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# Role of miRNAs in Precancerous Oral Lesions and Oral Squamous Cell Carcinoma: A Current Update

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## Abstract

Oral cancer can originate from any mouth tissue, including tongue, lip, cheek, floor of the mouth, sinuses, pharynx (throat), and hard & soft palate. Delayed clinical detection, poor prognosis and lack of appropriate treatment strategies are some of the characteristics of this cancer. Epigenetic alteration is a common event in neoplasia. A significant number of current scientific articles have investigated the role of genetic and epigenetic changes in cancer. Among various types of epigenetic events, we sought to explore the epigenetic regulation of miRNAs which has an established role in various cancers. The present article provides a comprehensive review of the literature regarding epigenetic regulations of miRNAs in precancerous oral lesions and oral squamous cell carcinoma.

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## INTRODUCTION

Oral cancer originates from any mouth tissues, including the tongue, lip, cheek, floor of the mouth, sinuses, pharynx (throat), and hard & soft palate. Delayed clinical detection, poor prognosis and lack of appropriate treatment strategies are some of the characteristics of this cancer. Although some potentially malignant precancerous lesions of oral mucosa like leukoplakia, lichen planus, and erythroplakia can be easily diagnosed early recognition of these lesions is often challenging.

Mouth cancer, also referred to as oral cancer, is a type of head and neck cancer and is defined as any cancerous tissue growth positioned in the oral cavity.[1] American Cancer Society's most current figures for 2022 showed that 54,000 people will be diagnosed with oral cancer or oropharyngeal cancer, and 11, 230 of these cases will be fatal in United States.[2] Global variation in the incidence of oral squamous cell carcinoma with geographic, ethnic, and cultural differences indicates a role for important genetic and environmental factors during the emergence and development of the disease. Genetic and epigenetic changes play an important role in the etiology of the disease. Epigenetic regulatory mechanisms of miRNA play an important role in cancer.

## ORAL SQUAMOUS CELL CARCINOMA (OSCC)

Oral cancer, also known as oral squamous cell carcinoma (OSCC), makes up 90% of all oral malignancies and 38% of head and neck cancer cases[3]. It is the most prevalent malignant cancer in the head and neck region, ranking 16th among all malignancies and 15th as a cause of death globally. The age-adjusted global incidence of oral cancer is four cases per 100,000, with significant variations based on gender, age group, country, race, ethnic group, and socioeconomic conditions.[4] Oral cancer is traditionally defined as oral squamous cell carcinoma (OSCC), which accounts for 90% of all oral malignancies and 38% of head and neck cancers.[3] Oral cancer is the most common cancer of the head and neck). group, country, race, and ethnicity.

Oral cancer is the most commonplace head and neck malignancy in the world, with a projected 34,864 new cancer patients reported worldwide in the year 2018 and this probably increase to 377,713 in 2020, more than doubling the 185,976 cases detected in 1990 and demonstrating its rapid growth.[5,6] The incidence of oral cancer is highest in Melanesia

and South-Central Asia - about 52% of all cases worldwide, tracked by Eastern Europe and then Australia/New Zealand.[6]

The estimated global incidences of OSCC cases are around 2, 75,000 per annum, out of which 2/3rd occur in developing countries.[7] The 20-fold wide variations in the geographical distribution of this disease are reported and the incidence in India and the whole of South and Southeast Asia is the highest in the world[8]. However, it is now increasing in Western and Eastern Europe, Latin America and the Pacific regions also.[9] The Indian subcontinent, especially India is considered the global epicentre of OSCC.[10] OSCC ranks as the second most frequently diagnosed cancer in men in India, making up to 20% of all cancer cases. It also stands as the fourth most common cancer in women in the country.[11] The National Cancer Registry Programme, Indian Council of Medical Research, New Delhi, India, has generated a forecast indicating an increase in the number of OSCC cases and deaths by 2020.[12] The Indian subcontinent, especially India regarded as the global epicentre of OSCC.[10] The National Cancer Registry Program, Indian Council of Medical Research, New Delhi, predicts an increase in OSCC cases and deaths by 2020.[12]

OSCC is the second most prevalent cancer in India, with around 120,000 new cases diagnosed annually according to Globacon-2018 data.[5,13] Among Indian males, it is the primary cause of cancer-related fatalities, claiming over 72,000 lives each year in the country.[14]

