Original Article

The lncRNA XIST/miR-29b-3p/COL3A1 axis regulates central carbon metabolism in head and neck squamous cell carcinoma and is associated with poor tumor prognosis

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Background: Recent evidence shows that COL3A1 promotes the progression of many types of cancer. The purpose of our study is to explore the correlation between COL3A1 and the prognosis of patients with head and neck squamous cell carcinoma (HNSCC) and its potential mechanism.

Methods: We initially screened the differentially expressed gene COL3A1 in The Cancer Genome Atlas (TCGA) database, and the association between the expression level of COL3A1, prognosis, and the clinical parameters of HNSCC patients was verified. A nomogram was constructed according to the multivariate analysis results. Next, a heatmap of COL3A1 co-expressed genes was constructed in TCGA database. The TargetScan database is used to explore the microRNAs (miRNA) related to COL3A1. The starBase database was used to explore and predict the long non-coding RNAs (lncRNAs) that the candidate miRNAs might bind to. Finally, the potential mechanism of action was investigated using Gene Set Enrichment Analysis (GSEA).

Results: COL3A1 expression is elevated in HNSCC tumor tissues, and HNSCC patients with high COL3A1 expression have worse prognostic factors. COL3A1 was positively correlated with the central carbon metabolism-related proteins: epidermal growth factor receptor (EGFR), phosphoglycerate mutase 1 (PGAM1), hexokinase 3 (HK3), and phosphofructokinase, platelet (PFKP). The TargetScan database showed that the best candidate miRNA for binding to the three prime untranslated region (3'UTR) end of COL3A1 mRNA was *hsa-miR-29b-3p*, which was negatively correlated with COL3A1. The starBase database showed that the lncRNA X Inactive Specific Transcript (lncRNA XIST) was the best candidate upstream non-coding RNA for regulating *hsa-miR-29b-3p*. GSEA showed that COL3A1 may be involved in the poor prognosis of HNSCC by participating in carbon metabolism, glucose metabolism, oxidative stress, and the Wingless-Type MMTV Integration Site Family (Wnt) and vascular endothelial growth factor A-vascular endothelial growth factor receptor 2 (VEGFA-VEGFR2) pathways.

Conclusions: Low COL3A1 expression can be employed as a new HNSCC predictive biomarker, and the prognosis of HNSCC patients with lower COL3A1 expression can be greatly improved. At the same time, we found that the lncRNA XIST/*miR-29b-3p*/COL3A1 axis may regulate the central carbon metabolism of HNSCC and is associated with poor prognosis. These findings point to a potential target for developing HNSCC anticancer therapies.

Keywords: Long non-coding RNA XIST (lncRNA XIST); *miR-29b-3p*; COL3A1; head and neck squamous cell carcinoma (HNSCC); bioinformatics analysis

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Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide and one of the leading causes of cancer death, with an estimated 600,000 people living with HNSCC every year (1,2). HNSCC includes numerous tumors from the larynx, pharynx, nasal cavity, sinuses, oral cavity, and tonsils (3). Its symptoms are mainly oral ulcers that do not heal for more than 2 weeks; foreign body sensation in the lips, mouth, or throat; difficulty chewing or swallowing; persistent nasal congestion or nosebleeds; neck swelling; persistent hoarseness or change in voice; unprovoked ear pain; restriction of tongue sticking out; facial or maxillary pain; and abnormal white or red plaques on the mucosal surface of the oral cavity. The position of the primary tumor of HNSCC is hidden and the early symptoms are not obvious (4), and it is mostly found in the middle to advanced stages. Despite the progress in the treatment of HNSCC with radiotherapy, chemotherapy, immunotherapy, and combination therapy, its prognosis is not optimistic. The survival time has not been greatly improved (5), with a 5-year survival rate of less than 50% (6). Recently, high-throughput and mass spectrometry technologies have been consistently applied in tumor research. Transcriptomics, proteomics, and metabolomics have promoted the precise treatment of tumors. Multi-omics integrated analysis improves a comprehensive understanding of complex carcinogenesis processes and makes up for the insufficiency of single-omics. At the same time, there are many related studies to explore biomarkers for diagnosis and treatment of HNSCC, such as identification of SERPINE1, PLAU and ACTA1 as biomarkers of HNSCC based on comprehensive bioinformatics analysis (7). LINC00958 and HOXC13-AS as key candidate biomarkers of HNSCC through comprehensive bioinformatics analysis (8). CD247 is an independent protective factor for the prognosis of HNSCC patients. Promote interferon by activating hsa04650 and hsa04660 pathways γ. The expression of interleukin (IL)-2 and IL-10 can improve the body's tumor immune monitoring ability, induce tumor cell apoptosis and inhibit tumor cell growth (9). Metabolic reprogramming is considered to be a tumor landmark (10). The acidic and hypoxic tumor microenvironment generated by reprogramming can promote tumor metastasis (11). Tumors have outstanding metabolic adaptability and can rapidly provide energy without using oxygen, thereby promoting tumor progression (12). Regardless of the specific perspectives, the starting point of all research is to enhance the prognosis of cancer patients. Therefore, active screening of markers associated with poor prognosis and early diagnosis of HNSCC is essential to improve the therapeutic effect and prognosis of HNSCC.

As a type III collagen, collagen type III alpha 1 chain (COL3A1) mainly exists in the extracellular matrix and is abundant in connective tissues such as skin, vascular intima, and muscle in the human body (13). Type III collagen deficiency can lead to tearing, perforation, breaking, and even fragmentation of structures associated with connective tissue in the body (14,15). The *COL3A1* gene is about 44 kb in length and has a total of 52 exons; it is situated in the

Highlight box

Key findings

- The prognosis of head and neck squamous cell carcinoma (HNSCC) patients with lower collagen type III alpha 1 chain (COL3A1) expression can be greatly improved, and it can be employed as a new HNSCC predictive biomarker.

What is known and what is new?

- Some small RNAs (such as miR-29a, miR-29b, miR-29c, and let-7d) use COL3A1 as the targeting molecule to involve in the growth and occurrence of tumors;
- The effect of COL3A1 on tumor cell behavior and its potential mechanism were investigated by bioinformatics analysis.

What is the implication, and what should change now?

- This work delivers various layers of evidence for the significance of COL3A1 in HNSCC development and its potential as a biomarker of HNSCC disease progression, and these results point to a possible target for the development of anticancer strategies in HNSCC.

human chromosome's q24.3–q3 region 2 and has obvious polymorphisms with multiple alleles (16). The COL3A1 gene polymorphism and expression are highly correlated with the growth and occurrence of aneurysms. The imbalance of COL3A1 expression will lead to aneurysm-like expansion and seriously affect the survival rate of patients. High expression of COL3A1 in lung fibroblasts will increase the risk of carcinogenesis (17,18). Interaction with G-protein coupled receptor 56 (GPR56), the membrane receptor of COL3A1, inhibits neuronal migration, and down-regulation of GPR56 promotes the metastasis of melanoma cells (19-21). Some small RNAs [such as *microRNA-29a* (*miR-29a*), *miR-29b*, *miR-29c*, and *miR-let-7d*] are involved in the growth and occurrence of tumors via COL3A1 as the targeting molecule (22-27). At present, there are few related studies on the role of COL3A1 in HNSCC. One study reported that COL3A1 has a good role in evaluating the immune cell infiltration, immune activity and gene expression of immune checkpoint of HNSCC lesions (28). At the same time, the head and neck are rich in connective tissue, so it is also of anatomical significance to study the role of COL3A1 in HNSCC.

