Original Article
Running Title: *SNHG3* and *HOXB8* in OSCC Progression
Received: July 7, 2026; Accepted: November 3, 2026

Upregulation of LncRNA *SNHG3* and *HOXB8* in Oral Squamous Cell Carcinoma: Association with Metastasis and Poor Histological Differentiation

Elham Rezaei[*,**], MD, Habib Zarredar[*], PhD, Fatemeh Manafzadeh[*], MD, Milad Asadi[***], PhD, Dariush Shanehbandi[****], PhD, Azam Safary[*****], PhD, Haleh Mikaeili[*], MD, Shahram Ghasembaglou[******♦], MD

[*]*Tuberculosis and Lung Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
[**]*Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran*
[***]*Department of Basic Oncology of Health Institute of Ege University, Izmir, Turkey*
[****]*Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
[*****]*Connective Tissue Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
[******]*Otolaryngology Head and Neck Surgery Department, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*

♦Corresponding Author
Shahram Ghasembaglou, MD
Otolaryngology Head and Neck Surgery Department,
School of Medicine, Tabriz University of Medical Sciences,
Tabriz, Iran
Email: sh.gasembeglo@yahoo.com

Abstract

Background: Oral squamous cell carcinoma (OSCC) is an aggressive head and neck malignancy. Long non-coding RNAs (lncRNAs) and developmental regulatory genes have been implicated in tumor initiation and progression. The present study aimed to evaluate the expression level of long non-coding RNA (lncRNA) small nucleolar RNA host gene 3 (*SNHG3*) and homeobox B8 (HOXB8) in OSCC tissues compared with paired normal marginal tissues, as well as their correlation with clinicopathological parameters and potential diagnostic value.

Method: A total number of 50 paired tumor and marginal tissues were collected in this case-control study. Quantitative real-time polymerase chain reaction, cDNA synthesis, and RNA extraction were completed, and levels of expression were normalized to *GAPDH*. Data were analyzed using the Shapiro-Wilk test for normality, paired Student's t-tests, Wilcoxon tests, and receiver operating characteristic curve analysis via GraphPad Prism software (version 9.5.1), with a significance level set at $P < 0.05$.

Results: *HOXB8* and *SNHG3* were significantly overexpressed in tumor tissues (*HOXB8*: fold change = 1.281, $P = 0.0092$; *SNHG3*: fold change = 1.174, $P = 0.0453$). *SNHG3* showed weak diagnostic value (Area under the curve (AUC) = 0.59), while *HOXB8* demonstrated limited discriminatory performance (AUC = 0.6059). Both genes were considerably related with markers of tumor aggressiveness, including lymph node metastasis, distant metastasis, and poor differentiation. The combined multi-marker panel successfully enhanced diagnostic accuracy, yielding a superior and statistically significant AUC of 0.6462 (95% confidence interval: 0.5881–0.7043, $P = 0.0068$).

Conclusion: *SNHG3* and *HOXB8* are upregulated in OSCC and were correlated with aggressive clinicopathological features in OSCC malignancy.

Keywords: Oral squamous cell carcinoma, SNHG3, HOXB8, lncRNA, Biomarkers

Introduction

Oral squamous cell carcinoma (OSCC) is the most prevalent histological subtype among oral malignancies, accounting for over 90% of all malignant tumors of the oral cavity and its adjacent structures. OSCC usually progresses insidiously, and most patients are diagnosed when the disease has reached advanced stages. In 2020, the Global Cancer Observatory estimated about 377,713 new cases of OSCC making it one of the most common cancers of the head and neck, worldwide.[1] Statistical evidence shows that the incidence rate of this disease has increased from 3.26 to 5.34 cases per 100,000 population between 1990 and 2021, confirming a significant upward trend. According to international reports, the death rate from oral cancer in 2020 is estimated to be 177,757 people and the five-year survival rate remains restricted to 40%-50%, highlighting an urgent need for early detection strategies and targeted biomarkers.[2] Over the past ten years, molecular research has focused more on the genetic and epigenetic complexity of OSCC, including the dysregulation of non-coding RNAs such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs).[3,4] LncRNAs are a family of transcripts longer than 200 nucleotides (nt) that have a variety of regulatory roles in gene expression which lack protein-coding potential.[4,5] In OSCC, the dysregulation of lncRNAs and miRNAs which play an important role in regulating oncogenic signaling pathways, interactions between tumor cells and tumor microenvironment have been considered as potential biomarkers and novel therapeutic targets.[6, 7]

Evidence suggests that small nucleolar RNA host gene 3 (*SNHG3*) has been reported as an oncogene can play a role in many malignancies including gastric,[8] hepatocellular,[9, 10] lung cancers,[11, 12] and colorectal malignancy.[13] Lnc SNHG3 contributes to the tumorigenesis process in a variety of ways, including interaction with transcription factors, altering DNA methylation patterns. As a competing endogenous RNA (ceRNA), it acts as a molecular sponge for miRNAs. Studies conducted in the field of OSCC have shown that *SNHG3* enhances tumor cell migration and growth by participating in ceRNA networks and affects targets such as *HOXB8* through interaction with *miR-2682-5p*.[14, 15] *HOXB8* is a member of the highly conserved homeobox (HOX) gene family, which encodes a homeodomain-containing transcription factor which is critically involved in embryonic development and cell differentiation. Aberrant regulation of HOX genes has been observed in many cancers, and their abnormal expression leads to increased metastasis, invasion, cell proliferation, and therapeutic resistance.[16] *HOXB8* is significantly upregulated in OSCC cancer tissues and cell lines and promotes cell proliferation and migration. Based on mechanistic studies, *SNHG3* positively regulates *HOXB8* expression through a ceRNA mechanism and in collaboration with *miR-2682-5p*, thus indicating an emerging pathway in the regulation of OSCC progression by lncRNAs.[14] Although these new findings have been raised, the clinical significance of *SNHG3* and *HOXB8* in tissue samples from OSCC patients, especially in relation to tumor size, lymph node status, metastasis and degree of differentiation, is still unclear. To address this gap, the present study quantified the tissue expression levels of *SNHG3* and *HOXB8* in OSCC patients, investigated their correlation with clinicopathological parameters, and assessed their individual and combined diagnostic performance. The purpose of this study was to compare the expression level of the *HOXB8* gene and lncRNA *SNHG3* in OSCC

tissue compared with paired adjacent non-tumor marginal tissues.

