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Elucidating the Role of *TP53* in Oral Squamous Cell Carcinoma: A Cross-Sectional Observational Study of Integrated Analysis of Expression Patterns and Functional Importance

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Abstract

Introduction Oral squamous cell carcinoma (OSCC) is one of the major contributors of cancers associated with carcinoma, specifically among alcohol and tobacco consumers. Alterations in the *TP53* gene are known to contribute to aggressive features of tumors. The present study explores miR-21, a microRNA targeting *TP53* gene, focusing on the potential role of miR-21 in the regulation of OSCC pathogenesis at both computational and experimental levels.

Objective This article aims to evaluate the expression and regulatory relationship between miR-21 and *TP53* in OSCC, and to determine the potential of miR-21 as a therapeutic target.

Materials and Methods This was a cross-sectional observational study designed to investigate the regulatory relationship between miR-21 and *TP53* gene in OSCC. A total of 30 OSCC tissue samples and normal tissue samples were collected from patients undergoing surgical resection at Saveetha Dental College and Hospitals, following approval by the institutional ethics committee and after obtaining informed consent. Tissue specimens were immediately snap-frozen in liquid nitrogen and stored at −80 °C until further use.
The bioinformatics tools, TargetScan and miRDB, predicted the miRNAs that regulate *TP53*. The OSCC tissue samples were used to perform qRT-PCR following the extraction of RNA with TRIzol reagent for the expression profiling of miR-21 and *TP53* gene.

Results While *TP53* gene expression was noticeably downregulated, the research showed that miR-21 was considerably increased in OSCC tissues. Given their inverse association, miR-21 may enhance carcinogenesis by suppressing *TP53* activity.

Conclusion The findings underscore the critical interplay between miR-21 and *TP53* in OSCC pathogenesis. By regaining *TP53* function, targeting miR-21 may offer a cutting-edge therapeutic approach for bettering patient outcomes. The clinical consequences of these interactions should be investigated further to provide tailored treatments for the management of OSCC.

Keywords
- oral squamous cell carcinoma
- microRNAs
- gene expression
- computational biology
- biomarker

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Introduction

Oral squamous cell carcinoma (OSCC) constitutes >90% of all oral cancers and is a serious health issue globally, as it is highly aggressive. OSCC heavily affects regions like parts of Southeast Asia and parts of Europe, especially with India accounting for 45% of all cases around the world.[1,2] The rising incidence of OSCC is largely attributed to lifestyle-related risk factors such as tobacco use, alcohol consumption, and betel quid chewing. It is now recognized as the sixth most common cancer among women and the fourth most common in men worldwide.

Despite advances in surgical and radiotherapeutic approaches, OSCC remains difficult to treat due to its complex molecular landscape, high rates of local invasion, and a considerable tendency for recurrence. These clinical challenges highlight the urgent need for molecularly targeted therapies that can improve prognosis and reduce mortality.[3]

Among targeted therapies, cetuximab—an estimated glomerular filtration rate inhibitor—was introduced for OSCC and initially showed promise in combination with chemotherapy or radiation. However, its overall impact on long-term survival and disease progression has been limited, and it has not significantly changed the poor prognosis associated with advanced-stage OSCC. This underscores the need for new, more effective therapeutic strategies and biomarkers in this disease.[4] One of the most commonly altered tumor suppressor genes, *TP53*, also known as the "guardian of the genome," is among the numerous genetic changes seen in OSCC.[5–7] Uncontrolled cellular proliferation, metastasis, and treatment resistance are all impacted by the *TP53* gene mutations or dysregulation, which interfere with normal cell cycle regulation, DNA repair processes, and apoptosis. The regulating role of microRNAs (miRNAs) in the development of cancer has been highlighted by new research.[8] Specifically, miR-21 has drawn notice for its carcinogenic potential in several solid tumors, including OSCC. Through the inhibition of important suppressor genes like *TP53*, increased expression of miR-21 has been associated with increased tumor development, invasiveness, and resistance to chemotherapy.

To find new biomarkers and explore treatment options that can enhance clinical outcomes for patients with OSCC, this study intends to examine the molecular interactions between miR-21 and *TP53* gene in OSCC by combining bioinformatics predictions with experimental confirmation.

Materials and Methods

Study Design

This was a cross-sectional observational study aimed at evaluating the regulatory relationship between miR-21 and *TP53* gene in OSCC. A total of 30 OSCC tissue samples and normal tissues were collected from patients undergoing surgical excision at Saveetha Medical College and Hospitals. The sample size ($n = 30$) was determined based on the availability of suitable specimens during the study period and the exploratory nature of the investigation, in line with similar gene expression studies.