Our study mainly explored the expression of COL3A1 in The Cancer Genome Atlas (TCGA) database and its role in estimating HNSCC patient survival rates. Furthermore, we performed bioinformatics analysis to predict upstream regulatory long non-coding RNAs (lncRNA) and microRNA (miRNA) genes to explore the effect of COL3A1 on tumor cell behavior and its underlying mechanisms. We present the following article in accordance with the TRIPOD reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-23-30/rc).

Methods

Data analysis using TCGA database

The expression of COL3A1 in pan-cancer was explored in TCGA database, and we also determined the expression of COL3A1 in both the HNSCC and adjacent tissues. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Correlation between clinical parameters and COL3A1 in HNSCC patients in TCGA database

We extracted the clinical data of HNSCC patients from TCGA database and then comparatively studied the correlation between these prognostic and clinical parameters and COL3A1.

Nomogram construction and assessment

In order to personalize the predicted survival probability of 1, 3 and 5 years, we built a nomogram based on the results of multivariate analysis. The RMS R package is used to generate the nomogram, including the clinical features related to COL3A1 and the calibration chart. Calibration and discrimination are the most commonly used methods to evaluate the performance of the model. In this study, the calibration curve is graphically evaluated by mapping the predicted probability of the nomogram and the observed ratio. The 45-degree line represents the best prediction measured value. The consistency index (C-index) is used to determine the discrimination of the nomogram, which is calculated by the bootstrap method with 1,000 resamples. In addition, the prediction accuracy of the nomogram and individual prognostic factors was compared using the C-index.

Screening of co-expressed genes of COL3A1

We identified positively or negatively correlated genes co-expressed with COL3A1 by data mining of TCGA database.

Predicted miRNAs regulated upstream of COL3A1 in the TargetScan database

To understand whether COL3A1 is regulated by upstream genes, we further explored the related miRNAs that may be bound to the three prime untranslated region (3'UTR) end of COL3A1 messenger RNA (mRNA) using the TargetScan database.

Predicted lncRNAs that candidate miRNAs may bind to in the starBase database

To better comprehend the signaling axis that regulates the upstream of COL3A1, we used the starBase database to predict and explore the lncRNAs that the candidate miRNAs may bind to.

Gene Set Enrichment Analysis (GSEA)

Online functional analysis was performed using Metascape (https://metascape.org/gp/index.html#/main/step1). As for functional analysis, the differential genes were included in

Metascape.

Statistical analysis

R (version 3.6.3) (https://www.r-project.org/) was utilized software for statistical analysis, and GSEA was employed to explore the possible cellular pathways of COL3A1. The Kaplan-Meier approach was employed to assess patient survival, and the log-rank statistical method was applied for the significance test. P<0.05 was considered statistically significant.

Results

The expression of COL3A1 is up-regulated in HNSCC

We found the COL3A1 molecule in TCGA database, and ascertained that the expression of COL3A1 was up-regulated in several tumors by pan-cancer analysis (*Figure 1A*). Also, the expression of COL3A1 was higher in the paired and unpaired HNSCC tumor tissues than that in the adjacent tissue. Using a receiver operating characteristic (ROC) curve, we determined that the area under the curve (AUC) was 0.888, indicating that COL3A1 had a high predictive research value (*Figure 1B-1D*).

Correlation between COL3A1 in TCGA database and prognosis of HNSCC patients

We observed that HNSCC patients with low COL3A1 expression had better disease specific survival (DSS) in TCGA database (*Figure 2A*). Further stratified analysis showed that in T3, N2, stage III, female, G3 and smoking history, patients with low expression of COL3A1 still had a superior prognosis to those with high expression (*Figure 2B*).

The relationship between COL3A1 expression in TCGA database and the clinical data of HNSCC patients

Compared with the clinical data of HNSCC patients, we found that patients with higher COL3A1 expression had a higher Tumor Node Metastasis (TNM) stage and pathological grade. This is also consistent with the previous research results (*Figure 3*).

Development of the nomogram

To individually predict the 1-, 3-, and 5-year survival of HNSCC patients, we designed a nomogram using TCGA database according to the multivariate analysis findings, with a nomogram concordance. index (C-index) of 0.570 (0.549–0.592) (*Figure 4A*). At the same time, the calibration curve indicated that the nomogram also had some predictive value (*Figure 4B*).

Screening of co-expressed genes of COL3A1

We then identified both the negatively and positively associated genes co-expressed with COL3A1 using the HNSCC results in TCGA database and constructed heatmaps of the top 50 positively (*Figure 5A*) and negatively (*Figure 5B*) correlated co-expressed genes, respectively.

Correlation between COL3A1 and carbon metabolism-related proteins in tumor centers

The carbon metabolism pathway in the tumor center is critical for the growth and existence of tumors. Therefore, we explored the correlation between COL3A1 and carbon metabolism-related proteins in the tumor center. We found that COL3A1 was positively correlated with epidermal growth factor receptor (EGFR), Phosphoglycerate Mutase 1 (PGAM1), Hexokinase 3 (HK3), and phosphofructokinase, platelet (PFKP) (*Figure 6*).

Predicted miRNAs regulated upstream of COL3A1 in the TargetScan database

To understand whether COL3A1 is regulated by upstream genes, we further explored the related miRNAs that may be bound to the 3'UTR end of COL3A1 mRNA using the TargetScan database. In the data analysis, we found that *hsa-miR-29c-3p*, *hsa-miR-29a-3p*, and *hsa-miR-29b-3p* are the possible upstream regulatory miRNAs, and the details of these miRNAs are shown in *Table 1*. Meanwhile, we also explored the association between these candidate miRNAs and the prognosis of HNSCC patients in the starBase database (*Figure 7*). At the same time, we also found that *hsa-miR-29b-3p* was negatively correlated with COL3A1 in the StarBase database (*Figure 8*). Based on the competitive endogenous RNA (ceRNA) hypothesis, lncRNA competitively combines with tumor suppressive miRNA to reduce the inhibitory effect of miRNA on target mRNA. Therefore, there should be a negative correlation between mRNA and target miRNA. Combined with ceRNA hypothesis, survival analysis and correlation analysis, we determined that *hsa-miR-29b-3p* was the best candidate miRNA.

