**MICRONRNAS AS A NOVEL BIOMARKER IN ORAL CANCER**

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**ABSTRACT**

Oral cancer is one of the commonest cancers in the world which spread from oral cavity to neck lymph nodes. However, the oral cancer is not easily diagnosed until their advanced stage which is the main cause for the low survival rate of patients. In recent years, the growing interest of researchers to investigate the role of microRNAs (miRNAs) in various biological processes as well as many diseases including carcinomas. With the advent of next generation sequencing technique, it has been determined that about 1400 miRNAs act as oncogenes and tumor suppressor genes; these play a pivotal role in developmental processes, growth control and in various human diseases like cancers. With the altered expression of tumor suppressor as well as oncogenic miRNAs, various processes such as pathological and biological have been found in altered form in various studies. Alteration in gene expression helps in the diagnosis of cancers at early stage. In this review, we focused on the role and significance of tumor suppressor as well as oncogenic miRNAs as an emerging novel biomarker for oral cancer.

**KEYWORDS:** MicroRNA, OSCC, Oncogene, Gene expression.

**INTRODUCTION**

Oral cancer is the sixth most common human cancer in men and women,[¹] which signify that three percent of all types of human carcinoma. Oral cancer exhibits precancerous lesions like Leukoplakia & Erythroplakia. It has been found that >300000 new cases of oral squamous cell carcinoma (OSCC) are diagnosed yearly.[²] Its incidence rate is 650000 while 350000 death occur in United State alone yearly.[³] In the developed countries, the overall incidence rates are very high with 4.0–6.8 per 100,000 in males and 0.8–4.5 per 100,000 in females.[⁴]

In India as well as south and Southeast Asian countries all have highest incidence rate of oral cancer. With 90% to 95% of oral squamous cell carcinoma in oral cancer in Indian population[⁶] This data shows that Head & Neck Squamous Cell Carcinoma (HNSCC) is the 4th most common cancer in men while 9th most common cancer in women.[⁷] Oral cancer begins in the mouth or oropharynx. It is particularly dangerous because patients do not notice in the early stage. It frequently prospers without producing pain or symptoms with high recurrence rate. Often oral cancer appears when the cancer metastasized to another location most likely to lymph nodes of the neck due to this, its prognosis at this stage is significantly worse.[⁸]

Nowadays early detection of oral cancer is attracting a lot of researchers and clinicians towards the identification of altered miRNAs. Many recent studies have been identified that miRNAs are playing a major role in different types of biological and pathological processes, mainly in the progression of cancers and also have been revealed that all types of cancer with their tumor staging and treatments are associated with the altered expression of miRNAs. Consequently, miRNAs are considered as an emerging diagnostic and prognostic biomarker in addition to cancer therapeutic marker. There are numerous approaches to know the molecular basis of oral cancer.[⁹–¹¹] These are microarray technology, methylation microarrays, gene expression microarrays, mitochondrial array and miRNA arrays. In recent years miRNAs array are in use to investigate oral cancer in solid tissue and biofluids like plasma, serum, saliva[¹²] and urine.[¹³] On the basis of miRNAs present in different types of solid tissues and fluids, it is classified as tumor tissue miRNAs and circulating miRNAs, while the biopsy procedure of tumor tissue is painful and higher risk procedure for cancer patients so circulating miRNAs are identified as an non invasive biomarker to detect oral cancer at their early stage.

**Risk Factors for Oral Cancer**

Use of tobacco and alcohol consumption are the major risk factor for oral cancer. Drinking and smoking both are independent factors but they have synergistic effect.
and significantly increase the risk of oral cancer.\textsuperscript{14,15}

**Micro –RNA**

MicroRNAs (miRNAs) are small non-coding RNAs which involve in the post transcriptional modification of coding RNA. These non coding small regulatory RNA are evolutionarily conserved and widely spread among different species (June et al., 2011). Lee et al. discovered the first micro RNA in \textit{C. elegans} in 1993 which was known as LIN-4.\textsuperscript{16} Molecular markers play significant role in clinical diagnosis due to progression of advance techniques in molecular biology (Hui et al. 2010).\textsuperscript{17} When miRNAs expression pattern of cancer and normal tissue were compared by Krutovskikh et al. in 2010, found that it may help in monitoring different types of carcinoma.\textsuperscript{18} In many studies MiRNAs are considered as a potential biomarker for prognosis, cancer onset at early stage and categorization of different types of cancer.\textsuperscript{19} MiRNAs are stable biomarker because they are resistant to degradation because they get packaged into microvesicle, exosomes, apoptotic bodies or they form the miRNA– Protein complex (Li et al. 2007).\textsuperscript{20} These miRNAs are found in significant amount in different fluids such as saliva, peripheral blood, urine and semen (Mitchell et al., 2008; Hanke et al., 2009; Park et al., 2009; Zubakov et al. 2010).\textsuperscript{21,22}

