




RESEARCH ARTICLE

ITGA5 is an independent prognostic biomarker and potential therapeutic target for laryngeal squamous cell carcinoma

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Abstract

Background: Integrin $\alpha 5$ (*ITGA5*) was involved in a variety of cancers. However, the role of *ITGA5* in laryngeal squamous cell carcinoma (LSCC) remains unknown.

Methods: The expression of *ITGA5* and the corresponding clinicopathological parameters of LSCC patients the TCGA database. Five datasets (GSE51985, GSE59102, GSE84957, GSE27020, and GSE65858) were downloaded from the GEO database as validation sets. Kaplan–Meier plotter, Cox regression analysis, and nomogram were performed to determine the prognostic value of *ITGA5* in LSCC. GO, KEGG, and GSEA were used to explore the underlying biological functions of *ITGA5* in LSCC. The algorithms ESTIMATE and CIBERSORT were adopted to evaluate the association between *ITGA5* and the infiltration of the immune cells. The algorithm pRRophetic was used to estimate the response to chemotherapeutic drugs.

Results: The expression of *ITGA5* was higher in the LSCC samples and linked to poor overall survival and recurrence-free survival. Further, the Cox regression analysis confirmed that high expression of *ITGA5* was an independent unfavorable prognostic factor. The predictive performance of nomogram based on the expression of *ITGA5* was accurate and practical. The functional enrichment analysis confirmed that *ITGA5* was related to the construction of the components and structures of the extracellular matrix. Finally, patients with high *ITGA5* expression were more likely to benefit from docetaxel and gemcitabine.

Conclusion: The expression of *ITGA5* was elevated in the LSCC and was a predictor for prognosis and chemotherapeutic response in LSCC patients.

KEYWORDS

chemotherapy, *ITGA5*, laryngeal squamous cell carcinoma, nomogram, prognosis

1 | INTRODUCTION

Laryngeal cancer is one of the most common cancers in the respiratory system. Recent data reported that, in 2021, an estimated

12,620 new cases were diagnosed with about 3770 patients dying from laryngeal cancer in the United States.¹ Based on the pathological cases, approximately 98% of laryngeal cancer cases are laryngeal squamous cell carcinoma (LSCC). Although progress has been

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made in the comprehensive management of patients with LSCC, the 5-year overall survival (OS) rate had reduced over the past 50 years, owing to the insidious symptoms and lack of effective diagnostic methods for early stages.² Approximately 60% of the patients with LSCC are diagnosed at an advanced stage or even with metastasis of the lymph node, and have had a poor prognosis.³ Recently, more and more evidence has demonstrated that the prognosis and chemotherapeutic efficacy of patients with tumor was largely affected by the infiltration of the immune cells.^{4–8} Therefore, the identification of novel potential biomarkers and their correlation with immune infiltration will potentially improve outcomes and direct individualized treatment for patients with LSCC.

Integrins, consisting of α and β subunits, are heterodimeric transmembrane proteins that function as surface adhesion receptors in different cells or cells and extracellular matrix communication.⁹ Integrin $\alpha 5$ (*ITGA5*) is a member of the integrin alpha chain family and is located on chromosome 12q13.13 in *Homo sapiens*.¹⁰ *ITGA5* mainly recognizes its specific ligand through the formation of an $\alpha 5\beta 1$ heterodimer and combines with integrin $\beta 1$ (TGF $\beta 1$).¹¹ Increasing evidence has proved that *ITGA5* is highly expressed in multiple cancers and is related to the progressions of tumor, such as cell proliferation, migration, and invasion. For instance, *ITGA5* was upregulated in pancreatic ductal adenocarcinoma. Silencing *ITGA5* could suppress the differentiation of human pancreatic stellate cells and reduce desmoplasia.⁹ In oral cancer, *ITGA5* facilitates cell growth, migration, and invasion by epithelial-mesenchymal transition.¹² According to Yu et al.,¹¹ *ITGA5* was overexpressed in colorectal cancer and promoted cell proliferation and tumorigenesis and reduced cell apoptosis. Additionally, *ITGA5* was shown to maintain chemotherapeutic resistance^{9,13} and cancer cell stemness.¹⁴ Russano et al.¹⁵ reported that *ITGA5* acts as a mediator of breast-to-bone metastasis and might improve the efficacy of volociximab/M200 in patients with breast cancer along with bone metastasis. These findings implied that *ITGA5* may be a potential therapeutic target for tumor angiogenesis, lymphangiogenesis, and metastasis. However, the expression and potential functions of *ITGA5* in the progression of LSCC remain largely unknown.

In this study, we explored the expression and prognostic value of *ITGA5* in patients with LSCC, and then performed functional enrichment analysis. More importantly, we revealed the relationship between the expression of *ITGA5* and tumor immune microenvironment (TME) as well as the infiltration of the immune cells in LSCC. Our results revealed that *ITGA5* was highly expressed in LSCC and might appear as an independent prognostic factor and therapeutic target for LSCC.

2 | MATERIALS AND METHODS

2.1 | Data preparation

The Cancer Genome Atlas (TCGA) database (<https://portal.gdc.cancer.gov/>) was used to download the *ITGA5* mRNA expression (FPKM form) and the corresponding clinicopathological parameters of 111 LSCC tissues and 12 normal tissues. GSE51985 (including 10

LSCC tissues and 10 adjacent normal tissues), GSE59102 (including 29 LSCC tissues and 13 normal tissues), and GSE84957 (including 9 LSCC tissues and 9 adjacent normal tissues), and the corresponding platform annotation files were downloaded from the Gene Expression Omnibus (GEO) database to validate the expression levels of *ITGA5* in LSCC. Additionally, although there were no normal tissues in the GSE65858 (including 48 LSCC samples with OS and recurrence-free survival [RFS] information) and GSE27020 (including 109 LSCC samples with RFS information), these two datasets with survival data were also downloaded to validate the prognostic value of *ITGA5* in LSCC. The clinicopathological features of the patients in this study are listed in Table 1.

2.2 | The expression of *ITGA5* and its association with the clinical characteristics of patients with LSCC

“Limma” R package was applied to estimate the expression of *ITGA5* between LSCC and non-tumor samples in the TCGA cohort. GSE51985, GSE59102, and GSE84957 were the validation cohorts. Further, the association between the expression of *ITGA5* and the clinical characteristics was also explored in patients with LSCC.

