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RESEARCH ARTICLE

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High eukaryotic initiation factor 5A2 expression predicts poor prognosis and may participate in the SNHG16/miR-10b-5p/EIF5A2 regulatory axis in head and neck squamous cell carcinoma

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Abstract

Background: This study attempted to investigate the significance of eukaryotic initiation factor 5A2 (EIF5A2) in the prognosis and regulatory network of head and neck squamous cell carcinoma (HNSCC).

Methods: EIF5A2 expression, prognostic information, and methylation levels of HNSCC were collected from the Cancer Genome Atlas (TCGA) database. Quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) and Western blot analyses were performed to determine EIF5A2 levels in HNSCC and normal tissue samples. R software was employed for expression analysis and prognosis assessment of EIF5A2 in HNSCC. A competing endogenous RNA (ceRNA) network was generated with the starBase database. Gene set enrichment analysis (GSEA) was used to determine the enriched physiological functions and network related to high expression of EIF5A2 in HNSCC. Immune infiltration-related outcomes were acquired from the CIBERSORT and Tumor Immune Estimation Resource (TIMER) database.

Results: EIF5A2 overexpression was observed in HNSCC and linked to poor progression-free survival and overall survival time. Cox regression analyses showed that EIF5A2 level was a stand-alone indicator of HNSCC patients' prognosis. A ceRNA network analysis highlighted the SNHG16/miR-10b-5p/EIF5A2 axis in EIF5A2 regulation. The GSEA results indicated that EIF5A2 was involved in complex signaling pathways. The CIBERSORT and TIMER databases revealed significant associations between EIF5A2 expression and immune cell infiltration.

Conclusion: EIF5A2 overexpression may be a risk factor for prognosis in HNSCC and may be regulated by the SNHG16/miR-10b-5p/EIF5A2 axis.

KEYWORDS
competing endogenous RNA (ceRNA) network, eukaryotic initiation factor 5A2, head and neck squamous cell carcinoma, prognosis, The Cancer Genome Atlas

1 | INTRODUCTION

Globally, increased attention has been given to HNSCC, and over 800,000 new reports and about 380,000 mortality incidences due to HNSCC have been reported recently.[1] HNSCC commonly involves the oral cavity, salivary glands, nasal cavity, paranasal sinuses, oropharynx, hypopharynx, and larynx,[2] whose main pathological type is squamous cell carcinoma, accounting for 97%. Tobacco (smoke and smokeless) ranks the first of major risk factors of HNSCC, followed by alcohol intake, areca nut consumption and human papillomavirus (HPV) infection.[2] With comprehensive treatment for HNSCC, namely, surgery, radiotherapy, chemotherapy, and biotherapy, HNSCC patient prognosis remains unsatisfactory.[3,4] Therefore, we aimed to identify a new biomarker of HNSCC to better understand its pathogenesis and prognosis for new therapeutic treatments.

EIF5A2 is overexpressed in a variety of cancers[5–7] and is critical for inducing cell proliferation, infiltration and metastasis ability, acting as a useful bioindicator for the identification and outcome of cancers.[8,9] In nasopharyngeal carcinoma (NPC), EIF5A2 was highly expressed and promoted NPC cell proliferation and motility, which was a stand-alone adverse prognostic indicator of failure-free survival (FFS), overall survival (OS) and distant FFS in NPC patients.[10] Nevertheless, the physiological role and prognostic significance of EIF5A2 in HNSCC are poorly understood.

RNAs include coding RNAs (messenger RNAs, mRNAs) and noncoding RNAs (ncRNAs). NcRNAs is the umbrella terminology for long noncoding RNAs (lncRNAs), microRNAs (miRNAs) and have key implications for human health.[11] MiRNAs, approximately 22 nucleotides in length, function in downregulating the expression of target genes and affect cell biological behaviors.[12,13] In 2011, Salmena et al. proposed ceRNAs as a regulatory factor among mRNAs and ncRNAs,[14] indicating that ncRNAs can regulate target mRNA expression by competitively binding to shared common miRNA recognition elements (MREs) with miRNAs, thereby exerting their biological functions. In recent years, more ncRNAs are reported to contribute to the regulation of tumorigenesis and cancer progression.[11–13,15] The ceRNA network was found to be linked to tumorigenesis and breast cancer[16] and liver cancer[17] formation. Therefore, the ceRNA network associated with EIF5A2 overexpression in HNSCC needs further study.

The tumor immune microenvironment (TIME) is known to mediate physiological reactions in cancer cells and influence the response to immunotherapy.[18] Tumor immune infiltrating cells (TIICs) is a critical component of the tumor microenvironment and have attracted extensive attention, especially in immunotherapy. HNSCC is enriched in TIICs, and studies have confirmed that the differences in TIIC composition and localization are strongly associated with HNSCC patient prognosis.[19] Hence, it is critical to examine the association between immune cell invasion and EIF5A2 level in HNSCC.

Herein, we attempted to investigate the value of EIF5A2 expression in the prognosis and function of HNSCC using TCGA database-based bioinformatics analysis. We analyzed the underlying mechanism of abnormal expression of EIF5A2 and explored the biological significance of EIF5A2 in HNSCC by using enrichment analysis and immune infiltration correlation analysis.

2 | MATERIALS AND METHODS

2.1 | Data resources and clinical data collection

The TCGA database contains over 20,000 primary cancer and corresponding adjoining tissue samples from 33 distinct cancer types. Target information, including gene expression, prognosis information, clinicopathological characteristics, and DNA methylation of HNSCC patients, were acquired from the public TCGA platform (https://portal.gdc.cancer.gov). The collected clinical profile included patient sex, age, histologic grade, tumor (T) stage, node (N) stage, clinical stage, survival status, and disease progression. Gene profile of HNSCC patients was acquired from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/), including GSE65858 and GSE41613, were used as the verification set.

To confirm the bioinformatics results, 16 HNSCC tissues and corresponding adjoining nontumorous tissues were obtained from the Ningbo Medical Centre Lihuili Hospital from May 2019 to November 2021. All patients were untreated prior to surgery. Two independent pathologists validated individual specimen using histopathological evaluation. All specimens were maintained in RNA-fixer Reagent (Bioteke) prior to storage at −80°C for further analyses.

2.2 | Differential EIF5A2 levels in HNSCC

Gene Expression Profiling Interactive Analysis (GEPIA) is an online tool (http://gepia.cancer-pku.cn.) that uses a standard processing pipeline to assess RNA-Seq information gained from TCGA and Genotype-Tissue Expression (GTEx).[20] The GEPIA online website was employed for analysis of EIF5A2 expression between multiple tumor and paired normal samples. The differential EIF5A2 levels in HNSCC versus normal samples were statistically assessed via the R software "limma" package. Box plots were generated to visualize the "ggpubr" package results. Differential EIF5A2 protein expression in HNSCC and normal samples was verified using immunohistochemistry (IHC) staining data obtained from the Human Protein Atlas (HPA) website (http://www.proteinatlas.org/).

2.3 | Pretranscriptional modification/DNA methylation

The CpG sites of Illumina Human Methylation 450K in the promoter region of EIF5A2 were revealed via the University of California Santa Cruz (UCSC) Genome Browser (http://genome.ucsc.edu). Pearson correlation analysis was used to assess the link between the EIF5A2 methylation and expression in HNSCC using the "corrplot" package in R.

