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RESEARCH ARTICLE

ORAL CANCER, CARCINOGENESIS & ROLE OF MIRNAS IN OSCC: DIAGNOSTIC, THERAPEUTIC, PROGNOSTIC

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ABSTRACT

Oral squamous cell carcinomas (OSCC) represent the most frequent of all oral neoplasms & in India, OSCC is the leading cancer in men and fifth common cancer in women. The five year survival rate of 60-80% for OSCC has not improved in the last 3 decades inspite of improvements in therapy strategies. Research on cancer tissues has revealed that there may be a link between molecular level and tissue level changes that drive malignant changes in the tissue and play a pivotal role in disease progression. Therefore, the somatic mutations can be used as biomarkers to diagnose oral or other tumours. Research indicates that, for high accuracy in identifying early disease onset in saliva, multiple biomarker candidates are needed. Biomarkers are objective indicators which play an important role in distinguishing between the presence or absence of disease by genomic, proteomic, or metabolomic expressions. Biomarkers include nucleic acids, proteins, peptides, enzymatic changes, antibodies, metabolites, lipids, and carbohydrates derived from body fluids. Biomarkers can be used for: patient risk determination, patient assessment, recurrence detection. They are classified according to various ways. It has been estimated that over 30% of protein-coding genes are regulated by miRNAs either by complementary binding of target mRNA or binding to imperfect complementary sites in the 3′ untranslated regions (3′UTR), leading to control of expression of these genes at the level of translation. MicroRNAs are important in tumourigenesis due to their proximity to chromosomal breakpoints and their dysregulated expression levels in many malignancies. The identification of aberrantly expressed miRNAs which disrupt the normal regulatory mechanisms in cancer cells is an important first step towards elucidating the details of miRNA-mediated oncogenic pathways, which are essential to know to significantly improve diagnosis, therapy, and prevention of the disease.

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INTRODUCTION

Oral cancers account for 2–4% of all cancer cases worldwide. Oral squamous cell carcinomas (OSCC) represent the most frequent of all oral neoplasms, and more than 90% of all oral neoplasms are estimated to be OSCC. In 2012, OSCC accounted for 145,000 deaths worldwide, with less developed regions of the world sharing 77 % of the burden. In India, OSCC is the leading cancer in men and fifth common cancer in women (Maha Yakob, 2014 and OSCC, 2016). The 5-year survival rate of oral cancer is 60–80% when detected during its early stages. This poor survival rate has not been improved in the past three decades despite improvements in therapy strategies. The key challenge to reduce the mortality and morbidity of this disease is to develop strategies to identify and detect OSCC when it is at very early stage, which will enable effective intervention and therapy. Up to now, beyond conventional clinical oral & histopathological examination, there are no scientifically credible, reliable early detection techniques available (Pre-Validation of Salivary Biomarkers for Oral Cancer Detection, 2012). Oral cancer broadly encompasses tumours arising in the lips, hard palate, upper and lower alveolar ridges, anterior two-thirds of the tongue, sublingual region, buccal mucosa, retromolar trigone and floor of the mouth. OSCC is acquired from a combination of environmental risk factors and genetic predispositions. The widespread practise of smoking or chewing tobacco and alcohol drinking, apart from poor oral hygiene, poor diet and Human Papilloma Virus (HPV) infections, in combination with an individual's genetics has the ability to mutate oncogenes that are in charge of cell survival and proliferation & therefore, may explain the disproportionately higher incidence of OSCC in India (Maha Yakob, 2014 and OSCC, 2016). Emerging evidence suggests that OSCCs in non-

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smoking or no alcohol use individuals may have distinct disease characteristics exhibiting a female predominance, higher average age, and a preponderance of tumor presentation at the mandibular alveolar ridge and maxilla at diagnosis (MicroRNA, 2015). Also, clinically identified premalignant lesions of the oral mucosa have a higher oncogenic risk than normal oral mucosa. Lesions termed leukoplakia, erythroplakia, and leukoerythroplakia are potentially malignant lesions of the aerodigestive tract. These lesions are defined as dysplasia of variable grades when verified with cellular atypia but without invasion. However, the values that predict cancer occurrence are a matter of debate because OSCC can arise where any epithelial dysplasia is detected. However, the onset and development of malignancy in these lesions are related to somatic mutations of tumour-specific DNA, which can be found in the saliva, plasma or other body fluids (Maha Yakob, 2014). Recurrence occurs in 15–33% of patients, with local recurrence being more common, thus, an improved diagnostic tool to predict which patients are most at risk for OSCC recurrence is needed (Maha Yakob, 2014).

Carcinogenesis

Thus, OSCC is a result of Carcinogenesis which is a complex process that occurs at the phenotype and genotype levels. Cancer development is driven by the accumulation of genetic and epigenetic changes that disturb the homeostatic equilibrium between cell proliferation and cell death.