2.3 | Prognostic analysis of *ITGA5* in patients with LSCC

According to the optimal cut-off point by routing algorithms, the OS and the RFS curves were plotted by the Kaplan–Meier (K-M) method, and the difference in the survival rates between high-*ITGA5* and low-*ITGA5* groups was assessed using the log-rank test. Univariate and multivariate Cox regression analyses were used to evaluate the prognostic value of *ITGA5* in LSCC. Further, a nomogram was constructed based on the expression of *ITGA5* and the clinical features to predict the survival probability. The 1-, 3-, and 5-years calibration curves and receiver operating characteristic (ROC) curves were used to assess the prediction performance of the nomogram.

2.4 | Functional enrichment analysis

Patients with LSCC from the TCGA were separated into the high-*ITGA5* and the low-*ITGA5* groups according to their median value. The Gene Set Enrichment Analysis (GSEA, version 4.1.0) was performed in our previous study.¹⁶ The *c2.cp.kegg.v7.2.symbols.gmt* was utilized as reference gene sets. Pathways with false discovery rate (FDR) $p < 0.05$ were considered statistically enriched. Differentially expressed genes (DEGs) between the high-*ITGA5* and the low-*ITGA5* groups were identified with FDR $p < 0.05$ and $|\text{Log2FC}| > 1$. The Gene ontology (GO) analysis and the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were performed based on the DEGs to explore the *ITGA5*-related functional pathways in LSCC.

TABLE 1 Table listing the clinical characteristics of patients with LSCC in this study

Characteristics	TCGA	GSE65858	GSE27020	GSE51985	GSE84957	GSE59102
Gender						
Female	20	5	—	0	0	1
Male	91	43	—	10	9	28
Age						
≤60	47	18	43	6	—	—
>60	64	30	66	4	—	—
Smoking history						
Yes	102	41	—	—	—	—
No	6	7	—	—	—	—
Unknown	3					
Alcohol history						
Yes	70	43	—	—	—	—
No	39	5	—	—	—	—
Unknown	2					
Histologic grade						
G1	8	—	42	—	—	—
G2-3	99	—	65	—	—	—
Unknown	4		2			
Pathologic T						
T1-2	19	20	—	3	—	—
T3-4	79	28	—	7	—	—
Unknown	13					
Pathologic N						
N0	39	29	—	5	—	—
N1-3	53	19	—	5	—	—
Unknown	19					
Pathologic M						
M0	40	46	—	10	—	—
M1	9	2	—	0	—	—
Unknown	62					
Clinical stage						
Stage I-II	13	17	—	3	0	14
Stage III-IV	85	31	—	7	9	15
Unknown	13					
Radiation therapy						
Yes	65	—	—	—	—	—
No	23	—	—	—	—	—
Unknown	23					
Targeted therapy						
Yes	31	—	—	—	—	—
No	51	—	—	—	—	—
Unknown	29					
Survival status						
Dead	50	13	—	—	—	—
Alive	61	35	—	—	—	—
Recurrence						
Yes	42	19	34	—	—	—
No	69	29	75	—	—	—

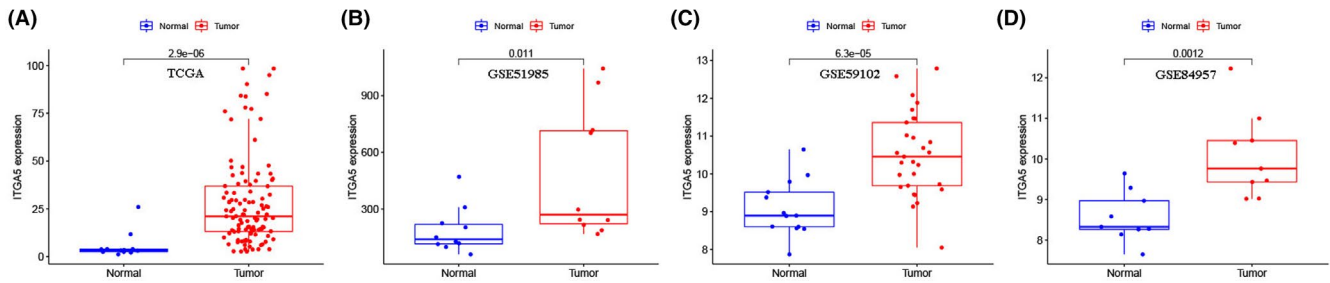


FIGURE 1 Expression of *ITGA5* in laryngeal squamous cell carcinoma (LSCC) and normal tissues from the Cancer Genome Atlas (TCGA) and the Gene Expression Omnibus (GEO) databases. (A) Expression of *ITGA5* in 111 LSCC and 12 normal tissues from TCGA cohort; (B) Expression of *ITGA5* in 10 LSCC and 10 normal tissues from GSE51985 cohort; (C) Expression of *ITGA5* in 29 LSCC and 13 normal tissues from GSE59102 cohort; (D) Expression of *ITGA5* in 9 LSCC and 9 normal tissues from GSE84957 cohort