The 5-year survival rate of OSCC in the world is approximately 50% and in India, it is around 40–45%.[15] According to SEER (Surveillance, epidemiology and end results, National Cancer Institute, USA), 62.2% were the overall 5-year relative survival for 2003-2009 from 18 SEER geographic areas. The 5-year relative survival by race and sex was 63.6% for white men and 64.6% for white women; 38.6% for black men and 53.0% for black women[16] (MD): National Cancer Institute; 1975-2002).[17] Like many forms of cancer, OSCC incidence also increases with age. This is mainly due to the longer exposure to potential risk factors which increase chances of damaging cellular DNA in the aging cells. It is reported that about 90% of the OSCC patients are over 40 years of age, with 65 as the average age at diagnosis.[18] Epidemiological data reported that the incidence of OSCC is increasing among youngsters rapidly over the past decades.[19] In India, a significantly high incidence is reported in the low- and middle-age groups mainly because the habit of high consumption of tobacco started at a relatively early age.[8] Moreover, it usually affects males more frequently than females. According to GLOBOCAN 2008 project, incidence in developed countries was 6.9% for male and 2.4% for females, whereas in developing countries, 4.6% for male and 2.6% for female. Males have a higher occurrence of OSCC, partly due to increased use of risk factors like tobacco and heavy alcohol. In India, where women also commonly chew tobacco, the incidence of OSCC is reported to be equal to or even greater than that among men[20]. India has many religious communities, with Hindus bearing the highest burden of oral cancer across the country. However, there is no national data available to confirm the same.[21]

## PRECANCEROUS ORAL LESIONS

The oral mucosa has several histologically distinguishable lesions that can be malignant and are called precancerous lesions (PCOLs).[22] The lesions identified are leukoplakia (LKP), erythroplakia (ERK), oral lichen planus (OLP), and submucous fibrosis (SMF).[23,24] Researchers have observed these lesions in connection with and prior to OSCC.[25] World Health Organization (WHO) in 2003 categorized PCOLs by the presence or absence of oral epithelial dysplasia as slight, moderate, or severe hyperplasia or carcinoma in situ, according to the cellular atypia and other morphological aspects of the epithelium.[26] The regular use of cancer-causing agents like tobacco, betel nut, and alcohol a co-carcinogen to the oral mucosa may lead to PCOLs transformation into OSCC. The spectrum of OSCC development is usually established by the multiple clonal, cytogenetic, and epigenetic alterations which reflect the varying ranges of malignant transformation and their incidence rate.

## microRNAs (miRNAs)

Lee *et al*. identified mature miRNAs as single-stranded, endogenous, non-coding RNAs that are evolutionarily conserved with a length of roughly 19–26 nucleotides. Target mRNAs' 3' untranslated regions (UTRs) contain complementary seed sequences that miRNAs preferentially interact with. Target mRNAs' 5' UTRs and coding regions may also contain miRNA binding sites.[27] The process essential for maintaining cellular balance is fundamental to biological systems and is mediated by miRNA gene silencing, this leads to inhibition of translation, which is then followed by deadening and degradation of mRNA.[28,29] miRNA-mediated gene silencing also plays an imperative role in both intra and intercellular communication via circulating cell-free miRNAs.[30,31]

Approximately 2600 mature miRNAs in humans are encoded by 1872 identified miRNA precursor genes, according to GENCODE data (v. 29).[32] The functions of many of these miRNAs are still unknown.[33] Up to a hundred different transcripts may be bound by a single miRNA.[32] In addition, a specific mRNA can attach to a variety of miRNAs at the same time or under different circumstances.[34]

The miRNA field has experienced a major detonation in recent years with thorough investigations by various innovative technologies. The miRNA gene family is constantly expanding with new members that were predicted around 2005, indicating that the human genome comprises approximately 1000 miRNA genes.[35] According to Sanger miRBase 2013, there are 2578 mature and 1872 precursor human miRNA sequences cataloged in the miRNA registry.[36] The average length of these is 22 nucleotides, ranging from 18 to 25

nucleotides evolutionarily conserved, noncoding RNA sequences that regulate gene expression in essentially all eukaryotic organisms.[37] The 1st miRNA was Lin-4, a small RNA, which did not encode the protein, but suppressed the expression of Lin-14 protein in the nematode Caenorhabditis elegans.[38] The miRNAs are involved in many biological regulations of cellular mechanisms with proliferation, differentiation, progression, apoptosis and metabolism.[39] Over 30 to 50% of gene expression is regulated by miRNAs at the post-transcriptional level with degradation or translational repression of their target mRNAs transcript.[40] The expression of numerous protein-coding genes can be regulated by each miRNA (10-100 or more), but every miRNA is not alike important. A small number of key functioning miRNAs have been identified in a variety of high throughput assays. Over the last few years, the functions and target genes of miRNAs have been relatively well studied, while many other genes are still being studied. Therefore, miRNAs have become the main regulators that can control a wide range of physiological and pathological functions for cells.

