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High Expression of *Myosin XVI* Predicts Poor Prognosis in Head and Neck Squamous Cell Carcinoma

Keerti Pranith Suryadevara*, MBBS student, Balachander Kannan, PhD candidate, Chandra Pandi**, PhD candidate, Anitha Pandi***, PhD candidate, Abilasha Ramasubramanian****, MDS, PhD, Vijayashree Priyadharsini Jayaseelan***, PhD, Paramasivam Arumugam***♦, PhD**

**Saveetha Medical College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India*
***Molecular Biology Lab, Centre for Cellular and Molecular Research, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India*
****Clinical Genetics Lab, Centre for Cellular and Molecular Research, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India*
*****Department of Oral Pathology and Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India*

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♦Corresponding Author:
Paramasivam Arumugam, PhD
Molecular Biology Lab, Centre for Cellular and Molecular Research, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India
E-mail: paramasivama.sdc@saveetha.com

Abstract

Background: Myosins, a superfamily of actin-dependent molecular motors, have emerged as crucial players in tumorigenesis. This study investigates the role of *Myosin XVI* (*MYO16*), an unconventional myosin, in head and neck squamous cell carcinoma (HNSCC).

Method: In this case-control study, we employed multiple databases to investigate the expression of *MYO16* in samples from the cancer genome atlas (TCGA), focusing on HNSCC along with associated clinicopathological features. Since HNSCC primarily includes oral squamous cell carcinoma (OSCC), we additionally validated the mRNA level of *MYO16* in OSCC samples using the real-time quantitative polymerase chain reaction (RT-qPCR) method. Moreover, we used various online databases to uncover the relationship between *MYO16* and tumor infiltration. Statistical analysis was performed using GraphPad Prism, and the significance was determined with student's t-test.

Results: Comprehensive analyses across diverse databases consistently reveal a significant upregulation of *MYO16* expression in HNSCC. RT-qPCR analysis revealed that *MYO16* is significantly upregulated in OSCC tumor tissue samples. Correlation with clinicopathological features and survival analysis underscores its potential prognostic value. Furthermore, *MYO16* interactions with immune cells within the tumor microenvironment are negatively associated with immune genes.

Conclusion: This study identifies *MYO16* as a potential biomarker associated with HNSCC development, and emphasizes its significance as a potential therapeutic target, aligning with its diverse roles across cellular processes. Further experimental studies are necessary to elucidate MYO16 functional implications and clinical relevance in HNSCC.

Keywords: Squamous cell carcinoma of head and neck, Health, Genetics, Cancer

Introduction

Head and neck squamous cell carcinoma (HNSCC) constitutes a prevalent malignancy originating from the mucosal surfaces of various head and neck regions. It ranks as the sixth most common malignant tumor, globally. HNSCC majorly consists of oral squamous cell carcinoma (OSCC) which is very common in India.[1] Etiological factors include alcohol, tobacco, human papillomavirus, and Epstein-Barr virus infections, resulting in phenotypic, etiological, biological, and clinical diversity within HNSCC.[2-4] Current treatment methods, encompassing surgery, radiation, and chemotherapy, often lead to cosmetic deformities and functional impairments, while the survival rate remains around 50%. Lymph node metastasis greatly impacts prognosis, highlighting the necessity of understanding the mechanisms behind HNSCC metastasis.[5] Cell migration, facilitated by protrusive structures like filopodia and lamellipodia, is a pivotal aspect of cancer invasion and metastasis; yet, the mechanisms underlying this in HNSCC remain unclear. Genetic factors also contribute to HNSCC development, with p53 mutations and chromosomal instability being common. The emergence of human papillomavirus-related malignancies, distinct in pathogenesis and prognosis, adds complexity. Multidisciplinary approaches are essential due to the diverse anatomical sites and critical nearby structures involved in HNSCC. Advances in genetic understanding, from microarrays to next-generation sequencing, have contributed to identifying mutated tumour suppressor genes and oncogenes, aiding the development of novel therapeutic strategies for HNSCC.[1,6,7]