Methods and Materials

Study design and ethical considerations

This case-control study evaluated data from patients diagnosed with lung adenocarcinoma who underwent surgical resection between December 2023 and December 2025 at Imam Reza Hospital. Clinical follow-up data were collected through regular postoperative check-ups, telephone interviews, and medical record reviews. The follow-up duration was defined as the time interval from the date of initial surgical resection to the date of the last known follow-up, disease progression, or death (censored at the date of last contact for surviving patients). All procedures and protocols were conducted in accordance with the Declaration of Helsinki and were approved by the Ethics Committee of Tabriz University of Medical Sciences (Ethics Code: IR.TBZMED.REC.1404.135). Written informed consent was obtained from all participants prior to inclusion in the study.

Patient selection and tissue collection

This study was performed on 50 patients with histopathologically confirmed OSCC who underwent surgical resection in Imam Reza Hospital affiliated with Tabriz University of Medical Sciences during 2023-2025. The inclusion criteria were: primary diagnosis of OSCC, no previous history of chemotherapy or radiotherapy and availability of complete clinical and pathological data. Patients with a history of other malignancies, recurrent OSCC, or significant comorbidities were excluded. During surgery, paired specimens including OSCC tumor tissues and adjacent grossly adjacent non-tumorous tissues (located at least 1.5–2 cm away from the visible tumor boundary) were collected. Specimens were immediately placed in RNase-free tubes, snap-frozen in liquid nitrogen, and stored at −80°C until further processing. The sample size of 50 patients with paired tumor and adjacent normal tissues was determined based on the availability of eligible pathologically confirmed OSCC tissue samples within the specified study timeframe (2023–2025) and following standard practices for similar molecular expression and tissue-based case-control studies.

RNA extraction, cDNA synthesis and quantitative real-time polymerase chain reaction (qRT-PCR)

In this study, total RNA was extracted from frozen tissue samples of TRIzol®reagent (Invitrogen, USA) according to the manufacturer's instructions. The quality and quantity of RNA extracted were assessed by optical density using a NanoDrop spectrophotometer (NanoDrop ND-2000C, Thermo Fisher Scientific, USA), and RNA integrity was confirmed by observing clear, sharp 28S and 18S ribosomal RNA bands on 1% agarose gel electrophoresis prior to cDNA synthesis. To ensure high reproducibility and specificity in qRT-PCR assays, reactions are typically configured in 20 µL volumes and subjected to a standardized two-step thermal cycling protocol comprising initial enzyme activation, 40 cycles of denaturation at 95°C, and annealing/extension at 60°C. Post-amplification melt curve analysis is essential to confirm product purity by checking for a single specific dissociation peak, while validated primer pairs must demonstrate an amplification efficiency between 90% and 110% over a dynamic dilution series. Furthermore, rigorous quality control is maintained using no template controls to detect reagent contamination and no-reverse transcription controls to verify the complete absence of genomic DNA carryover in the RNA template. *GAPDH* was used as an internal control, and relative expression levels of *SNHG3* and *HOXB8* were quantified using the $2^{-\Delta\Delta Ct}$ method. Threshold cycle (C_t) values of the target genes were first normalized to the internal reference gene

GAPDH within each sample to obtain ΔC_t (C_t target gene – C_t GAPDH). $\Delta\Delta C_t$ was then calculated by subtracting the mean ΔC_t of the paired adjacent normal tissues (calibrator) from the ΔC_t of each tumor sample (ΔC_t tumor-ΔC_t normal). Relative fold-change in expression was determined as $2^{-\Delta\Delta Ct}$. Primer sequences are provided in Table 1.

Statistical analysis

All data were checked for normal distribution using the Shapiro-Wilk test to determine the type of analysis (parametric or nonparametric). The relative expression values of *HOXB8* and *SNHG3* genes were obtained from the formula $2^{-\Delta\Delta Ct}$ after normalization with the internal control gene *GAPDH*. Comparisons between tumor and paired adjacent non-tumoral tissues were performed using the paired Student's t-test (for normal data) and the Wilcoxon test (for abnormal data). Depending on the distribution, the results were presented as mean ± standard deviation (SD) or Median. Correlation between two genes was evaluated using Pearson or Spearman coefficients. Binary logistic regression analysis was performed to evaluate the combined diagnostic performance of *HOXB8* and *SNHG3*. The dependent variable was tissue type (coded as 1 = OSCC tumor tissue, 0 = paired adjacent non-tumorous tissue). The independent variables included in the model were the relative expression levels of *HOXB8* and *SNHG3*.The diagnostic indices of the markers were estimated by drawing the receiver operating characteristic (ROC) curve and determining the area under the curve (AUC) along with 95% confidence interval (CI). Optimal cut-off values for diagnostic biomarkers were determined using the Youden index based on the maximum vertical distance from the ROC curve to the diagonal line of equality. GraphPad Prism software version 9.5.1 was used as the basis for all calculations (significance level: $P < 0.05$).

Results

Patient characteristics

This study comprised 50 patients with OSCC that had been histopathologically confirmed. A total of 50 tumor tissues and 50 paired adjacent non-tumorous tissues were collected from 50 patient during surgical resection. The study sample included male and female patients of diverse ages and clinicopathological traits. The clinical parameters evaluated were age, gender, tumor differentiation grade, lymph node metastasis, and the status of distant metastasis. The results showed that the level of gene expression was not significantly affected by demographic variables such as age and gender ($P > 0.05$), thus, the expression patterns of *SNHG3* and *HOXB8* were not significantly associated with age or gender in this cohort.