## miRNAs AND CANCER

The expression of approximately 60% of human genes is controlled by miRNAs as reported by Liu *et al*. in 2014. It is worth noting that a single miRNA molecule can bind to multiple target mRNAs. Several miRNAs can simultaneously inhibit a single mRNA molecule. The impact of the interaction with target mRNA molecules depends on the complementarity of the bond and the levels of expression of miRNA and mRNA, according to Alles *et al*. in 2019. In various diseases, including cancer, disruptions in miRNA expression have been linked to the absence or presence of specific miRNAs in tissue, as well as alterations in the expression of specific miRNAs.[41]

The miRNAs may be the largest class of gene regulation in carcinogenesis. 25.8% of the known human miRNAs expression is dysregulated in at least one type of cancer. The frequent chromosomal deletion of miR-15-a and miR-16-1 and their downregulation was the first evidence reported in chronic lymphocytic leukemia (CML).[42] Michael *et al*., 2003 reported miR-143 and miR-145 downregulation in the solid tumor of colorectal carcinoma.[43] The majority of the miRNAs are downregulated in cancer; however, some are reported to be upregulated. These variations were observed specifically for both tissue and tumor[44,37] and can cause changes in the expression of their target genes, resulting in cellular dysfunction and ultimately cancer.[45] The miRNAs may be a tumor suppressor or oncogenic in function. Tumor suppressor miRNAs (ts-miRs) target and regulate the proto-oncogenes, so when they are downregulated or silenced, the target oncogenes are overexpressed while, on the contrary, oncogenic miRNAs (onco-miRs) target TSGs when overexpressed.[44] The miR-21 was the first onco-miR that is universally overexpressed in cancer.[46]

## miRNA AND OSCC

The involvement of post-transcriptional modification and/or translational repression of gene expression has been characterized by driving cancer progression.[47-49] Furthermore, the modification of miRNA expression, which impacts the expression of numerous protein-coding genes, is significant for clinical cancer prognosis.[50,51] The recognition of biomarkers related to toxicity and transcriptomic signals associated with effects is crucial in developing drugs.[52] In OSCC, miRNAs exhibited elevated expression, rendering them potential candidates for cancer screening and diagnosis. The link between these markers and OSCC is being explored in primary tumors, biopsies, serum, and saliva.[51,53,54] Research aimed at improving the accuracy of patient categorization through the identification of novel prognostic biomarkers for oral cancer has demonstrated that elevated expression of miR-155 is positively connected with survival and that this impact is also substantially correlated with high numbers of CD8+ TILs. Furthermore, miR-185 was linked to a worse survival rate on its own.[55] Numerous aspects of oral cancer are linked to miRNAs, with epigenetic modifications and altered expression levels receiving particular attention.[56] The most extensively studied miRNAs in OSCC included miR-21 and miR-155, with miR-155e-5p contributing to tumor growth and showing a significant increase in OSCC tissues and cell lines.[57] Several studies have demonstrated the overexpression of miR-21 in OSCC.[58] MiR-770 was found to be an OncomiR, contributing to increased OSCC metastasis. Markedly elevated levels of miR-1237 were observed in OSCC tumor samples, particularly in the early stages (stages I and II), and this was associated with a poor prognosis for OSCC patients. It was revealed that oral cancers exhibited significantly lower expression of miR-205, and its overexpression reduced cell viability and induced cell death by activating caspase-3/caspase-7.[89]

