
21. Park NJ, Zhou X, Yu T, et al. Characterization of salivary RNA by cDNA library analysis. Arch Oral Biol 2006.
22. Lindsay F. Hofman Saliva Testing and Reference Laboratory, Inc., Seattle, Human Saliva as a Diagnostic Specimen, WA 98104: Dent Clin North Am. 2011 January ; 55(1): 159–178.
23. Franky D. Shah • Rasheedunnisa Begum • Bhairavi N. Vajaria • Kinjal R. Patel •Jayendra B. Patel • Shilin N. Shukla • Prabhudas S. Patel, A Review onSalivary Genomics and Proteomics Biomarkers in Oral Cancer ,Received: 4 July 2011 / Accepted: 4 July 2011 / Published online: 9 August 2011
24. Zeng Y. Principles of micro-RNA production and maturation. Oncogene 2006; 25: 6156–62.
25. Hammond SM, Bernstein E, Beach D, Hannon GJ. An RNA-directed nuclease mediates posttranscriptional gene silencing in Drosophila cells. Nature 2000; 404: 293–6.
26. Bentwich I, Avniel A, Karov Y, et al. Identification of hundreds of conserved and nonconserved human microRNAs. NatGe net 2005; 37: 766–70.
27. Berezikov E, Guryev V, van de BeltJ, Wienholds E, Plasterk RH, Cuppen E. Phylogenetic shadowing and computational identification of human micro- RNA genes. Cell 2005; 120: 21–4.
28. Lu J, Getz G, Miska EA, et al. MicroRNA expression profiles classify human cancers. Nature 2005; 435: 834–8.
29. Stadler BM, Ruohola-Baker H. Small RNAs: keeping stem cells in line. Cell 2008; 132: 563–6.
30. Phillips JR, Dalmay T, Bartels D. The role of small RNAs in abiotic stress. FEBS Lett 2007; 581: 3592–7.
31. Franzmann EJ, Reategui EP, Pedroso F, et al. Soluble CD44 is a potential marker for the early detection of head and neck cancer. Cancer Epidemiol Biomarkers Prev 2007; 16: 1348^ 55.

32. Nagler R,BaharG,ShpitzerT, et al.Concomitantanalysis of salivary tumormarkersKa new diagnostic tool for oral cancer.ClinCancerRes., 2006; 12: 3979^84.
33. Tavassoli M, Brunel N, Maher R, et al. p53 antibodies in the saliva of patients with squamous cell carcinoma of the oral cavity. IntJCancer, 1998; 78: 390^1.