SNHG3 and HOXB8 expression in OSCC tissues

The relative expression levels calculated by the $2^{-\Delta\Delta Ct}$ method showed an overall upregulation in most tumor samples. *HOXB8* expression was significantly higher in tumor tissues than in corresponding marginal tissues (fold change = 1.281, $P = 0.0092$) by qRT-PCR analysis, and *SNHG3* expression was significantly higher in OSCC tumor tissues than in paired adjacent marginal tissues (fold change = 1.174, $P = 0.0453$). The magnitude of *HOXB8* upregulation was more pronounced than observed for *SNHG3* (Figure 1).

Association of SNHG3 and HOXB8 expression with clinicopathological characteristics

Subgroup analyses were conducted to evaluate the clinical significance of *SNHG3* and *HOXB8* expression in OSCC according to known prognostic parameters, including lymph node status, distant metastasis, tumor differentiation, age and gender. High expression levels of both *SNHG3* and

HOXB8 were found to correlate significantly with lymph node metastasis. Gene expression was significantly higher in patients with nodal involvement than in patients without lymphatic spread ($P < 0.05$).

The results of the study showed that the upregulation of *SNHG3* and *HOXB8* genes is likely associated with tumor aggressiveness and regional metastasis. Likewise, the expression levels of *SNHG3* and *HOXB8* in the presence of distant metastasis were significantly higher than those without metastasis ($P < 0.05$). These results indicate that these genes may be involved in spreading of systemic tumors and the progression of severe disease. As for the analysis based on histological differentiation, the expression levels of both *SNHG3* and *HOXB8* genes were significantly higher in poorly-differentiated tumors than in moderately or well-differentiated tumors ($P < 0.05$). The presented results further confirm their possible role in OSCC progression by showing that increased expression of these genes is associated with tumor aggressive characteristics and reduced cellular differentiation. In contrast, there were no statistically significant associations between the expression levels of *SNHG3* or *HOXB8* and demographic variables such as age or gender ($P > 0.05$). Neither *SNHG3* nor *HOXB8* expression was significantly associated with patient demographic factors. Overall, the present results suggest that increased expression of *SNHG3* and *HOXB8* is associated with poor clinicopathological features, tumor aggressiveness and modest diagnostic performance (Table 2).

Diagnostic performance evaluation (ROC analysis)

To evaluate the diagnostic efficacy of *SNHG3* and *HOXB8* expression levels in differentiating OSCC tissues from adjacent non-tumorous marginal tissues, ROC curve analysis was performed. The results of *SNHG3* evaluation showed poor discrimination power with an AUC of 0.59 (95% CI: 0.5340–0.7592), which was not statistically significant ($P = 0.1148$) with an optimal cut-off value (determined by the Youden index) of > 1.021, yielding a sensitivity of 72.0% and a specificity of 58.0%. In contrast, *HOXB8* recorded poor but statistically significant discrimination power with an AUC of 0.6059 (95% CI: 0.5598–0.7706, $P = 0.025$) with an optimal cut-off value of > 1.091, yielding a sensitivity of 76.0% and a specificity of 66.0%. These findings suggest that either *SNHG3* or *HOXB8* alone has limited diagnostic discriminatory ability in this cohort. Therefore, it is suggested that future research should focus on developing combined and multi-indicator models to improve the discrimination of OSCC from non-tumorous tissues and potentially enhance diagnostic performance (Figure 2).

Evaluation of combined diagnostic performance

To improve diagnostic sensitivity and specificity, a binary logistic regression model was developed to integrate the expression levels of both *HOXB8* and lncRNA *SNHG3*. The multi-marker panel evaluation successfully improved the diagnostic accuracy, resulting in a higher and statistically significant AUC of 0.6462 (95% CI: 0.5881–0.7043, $P = 0.0068$). These findings indicate that although the tissue expression levels of each of the *SNHG3* and *HOXB8* genes alone have limited diagnostic value, their mathematical integration provides a much more robust and meaningful approach to distinguish malignant from normal oral tissues (Figure 3).

Correlation analysis between SNHG3 and HOXB8 expression in OSCC tissues

Pearson correlation analysis indicated a non-significant and poor negative correlation among *SNHG3* and *HOXB8* expression levels in OSCC tissues ($r = -0.072$, $P = 0.12$).

Despite the upregulation of both genes in tumor tissues, their expression patterns were not significantly associated, indicating that *SNHG3* and *HOXB8* may contribute independently to OSCC pathogenesis (Figure 4).

Discussion

Based on our findings, the significant increase in expression of *SNHG3* and *HOXB8* genes in OSCC tumor lesions and their direct correlation with clinical progression of the disease (metastasis to lymph nodes and distant sites, along with poor histological differentiation), reinforces the possible role of these two markers in tumor invasion. ROC curve analysis did not confirm an independent clinical value for *SNHG3* and *HOXB8* when analyzed individually, the novel approach of this study in mathematically integrating these two markers through combination Index was a turning point in improving the results. This integrated model was able to show better efficiency compared with the individual AUCs of each gene. ROC curve analysis showed that *SNHG3* (AUC = 0.59), *HOXB8* (AUC = 0.606), and their combination (AUC = 0.646) exhibit limited diagnostic performance and weak discriminatory ability. Therefore, these markers cannot be considered robust diagnostic tools on their own, and their clinical utility remains constrained.