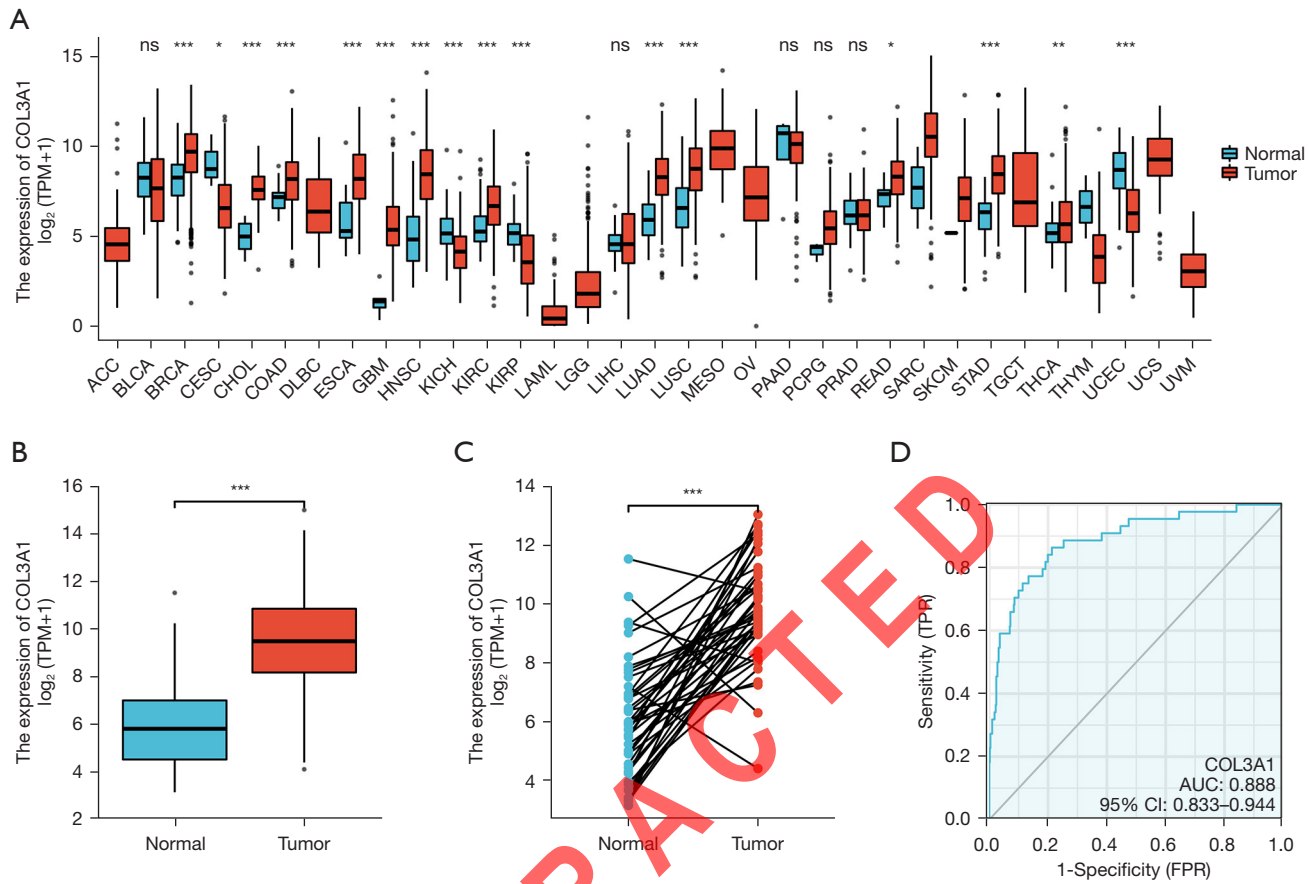

Figure 1 The expression of COL3A1 is elevated in HNSCC. (A) COL3A1 expression level in pan-cancer; (B) COL3A1 expression level in unpaired HNSCC cancer tissues (n=502) and paracancerous tissues (n=44) in TCGA database; (C) TCGA database expression levels of COL3A1 in paired HNSCC cancer tissues (n=502) and paracancerous tissues (n=44); (D) ROC curve analysis showed that COL3A1 has a good ability to discriminate between normal and tumor tissues. *, P<0.05; **, P<0.01; ***, P<0.001, the difference is statistically significant. ns, not significant; COL3A1, collagen type III alpha 1 chain; TPM, transcripts per million; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, Thyroid carcinoma; THYM, Thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma; TPR, true positive rate; FPR, false positive rate; AUC, area under the curve; CI, confidence interval; HNSCC, head and neck squamous cell carcinoma; TCGA, The Cancer Genome Atlas; ROC, receiver operating characteristic.

Predicted lncRNAs that hsa-miR-29b-3p may bind to in the starBase database

To better explore the signaling axis that regulates the upstream of COL3A1, we utilized the starBase database to indicate and explore the possible binding lncRNAs of *hsa-miR-29b-3p*. The details of these lncRNAs are shown in *Table 2*. Combined with previously published findings and the binding strength between base pairs, we believe that lncRNA X Inactive Specific Transcript (XIST) should be the best candidate upstream non-coding RNA for the

