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RESEARCH ARTICLE

The impact of microRNA-596 on oral cancer: Insights into tumor biology and treatment strategies

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Objectives: Despite advancements in treatment, oral squamous cell carcinoma (OSCC) remains a major global health issue with stagnant survival rates. MicroRNAs (miRNAs), particularly miR-596, play critical roles in cancer, acting as both oncogenes and tumor suppressors. This study aims to clarify miR-596's function in OSCC and assess its potential as a therapeutic target or diagnostic biomarker.

Methods: A thorough bioinformatics analysis utilising information from The Cancer Genome Atlas (TCGA) led to the selection of miR-596 for investigation. After extracting its sequence from miRBase, RNAfold was used to evaluate the secondary structure's functional characteristics. MiRNA expression levels were measured by quantitative reverse transcription PCR (qRT-PCR) on a total of 30 tissue samples, including OSCC and healthy controls. With TargetScan, the miR-596 gene targets were predicted.

Results: miR-596 is significantly downregulated in OSCC tissues compared to healthy controls. TargetScan analysis indicates that miR-596 regulates key genes involved in cell proliferation, metastasis, and apoptosis, suggesting its crucial role in these pathways. Additionally, the predicted secondary structure of miR-596 indicates high stability.

Conclusion: Based on its activity as a tumor suppressor, the study suggests that miR-596 may be an important factor in the development of OSCC. Its potential use as a therapeutic target or diagnostic biomarker is highlighted by the downregulation of miR-596 in OSCC. Validating these results and investigating the therapeutic applications of miR-596 in OSCC treatment require more investigation.

Keywords: oral squamous cell carcinoma, miR-596, biomarker, therapeutic target, gene expression

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Introduction

Oral Squamous Cell Carcinoma (OSCC) continues to cast a long shadow over global health, with 2020 marking a grim milestone of 377,713 new cases and 177,757 lives lost across the world [1]. Unfortunately, the 5-year survival rate for individuals with oral cancer has plateaued, despite significant advancements in diagnostic technologies and treatment measures. This impasse indicates a considerable vacuum in our knowledge of the molecular nuances underlying the initiation and development of this illness [2]. Within the field of molecular biology, microRNAs (miRNAs) have emerged as highly influential regulators. These small, non-coding RNA molecules play a crucial role in regulating gene expression and are essential for various cellular processes, including proliferation, apoptosis, and differentiation [3]. Their significance in cancer biology, including oral cancer, has been well-documented [4]. Of these, miR-596 is unique in that it intrigues scientists with its dual nature—it may function as an oncogene in certain cancer contexts or as a tumor suppressor [5]. Recent investigations have illuminated miR-596's tumor-suppressing potential in cancers such as gastric cancer [6] and melanoma [7]. However, its exact significance in oral cancer is yet unknown. Inconsistent data indicates that, in certain cases, miR-596 may also display carcinogenic properties [8]. This dual nature of miR-596 underscores the complex and context-dependent nature of miRNA function in cancer, where the same molecule can influence different outcomes based on the cellular environment.

This study aims to demystify miR-596's role in oral cancer, probing whether it could serve as a potent diagnostic biomarker or a therapeutic target. By unraveling the multifaceted actions of miR-596, particularly its involvement in key signaling pathways related to cell proliferation and apoptosis, this research aspires to deepen our comprehension of miRNA-driven dynamics in cancer biology. The insights gained could potentially revolutionize the development of novel diagnostic and therapeutic strategies, offering fresh hope for patients battling oral cancer.

Methods

Selection and characterization of miRNA

Our investigation of full-length miRNAs associated with OSCC was thorough, and we used information from The Cancer Genome Atlas (TCGA) (https://portal.gdc.cancer.gov/) [9]. We chose a miRNA that was not previously studied in relation to OSCC because of its bioinformatics analysis-identified differential expression patterns and possible regulatory importance. The target miRNA's sequence was obtained from well-known miRNA databases such as miRBase (https://www.mirbase.org/). We used RNAfold (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi) to analyse the secondary structure of the miRNA

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in order to estimate its functional features [10]. Understanding the stability and possible binding capacities of the miRNA is essential for comprehending its regulatory roles, and this investigation offered vital insights into these aspects.