The aforementioned data support the hypothesis that to overcome tumor heterogeneity in OSCC, relying on multifactorial combination panels rather than single markers would be a more logical and accurate approach for diagnostic purposes. The present findings of *SNHG3* overexpression are consistent with previous studies in various human cancers such as colorectal,[13] gastric,[8] hepatocellular,[17] and lung cancers,[11] in these cases, *SNHG3*, as an oncogenic lncRNA, increases the proliferation, migration, and invasion of cancer cells through ceRNA activity and interaction with RNA-binding proteins.[18] Previous mechanistic studies in OSCC cell lines have suggested that *SNHG3* may regulate *HOXB8* expression via a ceRNA network involving *miR-2682-5p*, thereby promoting tumor aggressiveness.[14]. Although, the increased expression of both genes was observed in clinical OSCC tissues, the direct correlation between *SNHG3* and *HOXB8* expression was not statistically significant, suggesting that the regulation of these genes in-vivo may be influenced by other context-dependent factors. Also, the increased expression of *HOXB8* in our OSCC patient cohort is consistent with findings reported in head and neck squamous cell carcinoma (HNSCC) and other malignancies; in these studies, *HOXB8* has been implicated in processes such as proliferation, migration, invasion, epithelial–mesenchymal transition (EMT), and activation of the *PI3K/AKT/mTOR* and *STAT3* oncogenic pathways.[19, 20] The current findings highlight the potential role of *HOXB8* as a marker of tumor aggressive behavior and unfavorable differentiation and are consistent with our findings in patients with OSCC. From a mechanistic perspective, *SNHG3* exerts its oncogenic effects through multiple pathways. By playing the role of a (ceRNA, this molecule interacts with specific microRNAs, thereby reducing the suppression of downstream oncogenes; a process that ultimately increases the proliferation, migration, invasion and EMT of cancer cells. *SNHG3* also interacts with RNA-binding proteins and chromatin regulators, modulating transcriptional and post-transcriptional networks supporting tumor growth and metastasis.[21] SNHG3 may function as a competing endogenous RNA and reduce the inhibition of *HOXB8* and other oncogenic genes by recruiting microRNAs, thereby promoting

proliferation, metastasis and EMT. *HOXB8* also contributes to tumor growth, invasion and metastatic potential by regulating key oncogenic pathways. Although we did not perform functional assays in this study, the consistency of our clinical data with the mechanisms suggested by previous studies supports the biological plausibility of this regulatory network in OSCC.[14, 22] The findings of this study support this association, as *SNHG3* and *HOXB8* expression was significantly increased in OSCC tissues and correlated with clinicopathological aggressiveness indicators including lymphatic metastasis, distant metastasis, and low grade of tumor differentiation. Although direct correlation between their expression levels in clinical samples does not always reach statistical significance, the functional interaction suggested in mechanistic studies highlights the potential role of the *SNHG3/HOXB8* axis in OSCC progression and introduces these genes as promising candidate biomarkers for further investigation as prognostic markers and potential therapeutic targets. Studies using the Cancer Genome Atlas Prostate Adenocarcinoma (TCGA-PRAD) database have shown that *SNHG3* expression was increased in prostate cancer patients with bone metastases compared with patients without metastasis.[23] In ovarian cancer cells, inhibition of *SNHG3* causes cell cycle arrest in the G1/G0 phase and thus attenuates the malignant traits of the cells.[24] Recent research has shown that *HOXB8* plays a role in cancer progression beyond OSCC. *HOXB8* is upregulated in tumor samples and associated with advanced disease stages and a poor prognosis, according to a multiomics study of HNSCC (including OSCC). The results of functional experiments showed that *HOXB8* gene knockdown significantly reduced the proliferation, invasion, and migration of HNSCC cells. At the mechanistic level, *HOXB8* is involved in the regulation of important oncogenic pathways including EMT, *PI3K/AKT/mTOR* signaling pathway, and tumor immune microenvironment. Taken together, these findings indicate that these findings are consistent with our clinical observations and support the potential association of elevated *HOXB8* expression with aggressive features such as metastasis and poor tumor differentiation.[19] Additional evidence from other cancer types further supports the oncogenic role of *HOXB8*. As an example, in colorectal cancer (CRC), *HOXB8* has been shown to promote chemoresistance by activation of the *STAT3* pathway, supporting a role in progression and therapy resistance.[25] Although the mechanisms may differ by tumor type, the consistent upregulation of HOXB8 in cancers with aggressive behavior echoes our findings in OSCC. Li. et al. suggested that knockout of the HOXB8 can inhibit the growth and metastases of CRC cells via the Wnt/β-catenin signaling pathway, and led to low expression of the c-myc, CyclinD1 and other factors.[26] Also, Ding et al. reported that the up regulation of the HOXB8 in metastatic tissues compared with non-metastatic tissues, and high expression of the HOXB8 in metastatic tissues can induce the invasion, migration, and EMT of gastric cancer cells.[27] A recent study indicates that HOXB8 gain of function act as tumor suppressor through controlling oncogenic MEK/ERK signaling. [28] In addition, knockdown of the *HOXB8* can suppress the growth and metastasis of osteosarcoma by controlling the *Wnt/β-catenin* signaling pathway.[29] These findings suggest that both genes may be associated with tumor aggressiveness, indicating their association with tumor aggressiveness and modest diagnostic performance. In ovarian cancer, elevated *HOXB8* expression has been associated with aggressive tumor behavior, including increased cellular proliferation, migration, invasion, and metastatic potential