All samples were confirmed as OSCC through histopathological examination by two independent oral pathologists. Only newly diagnosed, treatment-naive OSCC cases were included. Tissues were collected immediately post-resection, snap-frozen in liquid nitrogen, and stored at −80°C until further use.

Inclusion and Exclusion Criteria

Patients included in the study had histologically confirmed OSCC and provided sufficient, good-quality tissue samples for molecular analysis. Only treatment-naive individuals who had not received chemotherapy or radiotherapy were eligible. Exclusion criteria comprised previously treated or recurrent OSCC cases, inadequate or degraded tissue samples, and the presence of systemic inflammatory or immunological disorders that could confound molecular expression profiles.

Identification of a Critical Tumor Suppressor in OSCC and Regulatory miRNAs

To find a tumor suppressor gene known to be relevant in OSCC, a comprehensive study of the literature was undertaken. Because of its essential function in tumor suppression mechanisms like apoptosis, DNA repair, and cell cycle regulation, as well as its constant downregulation in OSCC, *TP53* gene was chosen. Potential miRNAs were predicted using two trustworthy online databases, miRDB and TargetScan, to investigate post-transcriptional control of *TP53* gene. Following the selection of miRNAs with a high target prediction score for the *TP53* gene, miR-21 was selected because of its propensity for overexpression in head and neck cancers and its recognized carcinogenic characteristics.[9,10] RNAfold, an online tool for predicting secondary structure and evaluating the stability of miRNA hairpin loops, was utilized to further analyze the sequencing and structural characteristics of miR-21. The stability of miR-21, which is essential for its effective binding to target mRNAs, was inferred from the minimum free energy (MFE) values produced.

RNA Extraction and Reverse Transcription

Thirty tissue samples were collected from patients with OSCC who had histological confirmation. TRIzol reagent (Invitrogen, Carlsbad, California, United States) was used to isolate total RNA from tissue samples in accordance with the manufacturer's recommended procedure. NanoDrop spectrophotometry from Thermo Fisher Scientific (Massachusetts, United States) was used to measure the amount and purity of the isolated RNA. RNA samples were deemed suitable for additional investigation if their 260:280 ratios fell between 1.8 and 2.0.[11] A commercial reverse transcription kit (Takara, Japan) was used to synthesize complementary DNA (cDNA), which was then kept at −80°C until it was needed for expression investigations.[12]

Quantitative Gene Expression Analysis

Using a BioRad CFX96TM real-time PCR machine (California, United States) and SYBR Green Master Mix (Takara, Japan), quantitative PCR (q-PCR) was used to analyze gene

expression. Eurofins Genomics developed and synthesized specific primers for miR-21 and *TP53* gene. *β-actin* served as a housekeeping gene for *TP53* gene normalization, while the short nuclear RNA U6 served as an internal control for miRNA normalization.

Forty amplification cycles of 95°C for 15 seconds and 60°C for 30 seconds were performed after initial denaturation at 95°C for 2 minutes in duplicate for each qRT-PCR experiment. Relative gene expression levels were computed using the $2^{-\Delta\Delta Cq}$ technique, which compares the fold-changes of normal and malignant tissue samples.[12]

Outcomes

Primary Outcome

To determine the relative expression levels of miR-21 and *TP53* in OSCC tissues using quantitative real-time PCR (qRT-PCR).

Secondary Outcome

To explore the potential inverse regulatory relationship between miR-21 and *TP53* based on expression trends and bioinformatics predictions using TargetScan (https://www.targetscan.org/vert_80/, TargetScan Release 8.0), miRDB (https://mirdb.org/, version 6.0), and RNAfold (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi).

Statistical Analysis

To guarantee reproducibility, every experiment was performed in duplicate. The data were presented using the standard error of the mean (SEM) ± the mean. To compare the expression levels of OSCC and control tissues, statistical analysis was performed using GraphPad Prism version 10.1.0 and the Student's *t*-test. Statistical significance was established at a *p*-value of less than 0.05.

Ethical Approval

The Institutional Ethics Committee approved this study (Approval No: 581/03/2023/UG/SRB/SMCH, Department of Medicine, Saveetha Medical College), and all methods followed the Helsinki Declaration's ethical guidelines. Written informed consent was obtained from all patients prior to tissue sample collection.