The molecular level changes that occur in carcinogenesis are:

- Cancer cell proliferation without external stimuli,
- Insensitivity to inhibitory growth signals,
- Evasion of apoptosis or cell death mechanisms and/or activation of anti-apoptotic genes,
- Unlimited replicative potential,
- Sustained angiogenesis,
- Invasion and metastasis ability,
- Genomic instability, and
- Protooncogenes mutation caused by defects in dna repair.

Research on cancer tissues has revealed that there may be a link between molecular level and tissue level changes that drive malignant changes in the tissue and play a pivotal role in disease progression. Thus, the inference that can be drawn is that a study of the biological molecules involved in the molecular mechanism of carcinogenesis could provide valuable diagnostic data, i.e., of biomarkers, on the cancer disease process. Thus, the somatic mutations can be used as biomarkers to diagnose oral or other tumours.

Diagnosis of OSCC

Although, the oral cavity is readily accessible for clinical examination, most tumours are not diagnosed until they have advanced or metastasized, thereby limiting the effectiveness of chemotherapy, radiotherapy and surgery. Moreover, the development of second primary tumours hamper the success of multimodal therapeutic procedures leading to poor prognosis and dismal 5-year survival rates. Hence, research directed towards the identification of biomarkers for early diagnosis of OSCC, indicators of good or bad prognosis, and determinants of treatment response/overall survival is undeniably essential (Mehrotra, 2011). Much effort has gone into improving lesion detection and diagnosis and one way is to remove the need for scalpel biopsy. This has been attempted using special scanning devices based on either infrared light or fluorescence. These approaches have the possibility of easing patient concerns about surgical biopsy while also potentially making it possible to detect and diagnose in one step. Others have used gene-based methods to determine changes in the oral mucosa indicative of cancer. First with mRNA, and then miRNA, wherein RNA signatures for OSCC have been developed using surgically obtained tissue. A second approach has looked for markers of OSCC in body fluids, such as blood or saliva, with interesting, but, variable results, likely due to low RNA concentrations, leading to variable results. The limited follow-up on published RNA classifiers for OSCC combined with the lack of standardized sample collection methods for RNA-based detection and diagnosis has also slowed validation for clinical purposes (Courthod, 2014).

For almost three to four decades, changes in protein coding tumor suppressor genes and/or oncogenes have been thought to be the main drivers of tumor development. However, the recent discovery of thousands of genes that transcribe noncoding RNAs (including miRNAs) makes it obvious that cancer biology is even more complex than initially expected. Several layers of molecular regulators (e.g., mRNA, miRNA, and protein) are involved in the development and maintenance of cancerous phenotypes. As the progression of premalignant lesion into a cancerous tissue is often distinguished by the identification of cytogenetic changes such as the loss of chromosomal 3p region (a critical tumor suppressor gene region), resulting in a 3.8 fold higher risk of carcinogenesis, so, molecular regulators like, miRNAs , the 18–25 nucleotides long, noncoding RNA molecules, have recently gained significant attention as potential regulators and biomarkers for human carcinogenesis (Anjie Min, 2015). Next generation sequencing (NGS) of such RNAs has been used to identify specific changes in circulating miRNAs in lung-, breast and nasopharyngeal cancer. Salivary biomarkers can be used between biopsies to assist in monitoring the disease status of dysplasia patients. Although, salivary diagnostics for OSCC are very promising due to the direct contact of saliva with premalignant or malignant lesions, yet, no single bio-molecule has been shown to meet the real world requirement for high accuracy in identifying early disease onset, suggesting multiple biomarker candidates are needed for high accuracy and sensitivity in detecting OSCC. In addition, extensive and rigorous biomarker validation will be crucial to the acceptance of newly discovered biomarker candidates prior to adoption for clinical utilization. However, the advantage is that in saliva, tumour-specific DNA is positive in 100% of patients with oral tumours in various studies (Yan, 2017 and Elashoff, 2012).

Biomarkers

According to the National Institutes of Health (NIH), a biomarker is a characteristic that is objectively measured and evaluated as an indicator of a normal biological process, pathogenic process, or pharmaceutical response to therapeutic intervention (Santosh, 2016). Biomarkers play an important role in distinguishing between the presence or absence of disease. The underlying tissue changes in the disease process could be categorized as genomic, proteomic, or metabolomic expressions. Biomarkers include nucleic acids, proteins, peptides, enzymatic changes, antibodies, metabolites, lipids, and carbohydrates. Biomarkers can be derived from one, or a

combination, of the following body fluids blood, serum, plasma, body secretions (sputum, saliva), or excretions (stool, urine). Body fluids sample for biomarker investigation can be obtained by noninvasive, minimally invasive or invasive methods. Nucleic acids (DNA/RNA) extracted from blood, saliva, oral exfoliative cells, or buccal smear cells are instrumental in identifying mutations and will help to correlate and confirm the diagnosis, monitor the disease progression, or act as prognostic indicators in treatment.

