Review Article

MicroRNA's – The vibrant performers in the oral cancer scenario

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SUMMARY

MicroRNAs (miRNAs) are a family of small non-coding (18–22 nucleotide) RNA molecules. These molecules regulate gene expression by either inhibiting mRNA translation or by degrading mRNA. A single miRNA can control the expression of target genes, and the expression of a target gene can be regulated by multiple miRNAs. They are key regulators of various biological and pathological processes. These include cell proliferation, development and tumorigenesis. Novel studies have discovered definite signature miRNAs in the initiation and progression of cancers. Interestingly, miRNAs have also been found in fragile genomic sites that are associated with increased cancer risk.

These micro RNAs regulate the expression of several genes that play a crucial role in the transition of normal oral mucosa through dysplasia to malignancy. The aim of this review is to recapitulate the current understanding of the many miRNAs that have been identified, the genes that they target and the role that they play in the carcinogenic pathway. The review also highlights the prospective role of miRNAs in the diagnosis, prognosis and treatment of oral cancers.

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1. Introduction

MicroRNAs (miRNAs) are a class of small mon-coding RNAs that regulate gene expression by repressing mRNA translation or by cleaving to target mRNA [1,2]. These small non-coding RNA molecules function as post –transcriptional regulators and are capable of causing both gene silencing and activation [3]. miRNAs are short (18–22 nucleotides) single stranded non coding RNAs. By base-pairing to the 3'-untranslated region (3'-UTR) of the target mRNAs, the mature miRNA is incorporated into the RNA-induced silencing complex (RISC) where it mediates gene expression by binding to the target mRNA [4,5]. It is believed that 50% of the human genes are regulated by miRNAs. These tiny RNAs govern many cellular, physiological, developmental and pathological processes [6]. There are numerous miRNA signatures that closely correlate with human diseases [7].

Dysregulations of microRNA have been reported in various cancers, and have been shown to play important roles in cancer initiation, progression, apoptosis, invasion and metastasis [8–13]. The miRNA signatures have been helpful at all levels right from diagnosis to treatment response [14]. Distinct miRNA expression profiles in OSCC have been demonstrated indicating that miRNAs may participate in the OSCC tumorigenesis [3,8]. This review summarizes the existing data on the expression and role of miRNAs in potentially malignant oral disorders and oral squamous cell carcinomas.

2. Analysis

The expression of miRNA is assessed through RNA extraction followed by reverse transcription- quantitative polymerase chain reaction. Cell proliferation assays and Western-blot analysis and cell culture methods have also been used to determine the expression of miRNAs. The miRNA levels in tissue sections can also be evaluated by in situ hybridization.

3. miRNA expression in oral leukoplakia

In a study by Brito et al. the expression of miR-21, miR-181-b and miR 345 was evaluated in 22 cases of oral leukoplakia and 17 cases of oral squamous cell carcinomas using quantitative polymerase chain reaction technique [14]. An increased expression

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Table 1
Association of the expression of microRNA with oral lecukoplakia, oral cancer and with various dysplastic features [14]. (Association as per chi square test).

Comparison	miR-21	miR-181-b	miR-345
OSCC vs. OL	0.02	0.02	0.0002
OSCC vs. Normal oral mucosa	0.001	0.05	0.005
Oral Leukoplakia vs. Normal oral mucosa	0.001	–	
Increased mitotic figures	0.02	0.01	
Abnormally superficial mitosis	0.01		
Increased nuclear-cytoplasmic ratio	0.03	0.02	0.005
Hyperchromasia	0.02	0.02	
Increased number and size of nucleoli			0.04

Table 2
The miRNA's, the target genes and their biological implications in oral submucous fibrosis [28].

miR	Target Genes	Experimental finding	Biological implication in OSF
miR-509-5p	BMPR2, CDH6, HAS3	Down regulated	Inhibits cell proliferation and migration Promotes apoptosis
miR-610	CDH1, DSC2, KRAS, MMP19, MAPK1, TIMP3	Down regulated	Has a role in forming defective collagen
miR-760	CDH4, COX10, IL6, Il6R, IGFIR, TIMP2, TGM2	Downregulated	As a predictive marker for pre-cancer
miR-455	BMP7, BMPR, DSC1, MAPK14, MAPK11, IGF1, TIMP2, TGM 3	Upregulated	Has a role in the molecular pathway of OSF
miR-623	MAPK1, MAPK11, MAPK4, MMP1, MMP8, TIMP2, IL10	Upregulated	Has a role in the excess formation of collagen

of miR-21 was evident in dysplastic lesions and oral squamous cell carcinomas compared to normal oral mucosa. An increased nuclear/cytoplasmic ratio was associated with a high expression of miR21, mir-181b and miR 345 (Table 1).

