ORIGINAL RESEARCH

# Expression Analysis of Circulating miR-486-3p and CDKN2A as biomarkers & regulators in Oral Squamous Cell Carcinoma: A Prognostic Study

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## Abstract

**Background :** Oral squamous cell carcinoma (OSCC) remains a significant global health burden with poor prognosis due to late diagnosis. Emerging evidence suggests microRNA-486-3p (miR-486-3p) plays an oncogenic role in various malignancies, but its diagnostic potential in OSCC requires further validation.

**Objective :** The objective of this study was to identify differentially expressed miRNAs associated with OSCC, to develop a diagnostic panel. This present study about investigating whether the circulating microRNA 486-3p is up regulated or down regulated in OSCC patients.

**Methods :** In this case-control study, we analyzed miR-486-3p expression in 15 histologically confirmed OSCC patients and 15 healthy controls matched for age (±5 years) and gender. All participants provided 2mL blood samples collected in EDTA tubes Analyzed gene expression using qRT-PCR.

**Result :** Results revealed significantly higher miRNA-486-3p expression in OSCC patients with habits like smoking or alcohol use, compared to controls. This increased expression in tumor samples suggests that miRNA-486-3p may contribute to OSCC tumor growth and migration, supporting its potential role in promoting tumorigenicity. Notably, previous evidence indicates that miR-486-3p may indirectly regulate the CDKN2A tumor suppressor pathway, which plays a crucial role in cell cycle control. Although a direct interaction is not yet confirmed, upregulation of miR-486-3p may influence CDKN2A-related mechanisms, further contributing to dysregulated cell proliferation in OSCC.

**Conclusion:** The study found that miRNA-486-3p is upregulated in OSCC, suggesting its role in tumor development, progression, and metastasis. This upregulation could serve as a biomarker for early OSCC detection. The findings highlight the potential of miRNAs in cancer diagnostics and therapy. Further research is needed to clarify miRNA-486-3p's mechanisms, including its relationship with key regulatory genes such as CDKN2A, and assess its potential as a diagnostic or therapeutic target.


**Key word:** Micro RNA – 486-3P, Oral squamous cell carcinoma, qRT -PCR.

## Introduction

Oral squamous cell carcinoma (OSCC) is recognized as one of the most common cancers worldwide.[1] As reported by GLOBOCAN in 2020, OSCC ranks as the seventh most common cancer worldwide, accounting for roughly 4.5% of all cancer cases. Each year, it is responsible for an estimated 450,000 deaths, which is about 4.6% of all cancer-related fatalities. OSCC tends to occur more frequently in men than in women and is most often diagnosed in individuals over the age of 50.[2,3]

Current treatments for oral cancers generally involve surgical tumor removal, often accompanied by neck dissection or chemotherapy, followed by radiation therapy. Despite these efforts, late-stage cases frequently result in poor outcomes, including tumor recurrence and distant metastasis. This study highlights the urgent need to enhance our understanding of tumor dynamics, heterogeneity, and molecular pathophysiology to support the development of more effective treatments.[4] The push for improved diagnostic methods and personalized therapies has led researchers to explore potential biomarkers and therapeutic targets, focusing on non-coding RNAs (ncRNAs), key cellular biology regulators.(5,6)

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**Conflict of Interest:** None

As an integral part of mammalian cells, microRNAs (miRNAs) make up a large family of regulatory elements,[5,6,7] with humans having roughly 2,000 identified microRNA genes.[8,9]

MiRNAs are differentially expressed in various cancers, including oral cancer, when compared to noncancerous tissues, indicating their potential key roles in tumorigenesis. Research has identified two main types of cancer-related miRNAs: oncogenic miRNAs and tumor suppressor miRNAs.[10] Researchers have identified miRNAs that act either as tumor suppressors or oncogenes, with their expression levels found to be decreased or increased in various cancers.[1]