[3031]. The study by Zhang et al. have shown that *HOXB8* is overexpressed in HNSCC and is associated with advanced stage, lymphatic metastasis, and poor prognosis of patients. The results of functional studies indicate that *HOXB8* has an enhancing role in proliferation, migration, invasion, tumor growth, and pulmonary metastasis in HNSCC, both in-vitro and in-vivo. Notably, *HOXB8* is also involved in modifying the tumor microenvironment and is associated with reduced CD8+ T lymphocyte infiltration and increased M2 macrophages, which reinforces the possibility of its role in immune evasion. Furthermore, a prognostic model designed based on *HOXB8*-related genes including *ADD2*, *SYT1*, *PXYLP1*, and *MRPL33* was able to well group patients according to risk, thus demonstrating the clinical significance of *HOXB8*. In their entirety, these results suggest that *HOXB8* may play an important role in the progression of HNSCC, and may be regarded as prognostic biomarker and potential therapeutic target.[19] Despite these alignments, in some tumor contexts, *SNHG3* expression has been proposed as a prognostic marker with strong predictive capability, whereas in our OSCC cohort, *SNHG3's* diagnostic performance (AUC ~0.59) was not statistically significant. Overall, our findings are concordant with a growing body of evidence indicating that *SNHG3* and *HOXB8* associated with aggressive clinicopathological features in cancer biology. The frequent association of high *SNHG3* and *HOXB8* expression with clinicopathological aggressive markers suggests that these genes may participate in key pathways of cancer progression, metastasis, and differentiation. Thus, previous and present study results demonstrate the potential of *SNHG3* and *HOXB8* as part of prognostic signatures, and emphasize the need for further investigation of their mechanistic roles and therapeutic potential.[19, 21] Importantly, the our study demonstrates that *HOXB8* and *SNHG3*, in a multivariable logistic regression model, show a relative improvement compared with individual markers, as evidenced by the higher AUC in the combined ROC analysis. The present study has certain limitations that should be considered. First, the sample size was relatively small, and survival data were not available, limiting assessment of long-term prognostic impact. Second, our analysis was based only on qRT-PCR data and functional studies in OSCC tissues were not performed. In the future, the prognostic significance and functional roles of *SNHG3* and *HOXB8* should be verified in larger cohorts, survival studies and in-vivo functional assays. Evaluation of multi-marker panels can also provide insight into their potential use in the development of targeted therapies and improvement of diagnostic accuracy. In final, although the combined ROC analysis achieved statistical significance, the observed AUC remained limited and is unlikely to be sufficient for clinical application as a standalone diagnostic model. Future multi-center studies with larger cohorts are warranted to validate these findings.

Conclusion

HOXB8 and *SNHG3* are overexpressed in OSCC tissues and are closely related to the malignancy indicators such as lymph node metastasis, distant metastasis and poor tumor differentiation. These results suggest the involvement of both genes in tumor progression and aggressiveness. Although their independent diagnostic power was limited, so these findings suggest that *SNHG3* and *HOXB8* are associated with aggressive clinicopathological characteristics and may represent candidate biomarkers for disease progression. Further studies including survival analyses are required to establish their prognostic value. [1]

Availability of Data and Material

All data generated or analyzed during the present study are included in this article and its supplementary information files. We also agree that the copyright for my/our article will be transferred to journal of Egyptian National Cancer institute when it is accepted for publication.

Statement on the Use of Artificial Intelligence

Artificial intelligence (AI) tools (Gemini) were used solely to check grammar, spelling, and sentence structure and to improve the clarity and readability of the manuscript.

Acknowledgments

We would like to appreciate the Clinical Research Development Unit of Imam Reza General Hospital, Tabriz, Iran for their cooperation to conduct this study.

Funding

This study was supported by the Tuberculosis and Lung Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

Authors' Contribution

E.R: Study design, analysis interpretation, and reviewing the manuscript; **H.Z**: Study design, writing the draft, and reviewing the manuscript; **F.M:** Data collection, processing and reviewing the manuscript; **D.SH**: Data gathering, drafting, analysis and interpretation; **M.A**: Data collection and processing; **A.S:** Writing the manuscript, Data gathering; **H.M**: Data collection, Writing manuscript, drafting; **SH.GH:** Study design, analysis interpretation, Writing the manuscript, and reviewing the manuscript. 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.

References

1. Tan Y, Wang Z, Xu M, Li B, Huang Z, Qin S, et al. Oral squamous cell carcinomas: state of the field and emerging directions. *Int J Oral Sci.* 2023;15(1):44. doi: 10.1038/s41368-023-00249-w. PMID: 37736748; PMCID: PMC10517027.
2. Choi SW, Sharma D, Ramamurthy P, Thomson P. Oral cancer: licensed to kill? *Faculty Dental Journal.* 2023;14(4):126-32. doi: 10.1308/rcsfdj.2023.29.
3. Shanehbandi D, Asadi M, Seyedrezazadeh E, Zafari V, Shekari N, Akbari M, et al. MicroRNA-based biomarkers in lung cancer: Recent advances and potential applications. *Curr Mol Med.* 2023;23:648-67. doi: 10.2174/2772432817666220520085719. PMID: 35619321.
4. Aftabi Y, Ansarin K, Shanehbandi D, Khalili M, Seyedrezazadeh E, Rahbarnia L, et al. Long non-coding RNAs as potential biomarkers in the prognosis and diagnosis of lung cancer: A review and target analysis. *IUBMB Life.* 2021;73(2):307-27. doi: 10.1002/iub.2430. PMID: 33369006.
5. Asadi M, Sadeghi-Mohammadi S, Shekari N, Zafari V, Soleimani Z, Mert U, et al. Therapeutic potential of circular RNAs as targets for cancer treatment. *Adv Pharm Bull.* 2025;15(3):533-51. doi: 10.34172/apb.025.45330. PMID: 41403739; PMCID: PMC12703386.
6. Azimi H, Zarredar H, Asadi M, Zafari V, Shanehbandi D, Sabbagh Jadid H, et al. Evaluation of potential miR-221, miR-93, miR-21, and miR-24 as molecular markers for laryngeal squamous cell carcinoma. *Middle East J Cancer.* 2025;16(3):249-57. doi: 10.30476/mejc.2024.102700.2105.