Sample Collection

A total of 30 tissue samples, including 15 OSCC and 15 normal instances, were collected from patients who provided informed consent. The target group consisted of people who were at risk for OSCC (confirmed by Department of Biochemistry, Saveetha Dental College and Hospitals) and resided in the Saveetha Dental College and Hospitals catchment region, where OSCC causes over 30% of all new cancer cases each year. Our sample size was calculated using G*Power and its representativeness is supported by this context. Normal tissues refer to adjacent non-cancerous tissue from OSCC patients, not from cancer-free individuals. Samples were collected over a six-month period from December 2023 – June 2024, using biopsy procedures. To ensure the integrity of RNA, samples were simultaneously converted into cDNA and stored at -80°C for long-term preservation.

RNA extraction and quantification

As directed by the manufacturer, total RNA was extracted from the tissue samples using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The concentration and purity of the collected RNA were evaluated using a Thermo Fisher Scientific NanoDrop 2000 Lite spectrophotometer [11]. For further examination, the RNA samples were afterwards kept at -20°C.

Reverse transcription [12]

The extracted RNA underwent reverse transcription. First, the RNA sample was mixed with nuclease-free water, deoxyribonucleotide triphosphates (dNTPs, 10 mM each) from New England Biolabs Inc., and an oligo (dT)18 primer (Promega, 50 µM) for genes and a universal adaptor for miRNAs. After 5 minutes of incubation at 65°C, this mixture was quickly cooled to produce a final volume of 10 µl. Next, 5x prime buffer (New England Biolabs Inc.), murine RNase inhibitor (New England Biolabs Inc.), reverse transcriptase (New England Biolabs Inc.), and nuclease-free water were added to bring the total volume to 20 µl. A PCR (MiniAmp Plus thermal cycler, ThermoFisher) was then used to incubate the mixture for 10 minutes at 30°C, 30 minutes at 42°C, and 5 minutes at 95°C. A final hold was then placed at 4°C [12]. For further investigation, the cDNA was kept at -20°C after being measured using a Nanodrop Lite spectrophotometer.

Expression using qRT-PCR

For expression analysis, the cDNA was examined using Sybr Green (Takara, Japan). U6 was employed as the housekeeping control for normalization of miRNA levels. For the qRT-PCR, the BioRad CFX96 Real-Time System was used, and primers were purchased from Eurofins (Table 1). With a final melt curve analysis, the thermal cycling settings comprised an initial 30-second denaturation phase at 95ºC, 40 cycles of 5 seconds at 95ºC for denaturation, and 30 seconds for annealing. Every measurement was carried out in duplicate, and the 2^-ΔΔCq technique was used to assess the levels of gene expression [13].

Statistical Analysis

The information is displayed as the standard error of the mean (SEM) paired with the mean of two independent experiments. To assess group differences, statistical analysis was conducted using the student t-test in GraphPad Prism 10.1.0, with significance established at a p-value less than 0.05 (*). To ascertain the precise statistical significance between the groups, Tukey's test was also utilised.

Results

Identification and characterisation of miR-596

Through a thorough review of data from TCGA, our study identified miR-596 related with OSCC. Based on bioinformatics investigation, this miRNA was chosen because of its possible regulatory importance and differential expression.

Sequence retrieval and structural analysis

The structural analysis of the miR-596 sequence, which was derived from miRBase, using RNAfold showed that it had a stable stem-loop shape that is necessary for the proper processing and operation of the miRNA (Figure 1). Its significant structural stability is highlighted by the projected lowest free energy of -36.40 kcal/mol, suggesting that miR-596 may play a role in OSCC (Table 2).

Gene expression of miR-596 in OSCC

The tissue samples from OSCC patients and those from healthy people differed significantly in terms of gene expression, with miR-596 levels much lower in OSCC patients. The noteworthy decline in miR-596 implies that it may have a crucial function in the advancement of OSCC. The development of the malignancy may be aided by the downregulation of miR-596, which may have an impact on important regulatory pathways related to metastasis, apoptosis, and cell proliferation – key processes that are often altered in cancer progression. For instance, pathways such as the Phosphatidylinositol 3-kinases-protein kinase

Table 1. Primer sequences used for qRT-PCR Analysis

miRNA/ Reference gene name	Forward Primer	Reverse Primer
U6	5'- CTCGCTTCGGCAGCACA-3'	5'- ACGCTTCACGAATTTGC-3'
miR-596	5' – AAGCCTGCCCGGCTCCT – 3'	5' – GCTGTCAACGATACGCTACGT – 3'