Xiao et al. extensively evaluated 27 cases of oral leukoplakias and oral leukoplakias that transformed to oral cancers using FISH analysis, Cell migration and invasion assay, plasmid reconstruction and qRT-PCR. They found that microRNA 31* down regulates FGF3 and facilitates the progression of oral leukoplakia to oral cancer [15].

4. miRNA's involved in the progression of oral leukoplakia to oral squamous cell carcinoma

In a study by Philipone et al. the expression of miRNA 129-2-3p, miRNA 204-5p, miRNA-208b-3p, miRNA 3065-5p were evaluated in oral leukoplakia cases that progressed to oral squamous cell carcinoma ($n = 40$ cases) and those oral leukoplakia cases that did not progress to oral squamous cell carcinoma ($n = 40$ cases) using quantitative real time polymerase chain reaction technique [16]. In this study there was an over expression of miR-208b-3p and miRNA-3065-5p, whereas miRNA 204-5p and miRNA 129-2-3p were under expressed in the cases that progressed to oral cancer. miR-208-3p has a presumptive oncogenic role and it's expression is associated with increased cellular proliferation, cell cycle progression and tumorigenitiy. Overexpression of miR208- 3p leads to down regulation of SOX-6 protein which results in downregulation of p21, upregulation of cyclin D1 and deregulation RB via phosphorylation [17].

miR3065-5p reduces cell migration and invasion and serves as a tumor suppressor [18]. Hypermethylation of the promotor region and silencing of miR-129-2-3p leads to overexpression of SOX4 and Cdk6 that result in tumorigenesis. Epigenetic alterations are a frequent event in oral carcinogenesis [19,20]. miR-204-5p is thought to have a tumor suppressor function, it's downregulation via hypermethylation is associated with increased metastasis and decreased overall survival. It is located at the genomic imbalanced 9q21.1-22.3 locus associated with genetic predisposition to head and neck cancer [21,22]. In addition, Maimaiti et al. found that 3-miRNA signatures (miR-129-5p, miR-339-5p and miR-31) were hubs that mediated the initiation and progression of oral leukoplakias from the non-malignant to the aggressive one via targeting various transcription factors [23].

5. miRNA expression in oral submucous fibrosis

Oral submucous fibrosis is a prevalent precancerous condition in the south-east Asian and the Indian subcontinent. This condition is etiologically linked to the habit of chewing betel quid. The obvious clinical sign of this condition is the development of progressive trismus. Chickooree et al. carried out an Agilent human miRNA microarray assay to determine the miRNA expression of oral submucous fibrosis in 3 cases of Oral submucous fibrosis and three normal oral mucosa samples [24]. In this study, miR455, miR-760, miR-623, miR-610 and miR-509-3-5p and their target genes were assessed (Table 2).

Chattopadhyay et al. investigated the expression of miRNAs and their target genes in oral potentially malignant oral disorders and oral cancers. It was evident that there was a significant increase in the expression of miR 31 in the precancerous lesions and oral cancers. There was a 3-fold increase in the expression of miR31 in oral submucous fibrosis [25].

6. microRNA expression in oral squamous cell carcinoma

In a study carried out by Liao et al. to analyze the expression of micro-RNA in 106 paired normal and tumor tissue samples using quantitative reverse transcriptase polymerase chain reaction, there was an increased expression of miR-1246 in oral squamous cell carcinoma tissues compared to non-cancerous tissue. The mean value of 3.47 was utilized to divide the oral squamous cell carcinoma group into high expression group and low expression group. There was a significant positive correlation between the expression of miR-1246 and the TNM stage, nodal status and the tumor grade. Patients with a higher expression of miR 1246 tended to have much lower survival rates than patients with lower miR 1246 expression [7].

miR 1246 can promote stem-cell like properties in tumor cells [26]. It can also specifically target the 3'-UTR of CADM1 and downregulates it's expression that leads to enhanced migration and invasion capacity [12]. Enhanced miR-1246 suppresses thombospondin-2 and facilitates tumor proliferation, invasion and migration [13].