7. Liang Z, Zhao Y, Wang X, Li Y. The role and targeting strategies of non-coding RNAs in immunotherapy resistance in oral squamous cell carcinoma. *Front Cell Dev Biol.* 2025;13:1642417. doi: 10.3389/fcell.2025.1642417. PMID: 41221298; PMCID: PMC12597961.
8. Xie Y, Rong L, He M, Jiang Y, Li H, Mai L, et al. LncRNA SNHG3 promotes gastric cancer cell proliferation and metastasis by regulating the miR-139-5p/MYB axis. *Aging (Albany NY).* 2021;13:25138-52. doi: 10.18632/aging.203732. PMID: 34898477; PMCID: PMCPMC8714158.
9. Shan DD, Zheng QX, Wang J, Chen Z. Small nucleolar RNA host gene 3 functions as a novel biomarker in liver cancer and other tumour progression. *World J Gastroenterol.* 2022;28:1641-55. doi:10.3748/wjg.v28.i16.1641. PMID:35581965; PMCID: PMC9048787.
10. Guo J, Zhang J, Xiang Y, Zhou S, Yang Y, Zheng J. Long noncoding RNA SNHG3 interacts with microRNA-502-3p to mediate ITGA6 expression in liver hepatocellular carcinoma. *Cancer Sci*. 2024;115:2286-300. doi: 10.1111/cas.16190. PMID: 38680094; PMCID: PMCPMC11247603.
11. Liu L, Ni J, He X. Upregulation of the long noncoding RNA SNHG3 promotes lung adenocarcinoma proliferation. *Dis Markers*. 2018;2018:5736716. doi: 10.1155/2018/5736716. PMID: 30154938; PMCID: PMCPMC6081568.
12. Peng Y, Long XD. The role of the ceRNA network mediated by lncRNA SNHG3 in the progression of cancer. *Discov Oncol.* 2024;15(1):514. doi: 10.1007/s12672-024-01184-w. PMID: 39349640; PMCID: PMC11442963.
13. Huang W, Tian Y, Dong S, Cha Y, Li J, Guo X, et al. The long non-coding RNA SNHG3 functions as a competing endogenous RNA to promote malignant development of colorectal cancer. *Oncol Rep.* 2017;38:1402-10. doi: 10.3892/or.2017.5837. PMID: 28731158; PMCID: PMC5549033.
14. Lu N, Yin Y, Yao Y, Zhang P. SNHG3/miR-2682-5p/HOXB8 promotes cell proliferation and migration in oral squamous cell carcinoma. *Oral Dis*. 2021;27:1161-70. doi: 10.1111/odi.13656. PMID: 32989886.
15. Liu Z, Tao H. Small nucleolar RNA host gene 3 facilitates cell proliferation and migration in oral squamous cell carcinoma via targeting nuclear transcription factor Y subunit gamma. *J Cell Biochem.* 2020;121:2150-8. doi: 10.1002/jcb.29421. PMID: 31762107.
16. Feng Y, Zhang T, Wang Y, Xie M, Ji X, Luo X, et al. Homeobox genes in cancers: From carcinogenesis to recent therapeutic intervention. *Front Oncol.* 2021;11:770428. doi: 10.3389/fonc.2021.770428. PMID: 34722321; PMCID: PMC8551923.
17. Zhao Q, Wu C, Wang J, Li X, Fan Y, Gao S, et al. LncRNA SNHG3 promotes hepatocellular tumorigenesis by targeting miR-326. *Tohoku J Exp Med*. 2019;249:43-56. doi: 10.1620/tjem.249.43. PMID: 31548493.
18. Xu B, Mei J, Ji W, Bian Z, Jiao J, Sun J, et al. LncRNA SNHG3, a potential oncogene in human cancers. *Cancer Cell Int.* 2020;20:536. doi: 10.1186/s12935-020-01608-x. PMID: 33292213; PMCID: PMC7640707.
19. Zhang JW, Gao XL, Wang J, Fan XL, Liang QW. Comprehensive analysis illustrating the role of HOXB8 in head and neck squamous cell carcinoma: evidence from multi-omics analysis and experiments validation. *BMC Cancer*. 2025;25:804. doi: 10.1186/s12885-025-14205-w. PMID: 40307753; PMCID: PMC12042558.
20. Wang T, Lin F, Sun X, Jiang L, Mao R, Zhou S, et al. HOXB8 enhances the proliferation and metastasis of colorectal

cancer cells by promoting EMT via STAT3 activation. *Cancer Cell Int*. 2019;19:3. doi: 10.1186/s12935-018-0717-6. PMID: 30622439; PMCID: PMC6317211.
21. Tang J, Su M, Xiao Y, Ou W, Wang W, Peng RW, et al. The role of LncRNA SNHG3 in human cancers. *Discov Oncol.* 2025;16:1812. doi: 10.1007/s12672-025-03640-7. PMID: 41042421; PMCID: PMC12494508.
22. Zhang H, Wei N, Zhang W, Shen L, Ding R, Li Q, et al. lncRNA SNHG3 promotes breast cancer progression by acting as a miR‐326 sponge. *Oncol Rep*. 2020;44:1502-10. doi: 10.3892/or.2020.7690. PMID: 32945476; PMCID: PMC7448486.
23. Xi X, Hu Z, Wu Q, Hu K, Cao Z, Zhou J, et al. High expression of small nucleolar RNA host gene 3 predicts poor prognosis and promotes bone metastasis in prostate cancer by activating transforming growth factor-beta signaling. *Bioengineered.* 2022;13:1895-907. doi: 10.1080/21655979.2021.2020393. PMID: 35030969; PMCID: PMC8805939.
24. Liu EL, Zhou YX, Li J, Zhang DH, Liang F. Long-chain non-coding RNA SNHG3 promotes the growth of ovarian cancer cells by targeting miR-339-5p/TRPC3 axis. *Onco Targets Ther.* 2020;13:10959-71. doi: 10.2147/ott.s249873. PMID: 33149611; PMCID: PMC7604867.
25. Liang Y, Lin H, Jiang Z, Zhao Q, Cui R, Li S. HOXB8 mediates resistance to cetuximab in colorectal cancer cells through activation of the STAT3 pathway. *Discov Oncol.* 2024;15:603. doi: 10.1007/s12672-024-01440-z. PMID: 39472327; PMCID: PMC11522251.
26. Li X, Lin H, Jiang F, Lou Y, Ji L, Li S. Knock-down of HOXB8 prohibits proliferation and migration of colorectal cancer cells via Wnt/β-catenin signaling pathway. *Med Sci Monit.* 2019;25:711-20. doi: 10.12659/msm.912218. PMID: 30677006; PMCID: PMC6357822.
27. Ding WJ, Zhou M, Chen MM, Qu CY. HOXB8 promotes tumor metastasis and the epithelial-mesenchymal transition via ZEB2 targets in gastric cancer. *J Cancer Res Clin Oncol.* 2017;143:385-97. doi: 10.1007/s00432-016-2283-4. PMID: 27761656; PMCID: PMC11819265.
28. Wilmerding A, Bouteille L, Rinaldi L, Caruso N, Graba Y, Delfini MC. HOXB8 counteracts MAPK/ERK oncogenic signaling in a chicken embryo model of neoplasia. *Int J Mol Sci*. 2021;22. doi: 10.3390/ijms22168911. PMID: 34445617; PMCID: PMC8396257.
29. Guo J, Zhang T, Dou D. Knockdown of HOXB8 inhibits tumor growth and metastasis by the inactivation of Wnt/β-catenin signaling pathway in osteosarcoma. *Eur J Pharmacol*. 2019;854:22-7. doi: 10.1016/j.ejphar.2019.04.004. PMID: 30954562.
30. Xiang L, Lou Y, Wang P, Hu Y, Wang D. HOXB8 promotes invasion and metastasis of high-grade serous ovarian cancer via suppression of the KDM6B/C/EBPα signaling axis. *Transl Cancer Res.* 2026;15(2):83. doi: 10.21037/tcr-2025-aw-2272. PMID: 41815138; PMCID: PMC12971560.
31. Stavnes HT, Holth A, Don T, Kærn J, Vaksman O, Reich R, et al. HOXB8 expression in ovarian serous carcinoma effusions is associated with shorter survival. *Gynecol Oncol.* 2013;129(2):358-63. doi: 10.1016/j.ygyno.2013.02.021. PMID: 23438671.