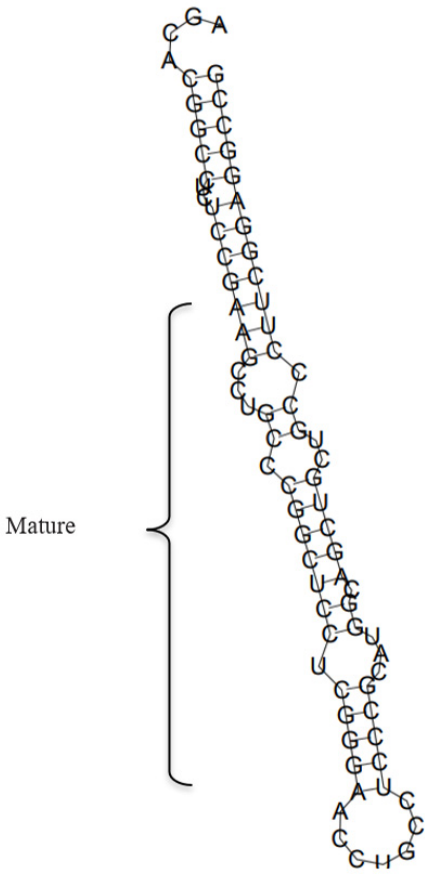

Fig. 1. Predicted secondary structure of miR-596 with corresponding minimal free energy of -36.40 kcal/mol

B (PI3K/Akt) and mitogen activated protein kinases/ extracellular signal-regulated kinases (MAPK/ERK) signaling cascades may be affected by changes in miR-596 expression levels. These pathways play pivotal roles in regulating cell survival and growth; thus, their dysregulation could contribute to the aggressive behaviour observed in OSCC (Figure 2).

Target Identification

The particular miRNA's probable targets were identified by TargetScan research in many places. This investigation showed that miR-596 targets several significant genes. An overview of these targets and the corresponding biological processes and molecular roles is provided in Table 3.

Discussions

The results are consistent with the body of research on the function of miRNAs in cancer biology, particularly the intricate roles that miR-596 plays in different forms of cancer. Depending on the circumstances surrounding the malignancy, prior studies have shown that miR-596 can function as an oncogene or a tumor suppressor. Studies have shown that in the case of gastric cancer, miR-596 acts as a tumor suppressor. Its downregulation is linked to a worse prognosis for patients as well as an increase in tumor growth [14]. For example, its downregulation has been linked to increased activation of oncogenic pathways such as Wnt/β-catenin, which promotes cell proliferation and metastasis [15]. Additionally, miR-596 has been shown to impede melanoma cell motility and invasion, highlighting the importance of understanding its unique roles across various cancer types [16].

In our study, we observed that miR-596 is significantly downregulated in OSCC tissues compared to healthy controls, which indicate that it plays a role in the advancement of the illness. The findings are consistent with previous observation that miR-596 downregulation is connected

Table 2: Characteristics of hsa-miR-596: Minimum Free Energy, Mature Sequence, Match Extent, and A+U Content

Source miRNA	Source organism	Minimum Free Energy	Mature Sequence	Match Extent	Strand	A+U%
miR-596	Homo sapiens	- 36.40 kcal	AAGCCUGCCCGGCUCCUCGGG	22/22	3p	27 (high GC content)

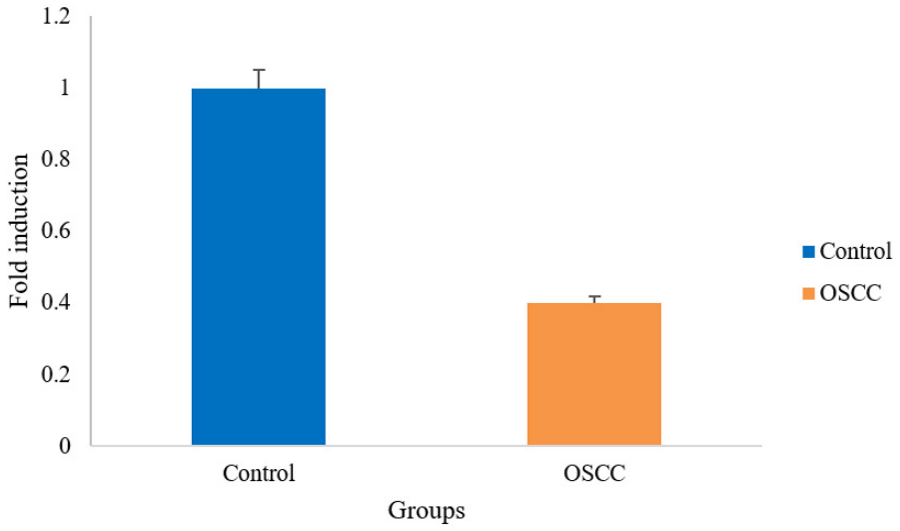

Fig. 2. Differential expression levels of miR-596 in oral squamous cell carcinoma (OSCC) vs. control samples (Statistical Significance: P <0.05)