2.4 | Assessment of EIF5A2 prognostic significance in HNSCC

Patients were separated into elevated expression cohorts (EEC) and reduced expression cohorts (REC), based on the median EIF5A2 levels in HNSCC patients. The Kaplan–Meier technique was employed for survival analysis. Univariate Cox analyses were employed to analyze the link between EIF5A2 levels and clinical profile, namely, age, sex, grade, and stage, with OS and PFS in HNSCC patients. Multivariate analyses were employed to estimate the stand-alone prognostic indicators in HNSCC. The survival analysis was conducted using the "survival" package of R software, and the analysis results were visualized using the "survminer." The forest plots were constructed using the "ggplot" package. OS was described as the duration between the diagnosis date and mortality from all-cause date. PFS was described as the duration between the diagnosis date and date of disease progression, death, or last follow-up contact.

2.5 | Generation and assessment of nomogram

To determine the independent nature of the estimated l-, 3-, and 5-year survival likelihood, a nomogram was generated using the multivariate data. Variables were assigned to the nomogram based on a point scale: A straight line was employed for variable point determination, and the total points of individual variables were rescaled on a range between 0 and 100. The variable positions were collected and documented as the total points. The HNSCC patient OS likelihood at 1, 3, and 5 years was assessed by drawing vertical lines from the total point axis down to the outcome axis. Next, a calibration curve was generated, based on the nomogram-estimated likelihoods against the observed rates, to assess the nomogram predictability. The bias-corrected line in the calibration plot was similar to the ideal curve (i.e., the 45-degree line), suggesting good agreement between the estimated and actual values. Lastly, receiver operating characteristic (ROC) curves and areas under the curve (AUCs) were generated to determine the prediction precision of the nomogram.

2.6 | Generation of the ceRNA axis

The StarBase platform is excellent for the examination of ncRNA association from degradome-seq, CLIP-seq, and RNA–RNA interactome data.[21] We selected the mRNA upstream miRNAs and performed correlation analysis, differential expression, and OS analyses on the obtained miRNAs using R software. The obtained miRNAs met the following requirements: negative association with EIF5A2 transcript levels, low levels in tumor tissues, and positive association with prognosis of patients. The miRNAs upstream of lncRNAs were also selected via starBase. The acquired lncRNAs met the following requirements: positive correlation with EIF5A2 transcript levels and negative association with miRNA levels, and high levels in tumor tissues and negative association with prognosis of patients. The aforementioned differences were highly significant. Prognostic analysis was performed using the same statistical methods as EIF5A2 prognostic analysis.

2.7 | Gene set enrichment analysis

GSEA software (version 4.0.1) was retrieved from the (http://software.broadinstitute.org/gsea/index.jsp) website and used to investigate the potential molecular mechanism of the EIF5A2 gene that contributed to HNSCC progression. By using the median of EIF5A2 expression as a cutoff standard, the software separated all HNSCC patients into EEC and REC. GSEA was utilized to determine the marked relevant functional and network differences between the EEC and REC in HNSCC. Individual pathway enrichment analyses were repeated at least 1000 times. A function or network term with a false discovery rate (FDR) < 0.05 was set as significant enrichment.

2.8 | Correlations between TIICs and EIF5A2

Head and neck squamous cell carcinoma patients were separated into EEC and REC based on the median EIF5A2 levels. The gene profile and CIBERSORT (https://cibersort.stanford.edu/) were employed to analyze the relationship between EIF5A2 levels and each immune cell type.[22] We analyzed the relationships between EIF5A2 levels and TIICs, including B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, and neutrophils, by using TIMER (https://cistrome.shinyapps.io/timer/).[23]

2.9 | Quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA was isolated from 16 matched HNSCC and normal samples using TRIzol (Invitrogen, USA) before conversion to cDNA via a reverse transcription kit (Novoprotein, China). qRT-PCR was carried out in a LightCycler 480 (Roche, USA) system mixed with NovoStart® SYBR qPCR (Novoprotein, China). The PCR conditions were as follows: heating at 95°C for 30 s, denaturation at 95°C for 10 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s; the reaction lasted for 35 cycles. The housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an endogenous control. The EIF5A2 primers were EIF5A2-F: 5′-TGTCCTTCTACTCACAACATGGA-3′, EIF5A2-R: 5′-CTCACGAACTTCACCAGTTTCT-3′, GAPDH-F: 5′-CAGTCAGCCGCATCTTCTT-3′, GAPDH-R: 5′-GACAAGCTTCCCGTTCTCAG-3′. The EIF5A2 relative expression was computed via the $2^{-\Delta\Delta Ct}$ formula. All experimentations were completed thrice.

2.10 | Western blot analyses

The tissues were collected and lysed, and the protein quantification was done via a BCA protein assay kit (Beyotime, China) following kit directions. After total protein extraction through enhanced RIPA

buffer (Beyotime, China), proteins were electrophoresed in a 10% sodium dodecyl sulfate–polyacrylamide gel, prior to transfer to a PVDF membrane (Millipore, USA) at 240 mA constant current for 2 h, followed by a 2 h blocking in 5% skim milk, and overnight (ON) incubation in anti-EIF5A2 primary antibody (Proteintech, China) at 4°C. This was followed by three TBST-rinses and subsequent 1 h incubation in secondary antibody (Proteintech, China). Finally, chemiluminescence (Advansta, USA) was used to identify the signal and visualize the target protein expression, which was analyzed via the Image Lab software.

2.11 | Statistical analysis

All data analyses employed the R 4.0.3 software. The Wilcoxon signed-rank test was employed for assessment of linkage between EIF5A2 levels and clinical profile using the SPSS software 20.0 (IBM, Chicago, USA). Uni- and multivariate analyses were conducted via Cox regression models. Inter-group comparisons of EIF5A2 expression were determined via independent Student's *t* test as appropriate. Overall, $p < 0.05$ was set as the significance threshold.

3 | RESULTS

3.1 | EIF5A2 expression was markedly elevated in HNSCC tissues

The EIF5A2 expression pattern between tumor and normal tissue samples in numerous types of cancers was obtained from the GEPIA database, as depicted in Figure 1A. EIF5A2 was markedly upregulated in HNSCC, colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), lung squamous cell carcinoma (LUSC), pancreatic adenocarcinoma (PAAD), skin cutaneous melanoma (SKCM), and thymic carcinoma (THYM) but downregulated in acute myeloid leukemia (LAML) and testicular germ cell tumor (TGCT). We also verified and visualized the EIF5A2 expression level individually from the TCGA database. Based on our analysis, the EIF5A2 levels in HNSCC samples were markedly elevated, compared to normal tissues ($p < 0.001$, Figure 1B). The detailed clinical profiles, namely, sex, age, histological grade, T stage, N stage, clinical stage, survival status, and disease progression, are shown in Table S1. HPA database was also employed to determine the EIF5A2 protein levels in HNSCC samples and normal tissues, which suggested that EIF5A2 protein was highly expressed in HNSCC samples from IHC staining analysis (Figure 1C). Furthermore, EIF5A2 transcript and protein expressions were assessed via qRT-PCR and Western blot analysis. Both EIF5A2 transcript and protein expressions were markedly upregulated in HNSCC tissues relative to adjacent normal tissues (Figure 1D,E).