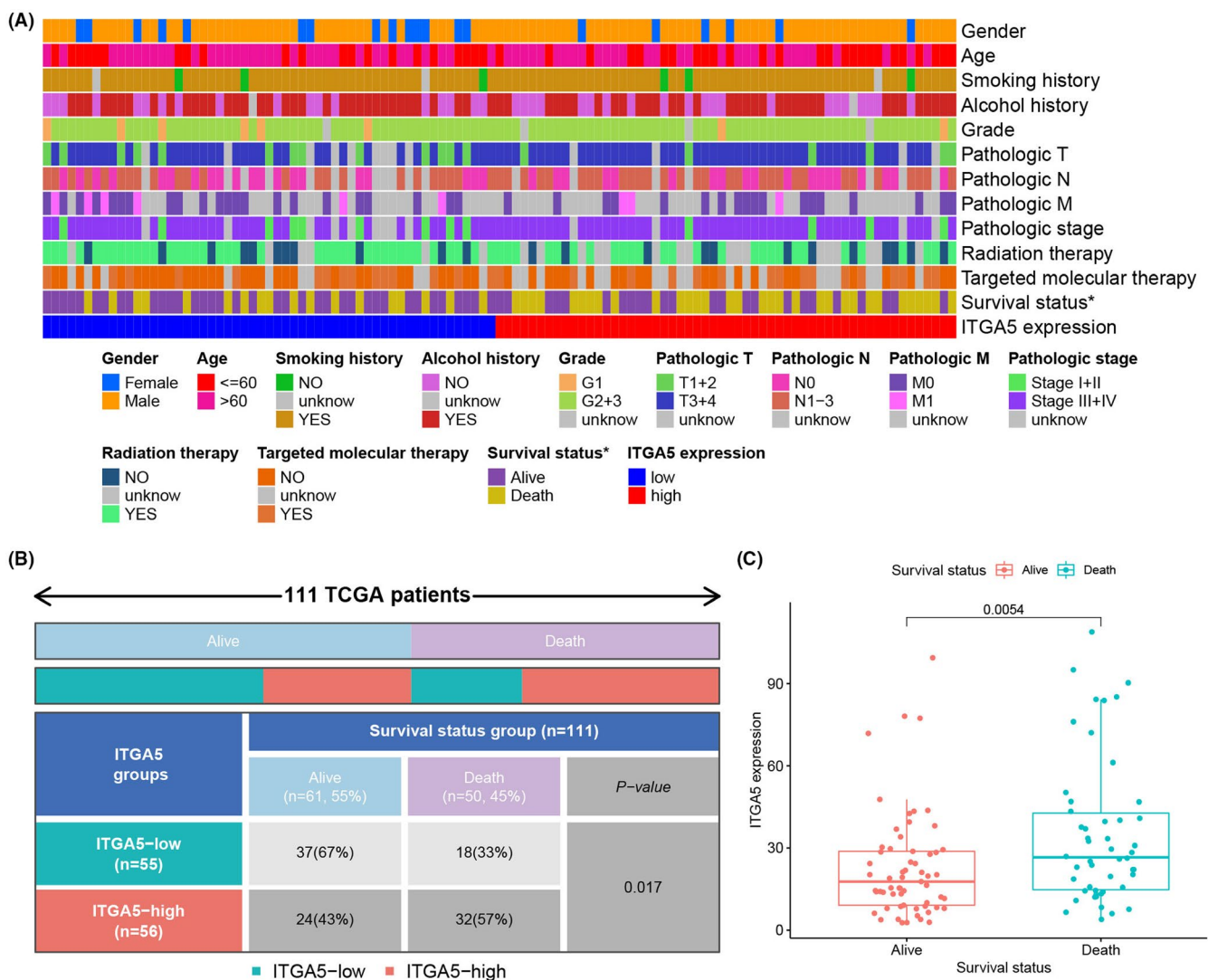


FIGURE 2 The association between the expression of *ITGA5* and the clinical characteristics in patients with laryngeal squamous cell carcinoma (LSCC). (A) Heatmap showing the distribution of the clinical characteristics of patients with LSCC between the high-*ITGA5* and the low-*ITGA5* expression groups; (B) The survival status in the high-*ITGA5* and the low-*ITGA5* expression groups were compared using Chi square test; (C) Significant statistical differences between the high-*ITGA5* and the low-*ITGA5* expression groups were assessed using Wilcoxon signed-rank test

2.5 | TME and the infiltration of the immune cells

The ESTIMATE algorithm was used to calculate the immuneScore, stromalScore, and estimate score of the LSCC samples.¹⁷ The CIBERSORT algorithm was adopted to quantify the absolute abundance of 22 tumor-infiltrating immune cell types in the LSCC samples.¹⁸ Then, the difference in the TME score and the infiltration of the immune cells between the high-*ITGA5* and the low-*ITGA5* groups were analyzed.

2.6 | Chemotherapeutic drug sensitivity analysis

In this study, we demonstrated whether *ITGA5* could influence the clinical response to treatment and could be considered as a potential biomarker for drug screening. The half-maximum inhibitory concentration (IC50) was estimated using the “pRRophetic” package¹⁹ to predict the chemotherapeutic response of four commonly used chemotherapeutic drugs (paclitaxel, gemcitabine, docetaxel, and cisplatin) in the high-*ITGA5* and the low-*ITGA5* groups.

2.7 | Statistical analysis

The R statistical language (version 4.1.0) was used for all statistical analysis and visualizations. To compare the expression of *ITGA5* among different groups, we performed Wilcoxon signed rank test or Chi square test where required. Spearman correlation analysis was used to examine the correlation analysis. Unless specified otherwise, $p < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | Expression of *ITGA5* in LSCC

By data mining using the TCGA database, *ITGA5* was determined to be elevated in LSCC compared with the normal tissues (Figure 1A, $p < 0.001$). Further, the differential expression of *ITGA5* in LSCC was verified based on three independent datasets from the GEO database, which revealed that the expression of *ITGA5* in LSCC was