These miRNAs remain stable in human peripheral blood and body fluids and display expression patterns that are specific to certain diseases. As a result, increasing evidence points to their potential as promising biomarkers for early cancer diagnosis and prognosis evaluation.[11] Changes in miRNA expression have now been recognized as a common characteristic across many human cancers, with tumor-suppressive miRNAs typically down-regulated and oncogenic miRNAs up-regulated in disease conditions.[12]

Due to the high prevalence and unfavorable prognosis of OSCC, there is a pressing need to discover molecular biomarkers that can aid in diagnosis, prognosis, and treatment. Among these, miR-486-3p stands out as a promising candidate because of its known role in influencing the PI3K/AKT signaling pathway and its involvement in controlling epithelial-mesenchymal transition (EMT)—both of which are key drivers of OSCC development and spread. Investigating miR-486-3p may therefore provide important insights into the biological mechanisms of OSCC and lead to the identification of new strategies for early detection and therapy.[13]

## Materials And Methods

### Clinical samples and patient characteristic

This institutional review board-approved study (Ref: MDCH/IEC/2022/17) employed a case-control design with consecutive sampling of OSCC patients presenting at our tertiary care centre between January 2022 and December 2022. Blood samples were collected from 15 patients of age from 45 to 54 years with histopathologically confirmed OSCC of all four stages in the site of tongue, buccal mucosa, maxilla and mandible following a detailed case history and intraoral examination. Additionally, blood samples were gathered from a control group of 15 individuals who apparently healthy and non smoker. The exclusion criteria ruled out participants with cardiovascular, hemorrhagic, or severe organ disorders, along with those undergoing surgical excision, radiotherapy, or chemotherapy.. Each participant provided a 2 ml blood sample in EDTA tubes, kept at 4–8°C during transport with ice packs. Upon arrival at the lab, samples were stored at –80°C for long-term preservation and analysis. Due to nature of intervention, blinding of participants, investigators and outcome assessors was not possible. However, data analysis was performed objectively based on predefined parameters.

### RNA extraction, reverse transcription and Real Time PCR (RT-PCR)

Using 500 µl of Trizol reagent which was added to 1 ml of blood. Trizol isolates high-quality RNA by dissolving cell components and preserving RNA integrity. After the sample becomes cloudy, 200 µl of chloroform was added, cap the tube, vortex for 15 seconds, and centrifuge at 12,000 rpm for 15 minutes at 4°C. This separates the mixture into three phases: organic, interphase, and an upper aqueous phase containing RNA, followed by 250 µl of isopropanol was added to precipitate RNA and centrifuge at 12,000 rpm for 10 minutes at 4°C. Supernatant was removed, wash the RNA pellet with 80% ethanol, and centrifuge at 7,000 rpm for 5 minutes at 4°C. Air dry the pellet for 5-10 minutes, then nuclease-free water was added to protect the RNA. Stored at -20°C or -4°C for stability. RNA concentration and purity was measured, aiming for an A260/A280 ratio above 1.6 for quality. Converting RNA to stable cDNA for PCR analysis using the Mir-X microRNA First-Strand Synthesis Kit, which contains reverse transcriptase, buffer, and primers. Quantified RNA (0.25 to 8 µg) to ensure sufficient cDNA yield. Mix buffer, enzyme, RNA are incubated for 37°C for 1 hour. The reaction was halted by heating at 85°C for 5 minutes. Quantified cDNA and analyzed gene expression using RT-PCR. Components (Cyber Green, primers, water, and sample) undergo denaturation at 95°C, followed by annealing for 35 cycles. Analyzed results with CFX Maestro software.