DNA methylation is a commonly investigated epigenetic modification leading to abnormal gene expression in cancer. Copy number variations (CNVs) are a strong cancer marker that is linked to oncogene activation and/or tumor suppressor gene inactivation in multiple malignancies. Ten CpG sites in the EIF5A2 promoter are shown in Figure 2A using the UCSC Genome Browser. Illumina Human Methylation 450K was downloaded from the TCGA data portal. Pearson correlation analyses showed that methylation modification is crucial for EIF5A2 differential expression in HNSCC (Figure 2B). CNVs may contribute to the high expression of EIF5A2 in HNSCC ($p < 0.001$, Figure 2C).

3.2 | EIF5A2 overexpression predicts poor prognosis in HNSCC

Survival analysis from the TCGA database suggested that HNSCC patients in the EEC had shorter OS durations, compared to the REC, which was consistent with data from the GSE65858 and GSE41613 datasets ($p < 0.05$, Figure 3A–C). Cox regression analysis highlighted that EIF5A2 expression (HR = 1.637, $p < 0.01$), age (HR = 1.027, $p < 0.001$) and stage (HR = 1.490, $p < 0.001$) served as stand-alone prognostic factors for survival (Figure 3D,E). Moreover, high EIF5A2 expression predicted a shorter PFS time than low EIF5A2 expression (Figure 4A,B). EIF5A2 expression (HR = 1.489, $p < 0.05$) and stage (HR = 1.332, $p < 0.01$) acted as stand-alone risk indicators of PFS in HNSCC (Figure 4C,D). The detailed clinical characteristics from TCGA, GSE65858 and GSE41613 of HNSCC patients are summarized in Table S1.

We also examined the link between EIF5A2 levels and the clinical profile of HNSCC patients. As shown in Figure S1, patients with more advanced T stage and N stage had higher EIF5A2 expression levels than those with lower stage (all $p < 0.05$).

3.3 | Generation and verification of a EIF5A2 level-based nomogram

To construct a quantitative method to predict HNSCC patient prognosis, a nomogram based on EIF5A2 expression was established and validated. The nomogram combined the risk scores of EIF5A2 expression, sex, grade, T stage, clinical stage, age, and N stage for clinical application (Figure 5A), which might improve the predictive sensitivity and specificity of OS predictions at 1, 3, and 5 years (Figure 5B). To validate the predictive ability of EIF5A2 expression combined with clinical features, ROC analysis was performed, and the AUC ranked first at 0.753 (Figure 5C). Overall, the nomogram is considered a superior predictive model for the prognosis of HNSCC patients.

3.4 | Generation of the lncRNA–miRNA-mRNA regulatory axis

To further investigate the modulatory network of EIF5A2 in HNSCC, we identified the miRNAs and lncRNAs upstream of EIF5A2 and

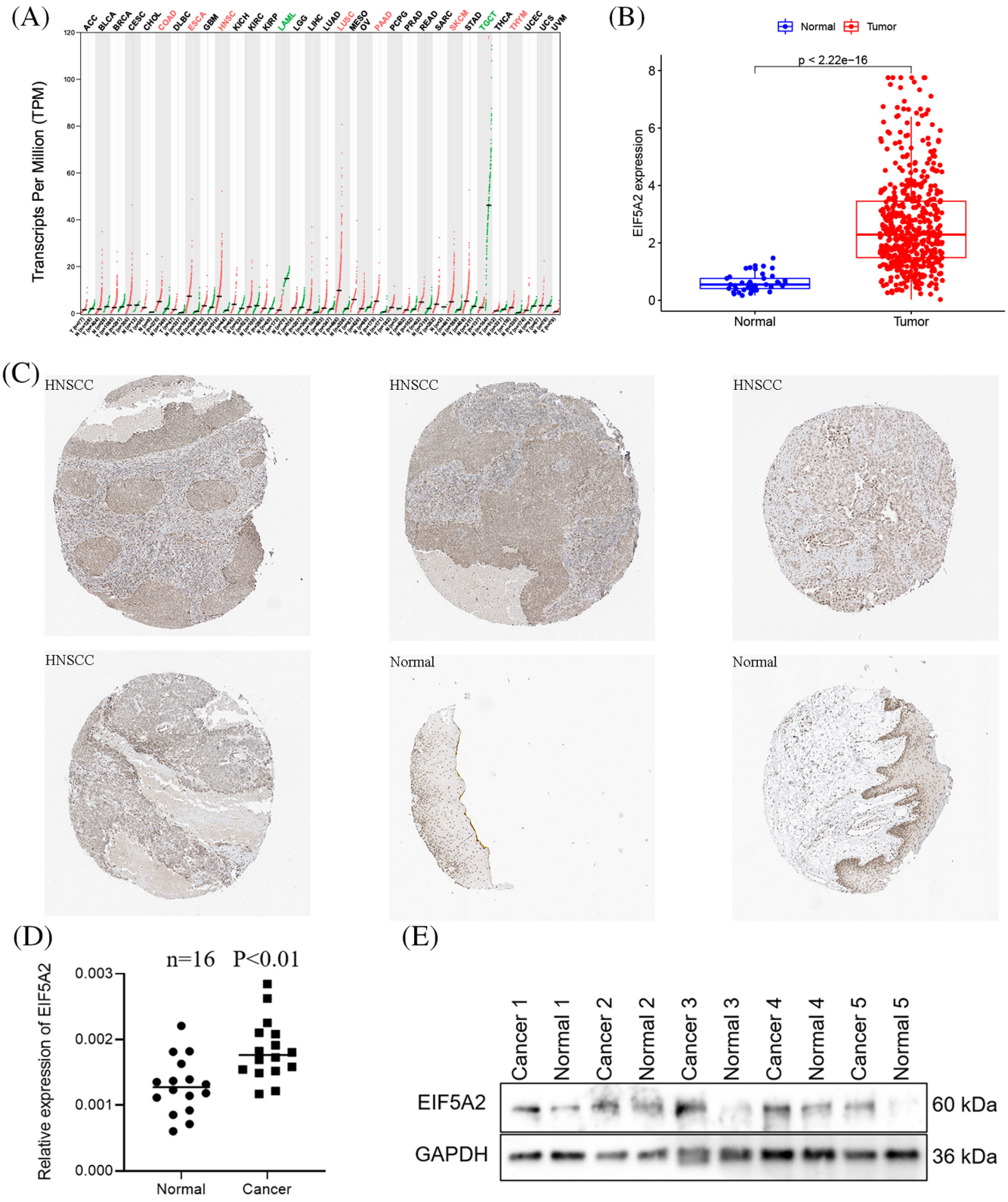

FIGURE 1 Expression levels of EIF5A2. (A) Differential EIF5A2 expression levels in various types of cancers. T: tumor tissues, N, normal tissues. Red abbreviations represent EIF5A2 significantly upregulated cancer types. Green abbreviations represent EIF5A2 significantly downregulated cancer types. (B) High EIF5A2 expression in HNSCC samples based on TCGA database. (C) IHC staining images of EIF5A2 protein levels in HNSCC versus normal samples from HPA database. (D) The relative expression levels of EIF5A2 mRNA of 16 paired samples were markedly upregulated in HNSCC tissues. (E) The EIF5A2 protein levels of five paired tissues were markedly upregulated in HNSCC versus normal samples.