In a study by Kawakita et al., the mechanism of action of miRNA-21 in 79 primary oral tongue squamous cell carcinomas was assessed using western blotting and in situ hybridization. An overexpression of miRNA -21 correlated with the pattern of tumor invasion ($p = 0.016$). They also found that miR-21 overexpression correlated inversely with the immunohistochemical expression of ß-catenin and Dickkopf-2 (Dkk-2). Dkk-2 expression was reduced in the area of miR-21 overexpression. They suggested that miR-21 inhibits tumor suppressor DKK-2 that in turns activates the Wnt/ß-catenin pathway and promotes tumor invasion in tongue oral squamous cell carcinomas. Also a miR-21 knockdown decreased the invasion potential with upregulation of Dkk-2 in tongue oral squamous cell carcinomas [27].

It is believed that miR-21 induces cell proliferation and inhibits apoptosis by regulating phosphatase and tensin homolog (PTEN) and Tropomysin-1 (TPM1) [28–32]. miR-21 overexpression is

Table 3
Summary of the role of miRNA's in oral squamous cell carcinomas.

MicroRNA	Target Genes/component	Role in the carcinogenic process
miRNA-21 (up-regulated)	• Inhibits a tumor suppressor DKK2 • It activates the ß catenin/WNT • Stromal fibroblast-like cells	• Promotes tumor invasion • Associated with increased risk of relapse
miR-1246 (up-regulated)	–	• Proliferation • Invasion and metastasis
miR-210 (up-regulated)	–	• Marker of Hypoxia • Loco-regional disease recurrence • Shorter overall survival time
miR-143 (Down regulated)	Hexokinase -2	• Suppresses cancer cell proliferation, migration and invasion
miR-223 (up-regulated)		• Associated with advanced size and stage of the tumor
miR-1297 (Down regulated)	PTEN	• Promotes tumor progression
miR-10 a (up-regulated)	Glut-1 upregulation	• Promotes Cell proliferation
miR-545 (up-regulated)	RIG -1	• Inhibits cell proliferation and migration

involved in many aspects of cancer including proliferation, invasiveness, metastasis and chemo-sensitivity [33–37].

Hedback et al. investigated the expression of miR-21 in stromal fibroblast-like cells and endothelial cells in 111 cases of tongue and floor of the mouth cancers using in situ hybridization and immunohistochemistry. They found a close association between miR-21 staining and alpha- smooth muscle actin staining. miR-21 expression was found in the center of the tumor and not at the invasive front. There was a correlation between a high expression of miR-21 in the stroma and an increased risk for relapse. The survival curve showed that patients with a high miR-21 expression had a significantly shorter disease free survival when compared to the patient having lower miR-21 expression [38].

Gee et al. investigated the expression of 3 microRNAs hsa-miR-210, hsa-miR-21, and hsa-miR-10b in 46 cases of head and neck cancers using real time reverse transcription Polymerase chain reaction and immunohistochemistry. High levels of hsa-miR-210 was associated with loco-regional disease recurrence and short overall survival. hsa-miR-21 and hsa-miR-10b had no prognostic significance. They suggested that hsa-miR-210, in addition to being a marker of hypoxia, may regulate critical pathways not detected at the transcript level, and have functions that are important in tumor behavior under hypoxic conditions, or in aggressive tumors that are hypoxic [39].

Sun et al. evaluated the expression of miR143 in 15 cases of oral squamous cell carcinomas using quantitative reverse transcription polymerase chain reaction and immunohistochemistry. It was found that miR143 suppresses OSCC proliferation, cell cycle and colony formation and promotes apoptosis. Overexpression of miR143 caused cell cycle arrest in tumor cells in the G1 phase. The colony formation assay on cancer cell lines revealed that the overexpression of miR-143 suppressed colony OCEM 1 and TCA 8113. miR 143 inhibited cell proliferation, invasion and glucose metabolism through directly targeting Hexokinase 2 [40].

Manikandan et al., analyzed the differential expression of 10 miRNAs in 61 primary oral squamous cell carcinomas using miRNA microarrays and reverse transcriptase polymerase chain reaction. They determined the association between the expression of miRNAs and the clinicopathological features of the patients. They found that let-7a, let-7d, let7f and miR-16 were downregulated. miR-

29b, miR142-3p, miR 144, miR 203 and miR-233 were upregulated. MiR-142-3p was the top upregulated miRNA which was overexpressed in 87% of the oral squamous cell carcinomas. A high level of miR-223 was associated with advanced tumor stage and size. They proposed that miRNA mediated repression may operate as an alternate mechanism of p53 inactivation [41].