### Statistical analysis

The sample size was calculated a beforehand using G*Power version 3.1 (Faul et al., 2007) for an independent t-test. Considering a one-tailed test, an effect size of 1.24, an $\alpha$ error probability of 0.05, and a power (1–β) of 0.95 the required sample size was estimated to be 30 participants (15 in each group). Data were expressed as mean ± standard deviation. Statistical analyses were performed using IBM SPSS Statistics Version 20 (IBM Corp., Armonk, NY, USA). Data were tested for normality using the Shapiro-Wilk test. Continuous variables were expressed as mean ± standard deviation. Differences between OSCC patients and controls were analyzed using an independent-samples t-test for normally distributed variables or the Mann-Whitney U test for non-normally distributed variables. Statistical significance was established at $p < 0.05$.

## Result

**Table 1 Clinicopathological Profile of OSCC Patients.**

Among OSCC patients with a smoking history, variability in clinical and pathological features was evident. The cohort consisted mostly of males (60%) and individuals above 51 years (67%). Tumor grades varied, with most being well-differentiated (60%), followed by moderately differentiated (33%), and only one poorly differentiated case (7%). Tumor sites included buccal mucosa (40%), tongue (27%), and other regions such as retromolar trigone, gingivobuccal sulcus, maxilla and mandible (33%)

Stage-wise, a considerable portion of patients presented with advanced disease, with 47% in Stage 4. Lateral distribution showed more right-sided lesions (53%), and a cohesive pattern of invasion was more common (57%) compared to non-cohesive patterns (53%). Invasion to adjacent sites was present in (40%) of patient.

Expression analysis of miR-486-3p showed variability

across these clinical subgroups. While all OSCC cases with smoking history demonstrated elevated miR-486-3p expression compared to controls, the extent of upregulation differed among patients, suggesting potential associations between expression levels and tumor site, grade, or stage.

**Graph 1: Bar Graph – Relative Expression of miR-486-3p and OSCC**

This bar graph compares the relative normalized expression levels of miR-486-3p between OSCC patients with smoking habit (green) and control group (pink). The expression of miR-486-3p is significantly upregulated in the habit group, with a notable increase in expression levels ($p < 0.01$), as indicated by the asterisk. This suggests a potential association between smoking and elevated miR-486-3p expression in OSCC patients, highlighting its possible role in smoking-related tumorigenesis or progression.

**Graph 2: Amplification Plot (qPCR)**

The amplification curve shows the cycle-by-cycle increase in fluorescence signal for miR-486-3p during quantitative PCR. The consistent and steep amplification curves after ~28–30 cycles reflect efficient and reproducible amplification across patient samples. The absence of early or erratic curves suggests high specificity and minimal background signal. These results confirm the reliability of the qPCR assay used to quantify miR-486-3p expression.

**Table : 1** Clinicopathological details of the patients.

| S. NO | Variables | Category | No of Patients (%) |
|---|---|---|---|
| 1 | GENDER | Male | 9 (60) |
| | | Female | 6 (40) |
| 2 | Age | < 50 years | 5 (33) |
| | | > 51 years | 10 (67) |
| 3 | Grade | Well differ. | 9 (60) |
| | | Moderately differ. | 5 (33) |
| | | Poorly differ. | 1 (7) |
| 4 | Site | Buccal | 6 (40) |
| | | Tongue | 4 (27) |
| | | Others RMT, GBS, Maxilla, Mandible) | 5 (33) |
| 5 | Stage | 1 | 2 (13) |
| | | 2 | 3 (20) |
| | | 3 | 3 (20) |
| | | 4 | 7 (47) |
| 6 | Laterality | Left | 7 (47) |
| | | Right | 8 (53) |
| 7 | Pattern of Invasion | Cohesive | 7 (47) |
| | | Non-cohesive | 8 (53) |
| 8 | Invasion to adjacent sites | Present | 6 (40) |
| | | Absent | 9 (60) |