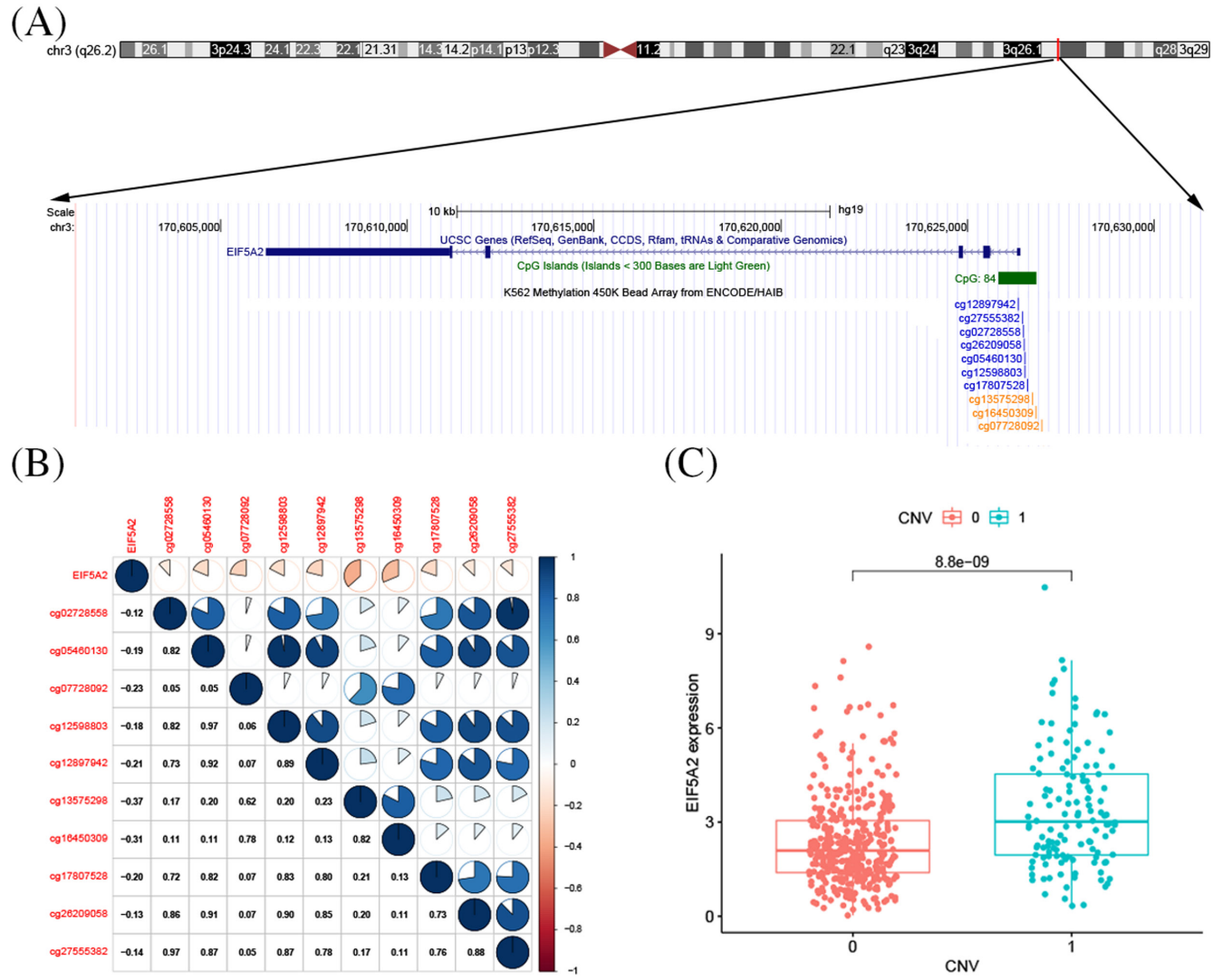

FIGURE 2 Underlying factors of EIF5A2 overexpression in HNSCC. (A) Genomic position of 10 available CpG in the EIF5A2 promoter by using UCSC Genome Browser. (B) Negative correlation between methylation modification and EIF5A2 expression in HNSCC. (C) Correlation between EIF5A2 expression and copy number variations.

generated a ceRNA axis. We first established the miRNA-EIF5A2 axis for better visualization from the starBase database (Figure 6A). Among these miRNAs, four upstream miRNAs negatively correlated with EIF5A2, including miR-155-5p, miR-10b-5p, miR-3150b-3p, and miR-200b-3p (all $p < 0.01$, Table S2). We further discovered that only miR-10b-5p was downregulated and strongly inversely associated with EIF5A2 mRNA levels in HNSCC tissues, as depicted in Figure 6B,C (all $p < 0.01$). Moreover, low miR-10b-5p expression predicted worse OS time in HNSCC patients (Figure 6D; $p < 0.05$).

Recently, the lncRNA–miRNA-mRNA axis has attracted increasing attention in various types of cancers, and we screened lncRNAs upstream of miRNAs by the same method. The lncRNAs SNHG16 and LINC02035 were found to potentially target miR-10b-5p (Figure 7A; Table S3). As shown in Figure 7B,C, SNHG16 and LINC02035 were both highly expressed in HNSCC, strongly inversely associated with miR-10b-5p and directly associated with EIF5A2 levels. Moreover, high SNHG16 expression predicted a shorter OS time in HNSCC patients, while LINC02035 expression had no significant correlation with prognosis (Figure 7D). Overall, the ceRNA regulatory network indicated that the SNHG16/miR-10b-5p/EIF5A2 network might function HNSCC prognosis.

3.5 | GSEA-based EIF5A2-associated networks

GSEA was performed to screen for EIF5A2-related networks in HNSCC. Based on our analysis, elevated EIF5A2 levels were strongly associated with “adherens junction,” “basal cell carcinoma,” “cell cycle,” “extracellular matrix (ECM) receptor interaction,” “focal adhesion,” “gap junction,” “mitogen-activated protein kinase (MAPK) signaling pathway,” “pathway in cancer,” “prostate cancer,” “renal cell carcinoma,” “small cell lung cancer,” “the transforming growth