miRNAs have a direct role in cancer since they can either activate or inhibit target mRNAs and cause their dysregulation inside the tumor.[90,91] The 1st report on the profile of miRNAs expression in the head and neck SCC (HNSCC) was reported in 2005 by Jiang *et al*. The aberrant miRNAs expression profiles were reported in OSCC tumors, cell lines and animal models[40] (another study by Wong *et al*., 2008 of tongue SCC with matched normal tissue, identified 8.3% downregulated and15.4% upregulated miRNAs, using a threshold of 3-fold changes in expression level.[81] Afterward, Childs *et al*., 2009 reported downregulation in 18.2% and upregulation in 2.5% of miRNAs using a 2-old expression change as a threshold in HNSCC.[92] In contrast, a miRNA profiling study showed only 3% of miRNAs are differentially expressed (8 overexpressed and under-expressed) in HNSCC compared to normal tissue.[93] The miRNA expression in OSCC is possibly tailored through many aspects like the tumor's location, its characteristics, and the presence of HPV infection or small-nucleolar RNAs all contribute to individual variations among patients. Research has shown significant statistical evidence

that miRNA expression in tumors and their metastases is similar, but the expression of the same tumor varies among different individuals. Additionally, the same tumor in different locations exhibits different expression profiles.[94]

The impact of HPV on miRNA expression could help in understanding the distinct clinical behavior of HPV-infected OSCC tumors. The miRNAs and HPV both mostly spread throughout non-coding sequences of the genome. The oncogenic high-risk HPV16/18 types are comparatively more common among HNSCC patients and preferences in oropharyngeal topographical sites than in other sites.[95] Wald *et al*. (2011) conducted a study on HPV positive and negative HNSCC cell lines and detailed the correlation between the expression of HPV-derived E6 oncogene and the overexpression of miR-363 or the under-expression of miR-181a, miR-218, and miR-29a in HPV positive HNSCC cell lines.[96] Inhibition of HPV16 oncogene E7 inhibits the proliferation, invasion, and migration of OSCC cells to upregulate miR-20a expression.[97] Afterward, Lejer *et al*. (2011) analyzed the miRNAs expression in OSCC and pharyngeal SCC patient samples to illustrate the effect of HPV and revealed 21 miRNAs perturbation in HPV-positive OSCC patients.[98] Further another study reported significant overexpression of miR-106-363 cluster in HPV-positive HNSCC.[99] The precise contribution of HPVs to miRNA imbalance and their role in OSCC development remains uncertain and requires additional research.

A variety of expression profiling experiments intended to detect dysregulated miRNAs in cancer and HNSCC demonstrated that miR-21 was the highest hit.[100] Hedback *et al*. (2014) performed in situ hybridization to recognize the role of miR-21; a most consistent overexpressed miRNA in many kinds of cancer including paraffin-embedded tumor tissue samples from primary OSCC patients.[101] The miRCURY LNA array with probes specific to 1168 miRNAs and validation of 10 miRNAs by TaqMan assays in OSCC primary tissue samples showed miR-1275association with lymph node invasion and the upregulation of miR-144 in OSCC.[58]

## miRNAs AND PCOLs

Precancerous lesions of the oral cavity are easily detected due to the accessibility of the habitat. The current inability to determine which PCOLs will evolve into OSCC is a major factor contributing to the poor prognosis of OSCC. So far, the histopathological study is incapable of delineating progression risk. Identifying miRNA markers that could indicate the likelihood of PCOLs becoming malignantly would be a very helpful tool for OSCC early diagnosis. An analysis of miRNA at the genome level was conducted on incisional biopsy tissues of oral leukoplakia patients preserved in formalin-fixed paraffin, which revealed the identification of 4 potential miRNAs - 208b-3p, 204-5p, 129-2-3p, and 3065-5p. Huang *et al*. (2007) performed miRNA profiling for a comparison of LKP with and without progression and foundmiR-21, miR-181b and miR-345 overexpression were related to high severity of precancerous lesions and suggested that these miRNAs might serve as biomarkers for the primary detection of progressive LKPs that are in danger for transmuting into malignant lesions.[59] The same observation was documented[60] in progressive and non-progressive LKP. The under expression of miR-155, miR-let-7i and miR-146a in OSCC were characterized the metastatic tumor progression.[61] In the contrary, more than 2-fold upregulation of miR-155 was observed in OSCC patients as compared to paired adjacent normal mucosal tissues.[62] These miRNAs were upregulated in OLP, which supported the hypothesis that different tumors exhibit different miRNA profiles.[63,64] Roy *et al*. (2016) revealed significantly downregulated of miR-26a and miR-29a expression in tissue samples of leukoplakia and oral cancer but upregulated in lichen planus tissues.[65] Ghallab *et al*. (2017) observed low miR-138 expression in erosive and atrophic OLP in contrast to reticular OLP. Further, they found miR-138 downregulation enhances the possible target CCND1's gene and protein expression in the OLP mucosa.[66] Another study reported miR-146a expression is significantly higher in CD4 (+) T cells of erosive OLP as compared to non-erosive OLP, suggested a possible role of miR-146a in the local immune disorder of OLP.[67] A genome-wide miRNA expression profiles represented 7 miRNAs expression are higher in OLP in comparison with the control and OSCC tissues.[68] A human miRNA microarray in buccal mucosa samples of SMF and normal tissues identified 11 differentially expressed distinct miRNAs. Target prediction with KEGG pathway demonstrated that predicted targets of these discovered miRNAs are primarily involved in binding, metabolic processes, cellular processes, and molecular processes.[69] miR-203 downregulates in SMF tissues as compared to healthy buccal mucosa tissues and those that are adversely controlled SFRP4 and positively regulated TM4SF1. A human adult skin keratinocyte cell line (HaCaT cells) showed decreased cell proliferation with significant miR-203 upregulation. This miR-203 upregulation significantly upsurges the expression of CK19 and ECAD proteins, and significantly downregulated the expression of NCAD and vimentin (markers of epithelial-mesenchymal transition), illustrating the role in genes may play a vital role in cutting-edge transformation of precancer into cancer besides may be functional oral cancer diagnosis and lichen planus from normal tissues.