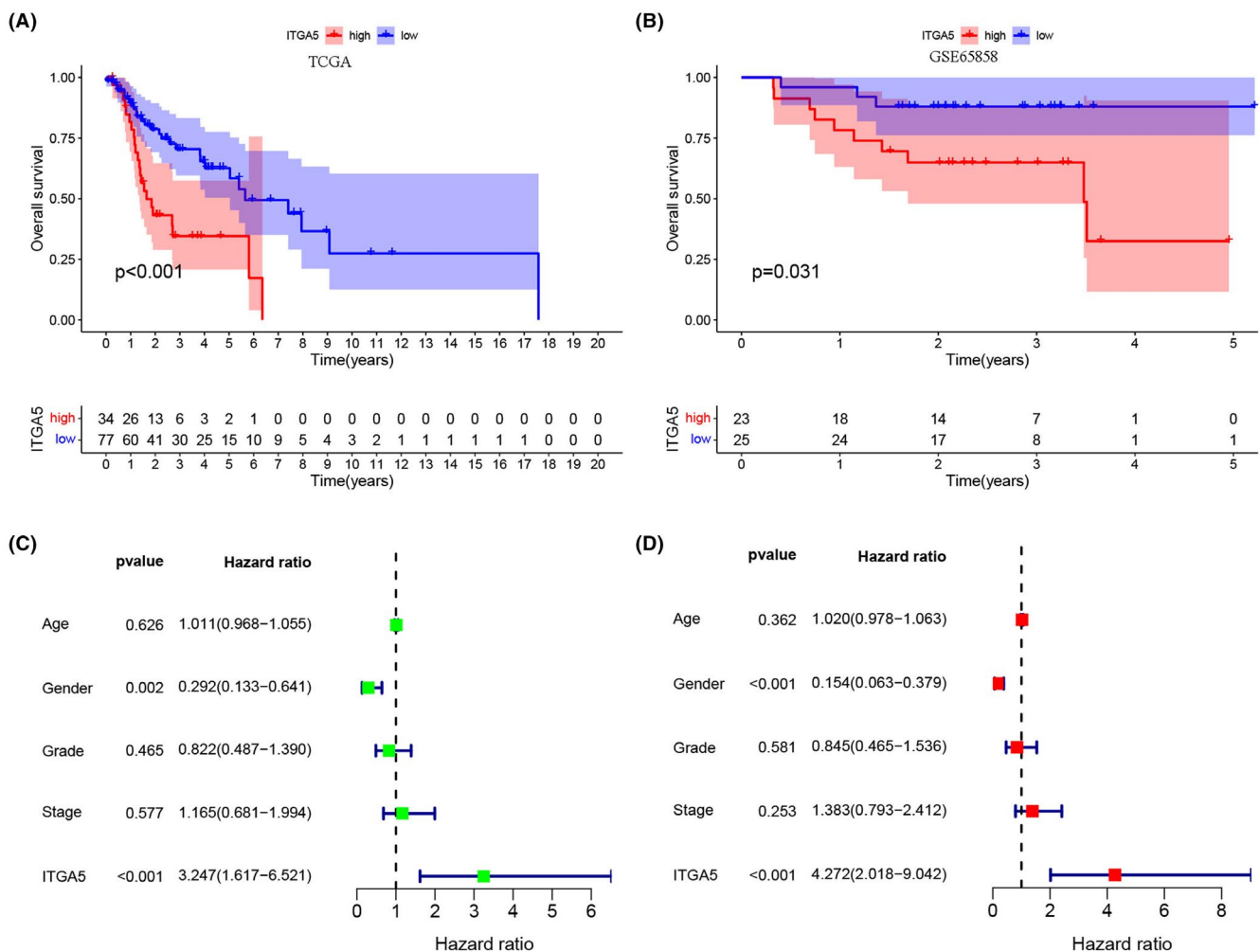


FIGURE 3 The expression of *ITGA5* for prediction of overall survival (OS) in patients with laryngeal squamous cell carcinoma (LSCC). (A) Survival curves of OS from the Cancer Genome Atlas (TCGA) cohort (n = 111); (B) Survival curves of OS from the GSE65858 cohort (n = 48); The univariate (C) and multivariate Cox regression analyses (D) of OS according to the expression of *ITGA5* after adjusting the clinical characteristics in the TCGA cohort

significantly higher than in the normal tissues (GSE51985 [Figure 1B, $p = 0.011$], GSE59102 [Figure 1C, $p < 0.001$], and GSE84957 [Figure 1D, $p = 0.001$]). Subsequently, we analyzed the correlations between the expression of *ITGA5* and the pathological parameters in LSCC (Figure 2A). Both Chi square test (Figure 2B, $p = 0.017$) and Wilcoxon signed rank test (Figure 2C, $p = 0.005$) showed that the dead patients had an elevated expression of *ITGA5* than the alive patients. However, no correlation between the expression of *ITGA5* and other clinical characteristics was found.

3.2 | *ITGA5* is associated with poor prognosis of patients with LSCC

The K-M curves and the log-rank test showed that patients in the high-*ITGA5* group had worse OS than the patients in the low-*ITGA5* group in the TCGA cohort (Figure 3A, $p < 0.001$). The patients with LSCC in the GSE65858 dataset were used as validation cohort and confirmed that the high-*ITGA5* group exhibited shorter OS than the low-*ITGA5* group (Figure 3B, $p = 0.031$). The univariate (Figure 3C, HR = 3.247, $p < 0.001$) and multivariate Cox regression analyses (Figure 3D, HR = 4.272, $p < 0.001$) demonstrated that *ITGA5* was an independent prognostic factor for OS. We also assessed the ability of *ITGA5* to predict the relapse in patients with LSCC. In the TCGA cohort (Figure 4A, $p < 0.001$), the K-M survival

curves indicated that patients with LSCC and high expression of *ITGA5* had significantly worse RFS compared with patients with low expression of *ITGA5*. Additionally, GSE27020 (Figure 4B, $p < 0.001$) and GSE65858 (Figure 4C, $p = 0.009$) datasets as validation cohorts confirmed this result. Further, the univariate (Figure 4D, HR = 4.442, $p < 0.001$) and multivariate Cox regression analyses (Figure 4E, HR = 4.741, $p < 0.001$) demonstrated that *ITGA5* was an independent predictive factor for recurrence in patients with LSCC.

3.3 | Establishment and validation of a prognostic nomogram

A prognostic nomogram was constructed based on age, gender, grade, stage, and expression of *ITGA5* for predicting 1-, 3-, and 5-years survival rates of patients with LSCC (Figure 5A). The calibration curves of 1-, 3-, and 5-years (Figure 5B) were all close to the reference line (at 45°), which represents the perfect prediction performance of the nomogram. The time-dependent ROC curve analysis evaluated the accuracy of the nomogram and the area under the curve at 1-, 3-, and 5-years which were 0.715, 0.726, and 0.649, respectively (Figure 5C), confirming that the predictive value of the nomogram was superior to any of the clinical factors (Figure 5D, age [0.425], gender [0.370], grade [0.475], and stage [0.502]).

