

Integrating expression profiling and computational modeling to elucidate PTEN-miR-141 interactions in oral squamous cell carcinoma

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Abstract. With a limited number of therapeutic options available, oral squamous cell carcinoma (OSCC) continues to pose a severe health burden, contributing to its high mortality rate. The tumor suppressor, phosphatase and tensin homolog (PTEN), is typically downregulated in OSCC, which aids in tumor growth. PTEN expression can be regulated by microRNAs (miRNAs/miRs), affecting the course of illness. In the present study, a possible PTEN regulator, miR-141, was found by computational analysis. PTEN and miR-141 expression profiling was performed on the samples of patients with OSCC and compared to that of healthy controls. The results revealed a significant inverse correlation between PTEN and miR-141 expression in OSCC tissues. Specifically, miR-141 levels were elevated, while PTEN expression was markedly downregulated in cancerous samples compared with the controls. This inverse correlation suggests that miR-141 may play a regulatory role in silencing PTEN, contributing to the progression of OSCC. These findings provide valuable insight into the molecular mechanisms underlying OSCC and suggest that miR-141 functions as an oncogenic miRNA in this context by targeting PTEN. The findings presented herein may have broad significance, since they may prove to be valuable for the development of new treatment approaches through the miR-141/PTEN axis. Restoring PTEN expression by targeting miR-141 may provide a novel treatment strategy for OSCC. PTEN and miR-141 may also function as prospective

biomarkers for early diagnosis, providing chances to enhance patient outcomes via individualized treatment plans. However, further research is required to further determine the therapeutic potential of this regulatory axis.

Introduction

A considerable percentage of head and neck malignancies are invasive and aggressive oral squamous cell carcinoma (OSCC). OSCC is a type of cancerous growth that arises from the stratified squamous epithelium of the oral cavity. It continues to pose a significant worldwide health concern and causes >90% of all oral cancers (1). In areas, such as Southeast Asia and portions of Europe where the disease is more common, OSCC has a major influence on world health. It poses a serious public health concern in these areas, since, for instance, OSCC accounts for ~45% of cases worldwide (2). As the population of the world ages and exposure to established risk factors, including alcohol, tobacco and chewing betel quid increases, the incidence of OSCC is expected to increase further. According to current statistics, OSCC is the sixth most prevalent type of cancer among females and the fourth most common among males (3). The condition can appear on the tongue, floor of the mouth, or the buccal mucosa, the latter of which is more frequently affected due to long-term irritation and exposure to cigarette smoke. The usual regulatory systems regulating cell growth and differentiation are disrupted by a complex multi-step process of genetic and epigenetic modifications that comprise the pathogenesis of OSCC. Currently, there is no effective treatment strategy for OSCC. Even though improvements have been made in surgical techniques, radiotherapy and chemotherapy, as these methods have limited success in improving long-term survival rates and reducing recurrence. Thus, in order to combat this condition, the primary objective is to develop novel therapeutic strategies that can effectively manage the diverse gene disturbances, as well as molecular abnormalities associated with the development of OSCC.

Human papillomavirus (HPV), in particular high-risk HPV-16, has also been linked to the etiology of OSCC in a growing number of patients, particularly those that do not have typical risk factors. As a result of the integration of

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HPV-16 into the host genome, tumor suppressor proteins become inactive and a buildup of genetic abnormalities that accelerate the development of cancer occurs (4). The process of field cancerization, in which a number of distinct regions of dysplasia develop inside the oral cavity and frequently result in the development of carcinoma *in situ* and invasive carcinoma, is another critical part of the pathophysiology of OSCC (5). The chance of tumor recurrence and treatment resistance is increased by this process, which is suggestive of extensive genetic instability in the afflicted tissue. The invasion of underlying connective tissues, angiogenesis stimulation and metastasis, which frequently involves nearby lymph nodes and distant organs, are the types of aggressive behavior displayed by OSCC as it progresses. Advanced-stage OSCC is associated with a poor prognosis due to its tendency for early metastases and local invasion (6). Moreover, among its counterparts implicated in OSCC, the phosphatase and tensin homolog (PTEN) gene stands out as it plays a crucial role in maintaining cellular homeostasis hence preventing malignant transformation. In the majority of cases of OSCC, the expression of PTEN, as a well-established tumor suppressor, is lost or frequently downregulated. Upon the loss of PTEN, patients exhibit accelerated cell growth rates and invasiveness that lead to resistance against standard care interventions, which expose them to poorer clinical outcomes (7). Recent studies have demonstrated the importance of epigenetic alterations, such as the methylation of the PTEN promoter, as major factors contributing to PTEN expression reduction, which further accelerates the progression of OSCC (8).