**Table 2 :** miRNAs expression between control and case samples of patients diagnosed with OSCC(The expression levels of miR-486-3p were analyzed in both healthy individuals and patients with oral squamous cell carcinoma (OSCC) using quantitative real-time PCR, with U6 small RNA serving as the internal reference. ΔCq values were determined by subtracting the U6 Cq from the miR-486-3p Cq, and relative quantification was carried out using the 2^-ΔΔCq method. Among the control group, ΔCq values ranged from approximately -1.82 to +1.95, with corresponding fold change values clustering around 1, indicating a low and stable expression of miR-486-3p in healthy tissues. In contrast, most OSCC patient samples showed significantly lower ΔCq values, often below -2, reflecting increased miR-486-3p expression. Notably, several patient samples exhibited markedly elevated fold changes—such as Patient 3 (17.74), Patient 5 (13.82), and Patient 15 (16.9)—when compared to the controls. These findings suggest a consistent upregulation of miR-486-3p in OSCC cases, highlighting its potential utility as a molecular biomarker for distinguishing cancerous from non-cancerous oral tissues.)

| Samples | MiRNA-486-3p | U6 | Δcq (miRNA. 486-3p - u6) | Δδcq | Fold change |
|---|---|---|---|---|---|
| **Controls** | | | | | |
| Control 1 | 30.03 | 28.46 | 1.57 | 1.45 | 0.366 |
| Control 2 | 29.81 | 31.58 | -1.77 | -1.86 | 3.63 |
| Control 3 | 30.15 | 31.58 | -1.43 | -1.52 | 2.867 |
| Control 4 | 30.15 | 30.22 | -0.07 | -0.16 | 1.117 |
| Control 5 | 30.01 | 30.22 | -0.21 | -0.3 | 1.231 |
| Control 6 | 31.01 | 29.9 | 1.11 | 1.02 | 0.493 |
| Control 7 | 30.83 | 30.12 | 0.71 | 0.62 | 0.65 |
| Control 8 | 31.05 | 30.32 | 0.73 | 0.64 | 0.641 |
| Control 9 | 30.55 | 30.22 | 0.33 | 0.24 | 0.846 |
| Control 10 | 30.68 | 29.46 | 1.22 | 1.13 | 0.456 |
| Control 11 | 30.05 | 28.15 | 1.95 | 1.86 | 0.275 |
| Control 12 | 29.83 | 31.165 | -1.82 | -1.91 | 3.758 |
| Control 13 | 30.18 | 31.12 | -0.94 | -1.03 | 2.042 |
| Control 14 | 30.24 | 30.42 | -0.18 | -0.27 | 1.205 |
| Control 15 | 30.25 | 30.09 | 0.16 | 0.07 | 1.049 |
| **Patients** | | | | | |
| Patient 1 | 30.47 | 33.07 | -2.6 | -2.819 | 7.056 |
| Patient 2 | 30.03 | 33.16 | -3.13 | -3.349 | 10.189 |
| Patient 3 | 30.05 | 33.98 | -3.93 | -4.149 | 17.74 |
| Patient 4 | 30.04 | 29.95 | -0.02 | -0.239 | 1.18 |
| Patient 5 | 30.25 | 33.82 | -3.57 | -3.789 | 13.82 |
| Patient 6 | 29.73 | 32.72 | -2.9 | -3.119 | 8.687 |
| Patient 7 | 29.92 | 32.19 | -2.27 | -2.489 | 5.613 |
| Patient 8 | 30.1 | 31.67 | -1.5 | -1.719 | 3.292 |
| Patient 9 | 29.73 | 32.06 | -2.3 | -2.519 | 5.731 |
| Patient 10 | 30 | 32.27 | -2.27 | -2.489 | 5.613 |
| Patient 11 | 30.68 | 33.27 | -2.59 | -2.809 | 7.007 |
| Patient 12 | 30.43 | 33.25 | -2.82 | -3.039 | 8.219 |
| Patient 13 | 30.25 | 33.82 | -3.57 | -3.789 | 13.823 |
| Patient 14 | 30.08 | 29.85 | 0.23 | 0.011 | 0.992 |
| Patient 15 | 29.07 | 32.93 | -3.86 | -4.079 | 16.9 |

**Table 2 : qRT-PCR Ct Values and Fold Change of miR-486-3p Expression in OSCC Patients and Controls**

The table presents Ct values (cycle threshold) for miR-486-3p and the internal control U6 in both control samples and OSCC patients with a smoking habit. These Ct values are used to calculate the ΔCt (difference between miR-486-3p and U6), then ΔΔCt (difference between patient ΔCt and control ΔCt), which finally gives the fold change in miR-486-3p expression relative to controls.