Table 1. Primer sequences and amplicon lengths used for quantitative qRT-PCR analysis

Gene	Primer sequence (5'-3')	Amplicon length	Tm °C
SNHG3	Forward: GAATCTGAATGGTACTGTTGAAG Reverse: CTCAACCCAGACTTACGGTC	164 bp	57
HOXB8	Forward: GACAGGTCAAAATCTGGTTCC Reverse: GCTTCTGTTTCTCCAGCTCCT	111 bp	58.5
GAPDH	Forward: GGAGCGAGATCCCTCCAAAAT Reverse: GGCTGTTGTCATACTTCTCATGG	197 bp	59.5

qRT-PCR: Real-time polymerase chain reaction

Table 2. Demographic and clinical characteristics of patients

Clinicopathological characteris		**Number**		***HOXB8* (*P* value)**	***SNHG3* (*P* value)**
Age		>60	31	0.081	0.096
		<60	19		
Sex		Female	24	0.096	0.12
		Male	26		
Lymph node Invasion		Positive	23	0.0012	0.0045
		Negative	27		
Distant Metastasis		Positive	4	0.0032	0.0047
		Negative	46		
Differentiation		Poor	18	0.025	0.017
		Intermediate	23		
		Good	9		

HOXB8: Homeobox B8; *SNHG3*: Small nucleolar RNA host gene 3

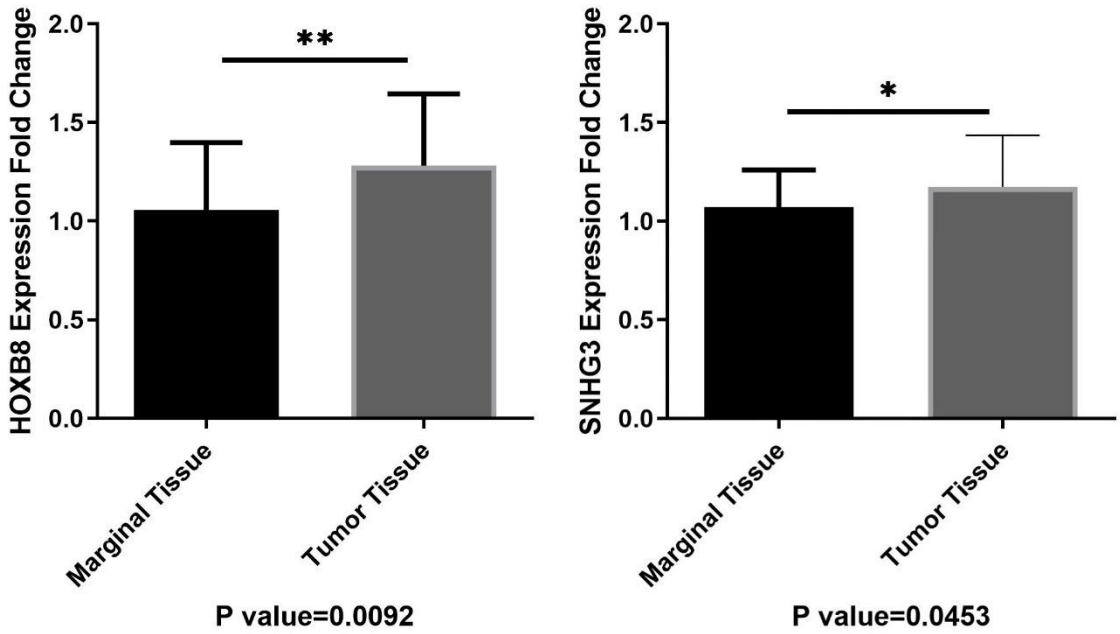


Figure 1. This figure shows the relative expression levels of *HOXB8* and *SNHG3* in OSCC tumor tissues compared with paired adjacent non-tumorous tissues. Expression was determined by qRT-PCR and calculated using the $2^{-\Delta\Delta CT}$ method, normalized to *GAPDH*. Data are presented as mean ± standard deviation. Statistical significance was determined by paired Student's t-test. Left panel: *HOXB8* expression (P = 0.0092). Right panel: *SNHG3* expression (P = 0.0453). ($^{*}P < 0.05$, $^{**}P < 0.01$).