The process of tumorigenesis involves various factors, including the emerging recognition of the significant roles of myosins. Myosins, actin-dependent molecular motors, convert adenosine triphosphate (ATP) hydrolysis energy into mechanical stress by interacting with microfilaments.[8] They are classified into 18 distinct classes with nearly 40 genes in the human genome. Myosins have three subdomains: the motor domain for actin binding and ATP hydrolysis, the neck domain with isoleucine (I) and glutamine (Q) (IQ) motifs for binding, and the tail domain for cargo transport along microfilaments.[8,9] Myosins have been suggested to play a role in reproductive system diseases , exhibit multifunctional roles in tumorigenesis, and are considered potential therapeutic targets.

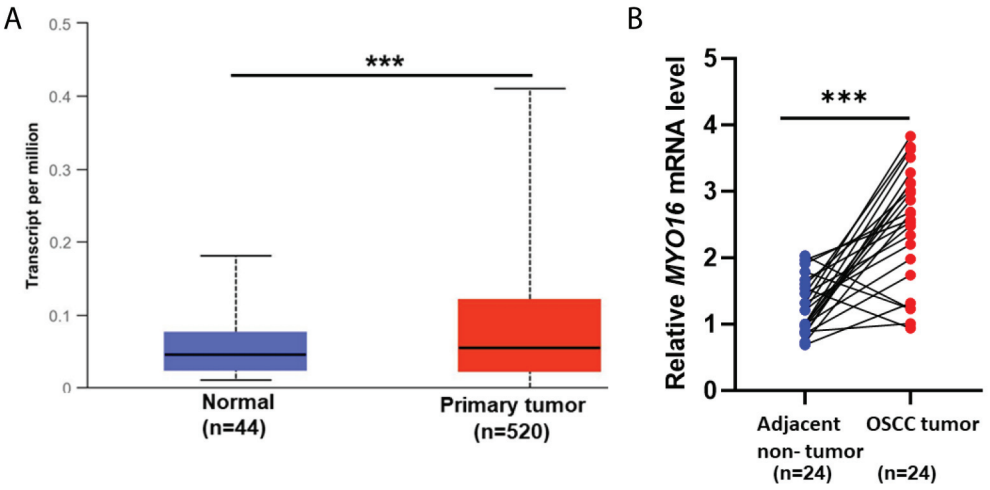

Figure 1. *MYO16* mRNA expression in HNSCC and OSCC. (A) UALCAN database showed that *MYO16* mRNA expression in HNSCC primary tumor and normal tissues (TCGA-dataset). (B) *MYO16* mRNA level analysed between matched OSCC tissues and adjacent non-tumor tissue from the same OSCC patient (Source: UALCAN (A), RT-qPCR (B)).

***Denotes significant difference $P < 0.001$; *MYO16*: *Myosin XVI* ; HNSCC: Head and neck squamous cell carcinoma; OSCC: Oral squamous cell carcinoma; mRNA: messenger ribonucleic acid; TCGA: The cancer genome atlas; UALCAN: The University of Alabama at Birmingham; RT-qPCR: Real time- quantitative polymerase chain reaction

Overexpression of specific myosins, such as myosin IE, II, Va, VI, VII, IX, and X, plays pivotal roles in various cancers, influencing processes like invadosome assembly, cell motility, DNA damage repair, and cell adhesion.[10-14] Further exploration of the involvement of myosins in cancer cell formation holds promise for future oncotherapy approaches.

The myosin superfamily encompasses actin-based mechanochemical machines converting ATP hydrolysis to mechanical work. Among these, *Myosin XVI* (*MYO16*), a neuronally expressed unconventional myosin, is associated with actin cytoskeleton regulation and neuronal functions. In Rat2 cells, MYO16 mRNA and protein peak during late G1 through the S-phase and decrease entering the M-phase. MYO16 depletion alters cell cycle distribution and triggers cell death. In DNA replication stress, MYO16 protein loss is evident, followed by recovery upon replication stress attenuation.[15] These findings collectively suggest a potential regulatory role for *MYO16* in cell cycle progression, warranting further exploration of its significance in cellular dynamics and its potential implications in disease contexts like HNSCC. In this study, we investigated the *MYO16* expression in OSCC patient sample tissue and HNSCC samples in the cancer genome atlas dataset.