In addition to genetic alterations, the regulation of PTEN is also influenced by microRNAs (miRNAs/miRNAs), which are small non-coding RNAs that modulate gene expression at the post-transcriptional level (9). miRNAs exert their effects by binding to complementary sequences on target mRNAs, leading to their degradation or inhibition of translation (10). Recent studies have identified several miRNAs that directly target PTEN, affecting its expression and contributing to tumor progression in various cancers, including OSCC (9). These findings suggest that miRNAs may serve as valuable tools for elucidating the molecular mechanisms underlying OSCC and for the development of novel diagnostic and therapeutic strategies (11).

The present study aimed to integrate these insights by employing a two-pronged approach. First, the present study utilized computational methods to identify miRNAs that target PTEN, focusing on those that exhibit significant regulatory interactions. Subsequently, the present study validated these miRNAs through experimental analyses, assessing their expression profiles in OSCC samples and evaluating their functional impact on PTEN. By combining computational predictions with empirical validation, the present study aimed to uncover miRNAs that may serve as biomarkers, thereby enhancing the accuracy of early OSCC diagnosis. These miRNAs may also aid in the exploration of novel therapeutic avenues by understanding the interplay between PTEN and its regulatory miRNAs.

Materials and methods

Identification of a key tumor suppressor in OSCC. The pathophysiology of OSCC was investigated through an

Table I. Characteristics of the patients in the present study.

Characteristic	Description
Age	30-75 years (mean, 55±10 years)
Sex	Males, 20; Females, 10
Tumor Stage	Stage I, 5; stage II, 10; stage III, 10; stage IV, 5
Metastasis	Yes, 8; no, 22
Additional factors	Smoking, 15 (yes); alcohol consumption, 12 (yes)

extensive literature review, which included identifying key genes involved in the disease. Among the various candidates, a well-established tumor suppressor of interest was selected for further research due to its critical role in maintaining cellular homeostasis and its frequent downregulation in OSCC. Although not a novel protein, the importance of this tumor suppressor (PTEN) in OSCC underscores its relevance for the study, aiming to deepen the understanding of its regulatory mechanisms and potential as a therapeutic target.

Selection and characterization of miRNAs. After identifying the gene, the present study concentrated on identifying possible miRNAs that target this gene, which may be critical for the progression of OSCC. TargetScan (https://www.targetscan.org/vert_80/) was used to anticipate miRNAs that could control PTEN computationally (12). A thorough list of potential miRNAs was supplied by this database, and particular miRNAs of interest were selected.

Sequence retrieval and structural analysis. Reputable miRNA databases, namely miRBase (<https://www.mirbase.org/>), provided the sequence of the selected miRNA. The present study utilized RNAfold (<http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi>) to analyze the secondary structure of the miRNA to gain a deeper understanding of its functional characteristics (13). The evaluation of the stability and possible binding abilities of the miRNA, two essential components of its regulatory roles, was made possible.

Sample collection. The present study received approval from the Institutional Ethics Committee, Department of Medicine, Saveetha Medical College (IHEC/SDC/PhD/O-PATH-1916/19/432) and all samples were collected during the time from period September, 2023 to January, 2024 in strict adherence to the Declaration of Helsinki. The sample size for the study was calculated using Gpower and a set of 30 tissue samples, including OSCC and adjacent normal tissues, were acquired from patients who provided informed consent through the Department of Medicine at Saveetha Medical College and Hospitals (Chennai, India) to validate the experiment. Informed consent was obtained from each individual (Table I). Patients >18 years of age with a confirmed diagnosis of OSCC and no notable medical disorders, such as hypertension or hypothyroidism were included in the present study. The diagnosis of OSCC was validated

Table II. miRNAs and reference gene primer sequences used in the gene expression analysis.

miRNA/ reference gene name	Forward primer	Reverse primer
β-actin	5'-GCACCACACCTTCTACAATG-3'	5'-TGCTTGCTGATCCACATCTG-3'
PTEN	5'-TGAGTTCCTCAGCCGTTACCT-3'	5'-GAGGTTTCCTCTGGTCCTGGTA-3'
U6	5'-CTCGCTTCGGCAGCACA-3'	5'-ACGCTTCACGAATTTGC-3'
miR-141	5'-GCGAAAGAGGCCCCG-3'	5'-AGTGCAGGGTCCGAGGTATT-3'

PTEN, phosphatase and tensin homolog.