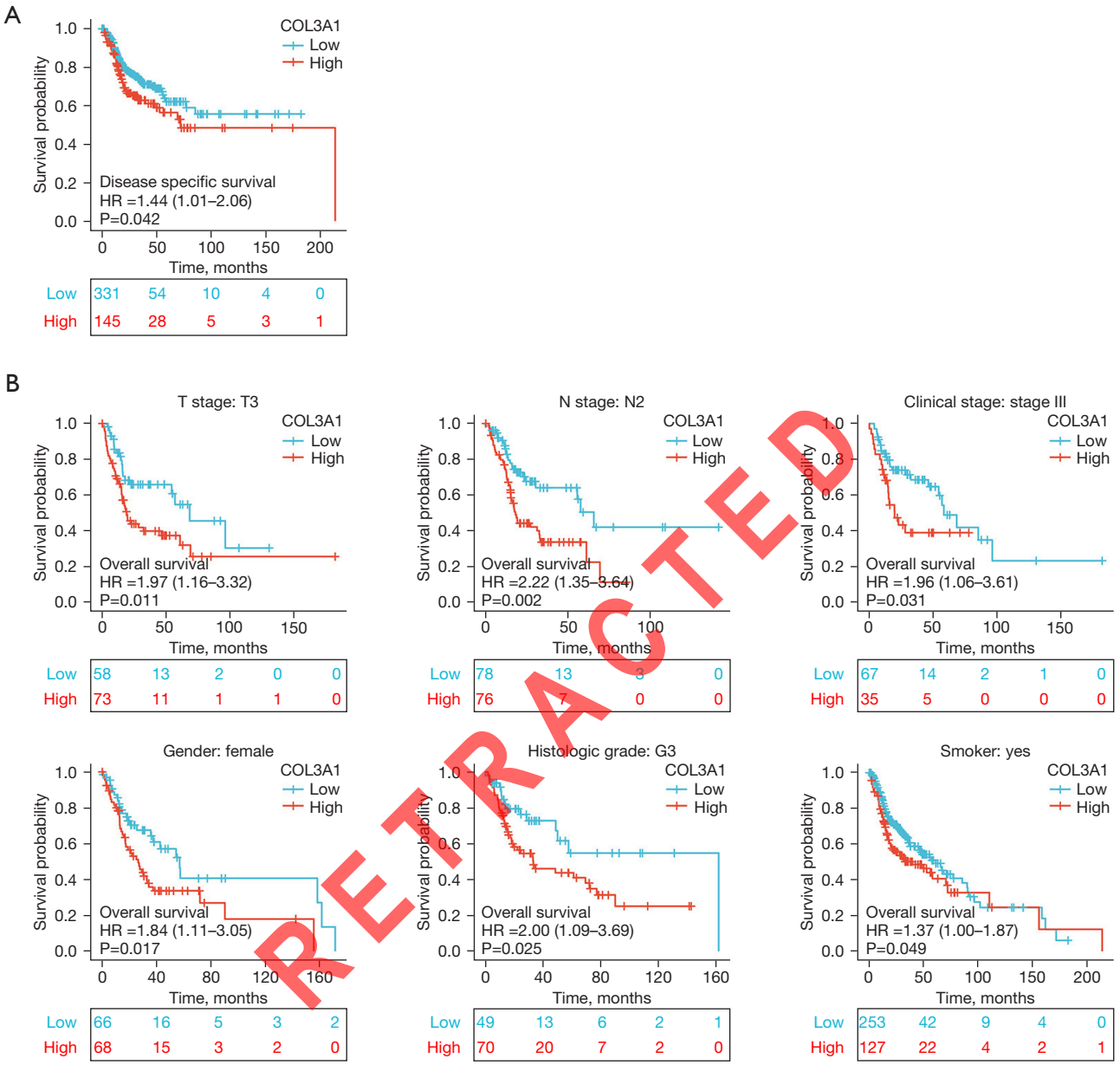

Figure 2 Correlation between COL3A1 with the prognosis of HNSCC patients in TCGA database. (A) Correlation between COL3A1 expression and the DSS of OSCC patients; (B) correlation between COL3A1 expression and the prognosis of HNSCC patients in the subgroup stratified analysis. P<0.05, the difference is statistically significant. COL3A1, collagen type III alpha 1 chain; HR, hazard ratio; DSS, disease specific survival; HNSCC, head and neck squamous cell carcinoma; OSCC, oral squamous cell carcinoma; TCGA, The Cancer Genome Atlas.

regulation of *hsa-miR-29b-3p*.

GSEA results

We also performed Kyoto Encyclopedia of Genes and Genomes (KEGG) functional analysis online using Metascape. We discovered six statistically significant and potentially relevant routes (*Figure 9*):

(I) REACTOME_METABOLISM_OF_CARBOHYDRATES;

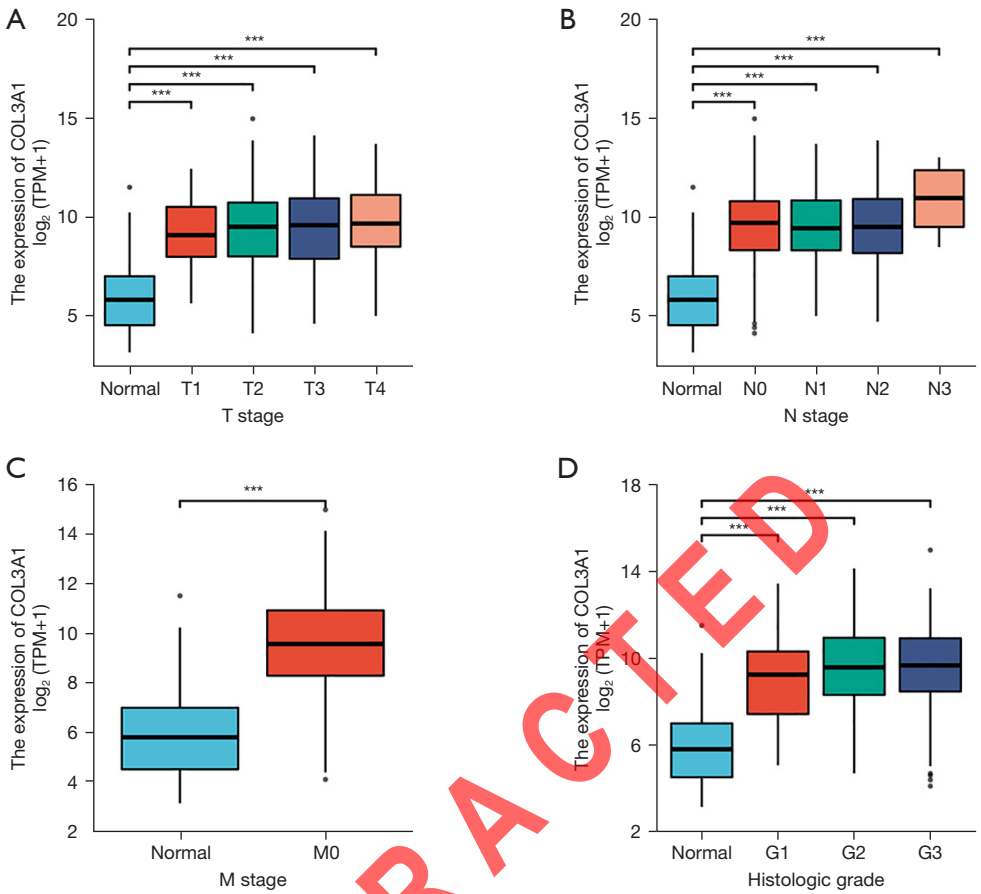

Figure 3 Correlation between COL3A1 expression and the clinical data of HNSCC patients. (A) The relationship between COL3A1 expression and T stage in clinical data of HNSCC patients; (B) the relationship between COL3A1 expression and N stage in clinical data of HNSCC patients; (C) the relationship between COL3A1 expression and M stage in clinical data of HNSCC patients; (D) the relationship between COL3A1 expression and histologic grade in clinical data of HNSCC patients .***, P<0.001. COL3A1, collagen type III alpha 1 chain; TPM, transcripts per million; HNSCC, head and neck squamous cell carcinoma.

(II) REACTOME_GLYCOSAMINOGLYCAN_METABOLISM;
(III) WP_ENERGY_METABOLISM;
(IV) REACTOME_OXIDATIVE_STRESS_INDUCED_SENESCENCE;
(V) KEGG_WNT_SIGNALING_PATHWAY;
(VI) WP_VEGFAVEGFR2_SIGNALING_PATHWAY.

Based on the above findings, we can speculate that the lncRNA XIST/miR-29b-3p/COL3A1 axis regulates the central carbon metabolism (CCM) of HNSCC and is associated with poor tumor prognosis.