Biomarkers can be used for

I patient assessment in multiple clinical settings by

- Estimating disease risk, screening for occult primary cancer.
- Distinguishing benign from malignant findings
- Distinguishing one type of malignancy from another,
- Determining prognosis,
- Acting as predictors/screening, and
- Monitoring disease status.

To either detect recurrence or determine progression/response to therapy

To help in the determination of a patient's risk of developing oral cancer & that can be done with risk reduction strategies and monitored with effective & higly sensitive screening methods & tools using biomarkers. These strategies when applied to high-risk groups are much more efficient than wholesale application to the entire population (Maha Yakob, 2014).

In saliva, Molecular signatures for the diagnosis of OSCC can be pursued in three levels:

- Changes in the cellular DNA, which result in,
- Altered mrna transcripts, leading to,
- Altered protein levels (intracellularly, on the cell surface or extracellularly (OSCC, 2016).

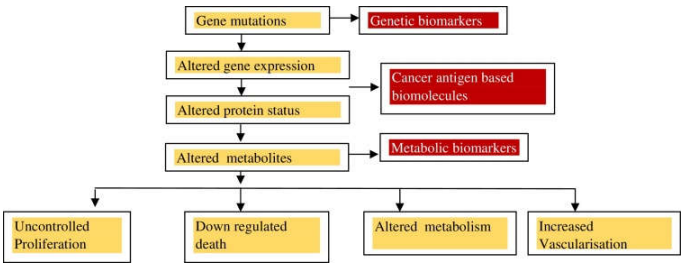

Fig. 1. Levels of molecular signatures for diagnosis of OSCC

In OSCC, biomarkers can be classified into groups based on their biological function as following:

Cellular Biomarkers

- Cell cycle progression & proliferation eg:EGFR ,Cyclin B1 overexpreesed indicate poor prognosis
- Tumor suppression & apoptosis eg:p53 & surviving overexpression indicate aggressive tumor & poor prognosis
- Hypoxia eg: GLUT-1, EPOR overexpreesed indicate poor prognosis
- Angiogenesis eg: VEGF & CD34 when positive indicate poor prognosis & LN metastasis
- Cell adhesion & Matrix degradation eg: MMP-7,-9,-13,-14 when positive indicate poor prognosis

SALIVARY BIOMARKER	USE
1) L-phenylalanine	screening biomarkers and help in the early diagnosis and monitoring of OSCC
2) Cloning of an acidic lactase gene 2 , a proteomic biomarker	used to differentiate between squamous cell carcinoma and adenocarcinoma.
3) CD34 , angiogenetic marker	serves as an important predicting tool for recurrent cases of OSCC.
4) ILs & TNF	Regulate and Mediate inflammation and angiogenesis ,so, deregulation in their production facilitates tumor growth, invasion and metastasis
5) HPV (mainly type 16)	may be a causative factor, chiefly among persons who do not smoke or drink alcohol
6) cyclin D1 gene amplification	Is associated with with poor prognosis in OSCC.
7) Ki67 marker levels	High in the saliva of patients with OSCC
8) Mammary serine protease inhibitor (Maspin) levels	found to be decreased in the saliva of patients with OSCC.

Humoral Biomarkers

- Parathyroid hormone related proteins
- Endothelins & their receptors eg: Endothelins
- Inflammatory cytokines & chemokines eg: IL-6

A biomarker must be verified and validated before it can be used in a clinical assay and have any impact or application in health risk assessment (Maha Yakob, 2014). Also, in biomarker research, the sensitivity and specificity of a marker must be determined in each study. Sensitivity is the true-positive rate, which is described by the percentage of the total number of people with the disease that test positive. Specificity is the true-negative rate, which measures the proportion of individuals that test negative for the disease that actually do not have the disease. The area under the receiver operating characteristics (ROC) curve (AUC) is also an important measurement when reporting biomarker performance. The AUC for a biomarker diagnostic test can range from 50%, which correlates to having no better insight than chance alone, to 100%, which denotes a perfect diagnostic test (Warnock, 2010 and Loo, 2010).