**Biogenesis of miRNA**

miRNAs are important key regulatory factors of gene expression, they involve in either repression or degradation of their target messengerRNAs (mRNAs).\textsuperscript{23-25} miRNAs represent 1%-3% of the whole mammalian genome.\textsuperscript{26} These micro RNAs are found in both introns as well as in exons. They are transcribed by RNA polymerase II and involve in the formation of the primary precursor miRNA (pri-miRNA) which is generated by stem loop precursor. Pri- mi RNA resembles with the messenger RNA in the features like 5’ cap and 3’poly AAA tail. In nucleus the stem –loop structure is processed by DROSHA and DiGeorge Syndrome Critical Region 8 (DGCR8) enzyme and produce as a precursor miRNA (pre-miRNA). Then these pre-miRNAs is transported from nucleus to cytoplasm through nuclear transporter known as Exportin-5 with the help of nuclear monomeric G protein (Ran). In the cytoplasm pre-mi RNA is processed by Dicer to produce duplex miRNA complex, after the processing of duplex micro RNA, one strand of miRNA is incorporated into RNA inducing silencing complex (RISC) to either suppress or degrade the target mRNA gene after that Argonaute (Ago) proteins joins with the RISC complex, which plays the important role in transcriptional and post-transcriptional gene silencing of targeted mRNA (Fig. 1).\textsuperscript{26-2}

**Tumor Tissue miRNA**

Various studies have been explored that the different expression profile of miRNAs play important role, to identify the tissue origin for tumors whose origin is not known. It is also involve in the diagnosis of subtypes of tumors or the tissues which are poorly differentiated on the basis of tissue specific deregulation of miRNAs.

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**Fig. 1.** This figure is showing microRNA processing and its role in mRNA degradation and Translational Inhibition (Taken from the review of Ahmad J et al. 2013).
Evaluations of miRNAs do not require large amount of tissue biopsy. According to recent researchers, it have been found that miRNAs can be measured in formalin-fixed paraffin-embedded tissues (FFPE) of papillary thyroid carcinoma, gall bladder cancer, lung cancer, renal tumor, hepatocellular carcinoma including oral cancer.

Circulating miRNA
In 2008, circulating miRNAs have been discovered in mammalian body fluids such as saliva, serum, plasma and sputum and also present in solid tissues. The National Cancer Institute defined miRNAs as biomarkers in blood, or other body fluids, that is used for the diagnosis of healthy or diseased person. Different studies have been described that, circulating miRNAs play as a regulatory roles in near about every physiological and pathological aspect of biology. These microRNAs are stable biomarkers and shows variability in expression patterns. Body fluids contain ribonuclease which can degrade miRNA so extracellular miRNAs evolve the new way to protect from the RNase digestion by packaging into vesicles and different proteins. These cancer miRNAs biomarkers should be diagnostic, prognostic and predictive with high sensitivity, specificity.

(a) Oncogenic miRNAs in Oral Squamous Cell Carcinoma
Micro RNA-21 in Oral Cancer
In oral cancer miRNA-21 acts as oncogene and it targets phosphate pensin (PTEN). In many studies it has been concluded that up-regulation of micro RNA 21 involves in the low expression of PTEN and their transcriptional regulator Grhl3. PTEN involves in the inhibition of phosphatidyl inositol-3 kinase pathway (PI3K). When expression of PTEN down-regulate, then PTEN is unable to inhibit the PI3K pathway.

microRNA-31
miRNA-31 shows up-regulation in oral leukoplakia and OSCC. They play oncogenic role in OSCC. Liu et al. (2010) suggested that ectopic expression of miR-31 involves in the repression of its target factor, inhibiting hypoxiainducible factor (FIH) expression to activate hypoxiainducible factor(HIF) under normoxic conditions, both in vitro and in vivo. The signal cascading of miRNA-31-FIH-HIF-VEGF affects many biological processes such as cell migration, proliferation and epithelial mesenchymal transition (EMT) in OSCC.

microRNA-134
MiRNA -134 is over-expressed in HNSCC patients when these samples are compared to normal controls.

microRNA-146a
MiRNA-146a is up-regulated in OSCC. Recent studies revealed that down-regulation of IL-1 receptor associated kinase-1(IRAk-1), NUMB and TNF receptor associated factor-6(TRAf-6) is related with oncogenic function of miRNA-146 in OSCC.

microRNA-155
MiRNA-155 is up-regulated in OSCC patient. The oncogenic role of miRNA-155 is linked with down-regulation of a tumor suppressor CDC73 in OSCC.