HOXB8: Homeobox protein B8; *SNHG3*: Small nucleolar RNA host gene 3; OSCC: Oral squamous cell carcinoma; qRT-PCR: Quantitative real-time polymerase chain reaction; *GAPDH*: Glyceraldehyde-3-phosphate dehydrogenase

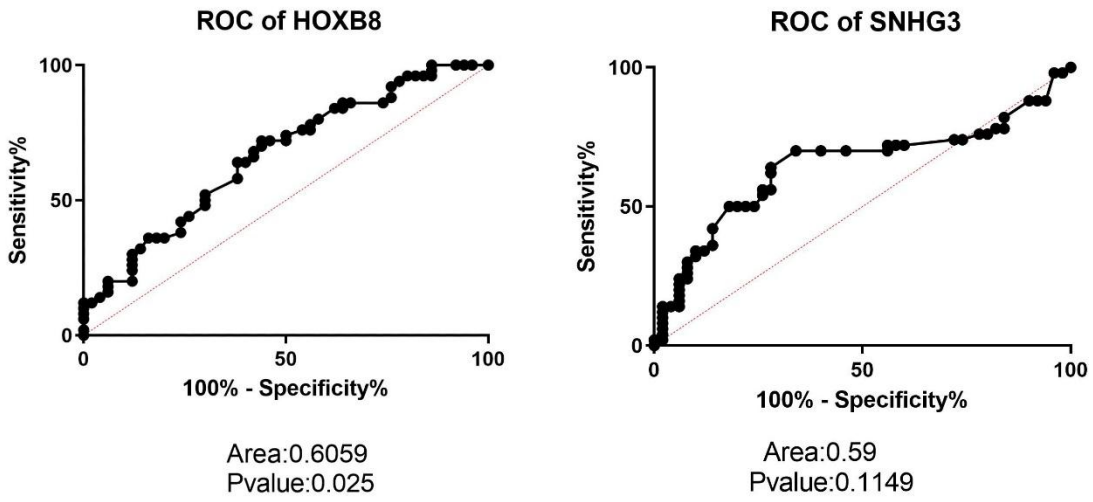


Figure 2. This figure shows the ROC curves evaluating the diagnostic performance of *HOXB8* and *SNHG3* expression in distinguishing OSCC tumor tissues from paired adjacent non-tumorous tissues. Left panel: ROC curve for *HOXB8* (AUC = 0.6059, P = 0.025). Right panel: ROC curve for SNHG3 (AUC = 0.59, P = 0.1149).
ROC: Receiver operating characteristic; *HOXB8*: Homeobox protein B8; *SNHG3*: Small nucleolar RNA host gene 3; OSCC: Oral squamous cell carcinoma; qRT-PCR: Quantitative real-time polymerase chain reaction; AUC: Area under the curve

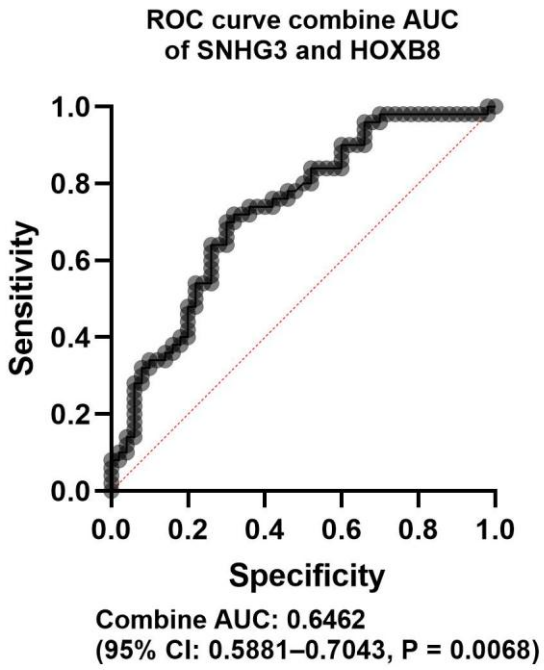


Figure 3. This figure shows the ROC curve analysis for the combined diagnostic performance of *SNHG3* and *HOXB8*. The combined predictive model was generated using multiple logistic regression analysis. AUC for the combined markers is 0.6462 (95% CI: 0.5881–0.7043, *P* = 0.0068). The dashed line represents the reference line (AUC = 0.50).

ROC: Receiver operating characteristic; *HOXB8*: Homeobox protein B8; *SNHG3*: Small nucleolar RNA host gene 3; AUC: Area under the curve

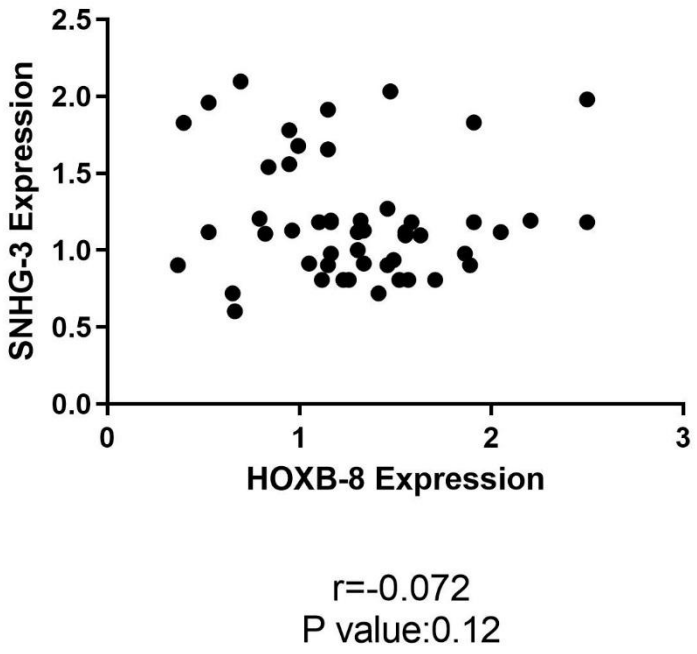


Figure 4. This figure shows the correlation analysis between *HOXB8* and *SNHG3* expression levels in OSCC tumor tissues. Pearson correlation analysis showed no significant correlation ($r = -0.072$, $P = 0.12$).
HOXB8: Homeobox protein B8; *SNHG3*: Small nucleolar RNA host gene 3; OSCC: Oral squamous cell carcinoma