Discussion

Lymph node metastasis and local invasion can occur in the early stage of HNSCC, leading to poor prognosis (29). The detailed molecular mechanism of HNSCC pathogenesis has not been fully elucidated, and further research is needed to improve the prognosis of HNSCC patients. In this study, we utilized bioinformatics analysis to evaluate the function of COL3A1 in the incidence and development of HNSCC and the expected mechanism through which it acts. Through the TCGA database, we discovered that the expression of COL3A1 was higher in HNSCC tumor tissues than that in paracancerous tissues and that the prognostic indicators for HNSCC patients with high COL3A1 expression were worse. The nomogram constructed in this study based on the multivariate analysis results also highlighted the clinical value of our research molecules—COL3A1. We then constructed a heatmap of the COL3A1 co-expressed genes

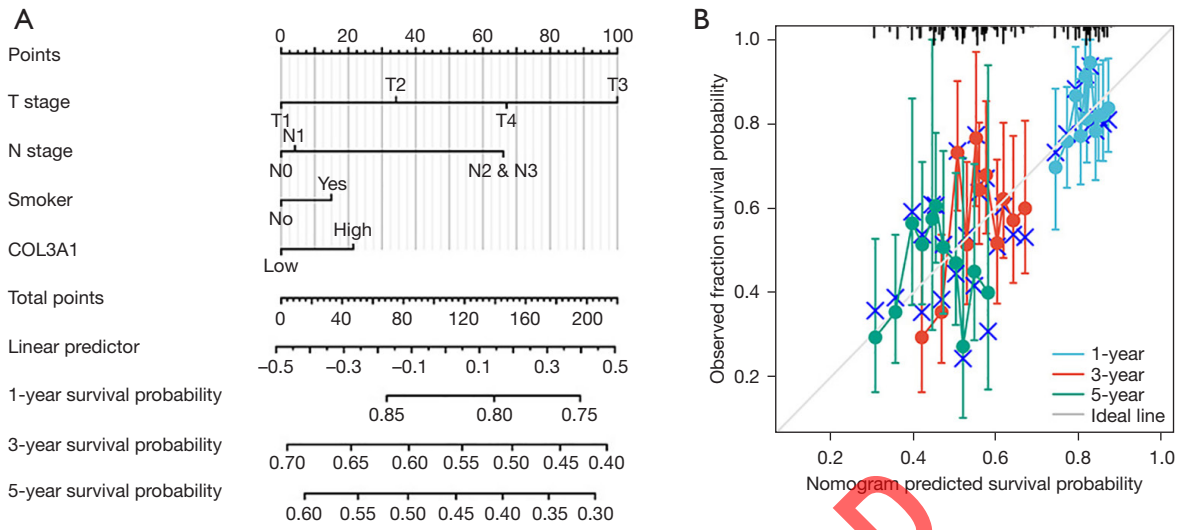

Figure 4 Nomograms and calibration plots for HNSCC patients. (A) Nomogram for predicting the 1-, 3-, and 5-year survival of HNSCC patients; (B) calibration plot of the nomogram for predicting the OS likelihood. HNSCC, head and neck squamous cell carcinoma; OS, overall survival.

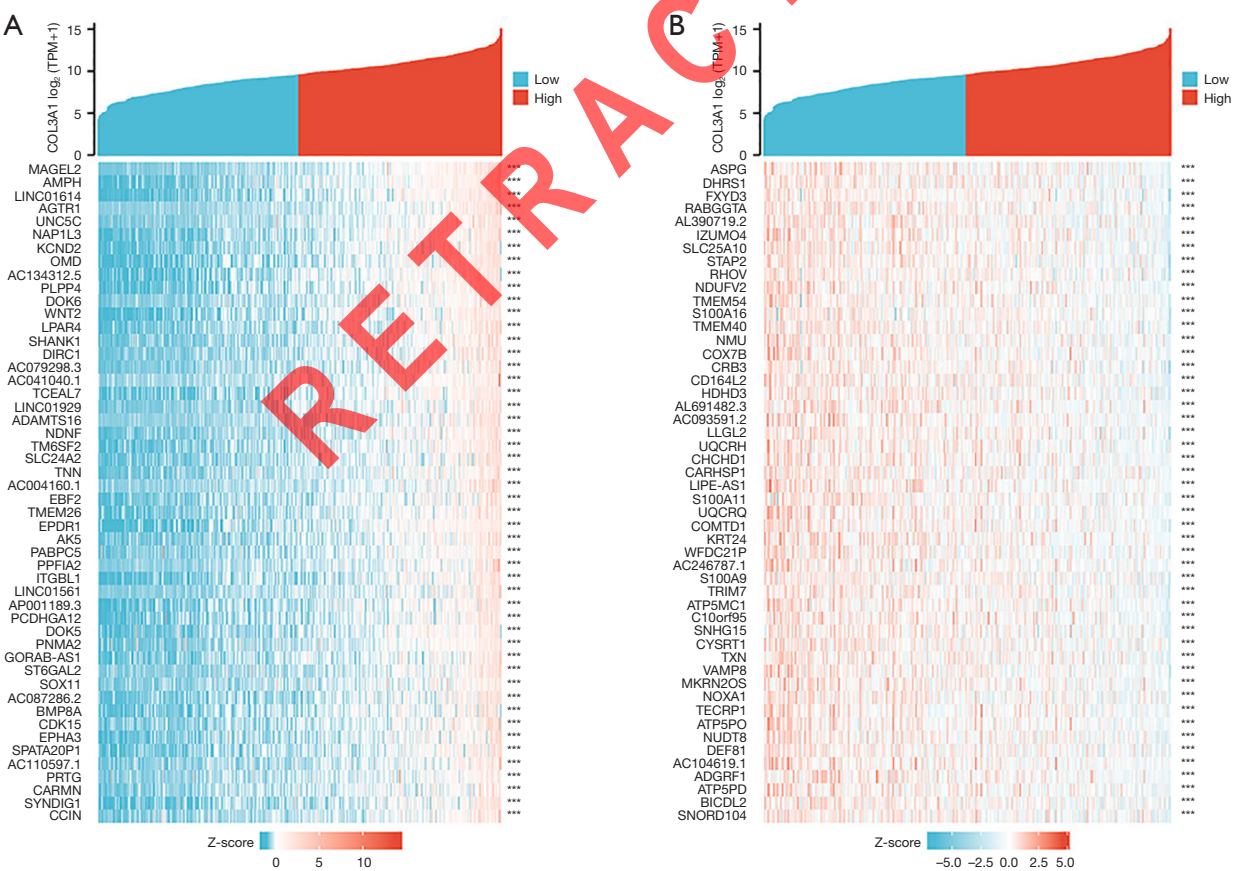

Figure 5 Heatmap of COL3A1 co-expressed genes. (A) Positively associated genes co-expressed with COL3A1 in HNSCC; (B) negatively associated genes co-expressed with COL3A1 in HNSCC. ***, P<0.001. COL3A1, collagen type III alpha 1 chain; TPM, transcripts per million; HNSCC, head and neck squamous cell carcinoma.