(b) Tumor Suppressor MiRNA in Oral Squamous Cell Carcinoma
microRNA-7
miRNA-7 plays the role as tumor suppressor in many human cancer like breast cancer, glioblastoma including oral squamous cell carcinoma. In many studies it have been confirmed that there are many protooncogenes, which work as target genes of miRNA-7 like insulin receptor substrate 1(IRS1), Insulin receptor substrate 2(IRS2), v-raf1 murine leukemia viral oncogene homologue(RAF1) and p21(CDC42/RAC1 activated kinase1(PAK1). Jiang et.al. concluded that miRNA-7 is involved in regulation of IGFIIR/IRS/P13K/Akt signaling cascade by regulation of insulin like growth factor 1 receptor at post-transcriptional level in cells of tongue squamous cell carcinoma (TSCC).

microRNA-99a
The target genes of miRNA-99a are IGFI1R and mTOR (mammalian target of rapamycin), that plays the crucial role in IGFI1R signaling pathway. miRNA-99a is downregulated in OSCC patient. It has been seen in many studies that miR-99a play a major role in lymphovascular invasion.

micro RNA-218
It works as a tumor suppressor by the regulation of mTOR in OSCC cases. It has been shown that miRNA-218 is epigenetically silenced in tissue specimens of OSCC.

microRNA- 9
It has been proved that hypermethylation of DNA is associated with decrease expression of MiRNA-9. In oral squamous cell carcinoma and oropharyngeal carcinoma the molecular process such as DNA hypermethylation downregulates the level of miR-9 and miRNA-9 targets CXC chemokine receptor 4 (CXCR4) genes and wnt/β-catenin pathway.

microRNA-138
It has been shown that miRNA-138 plays a major role in cell proliferation, migration and invasion in HNSCC derived cells. MiRNA -138 has been shown to regulate EMT related molecules like vimentin (VIM), Foslike antigen-1(FOSL-1), zinc finger E-box binding homeobox2 (ZEB2), RhoC and ROCK2.

Micro RNA-133 in Oral Cancer
In many studies it has been found that deregulation of gene expression is associated with the altered expression
of miRNA. It has been found in several studies that miRNA 133a and miRNA-133b are down-regulated in tongue Squamous cell carcinoma when they were compared with control tissue samples. MiRNA-133a and 133b are mainly found in muscle cells but their functions are still not known. Mirna-133a and MiRNA 133b play the main role as tumor suppressing miRNAs in squamous cell oral carcinoma. In many studies it has been found that micro RNA 133a and MiRNA 133b involves in the intrinsic apoptotic pathway. In the glycolytic pathway Pyruvate Kinase enzyme catalyzes the conversion of pyruvate from phosphoenol pyruvate (PEP). Pyruvate Kinase enzyme is specific for PKM1 and M2 and these isoforms are specific for tissue type. Pyruvate Kinase L (PKL) gene generates two isotypes via alternative promoters like Type L and Type R and the Pyruvate Kinase M genes involves in the generation of type M1 and type M2. These two isotypes have different sites where they play their important roles, the sites of action of Type M1 is brain, heart and muscle cells. Type M2 is found in proliferating cells like neoplastic cells. Pyruvate Kinase M gene have total 12 exons. At exon 9 and exon 10, Pyruvate Kinase M1 and M2 have difference like Exon 9 and Exon 10 determine sequence that are specific for PKM1 and PKM2. When the tumor progression occurs the PKM2 replaces the original tissue specific pyruvate kinase isoforms like Type L, R and M1. Tetrameric form of PKM2 have high affinity with phosphoenol pyruvate in the normal cell while in the cancerous cell PKM2 is found in the dimeric form which have lower affinity with the phosphoenol pyruvate. PKM2 inactivates the glycolytic pathway because it is not associated with the glycolytic complex. In the low glucose and oxygen environment PKM2 enhances the tumor progression and invasion. The oncoproteins such as pp60 kinase and E7 of human papilloma virus helps in the PKM2 dimerization. In many studies it has been concluded that PKM2 upregulates in many cancers like skin, gastric, colorectal and cervical cancers. PKM2 transcript was formed by exon 11 of pyruvate kinase M gene, MiRNA-133a and MiRNA-133b binds on the same exon segment of gene. Wong et al. (2008) concluded that upregulation of PKM2 expression involves in the downregulation of miRNA-133 in all types of tumors, they found that miRNA-133 targets the oncogene PKM2 in squamous cell oral carcinoma.

CONCLUSION
Recent studies of miRNAs research in the cancer biology, have shed light on the role of miRNAs as novel biomarker. However, these biomarker alterations affect diseases such as Cardiovascular disease, Alzheimer disease and cancer including Oral cancer. miRNAs is the non coding RNA, that regulates their target genes in many processes of cancer like cancer initiation, progression and metastasis, which will definitely help the researchers to do comprehensive mechanistic analysis of miRNAs. Future work should be undoubtedly focused on the miRNAs and their co-interaction associated with their target signaling pathways. More detailed study and better understanding of miRNAs will help the researchers to design miRNAs inhibitors for the creation of effective drug therapeutic against cancer including oral cancer.

ACKNOWLEDGMENT
The success and final outcome of this review article required a lot of guidance and assistance from many people and we are extremely fortunate to have got this all along the completion of this work.

Above all and the most needed, Mr. Sridhar Mishra PhD Scholar, Dr Ram Manohar Lohia Institute of Medical Sciences, Lucknow (RMLIMS) and Mr. Bilal Ahmed, Research Coordinator, Era’s Lucknow Medical College and Hospital, Lucknow who provided unflinching encouragement and support in various ways. Their truly scientific intuition has made a constant oasis of ideas and passion in science, which inspired and enriched the growth.

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