- **Control group (Control 1 to Control 15):** Fold change values mostly hover around or below 1, indicating low and stable expression of miR-486-3p with little variation between samples. This suggests that in healthy individuals (controls), miR-486-3p levels are consistent and not elevated.
- **Patient group (Patient 1 to Patient 15):** Fold change values vary widely, ranging from just below 1 to as high as 16.9 in some cases. This indicates that in OSCC patients with a smoking habit, miR-486-3p expression is significantly upregulated in many individuals, but with considerable variability between patients.

**Graph 3: Expression Variability Across Control and Patient Samples**

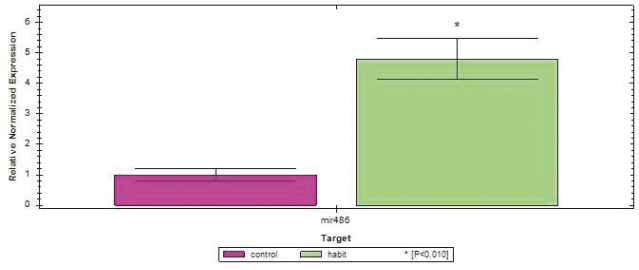
The orange bars represent fold change for each individual control and patient. Controls show consistently low bars, confirming stable and low miR-486-3p expression. Patients display tall and varying bars, reflecting large differences in miR-486-3p expression across individuals. Some patients have fold changes over 10 or even 15, indicating marked overexpression of miR-486-3p in these cases.

## Discussion

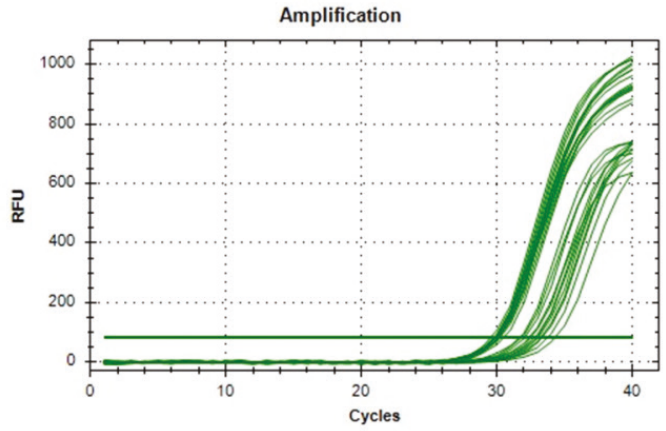
The main findings of this study indicated that tumor blood analysis from OSCC patients showed significantly high expression of miR-486-3p compared to the control group. OSCC ranks as the sixth most prevalent cancer worldwide, with over 300,000 new cases reported annually. As the most aggressive tumor affecting the oral and maxillofacial regions, OSCC constitutes 90% of all oral cancers. The prognosis remains grim, with a five-year survival rate of only 50%, primarily due to late-stage diagnosis in most patients.[21] Despite improvements in treatment modalities over the years, the overall 5-year survival rate for oral cavity cancer has remained relatively unchanged. For patients diagnosed at an early stage, the survival rate is about 89%. However, this rate significantly drops to approximately 39% for those diagnosed at a late stage. This stark contrast highlights the critical importance of early detection and intervention.[14]