## GENETIC ASSOCIATION OF miRNAs IN OSCC

The genetic polymorphisms of the genes that miRNAs target have the potential and affect the regulated multiple cellular pathways involved in carcinogenesis and other diseases.[70,71] Calin *et al*. (2004) reported that 50% of miRNAs are found in cancerous and vulnerable locations.[72] Human miRNAs mapping on fragile sites, translocation breakpoints, and transposons was performed by Lagana *et al*. (2010) and revealed the association of miRNA genes with delicate

locations.[73] The changes in chromosome 11 are further in OSCC. FRA11F (11q14.2) is frequently associated with the loss of distal 11q in over 50% of OSCC cases, while around 45% of OSCC cases exhibit amplification of chromosomal band 11q13, leading to a poor prognosis.[59,75-77] Additionally, FISH analysis indicated that reduced expression of certain miRNAs is linked to the loss of 11q and chromosome 21 copy number, potentially due to negative regulation by the CDX2 transcription factor, which is regulated by ATM.[78] The ATM gene is located at an 11q23.3 chromosomal location with decreased expression levels and copy number in OSCC cells lacking 11q.[79] The miRNA mapping revealed a decrease in the expression of miR-125b and miR-100 in OSCC cell lines and tumors compared to controls when comparing normal human oral keratinocytes with primary malignancies and cell lines from OSCC. The increase in their expression led to a reduction in cell proliferation and caused changes in the expression of target genes, either directly or indirectly.[78] Few groups observed the same results, whereas some of them found contradictory results. A miRNA array carried out by Tran *et al*. (2007) elucidated only miR-100 downregulation in HNSCC but not found decreased miR-125b expression.[80] This may be due to the different methodologies for expression studies or variation in geographical locations of the employed cell lines and malignancies. Tran *et al*. (2007) utilized samples from various topographical areas including the oropharynx, hypopharynx, larynx, tonsils, and oral cavity, whereas Wong *et al*. (2008) selected malignancies and cell lines that originated specifically from the oral cavity.[81]

Apart from the structural genetic changes, translocation of miRNAs target sites is also documented in the regulation of mRNAs target.[82] Single nucleotide polymorphisms (SNPs) were reported to impact the miRNA target sites in cancer-associated pathways.[83] KRAS-LCS6, an SNP of the let-7 family, is reported to be associated with lung cancer risk.