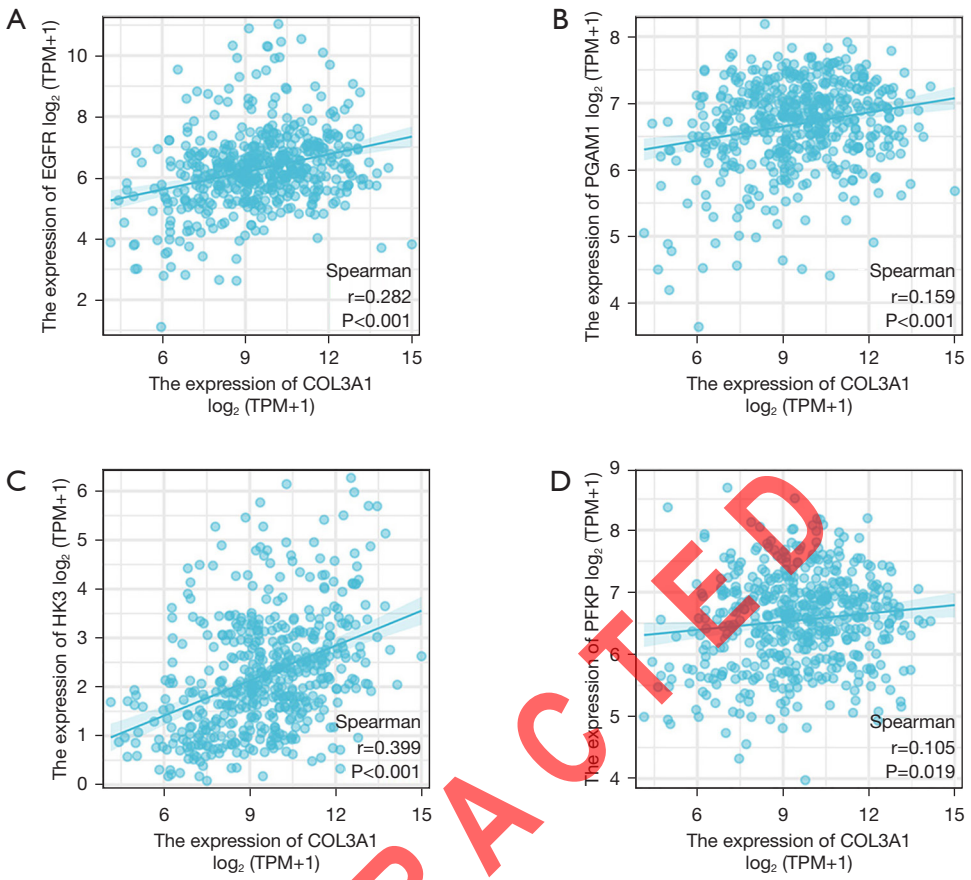

Figure 6 Correlation between COL3A1 and proteins related to carbon metabolism in tumor centers. (A) Correlation between COL3A1 and EGFR; (B) correlation between COL3A1 and PGAM1; (C) correlation between COL3A1 and HK3; (D) correlation between COL3A1 and PFKP. COL3A1, collagen type III alpha 1 chain; EGFR, epidermal growth factor receptor; HK3, hexokinase 3; PFKP, phosphofructokinase, platelet; PGAM1, phosphoglycerate mutase 1; TPM, transcripts per million.

Table 1 Predicted miRNAs in the TargetScan database that binds to the 3'UTR of COL3A1

Position 273–280 of CHD4 3'UTR	Predicted consequential pairing of target region (top) and miRNA (bottom)	Site type	Context++ score	Context++ score percentile	Weighted context++ score	Conserved branch length	PCT	Predicted relative KD
hsa-miR-29a-3p	5'-UUCAAAAUGUCUCAAUGGUGCUA-||||||	8mer	−0.86	99	−0.80	4.901	0.93	−5.205
	3'-AUUGGCUAAAGUCUACCACGAU							
hsa-miR-29c-3p	5'-UUCAAAAUGUCUCAAUGGUGCUA-|||||||	8mer	−0.86	99	−0.80	4.901	0.93	−5.404
	3'-AUUGGCUAAAGUUUACCACGAU							
hsa-miR-29b-3p	5'-UUCAAAAUGUCUCAAUGGUGCUA-|||||||	8mer	−0.86	99	−0.80	4.901	0.93	−5.404
	3'-UUGUGACUAAAGUUUACCACGAU							

"|" represents base pair expression representing the gene. COL3A1, collagen type III alpha 1 chain; 3'UTR, three prime untranslated region; PCT, percentile; KD, k-dimensional.

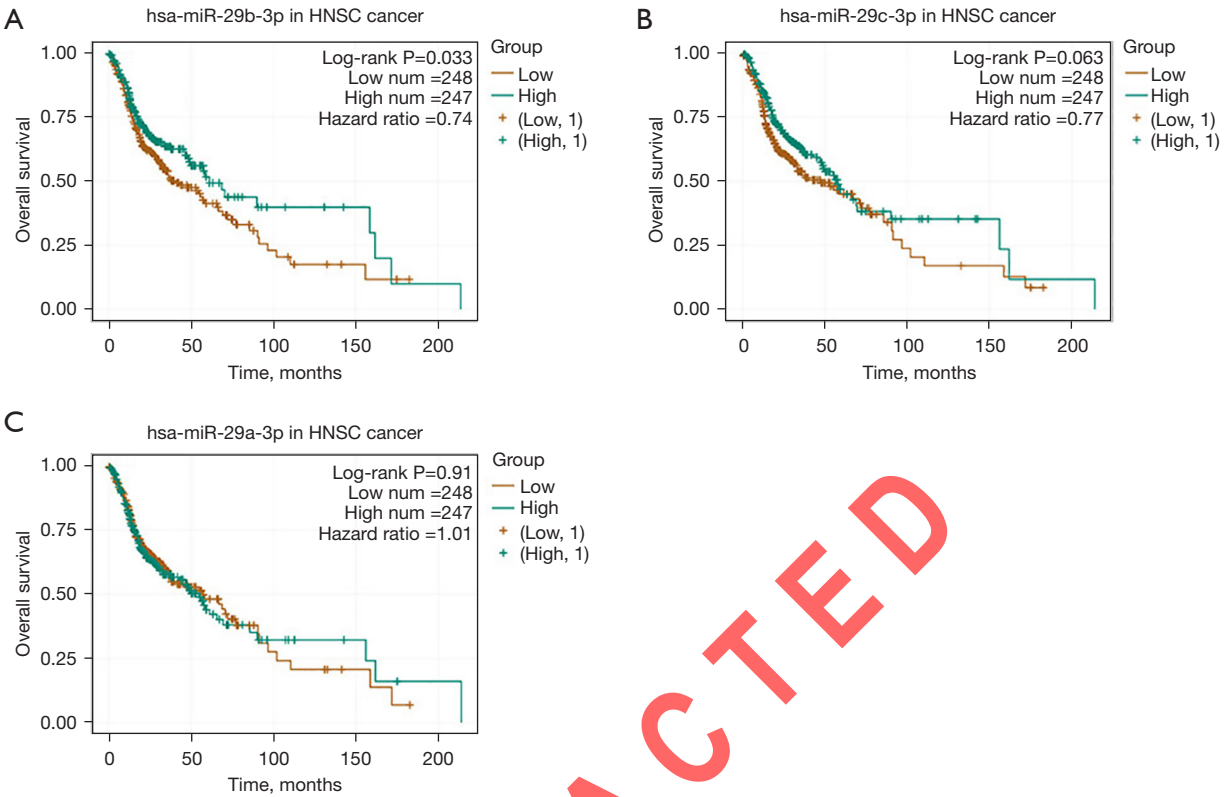

Figure 7 Correlation between the candidate miRNAs in the starBase database with the prognosis of HNSCC patients. (A) Correlation between *hsa-miR-29b-3p* and prognosis of patients with HNSCC; (B) correlation between *hsa-miR-29c-3p* and prognosis of patients with HNSCC; (C): Correlation between *hsa-miR-29a-3p* and prognosis of patients with HNSCC. P<0.05, the difference is statistically significant. HNSC, head and neck squamous cell.

in TCGA database. Our findings provide some references for subsequent cytological basic experiments.