Results

Identification and Structural Analysis of miR-21 as a Regulatory miRNA Targeting Gene

After *TP53* gene was discovered to be a major tumor suppressor gene linked to the pathophysiology of OSCC, we used bioinformatics prediction methods to find regulatory microRNAs that might affect *TP53* gene expression. Several potential miRNAs were found using miRDB and TargetScan. Because of its previously demonstrated carcinogenic functions in several malignancies, including OSCC, miR-21 stood out among these as a promising option (►**Fig. 1**). Previous publications have highlighted the overexpression of miR-21 in head and neck squamous cell carcinomas, indicating that it regulates genes involved in apoptosis, cell cycle, and tumor invasion.

The secondary structure and thermodynamic stability of miR-21 were predicted using RNAfold to assess its functional potential in more detail. miR-21 forms a distinctive and stable stem-loop structure, which is necessary for miRNA processing and function, according to the computational modelling (►**Fig. 2**). High thermodynamic stability and a great potential for successful interaction with target mRNAs like *TP53* gene were indicated by the estimated structure's MFE, which was −34.60 kcal/mol (►**Table 1**). These results lend credence to the theory that miR-21 may contribute to the molecular mechanisms of OSCC by acting as a strong post-transcriptional regulator of *TP53* gene.

Expression Profiling of *TP53* Gene and miR-21 in OSCC Tissues

The expression levels of miR-21 and *TP53* gene were measured in human OSCC tissue samples ($n = 30$) and compared with normal tissues ($n = 30$) using qRT-PCR to validate the in-silico predictions.

According to analysis, *TP53* gene expression was considerably lower in OSCC samples than in controls, which is in line with its well-established function as a tumor suppressor and the fact that it frequently mutates or is suppressed in

MicroRNA and Target Gene Description:

miRNA Name	hsa-miR-21-3p	**miRNA Sequence**	CAACACCAGUCGAUGGGCUGU
Previous Name	hsa-miR-21*		
Target Score	52	**Seed Location**	59, 460
NCBI Gene ID	7157	**GenBank Accession**	NM_001126114
Gene Symbol	TP53	**3' UTR Length**	1496
Gene Description	tumor protein p53		

Fig. 1 miRNAs predicted to target *TP53* gene: Computational prediction of putative miRNAs regulating *TP53* gene. miRDB was utilized to generate a comprehensive list of potential miRNAs, highlighting miR-21 as a significant candidate due to its biological relevance in OSCC and its expected interaction with *TP53* gene.

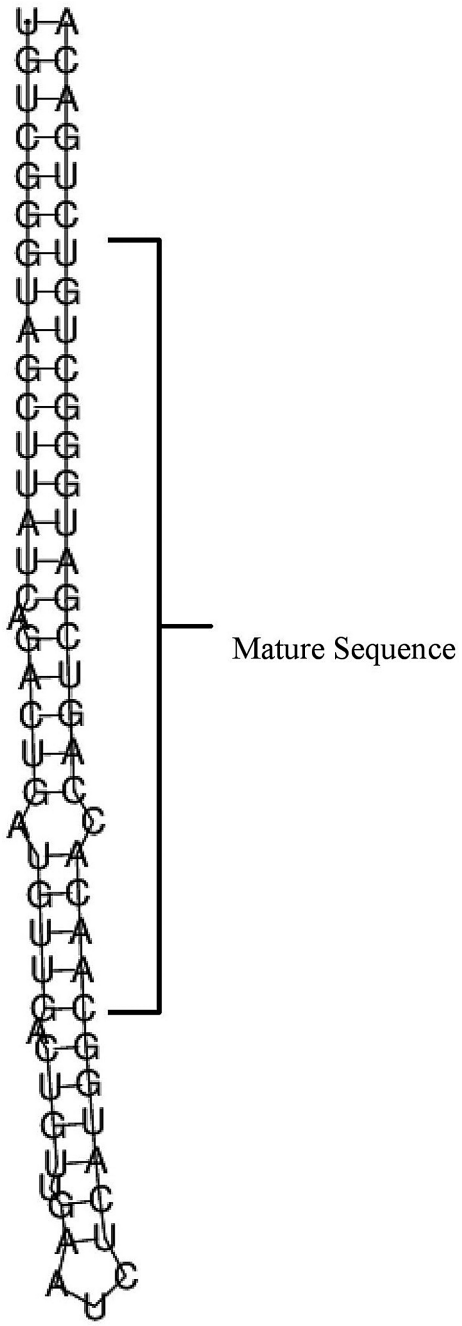

Fig. 2 Secondary structure analysis of has-miR-21–3p: RNAfold analysis predicts a stable stem-loop configuration essential for the proper processing and function of miR-21, with a measured free energy of −34.60 kcal/mol, indicating strong structural stability.

cancers (►**Fig. 3**). In the same OSCC tissues, miR-21 was also significantly elevated (►**Fig. 4**), supporting its suspected role as an oncogenic miRNA that could aid in *TP53* gene suppression.