Materials and Methods

Gene expression analysis using UALCAN database

To explore the expression pattern of *MYO16* in HNSCC, we first used publicly available transcriptomic data from The Cancer Genome Atlas (TCGA). The TCGA-HNSCC dataset containing HNSCC samples (n=520) and normal tissues (n = 44) was used. The UALCAN database (http://ualcan.path.uab.edu)[16] was employed for this analysis. We also examined the correlation of *MYO16* expression with various clinicopathological features, such as tumour stage, grade, nodal metastasis, and patient survival, to assess its potential prognostic significance.

Patient recruitment and sample collection

In this case-control study, a total of 40 OSCC patients were enrolled between March 2023 to November 2023 at Department of Oral and Maxillofacial, Saveetha Dental College and Hospitals, Chennai, to acquire primary OSCC tumor (n = 24) and adjacent non-tumor (surrounding tissues) (n= 24) tissue samples. Patients with no history of other systemic diseases or genetic diseases or recurrence were included. G power statistical software (version 3.1.9.6) was used to calculate the sample size for the present study, with the effect size, α error probability, and power. Samples were collected during surgery,

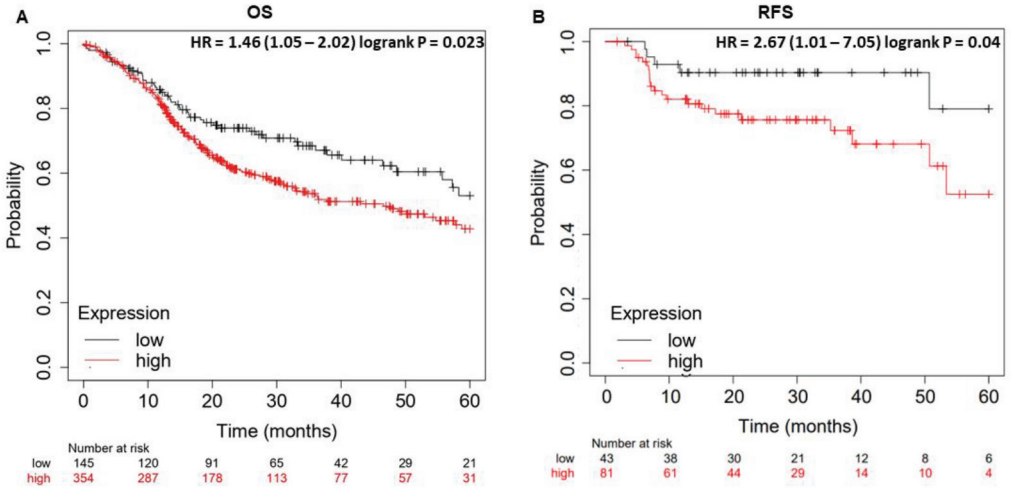

Figure 2. *MYO16* mRNA expression and HNSCC prognosis. (A) *MYO16* expression classified into low and high expression and analysed for OS up to 60 months. (B) *MYO16* expression classified into low and high expression and analysed for RFS up to 60 months (Source: Kaplan-Meier plotter).

HNSCC: Head and neck squamous cell carcinoma; OS: Overall survival; RFS: Relapse-free survival; HR: Hazard ratio; mRNA: messenger ribonucleic acid; *MYO16*: *Myosin XVI*

Table 1. Clinical features of patients with oral squamous cell carcinoma

Variable	Category	Number of patients (%)
Gender	Male	32 (80)
	Female	8 (20)
Age	≤ 50 years	17 (42.5)
	≥ 51 years	23 (57.5)
Grade	Well-differentiated	23 (57.5)
	Moderately-differentiated	15 (37.5)
	Poorly-differentiated	2 (5)
Site	Buccal	12 (30)
	Tongue	9 (22.5)
	Other (RMT, GBS, Maxilla, Mandible)	19 (47.5)
Stage	I	5 (12.5)
	II	8 (20)
	III	8 (20)
	IV	19 (47.5)
Laterality	Left	15 (37.5)
	Right	25 (62.5)
Lymph node metastasis	Yes	15 (37.5)
	No	25 (62.5)

RMT: Retromolar trigone; GBS: Gingivobuccal sulcus

histopathologically confirmed as tumor and non-tumor tissues, and promptly stored at -80 ºC until further processing. Corresponding clinicopathological data were recorded (Table 1). The Institutional Ethical Committee approved the study, adhering to the principles of the Helsinki Declaration, and informed consent was obtained from each patient.