Various classifications of cancer biomarkers (Giulia Courthod, 2014)

1. Based on biomolecules:
- DNA biomarkers
- RNA biomarkers
- Protein biomarkers
- Glyco biomarkers.

2. Based on disease state:
- Prediction biomarkers
- Detection biomarkers
- Diagnosis biomarkers
- Prognosis biomarkers

3. Based on other criteria:
- Pathological biomarkers
- Imaging biomarkers
- In silico biomarkers

Recent advances in oscc detection

Recent developments in proteomic technologies, such as mass spectrometry, liquid chromatography, and protein/peptide labeling technologies, allow the detection of low abundance molecules in the saliva proteome. Numerous studies have reported that the proteomic profile of saliva most of which are synthesized and subsequently secreted into the oral cavity by the salivary gland acinar cells is different in OSCC patients from the profile for OSCC-free controls. In 2008, 1166 salivary proteins were initially identified in a National Institute of Dental and Craniofacial Research (NIDCR)-funded project that sought to catalog and annotate the human salivary proteome. This project was an essential first step for saliva to be clinically useful in disease diagnosis and health monitoring (Maha Yakob, 2014 and Loo, 2010). Similarly, the identification of aberrantly expressed miRNAs which disrupt the normal regulatory mechanisms in cancer cells is an important first step towards elucidating the details of miRNA-mediated oncogenic pathways, which are essential to know because, despite recent advances in various treatment modalities, the survival rates of patients with OSCC have not markedly improved (Wikner, 2014). Therefore, understanding molecular oncogenic pathways based on the current genome-based approaches underlying OSCC could significantly improve diagnosis, therapy, and prevention of the disease.

miRNAs as diagnostic biomarkers

Some recent studies identified a potential role of some miRNAs as diagnostic biomarkers. Actually, miRNAs circulate stably in different human body fluids, such as blood, saliva, urine and breath, hence, they can be accessible with non-invasive methods (Fukumoto, 2015). The miRNAs in saliva have certain advantages as biomarkers compared with other salivary biomarkers like proteins, mRNAs, DNAs and bacterial products. The advantages of miRNA are that, aside from the distinctive function of miRNA as post-transcriptional regulator, miRNAs are stably present in saliva and the similarity between miRNA profiles of saliva and other body fluids provides high availability as biomarkers for various human diseases (Su-Hwan Kim, 2015). Also, a correlation between the expression of specific miRNAs, and outcome of SCHNC & OSCC patients has been reported. Therefore, it was noted that, low levels of miR-375 correlated with poor survival and distant metastases whereas, high levels of miR-210 correlated with locoregional recurrence (Fukumoto, 2015).

Micrornas as Biomolecules (miRNAs)

Micrornas (miRNAs) are endogenous, evolutionarily conserved, naturally abundant, relatively stable, small (about 22 nt long) non-coding RNA molecules that function as post-transcriptional gene regulation in most biological and pathological process (Bano, 2015 and Anjie Min, 2015), are also known as micro-coordinators of gene expression, which have been shown to be an effective tools to study the biology of diseases and to have great potential as novel diagnostic and prognostic biomarkers with high specificity and sensitivity. It has been estimated that over 30% of protein-coding genes are regulated by miRNAs either by complementary binding of target mRNA or binding to imperfect complementary sites in the 3′ untranslated regions (3′UTR), leading to control of expression of these genes at the level of translation. It has been also calculated that above 45,000 miRNAs target sites are present in the human 3′UTR and above 60% of human protein-coding genes are probably regulated by multiple miRNAs (8). miRNAs are transcribed in the nucleus as a long, capped, poly-adenylated precursor called primary miRNA (pri-miRNA) by a RNA polymerase II or III . This pri-miRNA is processed by the ribonuclease (RNase) III called Drosha or the double-stranded DNA-binding Protein called DGCR8/Pasha producing a precursor miRNA (pre-miRNA). The nuclear export receptor exportin 5/Ran GTP actively transports pre-miRNAs to the cytoplasm . Then, Pre-miRNAs are processed by the RNase III endonuclease Dicer along with the trans-activation-responsive RNA-binding protein (TRBP) and produced a small double-stranded RNA structure (22 nt). This duplex miRNA is unwound into mature single-stranded form and integrate into the RNA-induced silencing complex (RISC), which escorts the complex into the complementary 3′UTR of the target mRNA.