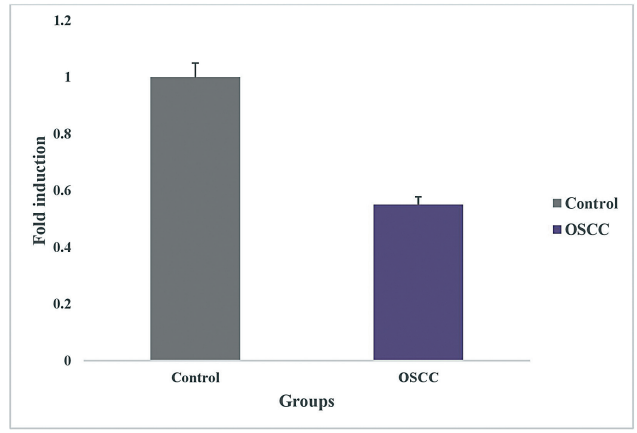

Fig. 3 Expression profiling of *TP53* gene in OSCC tissue samples: Comparative analysis reveals significant downregulation of *TP53* gene expression in patients with OSCC compared with healthy controls, suggesting its role as a critical tumor suppressor in OSCC pathogenesis.

Further evidence of a regulatory link was provided by the statistically significant inverse correlation between miR-21 overexpression and *TP53* gene downregulation ($p < 0.05$, Student's *t*-test). According to this pattern, increased levels of miR-21 may block *TP53* gene-mediated tumor suppressive pathways, which could promote tumor genesis, growth, and treatment resistance.

Expression of miR-21 and *TP53* in Relation to Clinicopathological Parameters

To evaluate the clinical relevance of miR-21 and *TP53* expression in OSCC, we analyzed their expression profiles in relation to tumor stage, patient age, tumor grade, and nodal metastasis status using data from TCGA (►**Figs. 5** and **6**) and patient samples (►**Figs. 7** and **8**). miR-21 expression is consistently elevated across all tumor stages, patient ages, grades, and nodal statuses compared with normal tissues. Although higher median levels tend to appear in advanced stages and grades, there is considerable variability within groups, indicating that miR-21 upregulation is a common feature of OSCC independent of these clinical factors. However, *TP53* expression is consistently reduced in HNSCC samples compared with normal tissues across all clinical categories. This downregulation shows no strong association with tumor stage, grade, age, or nodal status. In our study, correlation analysis was performed using Spearman's rank correlation to assess the relationship between gene expression and clinical parameters. Here, *miR-21* expression showed a strong positive correlation with tumor stage, size, and nodal involvement ($p < 0.001$). In contrast, *TP53* expression was strongly negatively correlated with these

Table 1 Minimum free energy, mature sequence, match extend, and A + U% of has-miR-21

miRNA	Organism	Minimum free energy	Mature sequence	Match extend	Strand	A + U%
miR-21	*Homo*	− 34.60 kcal	CAACACCAGUCGA	21/21	3p	50%
	Sapiens		UGGGCUGU			

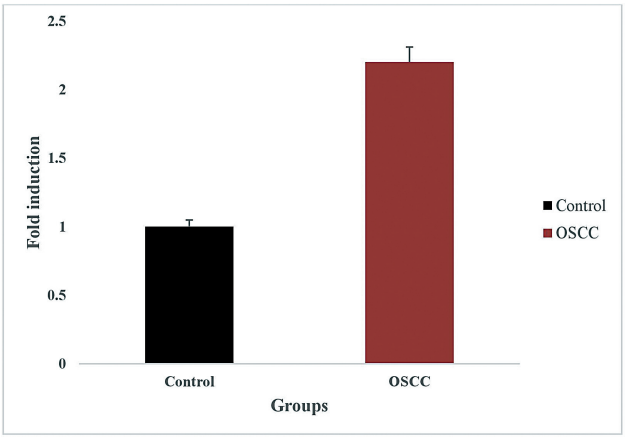

Fig. 4 Expression levels of miR-21 in OSCC tissue samples: Elevated levels of miR-21 are observed in patients with OSCC, indicating its potential oncogenic role and interaction with *TP53* gene in the development of oral squamous cell carcinoma.

clinical parameters ($p < 0.001$), suggesting inverse regulation with disease progression.