RNA extraction and real-time quantitative polymerase chain reaction (RT-qPCR) analysis

Total RNA was extracted from the tumor and adjacent non-tumor tissues using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA). RNA quality was assessed using Nanodrop One (Thermo Scientific, USA), followed by cDNA synthesis with Takara 1st strand cDNA synthesis

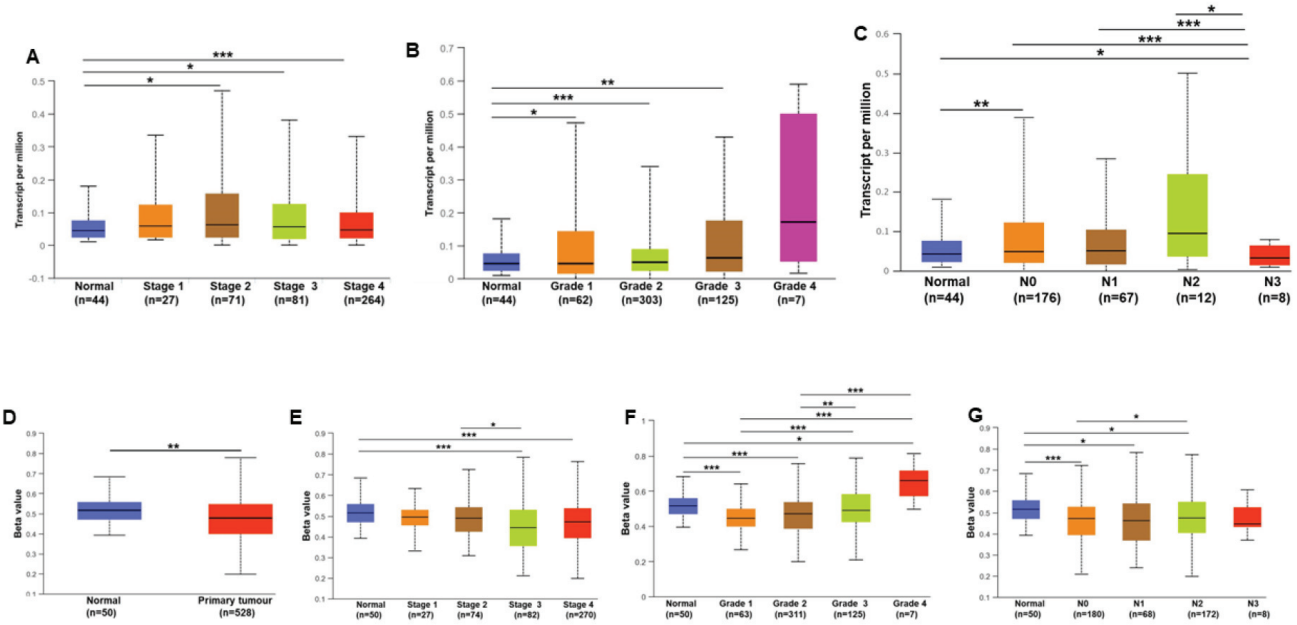

Figure 3. *MYO16* mRNA expression and DNA methylation in HNSCC. *MYO16* mRNA expression levels are significantly correlated with the clinicopathological features of HNSCC such as tumour stage (A), tumour grade (B), and nodal metastasis (C). The promotor methylation level of *MYO16* is also significantly decreased in HNSCC (D) and correlated with the clinicopathological features such as such as tumour stage (E), tumour grade (F), and nodal metastasis (G) (Source: UALCAN).