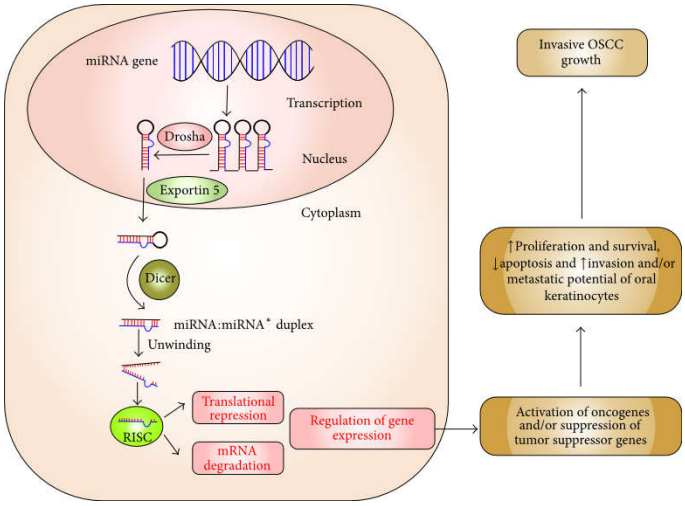

Figure 2. Transcription of miRNA

Role of MicroRNAs in oral cancer

MicroRNAs are important in tumourigenesis due to their proximity to chromosomal breakpoints and their dysregulated expression levels in many malignancies. Overexpression of certain miRNAs might result in the downregulation of tumour suppressor genes, while underexpression of other miRNAs might cause oncogene upregulation. Consequently, several studies evaluated the potential of miRNAs as diagnostic and prognostic biomarkers for cancers (Su-Hwan, 2015 and Momen-Heravi, 2014).

miRNAs in Tissues

miRNAs have an advantage that, they are stable due to the shorter lengths of miRNAs making them less susceptible to degradation caused by chemical and/or physical environmental factors. They can be easily isolated and measured from tissues and body fluids such as plasma, serum, saliva, milk, cerebrospinal fluids, so they are more useful. Expression motifs of serum miRNAs, are firmly linked to various diseases including cancer. Thus, interestingly, circulating miRNAs have many necessary features of ideal biomarkers (Faruq O, Vecchione, 2015). Also, a single miRNA can regulate expression and/or function of hundreds of target mRNAs and proteins and regulates several biological processes (e.g., cell proliferation, differentiation, migration, apoptosis, and signal transduction) important for cancer development Figure 2).