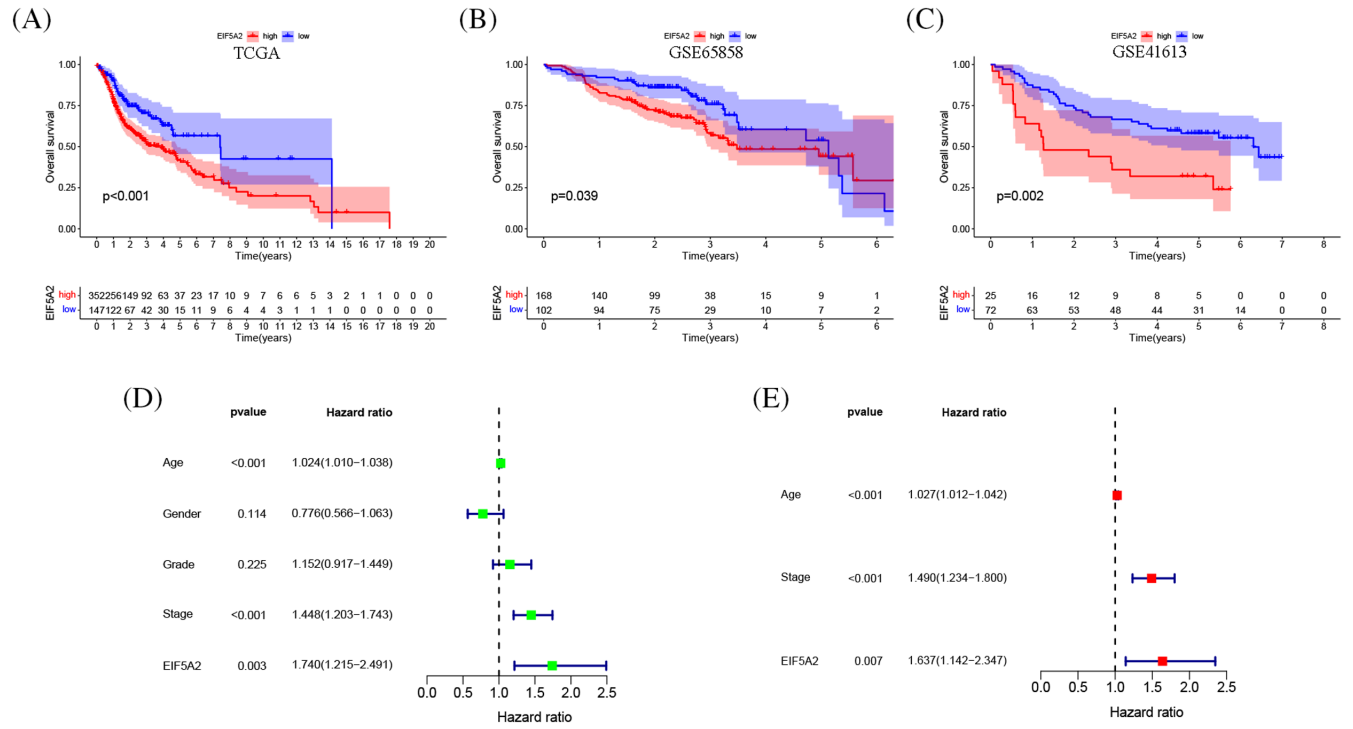

FIGURE 3 EIF5A2 overexpression predicts poor OS in HNSCC. (A–C) Kaplan–Meier survival analysis indicates that EIF5A2 overexpression predicts lower survival rate in HNSCC base on the TCGA database, GSE65858 database, and GSE41613 database. (D–F) Cox regression analysis suggests EIF5A2 expression as a stand-alone prognostic indicator of HNSCC patient OS.

factor-β (TGF-β) network," and "Wnt network" (Figure S2). Some of these axes like the Wnt, TGF-β and MAPK networks are closely correlated with tumorigenesis and tumor progression.

3.6 | Correlation between TIICs and EIF5A2 expression in HNSCC

In recent years, the immune microenvironment has received increasing attention in cancer progression. We further investigated the function of EIF5A2 in immune infiltration in HNSCC, showing patterns of correlations between EIF5A2 expression and TIICs. Ten TIICs were identified as differentially expressed and were visualized using a heatmap (Figure S3A). Incremental differences in $CD8^+$ T cells, follicular helper T cells, Tregs, gamma delta T cells, activated NK cells, and resting mast cells were observed in the REC, whereas the levels of memory resting $CD4^+$ T cells, resting NK cells and M0 macrophages increased in the EEC (Figure S3B). As shown in Figure S3C, correlation analyses demonstrated that EIF5A2 had markedly positive correlations with memory resting $CD4^+$ T cells, resting NK cells, activated mast cells, M0 macrophages, and activated dendritic cells. EIF5A2 had a strong negative association with $CD8^+$ T cells, Tregs, activated NK cells, memory B cells, resting mast cell follicular helper T cells, and gamma delta T cells. Our findings indicated that EIF5A2 can serve as a regulator of TIME in HNSCC. This information is invaluable for future HNSCC research.

4 | DISCUSSION

Head and neck squamous cell carcinoma is a highly prevalent worldwide malignancy.[1] Despite advances in HNSCCC interventions, the 5-year OS rate for advanced or recurrent patients has not significantly improved.[3,4] Hence, it is critical to identify novel biomarkers and potential therapeutic targets. Recently, EIF5A2 was shown to be strongly associated with multiple human cancer invasiveness and metastasis, namely hepatocellular carcinoma,[5] bladder cancer,[6] colorectal carcinoma,[7] gastric cancer,[24] lung cancer,[25] and breast cancer.[26] Huang et al. revealed that enhanced EIF5A2 expression in NPC cells augmented cell motility and growing capability, and it brought on 5-Fu chemoresistance in NPC cells.[10] However, reports on the levels and activity of EIF5A2 in HNSCC are limited. Thus, we conducted a bioinformatics-based assessment of EIF5A2 function in the progression and prognosis of HNSCC to offer novel clues for possible therapeutic targets.

Based on our analysis, the EIF5A2 transcript and protein expressions in HNSCC samples were both markedly elevated, compared with normal tissues, and high EIF5A2 levels were strongly associated with worse OS and PFS in HNSCC patients. Tang et al revealed that EIF5A2 transcript levels were markedly enhanced in hepatocellular carcinoma versus normal tissues, and excessive EIF5A2 expression was intricately linked to shorter OS durations in hepatocellular carcinoma patients.[5] Zhu et al. found that EIF5A2 was overexpressed in colorectal carcinoma tissues,