MicroRNAs offer a promising tool for cancer research due to their regulatory roles in gene expression. They can modulate the expression of specific genes and are capable of being transported between different cellular compartments, influencing processes such as translation and transcription. This ability to regulate gene activity in a nuanced manner adds a layer of complexity to their potential utility in diagnosing, monitoring, and treating cancer. By harnessing the insights gained from studying miRNAs, researchers aim to uncover new therapeu-

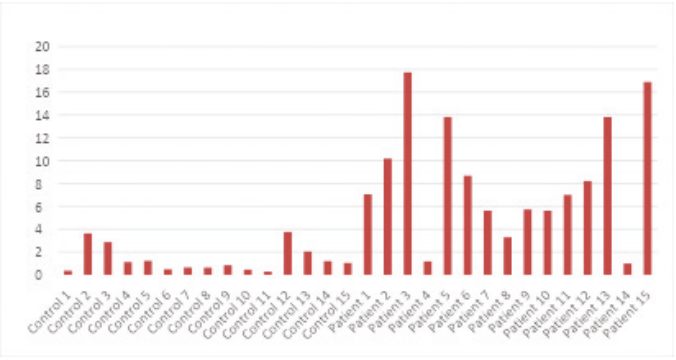
**Graph 1** – Represents gene expression analysis between normal and OSCC




**Graph 2 -** Amplication plot of miR-486-3p in control and OSCC patient




**Graph 3 :** The bar chart illustrates the relative expression levels (fold change) of miR-486-3p in control and OSCC patient samples (Among the control group, fold change values remain consistently low, generally below 5, with most samples clustering around 1, indicating stable and low expression of miR-486-3p in healthy individuals. In contrast, the patient group shows a marked increase in expression, with several samples—such as Patient 2, Patient 3 and patient 13 —exhibiting fold changes above 10 and patient 5, Patient 15 peaking near 18. This sharp contrast highlights a significant upregulation of miR-486-3p in OSCC tissues, supporting its potential as a diagnostic biomarker for distinguishing cancerous from normal oral tissue)



tic targets and improve outcomes for patients with OSCC and other cancers.[15]

Recent studies have increasingly highlighted the role of circular RNAs (circRNAs) as crucial regulators in cancer biology, including OSCC. One such circRNA, circ_0005320, has garnered attention due to its potential involvement in tumor progression through interactions with miRNAs. Utilizing bioinformatics tools such as the circinteractome database, researchers have predicted multiple miRNAs that can bind to circ_0005320, with five miRNAs identified as having multiple binding sites. Among these, miRNA-486-3p and miRNA-637 have been previously reported to participate in OSCC tumorigenesis, suggesting their functional relevance as targets of circ_0005320.

Experimental evidence supports this interaction; overexpression of miRNA-486-3p in OSCC cell lines (CAL27 and SCC25) significantly increases its cellular levels, and subsequent dual-luciferase reporter assays have confirmed that miRNA-486-3p directly binds to circ_0005320, reducing reporter activity in constructs containing the wild-type circRNA sequence but not in mutated versions. Furthermore, RNA immunoprecipitation assays demonstrated enrichment of both circ_0005320 and miRNA-486-3p in Ago2-containing complexes, substantiating the direct binding between these molecules.

Importantly, quantitative real-time PCR analyses have shown a marked downregulation of miRNA-486-3p in OSCC tissues and cell lines, implicating its reduced expression in the disease's pathogenesis. These findings collectively suggest that circ_0005320 may promote OSCC progression by sponging miRNA-486-3p, thereby disrupting its tumor-suppressive functions.(16)

Zujian et al. (2017) investigated oral tongue squamous cell carcinoma (TSCC) and the role of microRNAs in its pathogenesis. The researchers analyzed archived TSCC samples to identify miRNA biomarkers for early detection and identified 12 miRNAs with significantly altered expression in TSCC tissues compared with normal tissues. Among these, miR-486-3p demonstrated high diagnostic accuracy for the detection of TSCC.[17]