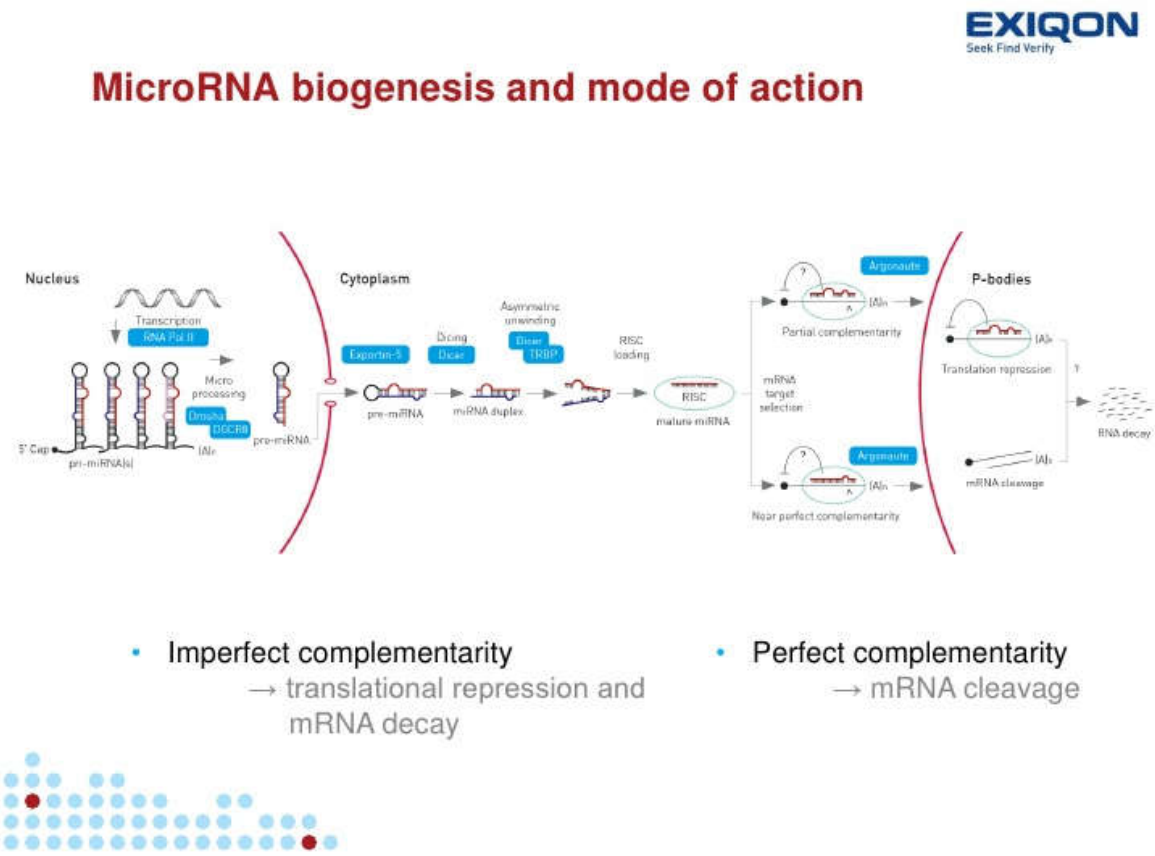

Fig. 3. Mode of Action of miRNAs

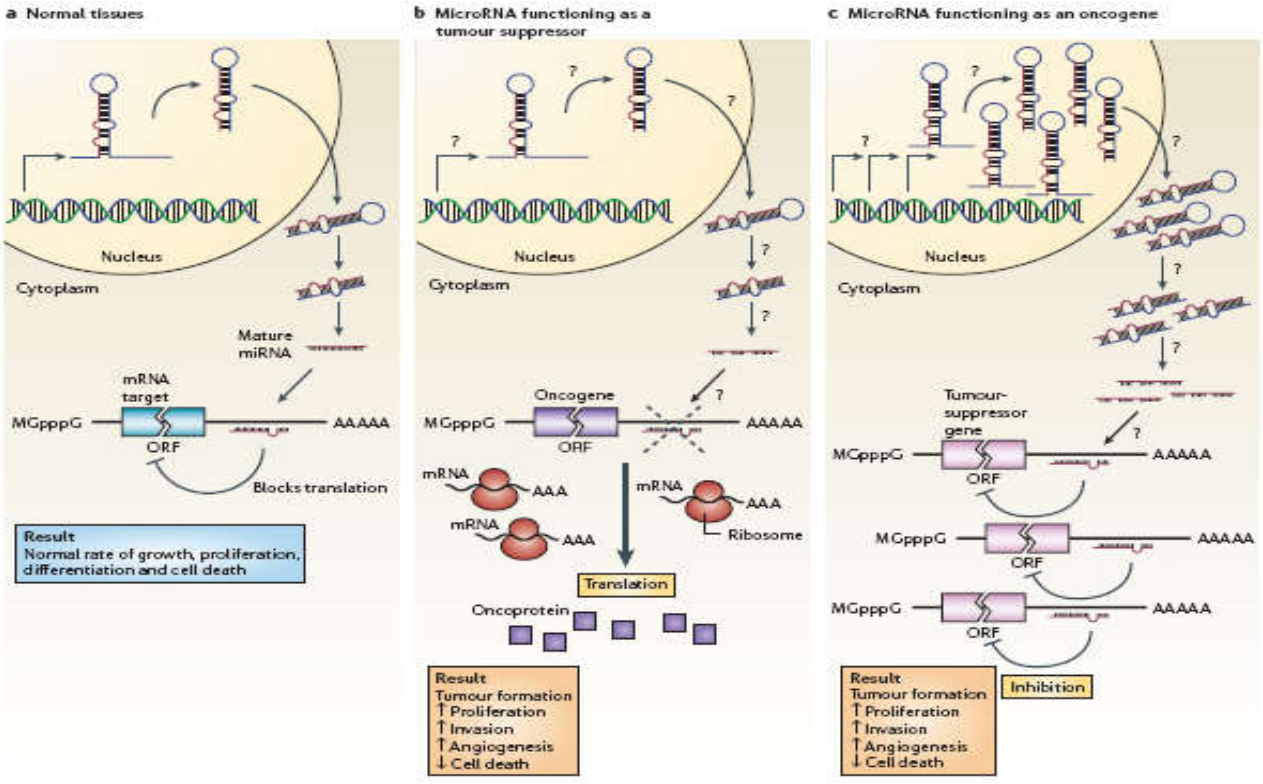

Fig. 4. MicroRNAs & Tumourogenesis

For almost three to four decades, changes in protein coding tumor suppressor genes and/or oncogenes have been thought to be the main drivers of tumor development. However, the recent discovery of thousands of genes that transcribe noncoding RNAs (including miRNAs) makes it obvious that cancer biology is even more complex than initially expected. Several layers of molecular regulators (e.g., mRNA, miRNA, and protein) are involved in the development and maintenance of cancerous phenotypes. Among them, miRNAs, have recently gained significant attention as potential regulators and

biomarkers for human carcinogenesis. Thus, in others malignancies, as well as in OSCC, miRNA regulates several oncogenes and tumor suppressors, driving the growth, proliferation, metastatic attitude and drug resistance also (Anjie Min, 2015 and Bartel, 2004).

development is highlighted and their potential value as diagnostic and prognostic markers for OSCC management is discussed. Normal cells have a finite life span and eventually reach replicative senescence in culture. Immortalization of such cells is the initial stage of tumorigenesis.