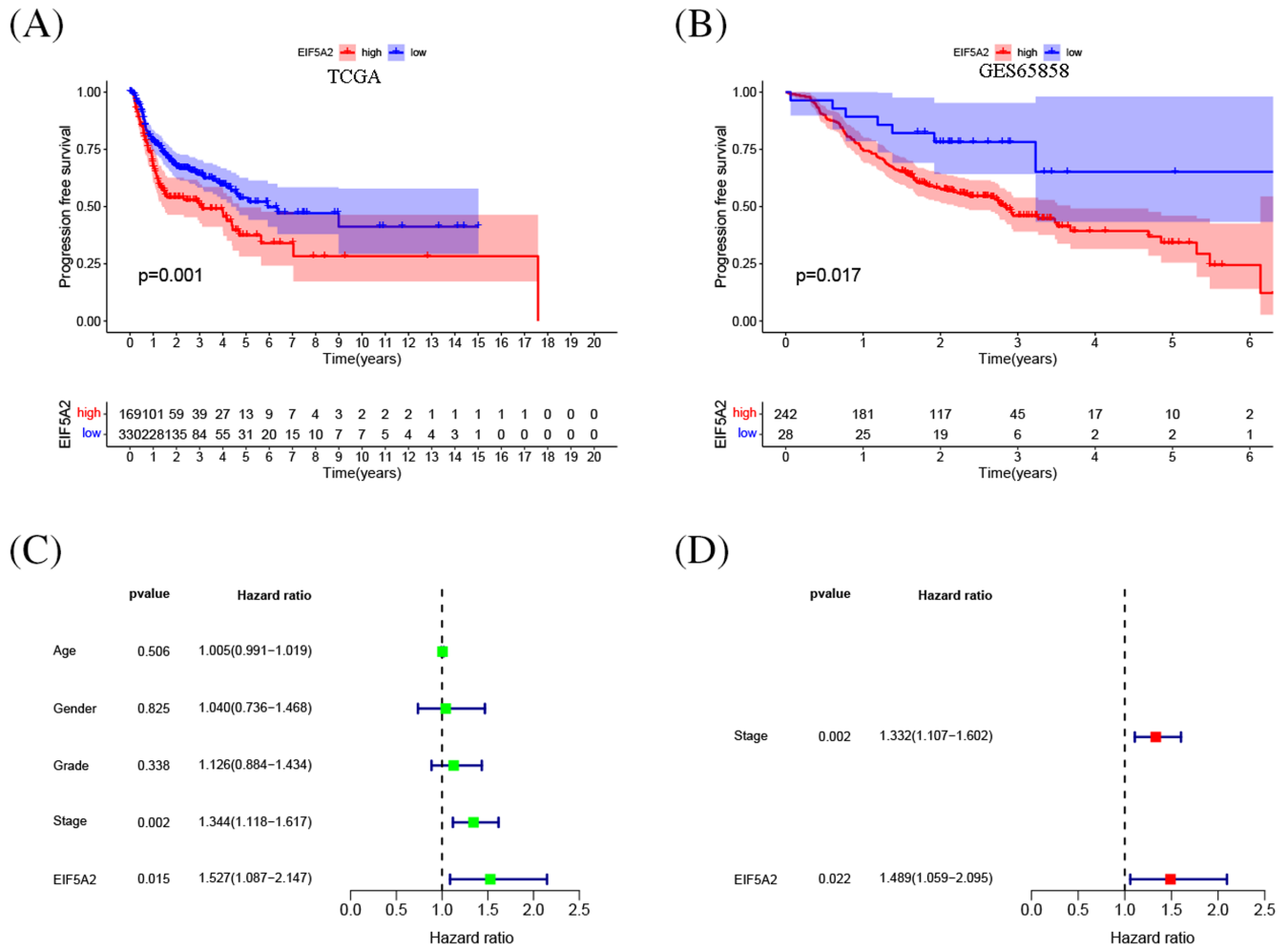

FIGURE 4 EIF5A2 overexpression predicts poor PFS in HNSCC. (A, B) Kaplan–Meier survival analysis indicates that EIF5A2 overexpression predicts higher recurrence rate in HNSCC base on the TCGA and GSE65858 database. (C, D) Cox regression analysis suggests EIF5A2 expression as a stand-alone prognostic indicator for recurrence in HNSCC patients.

as evidenced by an immunohistochemical evaluation, and EIF5A2 overexpression was associated with colorectal carcinoma metastasis and short median OS duration.[7]

Abnormal methylation of gene promoter cg sites could induce their aberrant expression to affect some crucial biological processes, such as cell proliferation, tumor invasion, and metastasis.[27,28] We evaluated the methylation status of promoter cg sites and found that the EIF5A2 levels in HNSCC was significantly inversely correlated with the EIF5A2 promoter methylation status, suggesting that aberrant methylation may be associated with abnormal EIF5A2 expression. Moreover, we also observed that elevated EIF5A2 levels in HNSCC was strongly correlated with CNVs. CNVs are tumor markers that may cause oncogene activation and/or tumor suppressor gene inactivation in malignant tumors and may represent a critical factor promoting tumor progression and determining sensitivity to antineoplastic treatments.[29] CNVs of EGFR[30] and PIK3CA[31] were shown to be linked to HNSCC progression and poor prognosis. Thus, we speculated that CNVs are critical for the aberrant EIF5A2 expression in HNSCC.

Furthermore, we revealed that EEC was associated with more advanced T stage and N stage, and high EIF5A2 expression was a stand-alone prognostic indicator of HNSCC survival and recurrence, based on uni- and multivariate analyses. He et al. reported that elevated EIF5A2 expression was associated with advanced T stage and local invasion in patients with non-small cell lung cancer, indicating that EIF5A2 may be a poor prognostic biomarker.[25] Yang et al. revealed that EIF5A2 overexpression was linked to advanced pT/pN stage and lymphovascular infiltration and worse OS in gastric cancer patients.[24] Taken together, EIF5A2 may be a poor prognostic indicator in HNSCC.

To evaluate the clinical value of EIF5A2, a nomogram was generated according to the EIF5A2 and clinicopathological features. We found that the nomogram performed much better in estimating patient OS, compared with individual prognostic factors in HNSCC patients, thereby enabling clinicians to predict the risk of individual patient death and guide patient evaluation and therapeutic decision making. Thus, we speculated that EIF5A2 may be a possible clinical bioindicator for HNSCC patients. Recently, the regulatory network in tumor development, including lncRNAs, miRNAs, and mRNAs, has been found to contribute to tumor development and progression.[14] We identified hsa-miR-10b-5p as an miRNAs upstream of EIF5A2, using correlation

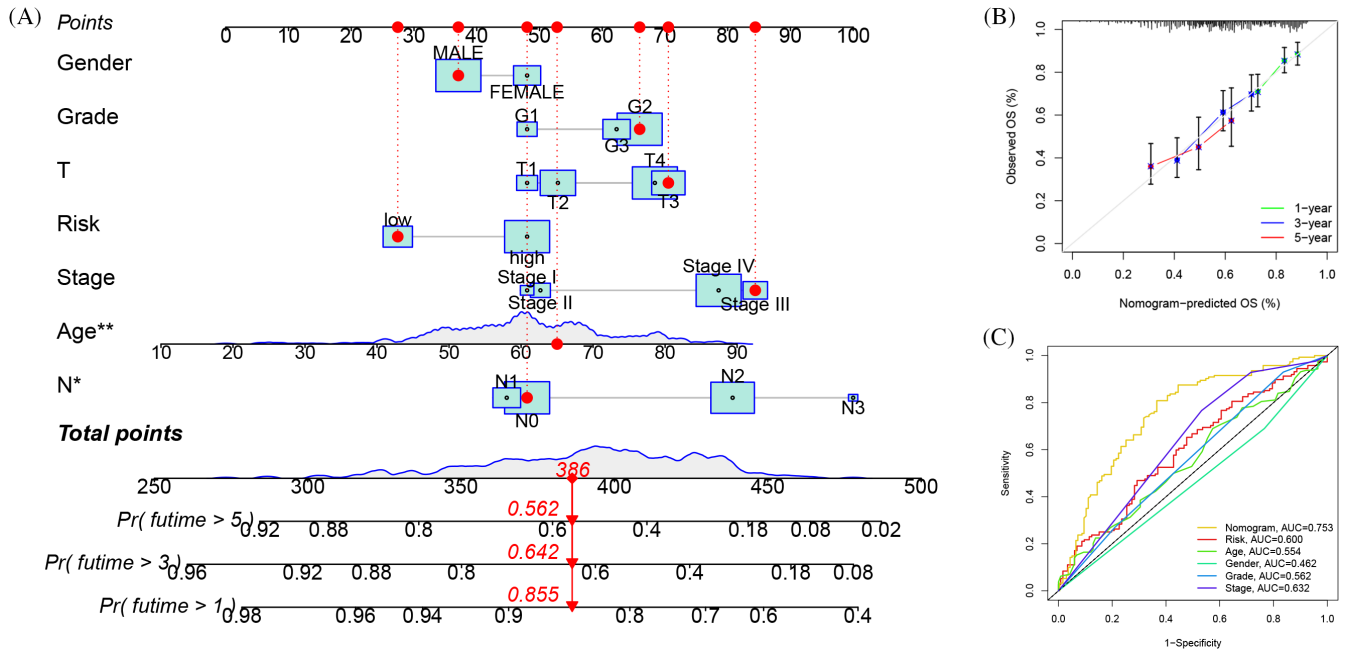

FIGURE 5 EIF5A2-related nomogram construction and validation. (A, B) Nomogram for estimating the likelihood of 1-, 3-, and 5-year OS for HNSCC patients (C) ROC curve of the EIF5A2 expression with combined nomogram and clinical characteristics.

analysis and the starBase database. We demonstrated that hsa-miR-10b-5p was inversely linked to EIF5A2 levels, and HNSCC patients with lower hsa-miR-10b-5p expression had shorter OS durations, relative to those with higher hsa-miR-10b-5p expression. These results indicated that hsa-miR-10b-5p may target EIF5A2 in HNSCC. Reports have supported the hsa-miR-10b-5p involvement in progression of multiple human cancers. Denkiewicz et al. confirmed that hsa-miR-10b-5p was correlated with the metastasis of breast cancer.[32] A previous study indicated that hsa-miR-10b-5p may modulate hepatocellular carcinoma cell proliferation by regulating SFRP1.[33] These studies partially supported the accuracy of our results.