In contrast, Chou et al. investigated the role of Discoidin Domain Receptor 1 (DDR1) in oral squamous cell carcinoma (OSCC) and its regulation by miR-486-3p. Their findings showed that DDR1 is significantly upregulated in OSCC, thereby promoting tumor growth and survival. The study further revealed that miR-486-3p, which is typically downregulated in OSCC tissues, directly targets DDR1 and suppresses its expression, resulting in reduced tumor growth and increased apoptosis. The authors also highlighted the role of epigenetic alterations, particularly methylation of the ANK1 promoter, in the suppression of miR-486-3p expression.[18]

Several factors may account for the discrepancy between these findings and the results of the present study. First, the present study evaluated circulating miR-486-3p levels in peripheral blood, whereas Chou et al. assessed miR-486-3p expression in tumor tissues. Second, variations in ethnicity, environmental exposures, tobacco use, disease stage distribution, and sample size may influence miRNA expression profiles. Finally, circulating miRNAs may originate from multiple cellular sources and therefore may not directly reflect tissue-specific expression patterns. Further studies incorporating paired blood and tissue samples are warranted to clarify these differences and better understand the biological significance of miR-486-3p expression in OSCC.

The overexpression of miR-486-3p resulted in suppressed cell proliferation and induced apoptosis, effects that were similarly observed upon DDR1 knockdown. In OSCC, frequent abnormal methylation of the ANK1 promoter contributes to tumor development by epigenetically silencing both ANK1 and its intronic microRNA, miR-486-3p. This revealed that miR-486-3p is transcriptionally co-regulated with its host gene ANK1 through epigenetic mechanisms. Notably, treatment with a DNA methylation inhibitor restored the expression of both ANK1 and miR-486-3p. They also found that the betel nut alkaloid arecoline promotes this methylation, further reducing miR-486-3p levels and enabling DDR1 to drive cancer progression.(18) Likewise, the CDKN2A gene, a known tumor suppressor, undergoes similar epigenetic silencing through promoter hypermethylation, further contributing to OSCC progression.

A recent study highlighted an important connection between miR-486-3p and TSP-2, a protein known to play a role in osteoclastogenesis, a key process in bone remodeling. The study found that TSP-2 enhances osteoclast differentiation via the RANKL-dependent signaling pathway. Additionally, TSP-2's effect on osteoclastogenesis is mediated by the activation of NFATc1, a transcription factor critical for this process, through the suppression of miR-486-3p expression.

Interestingly, restoring miR-486-3p expression reverses the effects of TSP-2, suppressing osteoclastogenesis and inhibiting osteolytic metastasis in experimental models. This discovery suggests that miR-486-3p could serve as a potential therapeutic target to counteract the damaging effects of TSP-2, especially in conditions like bone metastasis and excessive bone degradation. The multifunctional role of miR-486-3p across different cellular contexts makes it a promising candidate for further research, particularly in the treatment of malignancies and other diseases associated with bone and tissue remodeling.[19]

Sivakumar et al, (2025) explains, one of the major genetic disruptions linked to the development of OSCC involves changes in the CDKN2A gene. This gene, found on chromosome 9p21, produces two important tumor-suppressing proteins: p16(INK4a), which helps control the cell cycle, and p14(ARF), which plays a role in regulating apoptosis. When CDKN2A loses its normal function, these critical control mechanisms break down, contributing to the onset and progression of cancer. he concludes that CDKN2A plays a crucial role in the development and progression of oral squamous cell carcinoma (OSCC) by affecting cell cycle control and apoptosis. Its alterations lead to tumor aggressiveness, therapy resistance, and poor prognosis. Ongoing research is uncovering its potential as both a diagnostic and therapeutic target, though challenges remain.[20]