Discussion

This study highlights the roles of *TP53* gene and miR-21 in OSCC, with around 70% of patients having *TP53* gene mutations linked to aggressive tumors.[13] Shreya Reddy et al associate high miR-21 levels with advanced cancers, suggesting it promotes tumor growth by inhibiting *TP53* gene.[10] Our findings support Rajan et al, emphasizing the need for further research on the combined effects of multiple miRNAs on tumor behavior.[14] This study demonstrates the clinical relevance of miR-21 as a potential biomarker for early diagnosis and personalized treatment of OSCC. Targeting miR-21 in chemotherapy-resistant tumors using antagomirs or RNA-based therapeutics may enhance chemosensitivity and restore *TP53* gene activity. Recent advancements in RNA therapeutics show promise for clinical applications.[15] This research enhances understanding of *TP53* gene and miR-21 interactions in OSCC, paving the way for novel treatments to improve patient outcomes.

In addition to these molecular insights, recent advances in immunotherapy, particularly the introduction of immune checkpoint inhibitors such as nivolumab and pembrolizumab, have expanded the treatment landscape for OSCC. These therapies have shown promise in recurrent and metastatic cases by harnessing the immune system to target tumor cells.[16] However, despite these advances, the prognosis for patients with advanced-stage OSCC remains poor, with limited response rates and many patients not achieving durable remission. This underscores a considerable unmet need for more effective therapeutic strategies and predictive biomarkers in OSCC, especially for those diagnosed at later stages. The identification of molecular regulators such as

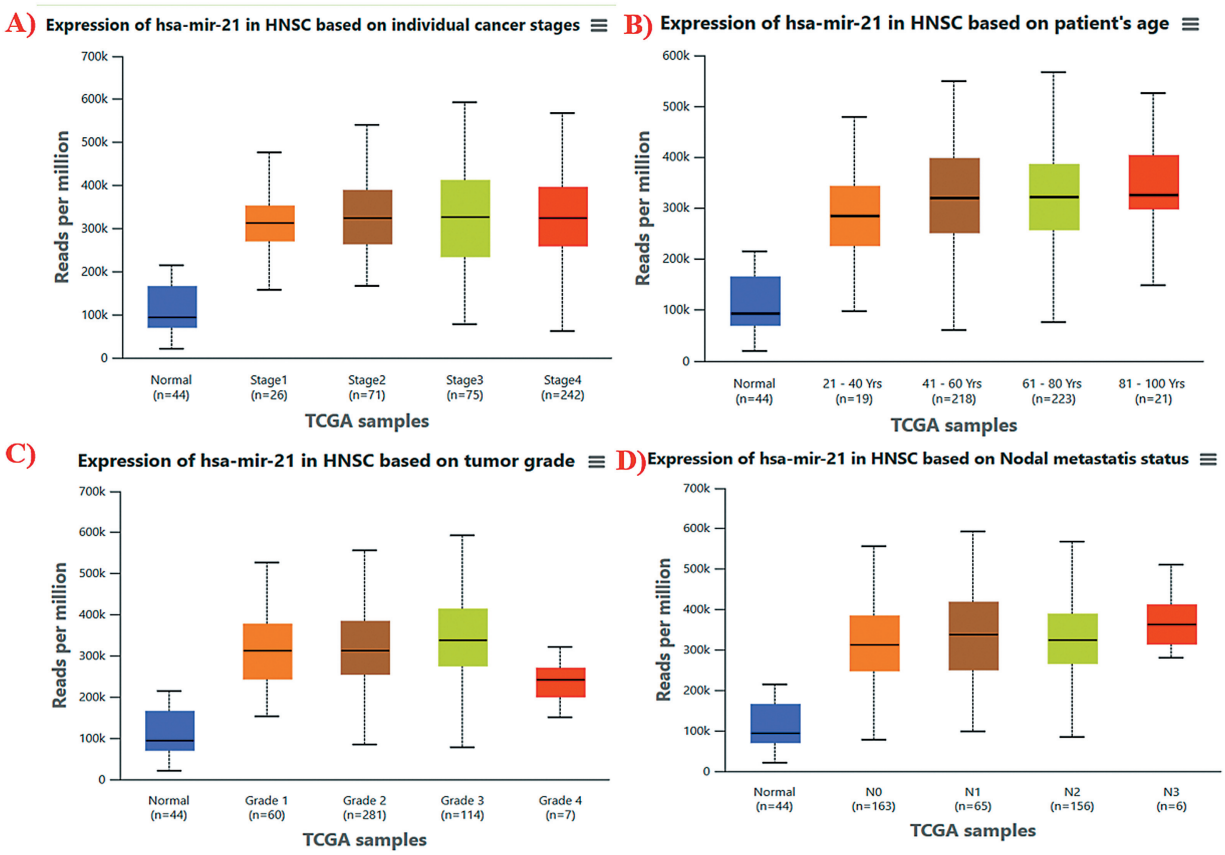

Fig. 5 (**A–D**) Expression of miR-21 in HNSCC samples stratified by tumor stage, patient age, tumor grade, and nodal metastasis status. Box plots show that miR-21 is consistently upregulated across all clinical subgroups compared with normal tissues, with substantial variability within each group.