Following the miRNA sponge theory, we identified the miRNA upstream lncRNAs, namely, LINC02035 and SNHG16. These two lncRNAs were ubiquitous in HNSCC, negatively correlated with hsa-miR-10b-5p expression and positively associated with EIF5A2 expression. However, only SNHG16 levels were significantly associated with HNSCC patient OS duration. A previous study concluded that c-Myc-mediated increase in SNHG16 promoted tumorigenesis and progression in oral squamous cell carcinoma.[34] Enhanced SNHG16 expression was correlated with tumor progression and worse prognosis in non-small cell lung cancer.[35] According to the ceRNA hypothesis, a lncRNA–miRNA-mRNA ceRNA axis was generated according to the EIF5A2 transcript levels. A preliminarily analysis of this network revealed the EIF5A2-associated network in HNSCC. However, further research is required to validate this finding.

The GSEA results indicated that high EIF5A2 expression was closely correlated with tumorigenesis- and progression-related networks like as the MAPK, Wnt, and TGF-β axes. Roy et al. found that the MAPK signaling pathway was an important transmitter of signal transduction from the cell surface to the nucleus and was found to be activated in most HNSCC cases.[36] The MAPK axis is critical for cell proliferation, differentiation, migration, apoptosis, and invasion; therefore, the development and progression of many tumors, including HNSCC, are associated with the abnormal modulation of the MAPK axis.[36,37] Yang et al. found that the Wnt axis is crucial for the development and progression of HNSCC, that is, promoting cell survival and invasive growth of HNSCC cells.[38] In addition, Kanwar et al. observed that MAPK had crucial roles in regulating the Wnt signaling pathway in HNSCC and maintaining the phenotype of cancer stem cells.[39] Roy et al. demonstrated that MAPK could regulate the Wnt signaling pathway in HNSCC through phosphorylation of glycogen synthase kinase 3β and promote chemoresistance in HNSCC.[36] Lu et al. found that overexpression of TGF-β1 in head and neck epithelia resulted in angiogenesis and epithelial hyperproliferation, leading to tumorigenesis and progression in HNSCC.[40] A review indicated that TGF-β was a multifunctional regulator in target cells, which contributed to a variety of cancers, including HNSCC.[41] In conclusion, our studies have revealed the potential molecular mechanisms and signaling pathways underlying the carcinogenicity of EIF5A2, but additional examinations are required to establish a detailed underlying mechanism.

HNSCC is enriched in TIICs, and most patients have positive responses to immunotherapy.[42] Thus, we aimed to examine the association between EIF5A2 expression and immune invasion in HNSCC. CIBERSORT analyses revealed that EIF5A2 had a substantial correlation with TIICs (especially $CD8^+$ T cells and Tregs), thus, indicating a relationship between EIF5A2 levels and the TIME in HNSCC. The T-cell immune response is critical for antitumor immunity, especially in HNSCC.[43] Prior investigations revealed that reduced $CD8^+$ T cells

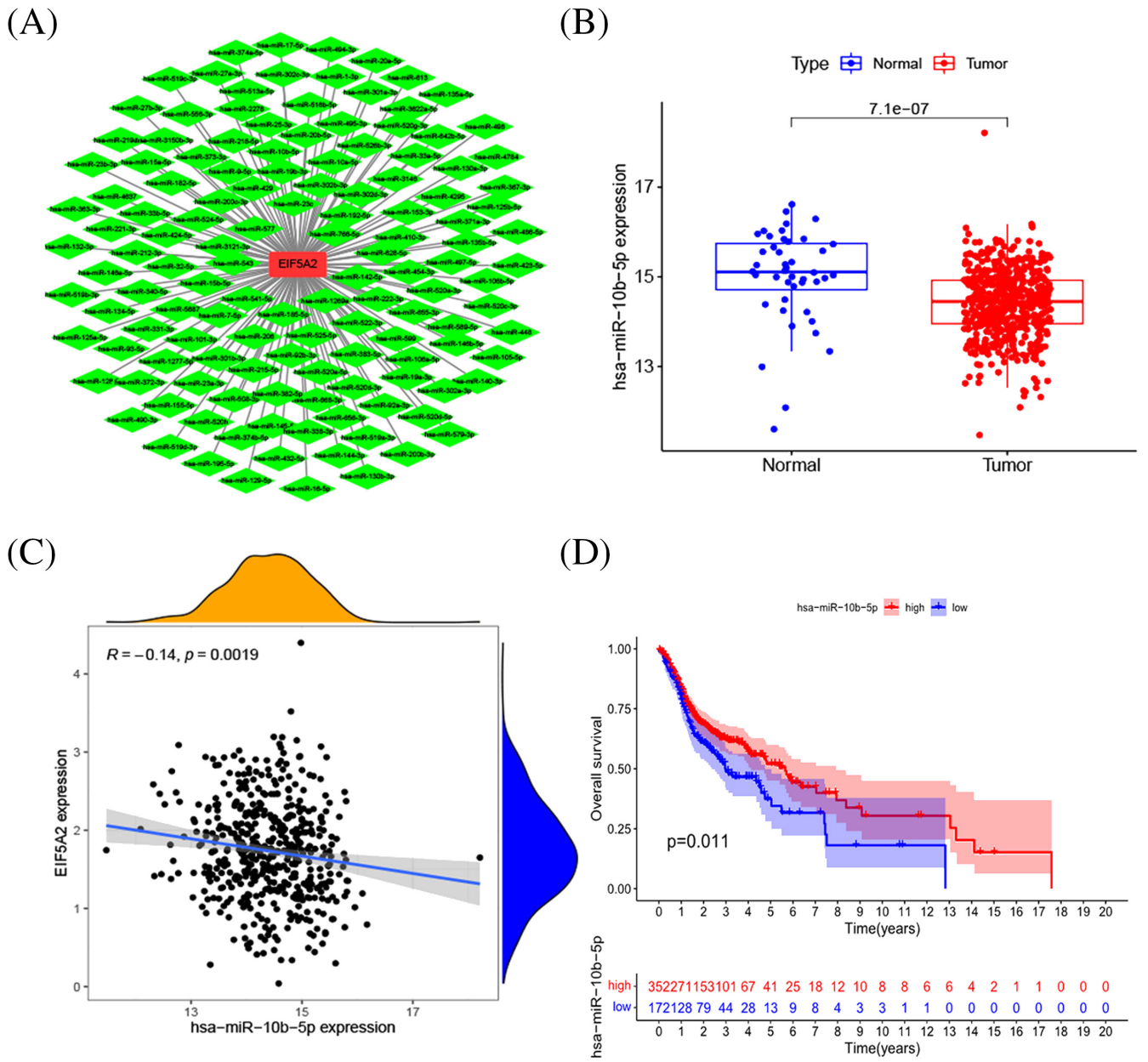

FIGURE 6 Potential regulatory mechanism of EIF5A2 in HNSCC. (A) Network mapping of EIF5A2-related miRNAs from the starBase database. (B) Expression level of miR-10b-5p between the HNSCC samples and normal tissues. (C) Correlation between EIF5A2 and miR-10b-5p expression. (D) Kaplan–Meier survival analysis of miR-10b-5p levels with OS in HNSCC patients in TCGA database.