Research published in Cancer Gene Therapy revealed that in lung cancer, the circular RNA FLNA functions as a molecular sponge for miR-486-3p. By binding to and reducing the availability of miR-486-3p, circFLNA influences the regulation of various downstream genes related to DNA repair and cell cycle

processes, such as XRCC1 and CYP1A1. While CDKN2A is not a direct target, this mechanism highlights how ceRNA-mediated regulation of miR-486-3p could have an indirect impact on signaling pathways associated with CDKN2A.[21]

A study indexed in PubMed on lung adenocarcinoma (LUAD) identified that miR-486-3p, along with its counterpart miR-486-5p, targets several genes essential for DNA replication—most notably GINS4, a critical component required for initiating the S phase of the cell cycle. Suppression of GINS4 leads to cell cycle arrest, mirroring the $G_1$/S phase inhibition typically regulated by CDKN2A. This indicates a potential functional overlap between the two pathways, even in the absence of direct interaction.[22]

Our study confirms that miRNA-486-3p linked to several cancers, including OSCC. In patients with OSCC, miRNA-486-3p expression is significantly elevated compared to healthy individuals. This increase suggests that miRNA-486-3p may contribute to the development and progression of OSCC, acting as a tumor promoters. The overexpression of miRNA-486-3p in OSCC may promote tumor growth, invasion, and metastasis by targeting genes involved in cell proliferation, apoptosis, and other cancer-related processes. Consequently, miRNA-486-3p is being explored as a potential biomarker for OSCC diagnosis and prognosis, as well as a therapeutic target.

## Conclusion

This study highlights a significant increase in miRNA-486-3p expression among individuals with OSCC, especially those with a smoking history. The elevated levels of this microRNA suggest its involvement in the molecular changes that contribute to the onset and advancement of OSCC. Its role in promoting abnormal cell growth, evading programmed cell death, and enhancing metastatic behavior underlines its potential as both a marker for diagnosis and a target for treatment.

Of particular interest is the possible indirect connection between miRNA-486-3p and the CDKN2A gene, a known regulator of the cell cycle. Although current data do not confirm direct interaction, it is plausible that miR-486-3p influences upstream elements or signaling pathways that ultimately impact CDKN2A function. Given CDKN2A's critical role in halting uncontrolled cell division, any alteration in its regulation could contribute to tumor development.

The findings also support the clinical value of miRNA analysis in the early identification of OSCC, particularly in populations at higher risk due to tobacco use. Early detection strategies based on molecular profiling could significantly improve treatment outcomes.

However, several limitations of the present study should be acknowledged. First, the relatively small sample size, comprising 15 patients with OSCC and 15 healthy controls, limits the statistical power and generalizability of the findings. In addition, the cross-sectional study design precludes the establishment of causal relationships between circulating miR-486-3p expression and disease progression. We recognize that subgroup analyses based on clinic-pathological parameters, including tumor stage, histological grade, and smoking status, are likely underpowered because of the limited number of participants within each subgroup. Consequently, the lack of statistically significant associations in certain comparisons may reflect inadequate statistical power rather than the absence of a true biological relationship. Likewise, statistically significant findings observed in small subgroups should be interpreted with caution and require validation in larger, well-powered cohorts before definitive conclusions can be drawn. Furthermore, potentially important confounding factors, including genetic predisposition, alcohol consumption, betel quid chewing, lifestyle factors, and environmental exposures, were not comprehensively evaluated and may have influenced circulating miR-486-3p expression levels. Future studies incorporating larger sample sizes, longitudinal follow-up, and comprehensive assessment of these variables are needed to validate and extend the present findings. To validate and extend these findings, future studies should include larger, diverse populations and follow participants over time.

Laboratory-based research on the biological effects of miRNA-486-3p, especially in relation to CDKN2A-associated pathways, could provide a clearer understanding of its role in OSCC. Ultimately, incorporating miRNA-based biomarkers like miRNA-486-3p into routine clinical screening could enhance early diagnosis and pave the way for more personalized therapeutic approaches in oral cancer management.

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