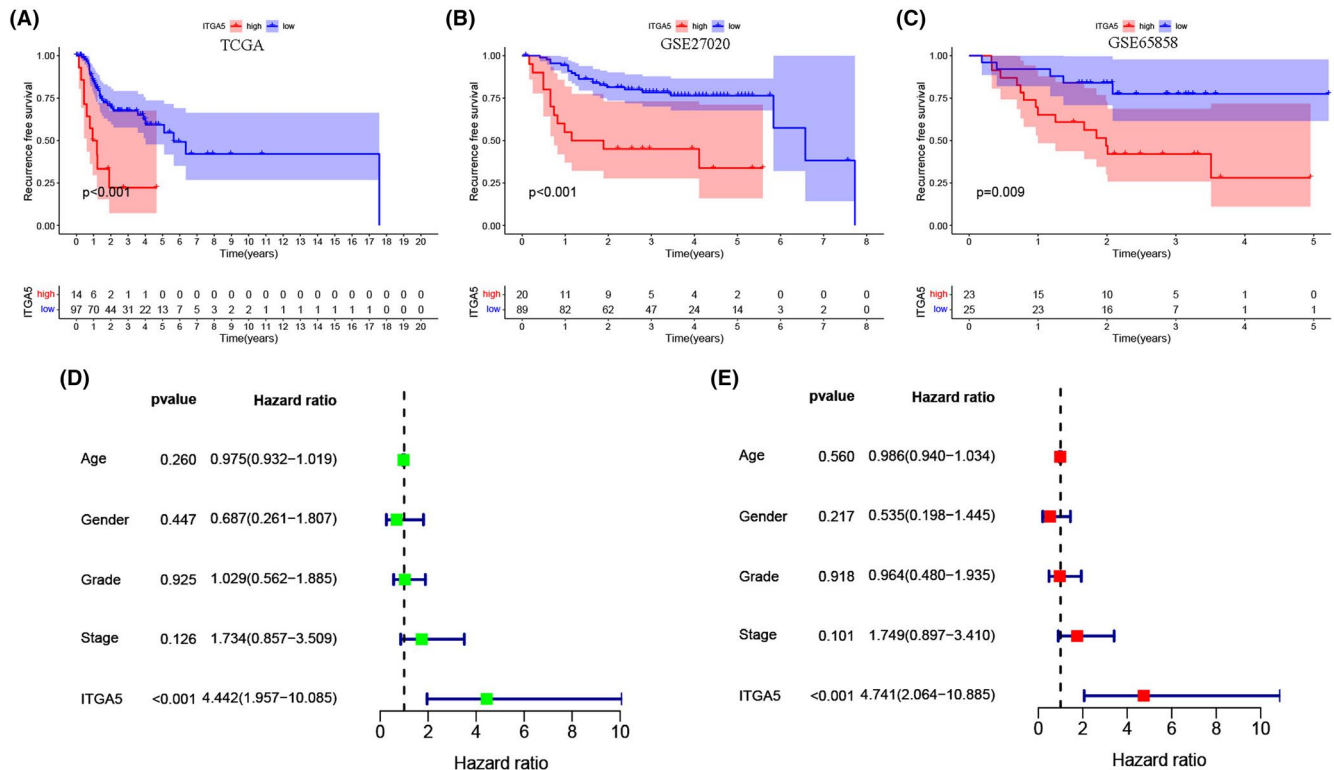


FIGURE 4 The expression of *ITGA5* for the prediction of recurrence-free survival (RFS) in patients with laryngeal squamous cell carcinoma (LSCC). (A) Survival curves of RFS from the Cancer Genome Atlas (TCGA) cohort ($n = 111$); (B) Survival curves of RFS from the GSE27020 cohort ($n = 109$); (C) Survival curves of RFS from the GSE65858 cohort ($n = 48$); The univariate (D) and multivariate Cox regression analyses (E) of RFS according to the expression of *ITGA5* after adjusting the clinical characteristics in the TCGA cohort

3.4 | Functional enrichment analysis of *ITGA5* in LSCC

To clarify the molecular mechanism of *ITGA5* in LSCC, we performed the GSEA analysis based on the high-*ITGA5* and the low-*ITGA5* expressions. There were six KEGG pathways significantly enriched in the high-*ITGA5* group (Figure 6A), including extracellular matrix (ECM)-receptor interaction, focal adhesion, glycosaminoglycan biosynthesis-chondroitin sulfate, glycosaminoglycan biosynthesis-keratan sulfate, leukocyte transendothelial migration, and regulation of the actin cytoskeleton. These significantly enriched the KEGG pathways that participated in a variety of cellular activities by regulating the construction of the ECM, such as adhesion, migration, differentiation, proliferation, and apoptosis. The GO and the KEGG

enrichment analysis were performed using 415 DEGs (Figure 6B, Table S1) between the high-*ITGA5* and the low-*ITGA5* groups. The results of the GO analysis (Figure 6C, D) and the KEGG analysis (Figure 6E, F) confirmed that the DEGs were related to the construction of the ECM components and structures.

3.5 | Association between *ITGA5* and TME as well as the infiltration of the immune cells in LSCC

According to the TME analysis, using the ESTIMATE algorithm, the results showed that patients with high-*ITGA5* had elevated stromal score than patients with low-*ITGA5* (Figure 7, $p < 0.01$), indicating that *ITGA5* might play important roles in the components of the ECM.