***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$; HNSCC: Head and neck squamous cell carcinoma; *MYO16*: *Myosin XVI*; mRNA: Messenger ribonucleic acid; UALCAN: The University of Alabama at Birmingham.

Table 2. Primer sequence for qPCR

Gene	Forward primer	Reverse primer
MYO16	5'- TGCTGAAAGCCGAAATTGCC-3'	5'- GTAACACCAGGGGACTGAGC-3'
GAPDH	5'-TCCAAAATCAAGTGGGGCGA-3'	5'-TGATGACCCTTTTGGCTCCC-3'

MYO16: *Myosin XVI*; *GAPDH*: Glyceraldehyde-3-phosphate dehydrogenase

kit (Takara, Tokyo, Japan) according to manufacturer's instructions.[17] RT-qPCR analysis using specific primers for *MYO16* and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was performed to quantify gene expression. The primers used in this study are listed in table 2. The Bio-Rad CFX Opus 96 system (Bio-Rad, Hercules, CA, USA) was used, with GAPDH as the reference gene, previous study protocol was used to analysis.[18]

Survival analysis with Kaplan-Meier plotter

Using the Kaplan-Meier plot (https://kmplot.com/),[19] survival analysis was conducted on HNSCC patient data from TCGA based on *MYO16* mRNA expression levels. The *MYO16* expression value classified low and high based on the cut-off value. The overall survival and relapse-free survival free survival rate were analyzed in this study.

Tumor immune regulator analysis

Using the vast dataset of TISIDB (http://cis.hku.hk/TISIDB/),[20] a premier resource for cancer-immune system investigations, our study employed a data-driven approach to investigate the potential association between *MYO16* expression and various elements implicated in the immune response, including distinct lymphocyte populations, diverse immune modulators, and chemokine signaling genes, within the context of HNSCC patients.

Ethical approval

The Institutional Ethical Committee of the Saveetha Dental College and Hospital approved this study (IHEC/SDC/FACULTY/20/PERIO/01).