CCM comprises the tricarboxylic acid cycle (TCA), the pentose phosphate pathway (PPP), and the Embden-Meyerhof-Parnas pathway (EMP); it is the main source of energy required by organisms and provides precursors for other metabolisms in the body. Redox reactions are major chemical reactions in the body, and oxidoreductases are the enzymes responsible for catalyzing these reactions. In metabolic pathways, including the PPP and TCA cycle, oxidoreductases act as key enzymes. Glucose is the most important energy substance, which provides the body's oxidative energy supply (main function), provides the required carbon source for the synthesis of lipids and proteins, and constitutes glycolipids and other physiological functions. Under normal circumstances, the metabolism in tumor tissue is higher than that in normal tissue, and there are many studies on CCM in tumors. For example, the activation of the nuclear factor erythroid-2 related factor 2 (NRF2) antioxidant program can lead to the imbalance of CCM in cancer (30). The PGC-1α/ERRα (PPARgamma coactivator 1alpha/estrogen-related receptor alpha) axis inhibits one-carbon metabolism in breast cancer and enhances the sensitivity to antifolate therapy (31). In this study, we found that COL3A1 was positively correlated with the carbon metabolism-related proteins, EGFR, PGAM1, HK3, and PFKP, in the tumor center.

A study has shown that HNSCC cells may exhibit marked differences in terms of energy metabolism compared with normal cells. Tumor cells absorb more sugar than normal cells. Even under oxygen-rich conditions, tumor cells still preferentially produce lactate. This phenomenon is also known as the Warburg effect or aerobic glycolysis (32). EGFR can activate CCM in tumor cells and plays a key function in aerobic glycolysis. A previous study has confirmed that EGFR can directly or indirectly regulate the functional glucose transporter 1 (GLUT1) to maintain glycolysis and the PPPs (33). EGFR regulates glycolysis by

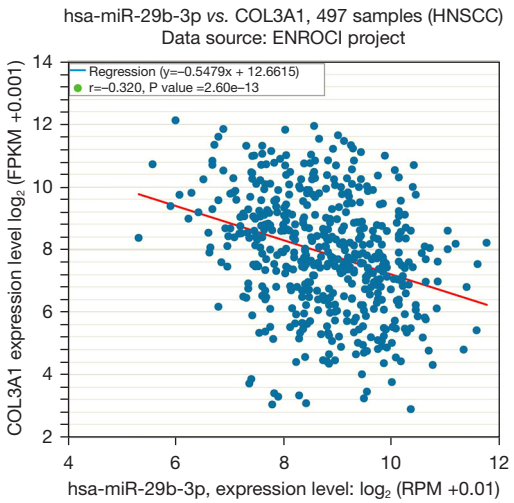

Figure 8 Correlation between *hsa-miR-29b-3p* and COL3A1 in the starBase database. P<0.05, the difference is statistically significant. HNSCC, head and neck squamous cell carcinoma; ENROCI, The Encyclopedia of RNA Interactomes; FPKM, Fragments Per Kilobase of exon model per Million mapped fragments; RPM, Reads of exon model per Million mapped reads.

regulating the downstream phosphatidylinositol 3 kinase (PI3K)/serine/threonine kinase (Akt)/mammalian target of rapamycin (mTOR) signaling mechanism and promotes the correct localization of GLUT1 to regulate tumor cells adhesion, proliferation, invasion, and migration, which are negatively correlated with prognosis (34). A study has also indicated that inhibiting the glycolytic pathway can increase the efficacy of targeting EGFR in the treatment of tumors, indicating that glycolysis may be the main oncogenic pathway of EGFR (35). PGAM1 changes 3-phosphoglycerate (3-PG) to 2-phosphoglycerate (2-PG) and is a key protein in the glycolytic pathway. The inhibition of PGMA1 results in decreased glycolytic activity, which inhibits tumor growth (36). After PGAM1 is attenuated, its substrate 3-PG is increased, which inhibits the PPP flux and nucleotide synthesis; also, the product 2-PG is decreased, inhibiting amino acid synthesis (37). Furthermore, the mTOR-PGAM1 signaling cascade also promotes the Warburg effect, and blocking PGAM1 inhibits mTOR-dependent glycolysis (38). Hexokinases (HKs) are involved in the first step of the glycolysis process, which catalyzes the phosphorylation of glucose to glucose-6-phosphate. HK3 is the first rate-limiting enzyme of glycolysis, which is overexpressed in numerous tumors and can affect the prognosis of tumor patients (39).

Table 2 Predicted upstream lncRNAs regulating *hsa-miR-29b-3p* in the TargetScan database

Gene ID	Gene name	Chromosome	clipExpNum	degraExpNum	RBP	merClass	miRseq	Align	PancancerNum
ENSG00000223734	AC098828.2	chr2	1	0	AGO1–4	7mer-m8	uuGUGACUAAAG-UUUACCACGAu	|:|| |||| :||||||	6
ENSG00000198221	AFDN-DT	chr6	2	0	AGO2	7mer-m8	uuGUGACUAAAGUU---UACCACGAu	::|||: | :|: :|||||	9
ENSG00000245532	NEAT1	chr11	10	0	AGO1–4	8mer	uugUGACUAA-AGU--UUACCACGAu	:|| ||| | | |||||||	1
ENSG00000248844	AP003555.1	chr11	1	0	AGO2	7mer-m8	uuGUGACUAAAGUUU---ACCACGAu	| :| || ||| | |||||	5
ENSG00000263126	AC040162.3	chr16	3	0	AGO1–4	7mer-m8	uuGUGACUAAAGUU-UACCACGAu	::|||| | || ||||||	1
ENSG00000225783	MIAT	chr22	1	0	AGO1–4	7mer-m8	uuGUGACUAAAGUUU--ACCACGAu	|::||| | | |: |||||	1
ENSG00000229807	XIST	chrX	8	0	AGO1, AGO2	7mer-m8	uuGUGACUAA---AGUUUACCACGAu	:::||||| ||| ||||||	2

"|" represents base pair expression representing the gene; the number of spaces in the Align column indicates the location of the mutation. LncRNAs, long non-coding RNAs; RBP, RNA binding protein; miRseq, microRNA sequencing.