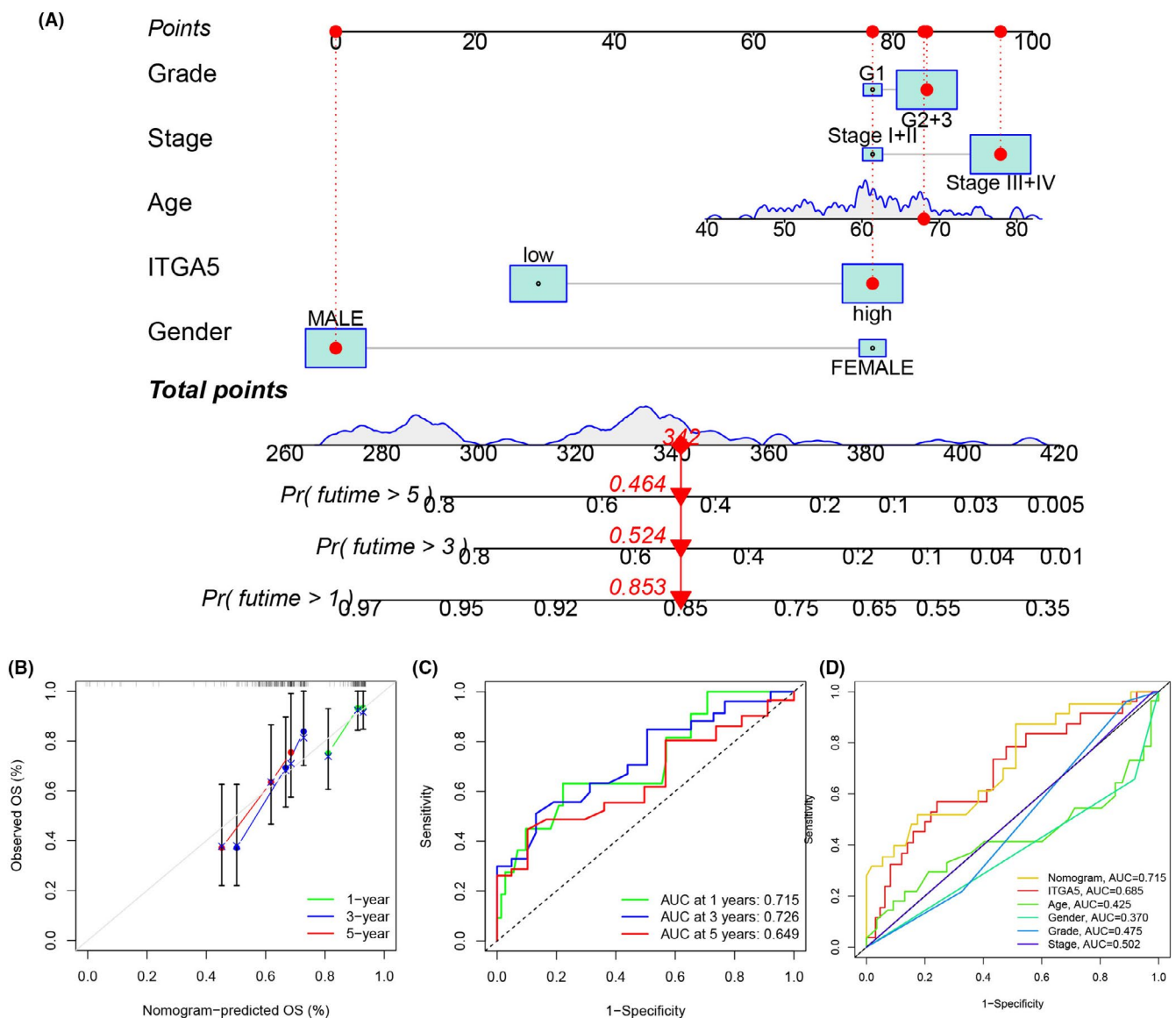


FIGURE 5 Construction and validation of a nomogram for patients with laryngeal squamous cell carcinoma (LSCC). (A) A nomogram was constructed to predict the 1-, 3-, and 5-years of OS for patients with LSCC; (B) Calibration curves of 1-, 3-, and 5-years of the nomogram; (C) Receiver operating characteristic (ROC) curves of 1-, 3-, and 5-years of the nomogram; (D) ROC curves of the nomogram and other clinical characteristics

Further, we evaluated the correlation between the expression of *ITGA5* and 22 immune cells. As shown in the violin plot (Figure 8A), patients with high-*ITGA5* had a higher abundance of M0 macrophages

and resting CD4 memory T cells and a lower abundance of CD8 T cells, naive B cells, plasma cells, and follicular helper T cells. Pearson correlation analysis revealed that high expression levels of *ITGA5* were directly

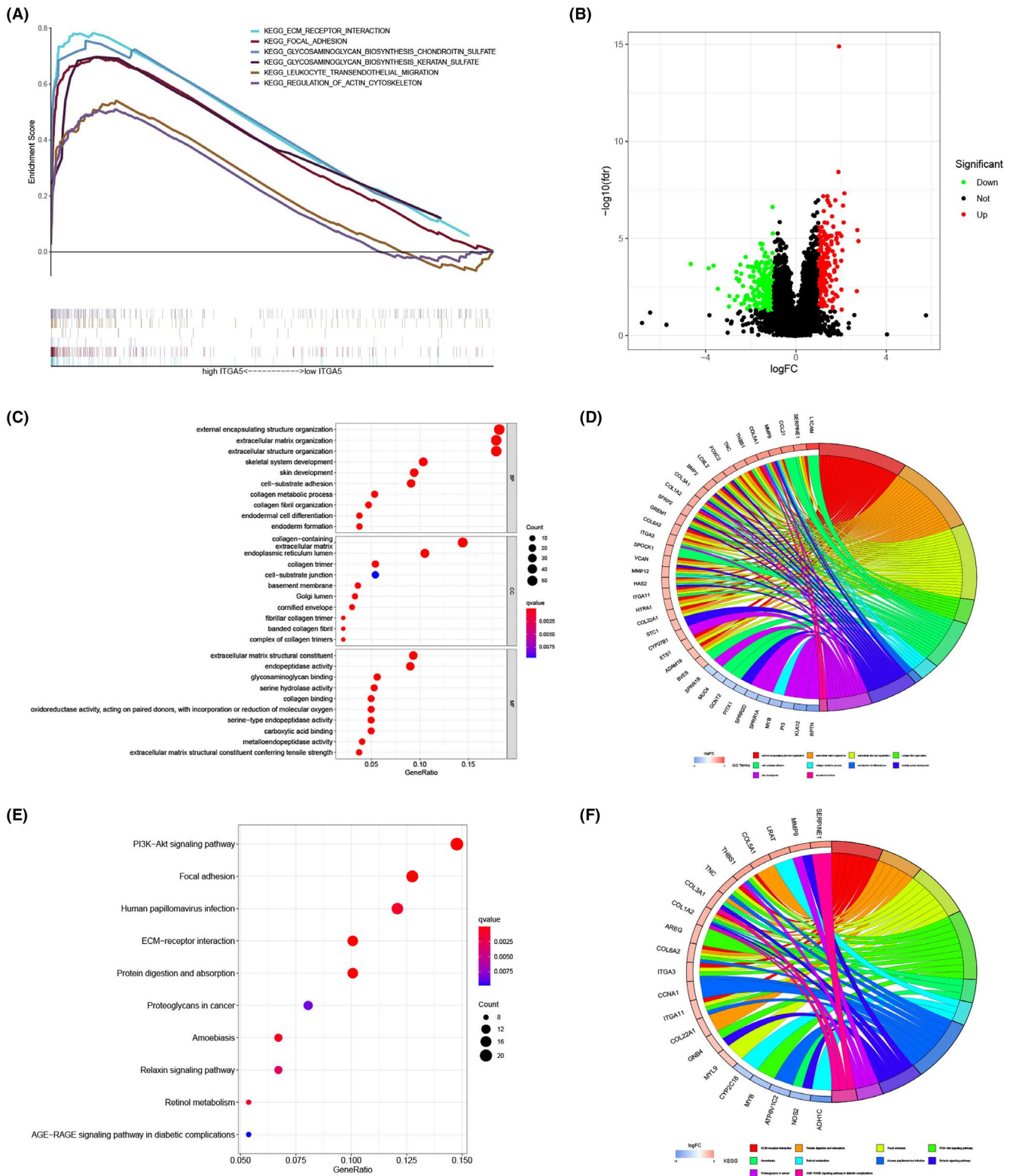


FIGURE 6 Functional enrichment analysis of *ITGA5* in laryngeal squamous cell carcinoma (LSCC). (A) Gene Set Enrichment Analysis (GSEA) between the high-*ITGA5* and the low-*ITGA5* expression groups; (B) Volcano plot of differential expression genes between the high-*ITGA5* and the low-*ITGA5* expression groups; (C) Bubble plot of the GO enrichment analysis; (D) Circos plot of the gene ontology (GO) enrichment analysis; (E) bubble plot of the KEGG enrichment analysis; (F) Circos plot of the KEGG enrichment analysis

proportional to M0 macrophages (Figure 8B) and resting CD4 memory T cells (Figure 8C) as well as resting natural killer (NK) cells (Figure 8D), and inversely proportional to CD8 T cells (Figure 8E), follicular helper T cells (Figure 8F) as well as plasma cells (Figure 8G).