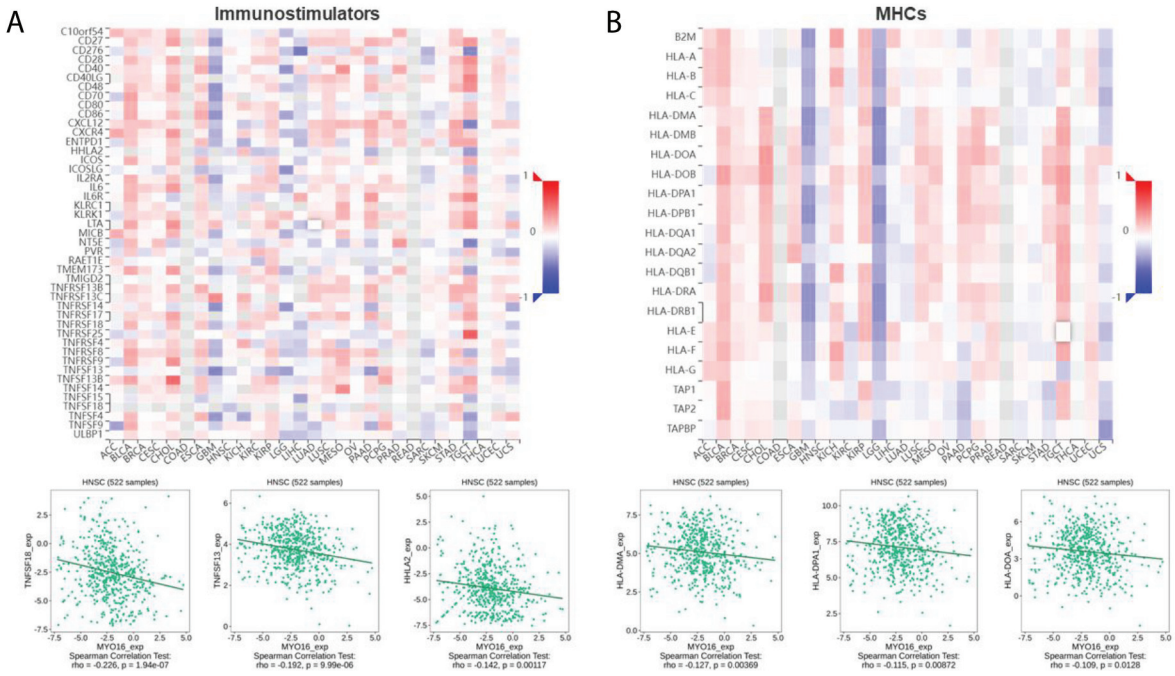

Figure 4. *MYO16* mRNA expression and Immune regulatory genes in HNSCC. (A) The *MYO16* mRNA expression and immunostimulator genes were analysed and top 3 negative correlated genes were plotted (TNFSF18, TNFSF13, and HHLA2). (B) The *MYO16* mRNA expression and MHCs genes were analysed and top 3 negative correlated genes were plotted (HLA-DMA, HLA-DPA1, and HLA-DOA) (Source: TISIDB database).

MYO16: *Myosin XVI* ; ; HNSCC: Head and neck squamous cell carcinoma; MHC: Major histocompatibility complex; TNFSF18: Tumor necrosis factor (ligand) superfamily, member 18; TNFSF13: Tumor necrosis factor (ligand) superfamily, member 13; HHLA2: Human endogenous retrovirus-H long terminal repeat-associating 2; HLA-DMA: Major Histocompatibility Complex, Class II, DM Alpha; HLA-DPA1: Major histocompatibility complex, class II, DP alpha 1; HLA-DOA: HLA class II histocompatibility antigen, DO alpha chain; TISIDB: An integrated repository portal for tumor-immune system interactions

All participants signed an informed consent form.

Statistical analysis

SPSS software version 25 (IBM, Armonk, NY, USA) and GraphPad Prism version 9.4.0 were used for statistical analysis, applying student's t-test or one-way ANOVA. Statistical significance was defined as $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Results

Upregulation of MYO16 in HNSCC and OSCC tumors

A comprehensive analysis across various databases revealed consistent upregulation of *MYO16* mRNA in HNSCC tumors compared with normal tissue (Figure 1A, $P < 0.001$). Our RT-qPCR analysis mirrored these findings, confirming significantly higher *MYO16* expression in OSCC tumor tissue than in adjacent non-tumor tissue (paired samples) (Figure 1B, $P < 0.001$).

High level of MYO16 predicts unfavorable prognosis in HNSCC and OSCC

Survival analysis using Kaplan-Meier plots underscored the adverse impact of high *MYO16* expression on the survival of HNSCC patients (Figure 2A, B). *MYO16* expression was classified as low or high based on the cut-off value for overall and relapse-free survival, respectively. The high expression of *MYO16* was negatively associated with overall survival, suggesting a poor prognosis (Figure 2A, $P = 0.023$), and a similar trend was observed for relapse-free survival (Figure 2B, $P = 0.04$).

Linking MYO16 with HNSCC clinicopathological features

Expanding our investigation through the UALCAN database, we explored the correlation between *MYO16* expression and various clinicopathological features in HNSCC. Notably, elevated *MYO16* mRNA expression was significantly linked to advanced tumor stage, higher grade, and nodal metastasis (Figure 3A-C, $P < 0.05$). Correspondingly, heightened *MYO16* DNA promotor methylation was decreased in HNSCC tumors and linked with tumor stage, grade, and metastasis (Figure 3D-G, $P < 0.05$).

MYO16 and immune regulators in HNSCC

Using the TISIDB database, we delved into the complex relationships between *MYO16* expression and immune regulatory genes. Our analysis focused on Immunomodulators (encompassing both immunostimulants and MHC molecule genes). The analysis of pan cancer samples revealed and identified the top three genes with significant negative correlations to *MYO16*. In the category of immunostimulators, *TNFSF18, TNFSF13,* and *HHLA2* exhibited negative correlations (Figure 4A, $P < 0.05$). Similarly, for MHCs, *HLA-DMA*, *HLA-DPA1*, and *HLA-DOA* displayed negative correlations with *MYO16* (Figure 4B, $P < 0.05$). Notably, all analyzed immune-regulating genes showed negative correlations with high *MYO16* expression in HNSCC patients.