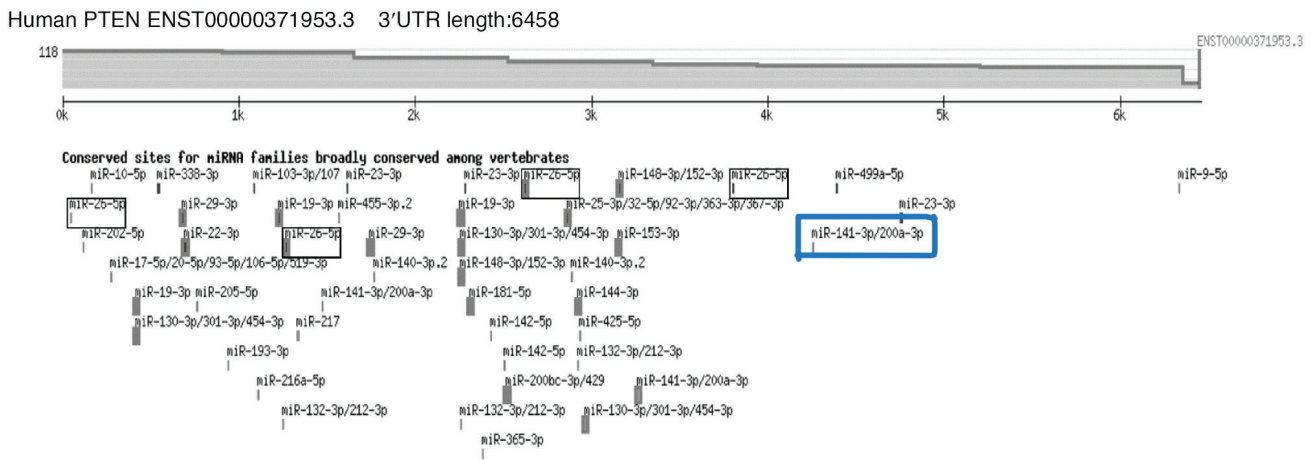


Figure 1. Predicted miRNAs targeting PTEN. The figure displays the miRNAs predicted to target PTEN, identified using TargetScan. Each miRNA is presented with its corresponding seed match type and conservation score. PTEN, phosphatase and tensin homolog.

by the Saveetha Medical College and Hospitals Department of Biochemistry. For further examination, the materials were stored at -20°C after being washed with PBS.

RNA extraction and quantification. Utilizing TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.), total RNA was isolated from the tissue samples in accordance with the manufacturer's recommendations. A Thermo Fisher Scientific NanoDrop 2000 Lite spectrophotometer (Thermo Fisher Scientific, Inc.) was used to evaluate the amount and caliber of the isolated RNA (14). Before being used again, the RNA samples were kept at -20°C.

Reverse transcription. Reverse transcription was performed on the isolated RNA to create complementary DNA (cDNA). For genes, an oligo(dT)18 primer (Promega Corporation, 50 μM) was employed, and for miRNAs, a universal adapter. Nuclease-free water and dNTPs (10 mM each) from New England Biolabs Inc. were added to the mixture. Following 5 min of incubation at 65°C, this mixture was rapidly chilled. Nuclease-free water, 5X prime buffer, reverse transcriptase (New England Biolabs Inc.), and murine RNase inhibitor comprised the final reaction mixture. The following temperatures were used for the reverse transcription process in a MiniAmp Plus heat cycler (Thermo Fisher Scientific, Inc.): 30°C for 10 min, 42°C for 30 min, and 95°C for 5 min, with a pause at 4°C in between (14). Using a Nanodrop Lite spectrophotometer, the cDNA was measured and stored.

Expression analysis using quantitative PCR (qPCR). The expression levels of the selected miRNA and PTEN gene were measured using qPCR with SYBR-Green (Takara Bio, Inc.). U6 and β-actin were employed as housekeeping controls for the expression of miRNA and genes, respectively. The primers were provided by Eurofins Genomics LLC, and the Bio-Rad CFX96 Realtime System was utilized to perform the expression analysis (Table II). Following a 30-sec initial denaturation phase at 95°C, there were 35-40 cycles of 5 sec at 95°C and 30 sec at the annealing temperature throughout the PCR cycling conditions. After the PCR cycles were completed, a melt curve analysis was carried out (15). Each test was run in duplicate, and the relative expression levels were determined using the 2^{-ΔΔCq} technique (16).

Statistical analysis. The means of the repeated experiments, along with the standard error of the mean (SEM) were used to display the results. For comparisons between two groups, the Student's t-test (paired) was used with GraphPad Prism 10.1.0 statistical software (15). All comparisons in the present study were made between two groups. To analyze the correlation between variables in the control and patient groups, Pearson's correlation was used. A value of P<0.05 was considered to indicate a statistically significant difference.

Results

miRNA selection and analysis. Computational predictions were made once PTEN was identified as a major gene of

Table III. Minimum free energy, mature sequence, match extent and A+U content of hsa-miR-141.

Source miRNA	Source organism	Minimum free energy	Mature sequence	Match extension	Strand	A+U%
miR-141	<i>Homo sapiens</i>	-37.30 kcal	UAACACUGUCUGGUAAGAUGG	22/22	3p	45.33

The table presents the minimum free energy, the mature miRNA sequence, the extent of sequence matching, and the A+U percentage content for hsa-miR-141.