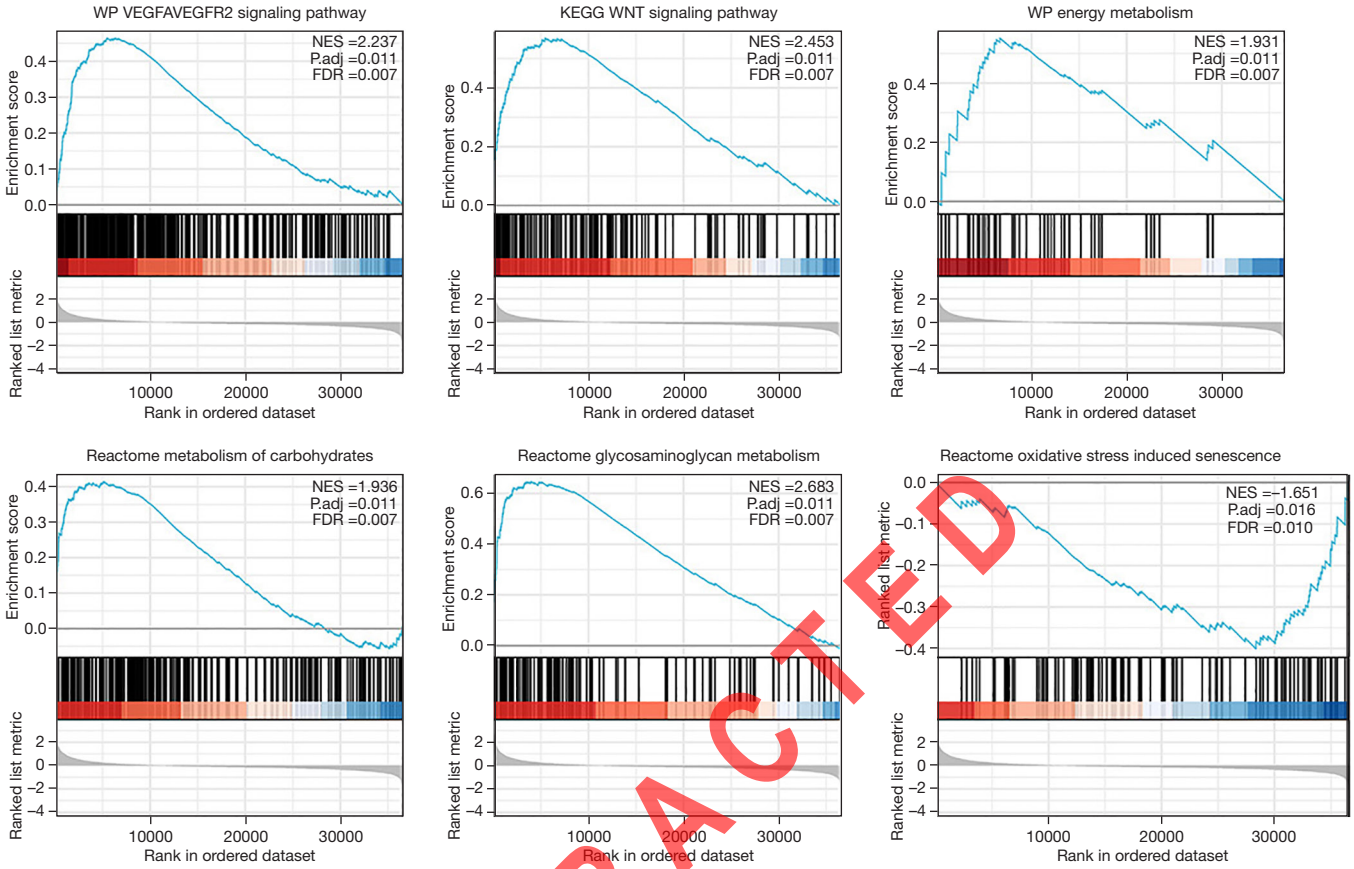

Figure 9 GSEA results. The Gene Ear Biology biological process gene set from MSigDB (Molecular Signatures Database) was used. There were 2,800 different random sample permutations. NES, normalized enrichment score; FDR, false discovery rate; WP, working path; KEGG, Kyoto Encyclopedia of Genes and Genomes; GSEA, Gene Set Enrichment Analysis.

HK3 increases cellular adenosine triphosphate (ATP) levels and reduces reactive oxygen species production. As highly glycolytic tumors are often accompanied by an immunostimulatory tumor microenvironment, HK3 may be involved in immune infiltration and might predict the immunotherapy response (40). Phosphofructokinase-1 (PFK-1) is a rate-limiting enzyme that converts fructose-6-phosphate to fructose-16, diphosphate. Among the three subtypes of PFK-1, the platelet type (PFKP) accounts for more tumors than the liver (PFKL) and muscle (PFKM) types. A study has confirmed that PFKP is overexpressed in tumors, which may indicate poor prognosis (41). When PFKP activity is enhanced, the glycolytic pathway is activated, which promotes DNA biosynthesis and damage repair, thereby stimulating tumor cell growth, migration, and invasion (42). Recent study has shown that the PFKP-Lactate dehydrogenase A (LDHA) axis mediates aerobic glycolysis by regulating lactate production in breast cancer cells (43). PFKP inhibition redirects glucose flux to the PPP, largely saving (rescuing) metabolic reprogramming and cell death. Our research molecule, COL3A1, is positively correlated with EGFR, PGAM1, HK3, and PFKP. To some extent, this research direction may also reflect an emerging field of HNSCC tumor treatment.

We also explored related miRNAs that may be bound to the 3'UTR end of COL3A1 mRNA using the TargetScan database. In the data analysis, we observed that *hsa-miR-29b-3p* was the best candidate miRNA, and *hsa-miR-29b-3p* was the potential candidate miRNA in the starBase database. *MiR-29b-3p* was negatively correlated with COL3A1. At present, there have also been some studies on the role of this miRNA in tumors, such as Linc00511, which is a ceRNA regulating VEGFA expression in pancreatic ductal adenocarcinoma by sponging *hsa-miR-29b-3p* (44). To better comprehend the signaling axis that regulates the upstream of COL3A1, we utilized the starBase database

to explore the possible binding lncRNAs of *hsa-miR-29b-3p*. Combined with the previously published findings and the binding strength between base pairs, we believe that lncRNA XIST could be the best candidate upstream non-coding RNA regulating *hsa-miR-29b-3p*. For example, lncRNA XIST induces the apoptosis of thoracic aortic aneurysm arterial smooth muscle cells via the *miR-29b-3p*/Elastin (Eln) pathway (45). LncRNA XIST promotes extracellular matrix synthesis, proliferation and migration by targeting *miR-29b-3p*/COL1A1 in human skin fibroblasts after thermal injury (46). Moreover, it also encourages osteoporosis by preventing the differentiation of bone marrow mesenchymal stem cells by sponging miR-29b-3p, which inhibits nicotinamide N-methyltransferase (47). Finally, we performed GSEA, and we found six potentially relevant pathways with statistical significance:

(I) REACTOME_METABOLISM_OF_CARBOHYDRATES;
(II) REACTOME_GLYCOSAMINOGLYCAN_METABOLISM;
(III) WP_ENERGY_METABOLISM;
(IV) REACTOME_OXIDATIVE_STRESS_INDUCED_SENESCENCE;
(V) KEGG_WNT_SIGNALING_PATHWAY;
(VI) WP_VEGFAVEGFR2_SIGNALING_PATHWAY.

Based on the above findings, we can speculate that the lncRNA XIST/miR-29b-3p/COL3A1 axis regulates the CCM of HNSCC and is associated with poor tumor prognosis.

Conclusions

In conclusion, this work provides various layers of evidence highlighting the significance of COL3A1 in the development of HNSCC and its potential as a biomarker of HNSCC disease progression. The above results point to a possible target for the development of anticancer strategies in HNSCC.

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Footnote

Reporting Checklist: The authors have completed the TRIPOD reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-23-30/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-23-30/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are 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. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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