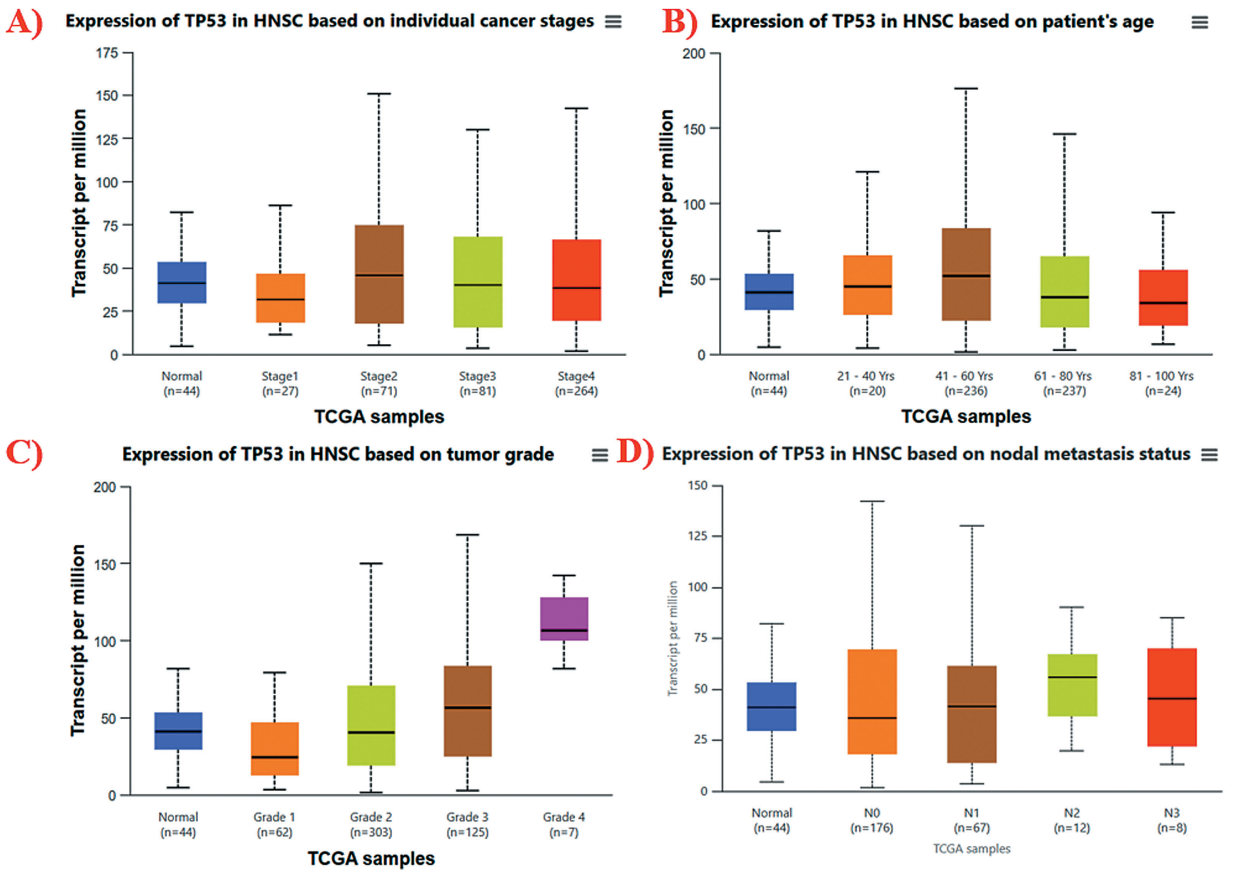

Fig. 6 (A–D) Expression of *TP53* in HNSCC samples stratified by tumor stage, patient age, tumor grade, and nodal metastasis status. Box plots demonstrate that *TP53* is consistently downregulated across all clinical subgroups compared with normal tissues, with no strong association between expression levels and specific clinicopathological features.