3.6 | Chemosensitivity analysis

To explore the potential role of *ITGA5* in the clinical treatment strategy, we estimated the IC50 of four common chemotherapeutic drugs recommended for laryngeal cancer therapy. Patients with low expression of *ITGA5* had higher IC50 of docetaxel (Figure 9A) and gemcitabine (Figure 9B), indicating that patients with high expression of *ITGA5* were more likely to respond to these two chemotherapeutic drugs. However, there was no difference in IC50 between the high-*ITGA5* and the low-*ITGA5* groups for cisplatin (Figure 9C) and paclitaxel (Figure 9D) therapies.

4 | DISCUSSION

Laryngeal squamous cell carcinoma is the second most common malignant tumor of the upper respiratory system as well as the head and neck.²⁰ The current treatment for LSCC is a comprehensive protocol that is based on surgical operation combined with chemotherapy, radiotherapy, targeted therapy, and immunotherapy.²¹ However, LSCC is a heterogeneous disease, resulting in the treatment outcome to be significantly different. Therefore, more biomarkers for prediction and risk stratification are needed urgently for patients with LSCC. *ITGA5* is an important member of the integrin family, which acts as an adhesive receptor for the ECM proteins. Recent research indicates that *ITGA5* is overexpressed in various types of tumors and promotes proliferation, migration, invasion, and metastasis of cancer cells.^{14,22–24} However, the role of *ITGA5* in LSCC remains unclear. Hence, we investigated the expression profile and clinical significance of *ITGA5* in LSCC by analyzing the TCGA and the GEO databases.

In this study, we combined results from the TCGA database with three independent datasets from the GEO database as validation cohorts, and confirmed that the expression levels of *ITGA5* were upregulated in LSCC, which was consistent with the findings of previous studies in other cancers.^{12,24} After the analysis of the clinicopathological data, it was found that the expression level of *ITGA5* was correlated with the survival status of patients with LSCC. Breuksch et al. also indicated *ITGA5* as a potent biomarker to discriminate the prognosis of patients with renal cell carcinoma.²⁵ Further, we evaluated the prognostic value of *ITGA5* in LSCC. Both TCGA cohort and GSE65858 dataset showed that high expression of *ITGA5* was strongly associated with worse OS in patients with LSCC. Regional recurrence was an important factor associated with the prognosis of patients with LSCC.²⁶ Interestingly, we found the expression level of *ITGA5* was significantly associated with poor RFS in the TCGA cohort, which was validated by GSE27020 and GSE65858 datasets. Moreover, the univariate and multivariate Cox regression analyses demonstrated that *ITGA5* was an independent predictive factor for death and recurrence in patients with LSCC. Collectively, all the above results strongly supported that the expression of *ITGA5* is a perfect prognostic biomarker for LSCC. Therefore, we developed a nomogram based on the expression of *ITGA5*, which can be used to precisely predict the survival rate of patients with LSCC. Calibration curves and ROC curves confirmed the accuracy of the nomogram prediction, which was superior to any single factor. However, in the near future, further studies will be needed to validate the nomogram using a larger sample size.

To further explore the potential mechanisms that might be mediated by *ITGA5* in LSCC, we performed the GSEA, GO, and KEGG enrichment analysis. The functional enrichment analysis showed that *ITGA5* was involved in pathways related to the construction of the components of the ECM and intercellular signaling within the matrix. Additionally, ESTIMATE analysis also confirmed that stromalScore increased significantly in the high-*ITGA5* expression group than in the low-*ITGA5* expression group. The components of the ECM have been gaining attention due to their extremely important functions in carcinogenesis and tumor progression.²⁷ Increasing

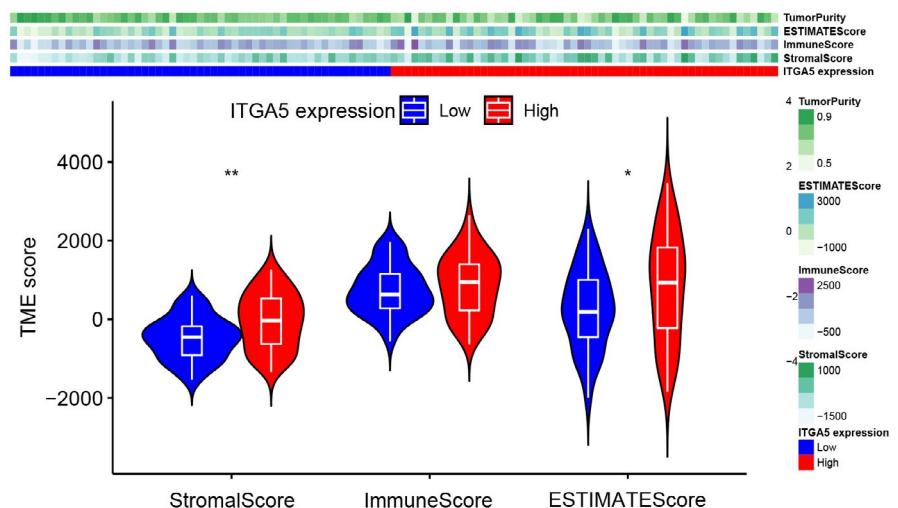


FIGURE 7 The stromal, immune, and ESTIMATE scores between the high *ITGA5* and the low *ITGA5* expression groups using the ESTIMATE algorithm