## FUTURE PROSPECTS

The miRNAs are involved in each phase of the cell cycle and breaking down their dysregulation might be useful in drug-targeted treatment. Recently, there has been a lot of interest in the potential unique therapeutic method of manipulating miRNA expression and function through the local and systematic administration of miRNA inhibitors, such as anti-miRNA oligonucleotides or miRNA sponges, or mimics.[27,44] This approach was coming into light after Janssen *et al*. (2013) reported miR-122 sequestered by Miravirsen and reduced HCV RNA levels in HCV-positive patients.[29] This study is in clinical phase 2a miravirsen as the first miRNA-targeted oligonucleotide drug accepted by FDA to receive Investigational New Drug (IND).[84]

The apoptosis pathway is another very important target for anticancer therapy. Usually, neoplastic cells are incapable of going through apoptosis. Pro-apoptotic agents that work possibly will promote apoptosis as a natural cell function and either directly or indirectly stop tumor growth. According to Yan *et al*. (2012), miR-99a mimics significantly reduced cell growth and promoted apoptosis in a tongue SCC cell line. Another study showed decreased apoptosis in LKP cell lines by miR-31-3p. Even more, the HNSCC cell lines' restoration of miR-100 increased apoptosis, which in turn inhibited cell migration and proliferation.[85] Above-discussed miRNAs are some recognized examples, nevertheless, it is still needed to check whether the functionally investigated miRNAs in OSCC are connected with apoptotic changes and apoptotic pathways to find out the new therapeutic target.

For locally advanced HNSCC, chemotherapy and radiation therapy are the usual course of treatment. However, therapeutic failure is frequently caused by resistance to anticancer drugs. There are certain reports of miRNAs as indicators that which patients will not respond well to radiation and chemotherapy in the treatment of cancer patients. Few miRNAs i.e., let-7family, miR-23a, miR-214, and miR-21 are reported as new agents modulating chemoresistance or radiosensitivity.[86] Yu *et al*. (2010) observed an unlike miRNAs expression in cisplatin-sensitive tongue SCC and cisplatin-resistant sublines: specific upregulation of let-7 family, miR-23a, miR-214, miR-518s, miR-608 and downregulation of miR-21 and miR-342.[87]

Further, several reports are available on the role of miRNAs in the advancement of radiosensitivity. A radio-resistant nasopharyngeal carcinoma cell line (CNE-2P) showed a significant increase in miR-205 levels.[88] The author further documented that 3'-UTR of PTEN encompasses a binding site for miR-205.PTEN is a greatly recognized inhibitor of cell cycle progression and may be responsible for radio-resistance through miR-205. Another study in OSCC observed low level of miR-125b was correlated with the proliferation and radio resistance and this was the downregulation of intracellular adhesion molecule 2 (ICAM2) that facilitates this process.[39]

## CONCLUSION

The posttranscriptional inhibition due to ts-miRNAs, onco-miRs, mutated miRs or epigenetically altered by methylation is extremely complex. The single miRNA can control multiple mRNAs or each mRNA may control by multiple miRNAs. The tumor suppressor and oncogenic effects may be present in miRNAs of the same family. Additionally, their expression levels can vary based on the tumor types. For example, miR-31 shows upregulation in OSCC and downregulation in gastric cancer. Epigenetic regulation governs each of these associations, leading to increased variability in the ultimate expression of the target mRNA.

The last decades shed some lights on the role epigenetic regulation of miRNAs in OSCC for early detection biomarker development and targeted therapy. It may give a possible answer to the undeciphered etiopathogenesis and behaviour of oral lesions and malignancies. Many trails have been performed for biomarkers, although many of them not supporting with high sensitivity and specificity. It is greatly accepted that miRNAs are the essential regulator of

proliferation, differentiation, apoptosis, survival, motility and morphogenesis of many tumors, including OSCC. Significant discrepancies in miRNAs expression amongst tumors and healthy tissues are reported. The changes in miRNAs expression have seen in precancerous lesions transforming into malignant, suggesting a role in cancer progression. While the extensive literature available on miRNAs and their involvement in tumorogenesis, still an association of miRNAs in OSCC is lagging. Among the many dysregulated miRNAs, several have been functionally validated and their potential target genes have been identified. However, many other miRNAs are known to be involved in cancer development with conflicting findings. Thus, additional studies will be needed to explore the exact mechanisms of OSCC. The miRNAs stability, presence in other biological samples (circulating miRNAs), and early changes during carcinogenesis make them a promising target in further investigation for better diagnosis and prognosis of OSCC. Up to now, there is a lack of clear data on incorporating miRNA expression analysis into current clinical treatment. Further research, including large, independent, well-defined studies involving families and populations, as well as validation studies, is needed to establish whether circulating miRNAs could be used as cancer biomarkers.

## CONFLICT OF INTEREST

The author declares no conflict of interest.

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