Kolokythas et al. investigated the micoRNAs in oral mucosal cells that were obtained through brush cytology from squamous cell carcinoma patients who never smoked ($n = 12$ cases) and smokers ($n = 8$ cases) through quantitative reverse transcription polymerase chain reaction. Through their investigation they found that miR 31-5p, miR-196a-5p and miR-503-5p were enriched in the tumor epithelial cells of tumors from both non smokers and tobacco users. This study showed that there was >2 fold change ($p < 0.05$) in the differential expression of miR196-5p in oral squamous cell carcinoma patients who smoked compared to patients who smoked but with non- malignant lesion [42].

Towle et al. also evaluated the dysregulation of miR-224, miR-135a, miR-143, miR223, miR155, miR-720 and miR-605 in 27 cases oral squamous cell carcinomas using tissue microarrays and in situ hybridization. In this study there was an upregulation of miR-21 and miR224. There was a down regulation of miR720, miR-375 and miR-605. miR-155 was found to be upregulated in both dysplasia and OSCC tissues. It exhibited a 4.14 fold change in the expression in dysplastic lesions compared to normal tissues and a 4.77 fold change in the expression in CIS/OSCC compared to normal tissues. They suggested that miR155 may represent an early and sustained driver of oral tumorigenesis in non- smokers and smokers [43].

Recently, Liang et al., evaluated the expression of microRNA-1297 in 16 cases of oral squamous cell carcinoma using western blotting and colony forming assays. They found that miR-1297 played a vital role in tumor cell growth by through regulation of PTEN. The expression of miRNA 1297 was lower in the tumor tissues compared to the non- tumor tissue. A down regulation of this miRNA was inversely related to PTEN expression. Secondly, an overexpression of miR-1297 resulted in proliferation of SSC-4 cells [44]. Chen et al. assessed the expression of miRNA 10 in 52 cases of oral squamous cell carcinomas employing reverse transcription polymerase chain reaction and cell proliferation assays. They found that miRNA 10a promotes cell proliferation in oral squamous cell carcinoma as an upstream activator of Glut 1 and by also promoting glucose metabolism [45]. Yuan et al. assessed the expression of microRNA 545 in 20 cases of oral squamous cell carcinomas through reverse transcription –Quantitative polymerase chain reaction. They found that microRNA-545 inhibits oral squamous cell carcinomas by targeting RIG 1. An overexpression of miRNA inhibited HSC 4 cells proliferation and migration [46].

Baghaei et al. evaluated the associated between the expression of PTEN and micro RNA-26b using RNA extraction and Quantitative-Real time polymerase chain reaction. They found that the proliferative activity induced by PTEN on tumor cells is regulated by micro RNA-26b [47]. A decrease in PTEN activity was also found to promote immunoresistance through activation of B7-H1 in glioma [48]. In addition miR -26b activity was found to block the Wnt/βcatenin pathway in intestinal carcinomas [49].

7. Therapeutic implication

Yu et al., reviewed miRNAs that were involved in the carcinogenic process of tongue squamous cell carcinomas. They deduced that miR-21 was an independent prognostic indicator of poor survival in tongue squamous cell carcinoma patients. The expression of miR-24 was also upregulated in these carcinomas, which led to enhanced proliferation and reduced apoptosis in the tongue squamous cell carcinoma cells. In addition the expression of miR184 was

higher in tongue squamous cell carcinoma patients when compared to normal individuals [50]. In a study by Yu et al., miR-21 served as a chemosensitive miRNA, while miR-214 and -23a served as chemoresistant miRNAs in the TSCC lines [51]. Petronacci et al. evaluated the role of miRNAs miR-497 5p and miR-4417. They found that an overexpression of miR-497 5p was associated with a better overall survival, while an overexpression of miR 4417 was associated with poorer overall survival [52]. A recent study multicentre study by Yan et al., it was evident that increased levels of circulating miR-486-5p, miR-375 and miR92b-3p in plasma were indicators of recurrence of OSCC [53].

8. Conclusion

Micro RNAs are distinct biological regulators with a "license to kill the messenger". Taken together, alterations in the cellular levels of miRNAs might have a major impact in the proteome of the cancer cell thereby contributing to oral carcinogenesis. miR-21 is one of the most common micro RNA that is overexpressed in leukoplakias and oral squamous cell carcinomas. miR-208b-3p and miRNA-3065-5p were found to be overexpressed in oral leukoplakias that progressed to oral cancers. A high expression of miR1246 has a significant effect on the overall survival of the patients. High level of hsa-miR-210 was associated with locoregional disease recurrence and a short overall survival time (Table 3). Identifying the differential expression of miRNAs in oral squamous cell carcinomas can lead to the development of novel therapeutics strategies.

Conflict of interest

None declared.

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