in tumor tissues are correlated with worse prognosis among HNSCC patients.[44] An additional study demonstrated that HNSCC patients with high tumor infiltration levels of Treg exhibited considerably enhanced OS[45] and disease-free survival (DFS).[46] It has been recently reported that gene expression alterations are important predictors of the response to immunotherapy.[47,48] In addition, a recent study showed that the interactions among KCNQ1OT1, hsa-miR-148a-3p, ITGA5, and native B cells might be closely related to HNSCC development and progression via coexpression analysis between ceRNAs and TIICs,[49] which revealed a more complex mechanism of tumorigenesis of HNSCC. Collectively, we hypothesized that EIF5A2 may influence patient prognosis by regulating immune invasion in HNSCC and may become a potential pharmaceutical target for HNSCC.

Even though the aforementioned findings highlight the association between EIF5A2 and HNSCC and elucidate the expression patterns and prognostic value of EIF5A2 in HNSCC, some limitations were present in the investigation. Firstly, the physiological roles of EIF5A2 require further validation using other freely available databases. Secondly, a larger sample size is warranted to enhance the credibility of results. Thirdly, further investigations are warranted into other clinical factors in order to enhance the clinical application of EIF5A2.

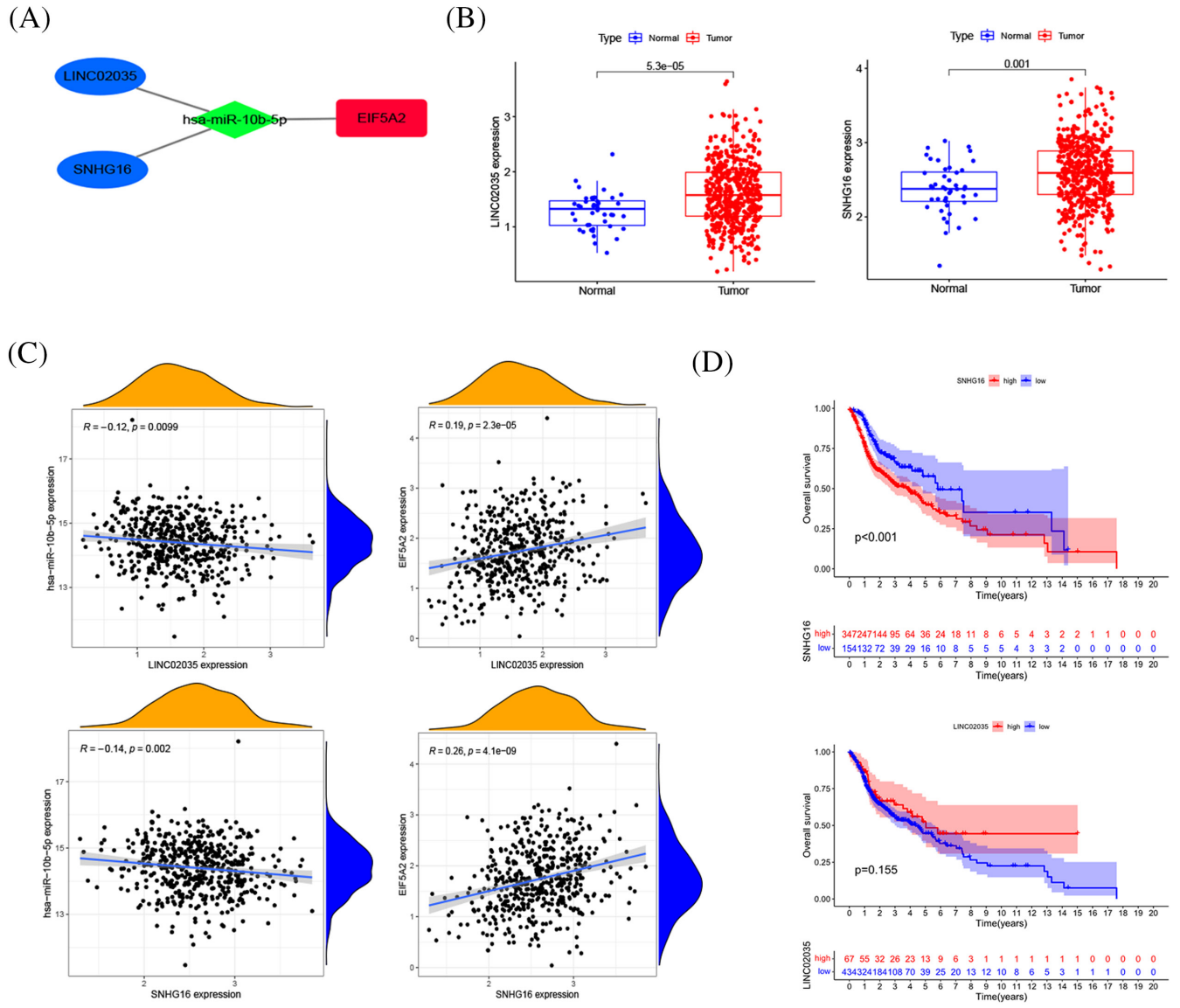

FIGURE 7 Construction of the EIF5A2-centered ceRNA network. (A) lncRNAs upstream of hsa-miR-10b-5p. (B) LINC02035 and SNHG16 and were both highly expressed in HNSCC. (C) LINC02035 and SNHG16 were found inversely associated with miR-10b-5p and directly associated with EIF5A2 levels. (D) Kaplan–Meier survival analysis demonstrated that elevated SNHG16 levels predicted worse OS time in HNSCC, and LINC02035 expression had no significant correlation with OS time.

5 | CONCLUSION

Our findings strongly suggest that EIF5A2 is highly expressed in the HNSCC tissues and may serve as a bioindicator HNSCC patient prognosis. The ceRNA network indicates that SNHG16/miR-10b-5p/EIF5A2 axis might play a regulatory role in EIF5A2 levels in HNSCC. The associations of EIF5A2 levels with several signaling pathways and TIICs as the mechanisms underlying the carcinogenicity of EIF5A2 in HNSCC need to be investigated further.

AUTHOR CONTRIBUTIONS

SY and DW organized and wrote the article. CCZ and KTL designed and produced the figures. MJ, DW, JD, HZ, and XC performed the analyses and contributed to the literature search. SF and KTL performed the qRT-PCR and Western blotting analysis. MJ and KTL revised the article. All authors reviewed the article and approved the article for publication.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data used for bioinformatics analyses in this study are freely available on The Cancer Genome Atlas (TCGA) program website (https://portal.gdc.cancer.gov), the UCSC Genome Browser (http://genome.ucsc.edu) and the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). The interpretation and reporting of these data are the sole responsibility of the authors.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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