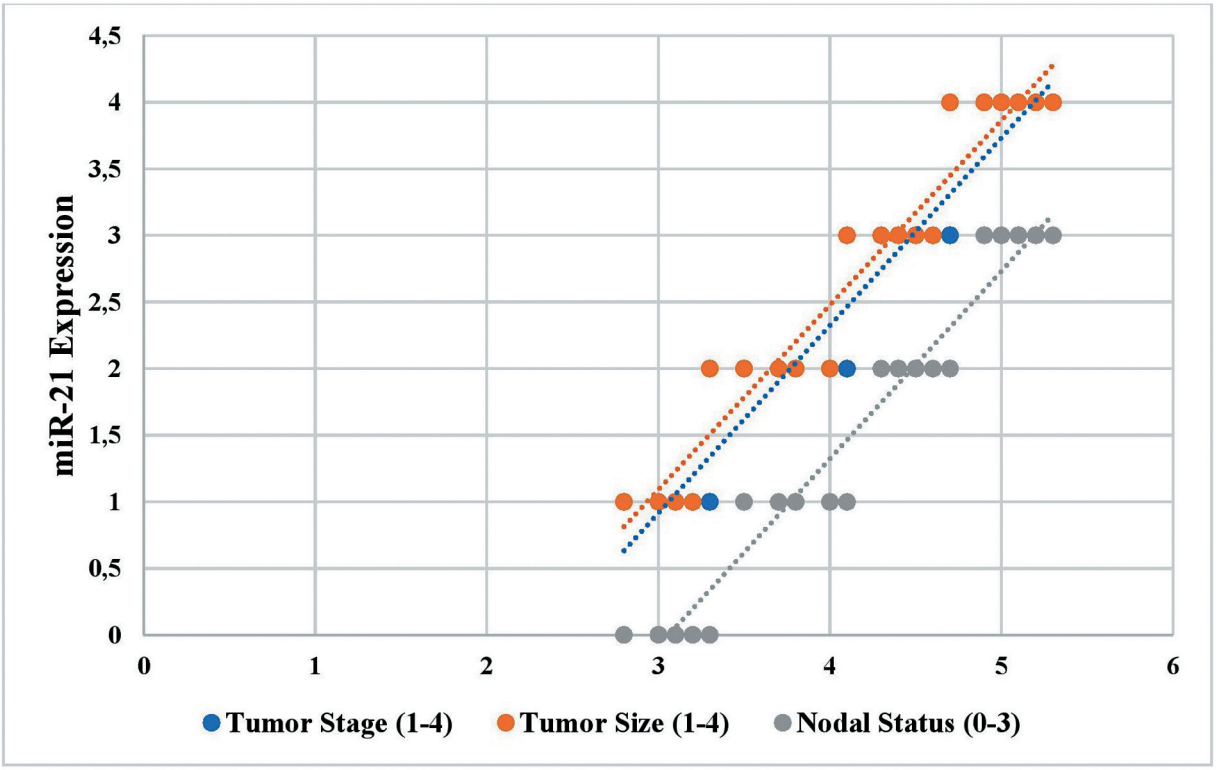

Fig. 7 Correlation of *miR-21* expression with tumor stage, tumor size, and nodal status in OSCC patient samples. *miR-21* expression shows a significant positive correlation with advancing clinical parameters. Scatter plots illustrate upregulation of *miR-21* with disease progression.