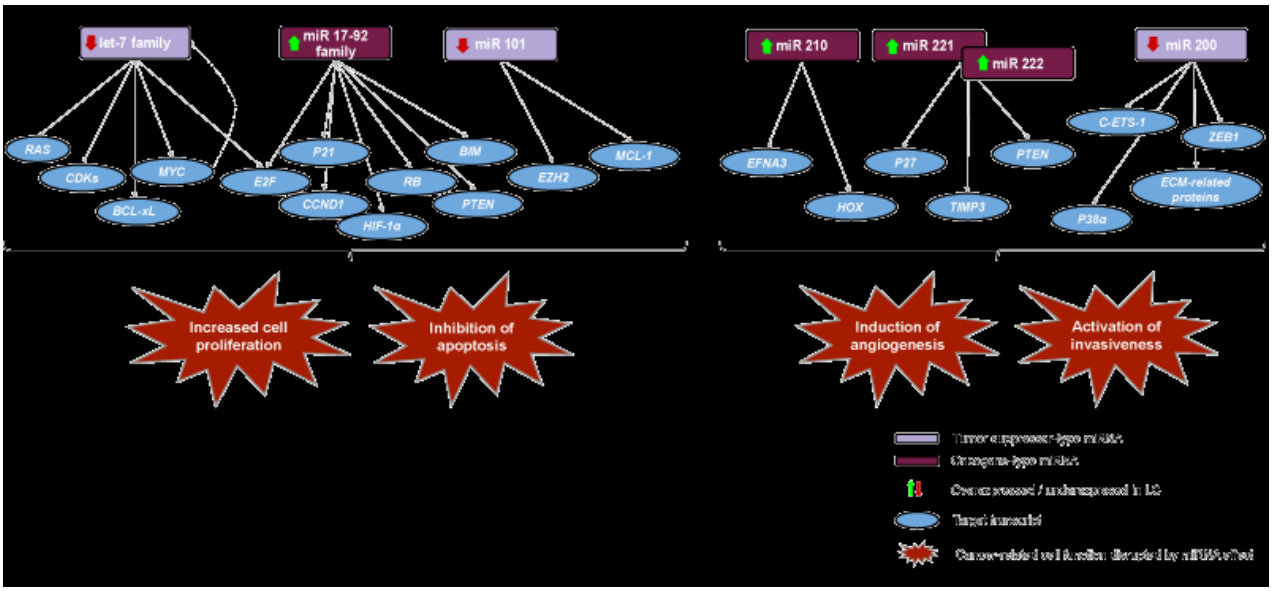

Figure 5. Multiple targets of single miRNA as shown in figure

Assessing miRNAs

The quantity of miRNAs can be easily estimated by various methods, such as Microarray, Hybridization, Deep-sequencing, qRT-PCR, and microbeads analysis. Therefore, circulating miRNAs have many necessary features of ideal biomarkers. Studies have shown that, saliva two miRNAs (miR-125a, miR-200a) were found reduced in oral squamous cell carcinoma patients (Li, 2009). Recent reports have also identified cancer specific miRNA signatures in OSCC cell lines and tissue samples, such as down-regulation of miR-137, miR-193a, miR- 375, miR-145 and miR-222 and up-regulation of miR-127, miR-21 and miR-10b. Thus, recent studies have shown deregulated expression of miRNAs in OSCC and OSCC-derived cell-lines compared to their normal counterparts, indicating their potential role in oral cancer development. Accordingly, several miRNAs have been shown to function either as tumor suppressors or as tumor promoters in OSCCs. In addition to their key biological functions in OSCC tumorigenesis, expression levels of several of miRNAs have been shown to correlate with clinicopathological variables and to have a diagnostic and prognostic value in OSCC (Yan, 2017 and Sethi, 2014). In a recently published study, significantly lower levels of miR-125a and mi-R200a were found in the saliva from 50 OSCC patients compared to 50 healthy control subjects . Salivary miR-31 increased significantly in patients with OSCC at all stages, and then decreased after the cancer had been excised. Along with the increased miR-31 in plasma, saliva and blood diagnostics may also lead to powerful OSCC biomarker prediction and disease progression (Maha Yakob, 2014 and Liu, 2012). Thus, salivary biomarkers can be used between biopsies to assist in monitoring the disease status of dysplasia patients. Although extensive and thorough biomarker validation is essential before any biomarker candidates can be tailored for clinical use, salivary diagnostics for OSCC are very promising due to the direct contact of saliva with premalignant or malignant lesions. In this review, the oncogenic and tumor suppressive roles of miRNAs in OSCC