Discussion

Our study unveils *MYO16* as a potential contributor to HNSCC and OSCC progression. We observed *MYO16* mRNA upregulation across databases and validated it through RT-qPCR, suggesting its involvement in HNSCC tumorigenesis. The association of high *MYO16* expression with poor prognosis and advanced clinicopathological features underscores its potential role in promoting aggressive disease. Interestingly, the observed decrease in *MYO16* promoter methylation hints at non-canonical regulatory mechanisms. Finally, the negative correlations between *MYO16* and immune regulators suggest its potential to suppress anti-tumor immunity. These findings position *MYO16* as a promising target for HNSCC and OSCC diagnosis, prognosis, and potentially, immunotherapy. However, further research is warranted to elucidate the precise mechanisms underlying MYO16 function and solidify its clinical implications.

The myosin superfamily consists of actin-based molecular motors that play essential roles in cell motility, intracellular transport, and cytoskeletal organization.[21] While MYO16 is a less-studied member, previous research has implicated other myosins in tumorigenesis and

cancer progression, making MYO16 an intriguing candidate for investigation in HNSCC.

The myosin family of genes holds a pivotal role in various tumours, both regulated by and regulating cadherin genes and oncogenes. Interestingly high expression of MYO1B promotes cell migration and lymph node metastasis in HNSCC.[22] Within this family, MYO II has been linked to tumour progression and invasion in melanoma, pancreatic and breast cancer.[23-25] MYO *V* is associated with gastric cancer through the control of apical and basolateral protein trafficking, vital for regulating epithelial cell polarity.[26] Myosin Va is tied to colorectal cancer, impacting the migration of metastatic cancer cells and the organization of the cytoskeleton.[27] Myosin VI contributes to DNA damage repair and tumour suppression, disseminating cancer cells, facilitating prostate cancer cell migration, and maintaining the Golgi structure and function.[28-30] Myosin IX down-regulates Rho activity and actin bundle assembly, influencing the collective migration of human epithelial cells.[31] Myosin X role involves responding to impaired p53, inhibiting cell adhesion, promoting protrusion formation, and contributing to tumor progression in breast cancer.[13] Moreover, it fosters filopodia formation and drives metastasis development in primary glioblastoma and acute lymphoblastic leukaemia.[32,33] A growing body of research suggests that myosin family genes contribute to tumorigenesis.

Our evidence suggests *MYO16* overexpression could be a prognostic marker for HNSCC, but further research is necessary before definitive conclusions can be drawn. Our study limitations included a small sample size, focus on mRNA levels, and reliance on in silico analysis. Moreover, we used only OSCC samples for validation, and more reliable results require other HNSCC subgroup samples. Large-scale studies incorporating clinicopathological analysis, protein expression, and functional assays are crucial for understanding how MYO16 drives HNSCC progression at the molecular level. Only through additional research can we solidify MYO16 clinical utility as a biomarker or potential therapeutic target.

Conclusion

Our study revealed the potential significance of *MYO16* in HNSCC and OSCC. Its upregulation, associated with poor prognosis, and potential role in immune evasion highlights its relevance as a diagnostic, prognostic, and therapeutic target. Further research is warranted to delve deeper into the precise mechanisms underlying *MYO16* functions and its potential as a biomarker or therapeutic target for aggressive cancers.

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Authors' Contribution

Keerti Pranith Suryadevara: Validation, formal analysis, investigation, data curation, writing original draft, review. Balachander Kannan: Data curation, formal analysis, investigation, writing original draft, and review. Chandra Pandi: Methodology, formal analysis, and review. Anitha Pandi: Data curation and methodology-review. Abilasha Ramasubramanian: Statistics, formal analysis and writing-review. Vijayashree Priyadharsini Jayaseelan: Methodology, formal analysis, writing-review. Paramasivam Arumugam: Conceptualization, methodology, formal analysis, writing, reviewing, and editing.

All authors have read and approved the final manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest

None declared.

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