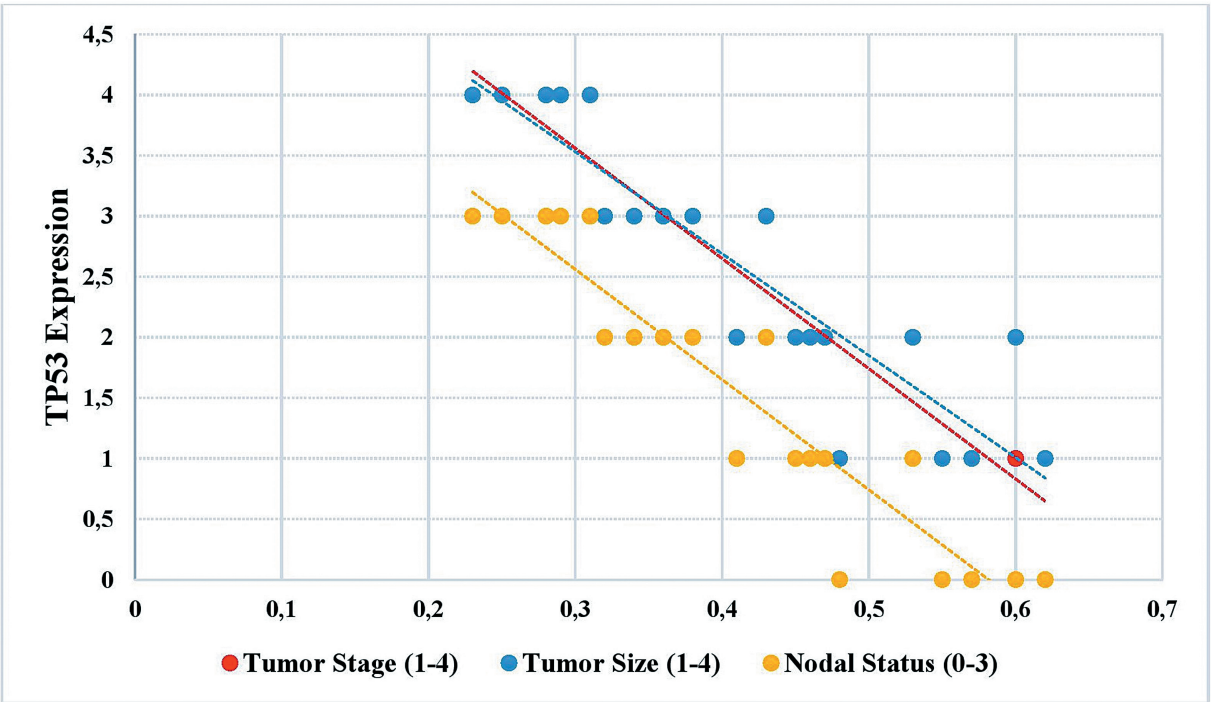

Fig. 8 Correlation of *TP53* expression with tumor stage, tumor size, and nodal status. *TP53* expression exhibits a significant negative correlation, and graphs demonstrate downregulation of *TP53* expression aligned with advancing disease.

miR-21 and *TP53* may contribute to the development of novel targeted therapies and improve clinical outcomes in this challenging disease. Although targeted therapies such as cetuximab have been used in OSCC management, their limited effectiveness in improving survival outcomes further highlights the necessity for innovative molecular targets, such as the miR-21/*TP53* axis explored in this study.

However, the study has certain limitations. The relatively small sample size may limit the generalizability of the findings. Additionally, the observational nature of the study precludes definitive conclusions about causality between miR-21 expression and *TP53* gene downregulation. Functional validation studies, such as gene knockdown or overexpression experiments, were not performed and are necessary to confirm the mechanistic link. Moreover, the exclusive focus on miR-21 does not account for the potential influence of other regulatory miRNAs or signaling pathways involved in OSCC. Future studies involving larger, multicentric cohorts and functional assays are warranted to validate and extend these findings.

While *TP53* dysfunction is a hallmark of many cancers, including OSCC, its presence or absence alone may not provide sufficient specificity or sensitivity as a biomarker for early diagnosis. However, evaluating *TP53* expression in conjunction with oncogenic miRNAs such as miR-21 may enhance diagnostic accuracy and better reflect the molecular mechanisms underlying OSCC progression. This combinatorial approach could improve the reliability of molecular biomarkers in clinical practice. The strength of this work lies in proposing an integrated biomarker strategy that moves beyond reliance on single markers, thereby offering a more robust and clinically translatable framework for improving early detection and understanding the molecular complexity of OSCC.

Conclusion

Our study examined the interaction between miR-21 and the tumor suppressor gene *TP53* in OSCC. We found that miR-21 is overexpressed, while *TP53* gene is downregulated in OSCC tissues, indicating that miR-21 inhibits *TP53* gene, which may promote cancer progression. This dysfunction of *TP53* gene contributes to uncontrolled growth and metastasis, worsening OSCC aggressiveness. Our dual approach highlights *TP53*'s role as a tumor suppressor and miR-21's oncogenic function, emphasizing the need for further research into miRNA-target interactions as therapeutic targets. Targeting miR-21 could improve treatment outcomes, and future studies should focus on clinical applications to enhance diagnostic and therapeutic strategies for OSCC.

Data Availability Statement

The datasets analyzed in this study are available from the corresponding author upon request.

Authors' Contributions

Conceptualization and formal analysis: D.S.; writing original draft: S.H.S.; data curation: A.K.P.; writing review and editing: D.S.

Patient Consent

Informed consent was obtained from all the participants before conducting the interviews, and anonymity and confidentiality were ensured throughout the research process.

Conflict of Interest
None declared.

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