This is followed by a transformation process that results in a frank neoplasm and then a further progression to fully invasive and metastatic malignancy. A series of molecular aberrances are involved in the malignant transformation and clonal expansion of neoplastic cells. Immortalization or partial transformation of normal oral keratinocyte (NOK) can be achieved by transfection with the HPV16 E6 and E7 oncogenes, which inactivates p53 and Rb, respectively. The human telomerase reverse transcriptase (hTERT) gene encodes the catalytic unit of telomerase that allows the maintenance of telomere length and also an escape from replicative senescence. Acquirement for hTERT expression is an early event in oral carcinogenesis. Moreover, hTERT expression increases as the severity ofOral premalignant disorder (OPMD) increases. Also, Jin Park *et al* found that, healthy saliva contains ~50 microRNAs. Two microRNAs, miR-125a and miR-200a, can discriminate oral cancer patients from control subjects ($P < 0.05$). The presence of microRNA in saliva is now substantiated, and it represents a third diagnostic alphabet in saliva, in addition to proteome and transcriptome (Park, 2009). Previous studies have confirmed the importance of miRNA alterations to head and neck squamous cell carcinoma (HNSCC) or OSCC carcinogenesis. miR-21**,** miR-31 and miR-184 have been found to be important oncogenic miRNAs, whereas various let-7 family members, miR-137 and other miRNA molecules are suppressor miRNAs in terms of HNSCC and/or OSCC.(5 ,27)

miR-21

A well-studied miRNA, the miR-21, has been shown to be overexpressed and to regulate several biological functions in OSCC. Overexpression of miR-21 has also been observed in oral premalignant lesions (oral leukoplakia) compared to normal oral mucosa, indicating that alteration in miR-21 could be an earlier event in OSCC progression. Experimental data have demonstrated an oncogenic role of miR-21 in OSCC by

promoting cell proliferation, invasion, antiapoptosis, and chemoresistance. These oncogenic functions were shown to be regulated by miR-21-mediated downregulation of several established tumor suppressor molecules, including PTEN, programmed cell death 4 (PDCD4), tropomyosin, reversion-inducing cysteine-rich protein with kazal motifs (RECK), and dickkopf 2 (DKK2). In addition to the functional roles in OSCC cells, a growing body of evidence suggests that miR-21 might be important in the regulation of carcinoma associated fibroblasts (CAFs) induction and their activity . Also, higher stromal expression of miR-21 was associated with poor prognosis in OSCC.

miR-31

miR-31 and its passenger strand miRNA (miR-) have been shown to be upregulated in oral leukoplakia (OLP) and OSCC and to have an oncogenic role in OSCC tumorigenesis. Experimentally, it repressed its target factor-inhibiting hypoxia-inducible factor (FIH) expression to activate hypoxia-inducible factor (HIF) under normoxic conditions & also affected several biological processes such as apoptosis , cell proliferation, migration, and epithelial-mesenchymal transition (EMT) in OSCC cells. It was shown to collaborate with human telomerase reverse transcriptase (hTERT) to immortalize normal oral keratinocytes (NOKs), indicating that it might contribute to early stage oral carcinogenesis .All these were seen to be mediated by the regulation of fibroblast growth factor 3 (FGF3) and RhoA expression levels. Salivary miR-31 (implicated in tumorigenesis) was appreciably superior in all stages of oral cancer, and salivary miR-31 was more copious than blood miR-31, , representing the oral tumor origin of this biomarker. Similarly, miR-146a overexpressed in OSCC enhances OSCC tumorigenesis with, concomitant downregulation of IL-1 receptor-associated kinase 1 (IRAK1), TNF receptor-associated factor 6 (TRAF6), and NUMB. Also, miR-134, miR=155 expression was upregulated in HNSCC tissue specimens and cells comapared to controls.

miRNAs as Tumor Suppressors in OSCC

Several miRNAs have been shown to be downregulated in OSCC.

- Mir-320 was downregulated in OSCC-derived cell-lines & tissue due to hypoxia & promotion of tumor angiogenesis.
- Downregulation of mir-99a was observed in OSCC patient specimens and cell-lines , especially in OSCC patients with lymphovascular invasion , suggesting a role for mir-99a in lymphovascular invasion. In addition, mir-99a induces apoptosis of OSCC cells.(30)
- Mir-218 has a tumor suppressive function but is epigenetically (DNA hypermethylation) silenced in OSCC tissue specimens
- Mirnas regulate .> EMT > EMT regulates tumor progression, invasion, and metastasis and acquisition of stem-like phenotype .
- Studies have shown that mirnas play a crucial role in the regulation of extracellular matrix (ECM) components, such as matrix metalloproteinases (mmps) and integrins.
- A mirna cluster, mir-17-92, including mir-17, mir-19b, mir-20a, and mir-92a, was found to be significantly downregulated in a more migratory OSCC-
- Mir-375, a tumor suppressor, was shown to be downregulated in HNSCC

Distinct expression profile of miRNA in OSCC and oral prelalignant tissue specimens compared to the normal controls offers the use of specific miRNA(s) signature for early stage diagnosis and prediction of OSCC prognosis (Yan, 2012). Firstly, they are abundantly expressed in OSCC and control tissues and hence their isolation and quantification are convenient and reproducible. Secondly, several OSCC-related miRNAs are secreted in bodily fluids such as serum, plasma, and saliva making them very useful for noninvasive clinical applications (Anjie, 2015; Li, 2009; Park, 2009; Elashoff, 2012).

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