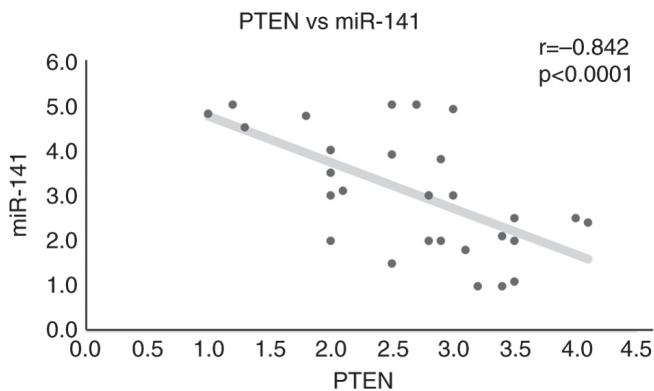


Figure 5. Inverse correlation between miR-141 and PTEN expression. The figure illustrates the negative correlation between miR-141 and PTEN expression levels in the samples, with a statistically significant P-value of <0.05. PTEN, phosphatase and tensin homolog.

Discussion

OSCC poses a major worldwide health burden, mostly due to its high mortality rates and rapid development (17). Known for its intricate interactions between genetic and epigenetic elements, OSCC frequently entails the suppression of key tumor suppressor genes, such as PTEN, which are essential for preserving cellular homeostasis and averting malignant transformation (18). The present study centered on the function of miRNAs in regulating the expression of PTEN, specifically focusing on miR-141, a pivotal regulator of PTEN found in several malignancies, including OSCC. In OSCC tissues, the data revealed a strong negative association between miR-141 and PTEN expression, with an increased expression of miR-141 corresponding to downregulated levels of PTEN. miR-141 has also been demonstrated to target and suppress PTEN in other types of cancer, including gastric and prostate cancer, which has resulted in increased tumor development and treatment resistance (19,20). These findings are in line with these studies. These comparative findings demonstrate the wider function that miR-141 plays in the oncogenesis of a number of cancer types and point to its potential as a therapeutic target that may be used universally.

This discovery also coincides with studies on hepatocellular carcinoma (HCC), where miR-141 has been linked to the down-regulation of PTEN, hence increasing carcinogenesis (21). On the other hand, these findings suggest that, in OSCC, miR-141 may potentially enhance invasiveness and metastatic potential by interfering with PTEN-mediated pathways, in contrast to HCC, where miR-141 primarily addresses cell proliferation. As

the functional impact varies throughout cancer types, different treatment approaches are required, highlighting the intricacy of miRNA regulation. The results presented herein have consequences that go beyond simple molecular understanding. In OSCC, the deregulation of the miR-141/PTEN axis may be a useful biomarker for prognosis and early identification. Comparative research on breast cancer has demonstrated that diagnostic accuracy is increased when miRNA profiling is combined with conventional markers. Comparably, early identification rates for OSCC may be increased by including PTEN expression analysis and miR-141 into the existing diagnostic methods, particularly for individuals lacking conventional risk factors. Furthermore, a viable treatment approach is to target the miR-141/PTEN pathway. Preclinical research on lung cancer has demonstrated that miRNA inhibitors can render tumors more sensitive to chemotherapy and restore PTEN expression (22). By reactivating tumor suppressor pathways, innovative medicines that overcome resistance to current medications may be developed if this technique is used to OSCC (23).

In conclusion, the present study statistically analyzed the expression of PTEN and miR-141 in OSCC in comparison to normal tissue samples. The findings presented herein provide a deeper understanding of the molecular interactions between these critical regulators, providing insight which may aid in the development of novel therapeutic approaches. miR-141 plays a crucial role in regulating PTEN expression in OSCC, with significant implications for both diagnosis and treatment. The present study highlights the potential of miR-141 as a universal therapeutic target by contrasting the results with research in other malignancies. Further studies are required however, to explore the therapeutic potential of targeting the miR-141/PTEN pathway in OSCC, particularly in combination with conventional therapies, in order to improve patient outcomes and reduce mortality rates.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

DS conceptualized the study, and was also involved in the study supervision, formal analysis and reviewing of the manuscript. AA was involved in the writing of the original draft of the manuscript, as well as in data analysis and interpretation. ASUPP was involved in the design of the study, in data curation and assisted with data analysis. AKP was involved in data collection and revised the manuscript. DS and AKP confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Ethics Committee of Department of Medicine at Saveetha Medical College and Hospitals (IHEC/SDC/PhD/O-PATH-1916/19/432). Written informed consent was obtained from all patients prior to the collection of tissue samples for research purposes.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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