Table 3. Molecular Functions and Biological Processes of Target Genes Regulated by hsa-miR-596

S.no	Target Protein	Uniprot Acc. No.	Molecular function	Biological process
1	Galectin-3-binding protein	NX_Q08380	Cargo receptor activity	Cell adhesion, signaling
2	ATP-dependent RNA helicase DDX3X	NX_O00571	RNA binding, hydrolase, ATP-dependent activity, GTPase activity	Chromosome segregation, signaling, differentiation, programmed cell death
3	Calnexin	NX_P27824	RNA binding	Protein folding, vesicle mediated transport, protein catabolic process
4	CSC1-like protein 1	NX_O94886	Transporter activity, molecular transducer activity, small molecule sensor activity	Transmembrane transport
5	Protein BTG2	NX_P78543	Molecular adaptor activity, transcription regulator activity	Mitotic cell cycle, DNA repair, regulation of DNA-templated transcription, cell differentiation

to tumor development and metastasis in other malignancies. Significant biological pathways linked to cell proliferation, apoptosis, and metastasis – all important stages in the formation of cancer – may be dysregulated as a result of the observed reduction in miR-596 levels in OSCC tissues. The data supports the hypothesis that miR-596 may function as a tumor suppressor in OSCC. A context dependent role driven by the tumor microenvironment and the unique molecular landscape of each cancer type is suggested by miR-596's dual nature, which shows it to have both tumor-suppressive and oncogenic qualities in various malignancies. Some research indicates that miR-596 may have carcinogenic potential in some circumstances, which is contrary to our findings. The structural study of miR-596 also reveals a low free energy of -36.40 kcal/mol, indicating a very stable stem-loop shape that is critical to its processing and function. Its capacity to control gene expression may be significantly influenced by its structural stability. Knowing these structural details can help us gain a better understanding of the interactions between miRNA and mRNA and how they affect the pathways linked to cancer.

Our use of TargetScan to identify putative miR-596 gene targets emphasizes the regulatory potential of this compound. These findings warrant further investigation into the precise mechanisms by which miR-596 impacts OSCC pathogenesis and experimental validation of its targets. Given these insights, miR-596 emerges as a promising novel diagnostic biomarker or therapeutic target for OSCC. Future research should focus on exploring the therapeutic potential of modifying miR-596 levels in clinical settings and confirming these results in larger cohorts. By validating the functional roles of its target genes and regulatory pathways, we can uncover new avenues for targeted therapeutics and deepen our understanding of miR-596's role in OSCC.

Conclusion

This study demonstrates that miR-596 is significantly downregulated in oral squamous cell carcinoma (OSCC) tissues compared to normal samples, suggesting its potential role as a tumor suppressor. The observed downregulation may disrupt critical cellular processes such as apoptosis and proliferation, contributing to the aggressive behavior of OSCC. Additionally, our findings identify key genes modulated by miR-596, indicating its extensive regulatory impact on pathways essential for cancer progression. However, it is important to note that these conclusions are based on a limited number of samples, and the study's limitations should be acknowledged. Further research is needed to clarify the precise mechanisms by which miR-596 influences OSCC pathogenesis and to validate these findings in larger cohorts.

Abbreviations

OSCC: Oral Squamous Cell Carcinoma;
TCGA: The Cancer Genome Atlas;
miRNA: microRNA;
SEM: Standard error of the mean;
dNTP: deoxyribonucleotide triphosphate

Authors' contribution

DS (Conceptualization; Data curation; Investigation; Methodology; Validation; Visualization; Writing – review) SF (Writing – original draft; analysis; data collection) AP (Data collection; Formal analysis; Writing) AP (Data collection; Validation; analysis). All authors read and approved the final manuscript.

Conflict of interest

None to declare.

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