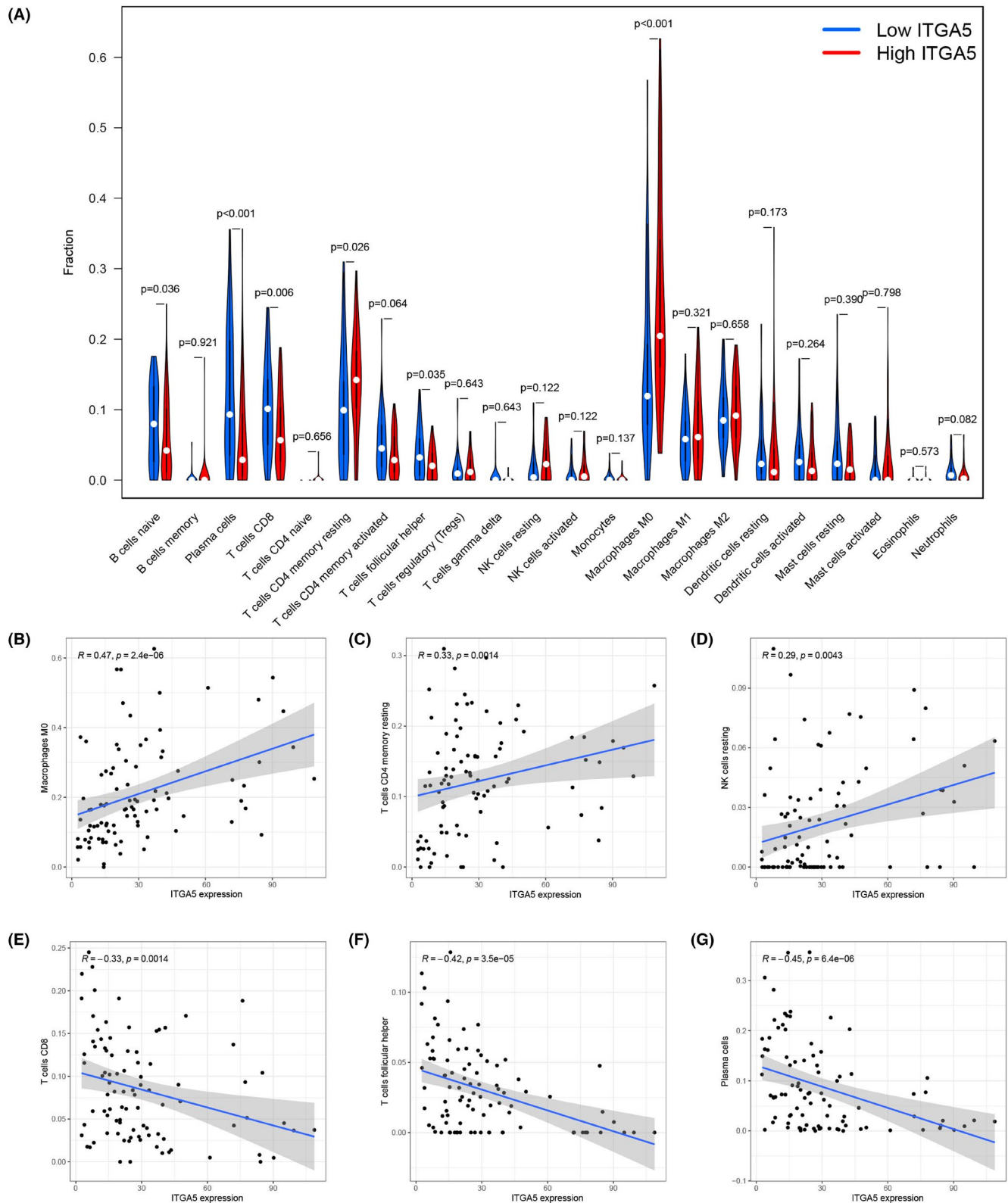


FIGURE 8 The correlation between the expression of *ITGA5* and tumor immune cell infiltrating using the CIBERSORT algorithm. (A) the fraction of immune cells between the high-*ITGA5* and the low-*ITGA5* expression groups are presented in the violin plot; A significant positive correlation was observed between M0 macrophages (B), resting CD4 memory T cells (C), and resting natural killer (NK) cells (D) with *ITGA5* expression; A significant negative correlation was observed between CD8 T cells (E), follicular helper T cells (F), and plasma cells (G) with *ITGA5* expression

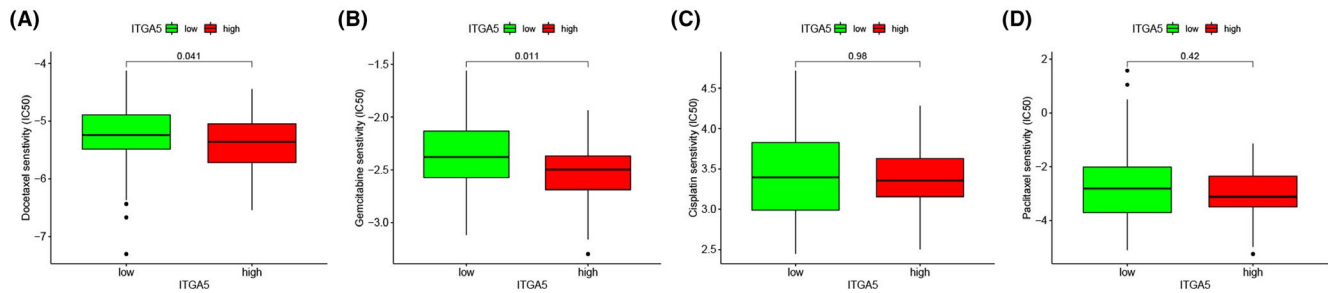


FIGURE 9 The correlation between the expression of *ITGA5* and chemosensitivity in laryngeal squamous cell carcinoma (LSCC). The IC50 values of docetaxel (A) and gemcitabine (B) decreased significantly in the high-*ITGA5* expression group; The IC50 values of cisplatin (C) and paclitaxel (D) were not significantly different between the high-*ITGA5* and the low-*ITGA5* groups

evidence has confirmed the crucial role of ECM in tumor metastasis.²⁸ Furthermore, *ITGA5* is an important member of the integrin family, which can provide a bridge for the mechanical adhesion of cells and increase the ability of tumor cells to metastasize and invade.²⁹ Therefore, we have a reason to believe that *ITGA5* might play a pivotal role in tumorigenesis, progression, and metastasis of LSCC by supporting the formation of the ECM and facilitating the tumor microenvironment.

The CIBERSORT algorithm was used to examine the relative proportions of infiltrating immune cells in each LSCC sample. Our results revealed that patients in the high-*ITGA5* group had higher proportions of immunosuppressive cells such as M0 macrophages and resting CD4 memory T cells than the low-*ITGA5* group, whereas the fraction of immune active cells such as CD8 T cells and follicular helper T cells were more abundant in the low-*ITGA5* group than the high-*ITGA5* group. Tian et al. showed that the high infiltration of M0 macrophages and resting CD4 memory T cells are associated with an unfavorable prognosis in glioma.³⁰ Conversely, previous studies have suggested that the infiltration of the CD8 T cells was positively correlated to improve prognosis and survival in several cancers, including melanoma,³¹ lung adenocarcinoma,³² and cutaneous angiosarcoma.³³ In line with these results, this might partly explain that the high-*ITGA5* group had a poorer prognosis than that of the low-*ITGA5* group in patients with LSCC.

Chemotherapy is one of the important treatments for patients with advanced LSCC, whereas the development of chemoresistance is rising and the efficacy of chemotherapy varies from person to person.^{34,35} Hou et al. reported that *ITGA5* was associated with chemoresistance in esophageal squamous cell carcinoma.³⁶ To evaluate the predictive value of *ITGA5* in clinical treatment for LSCC, we calculated the sensitivity of four common chemotherapeutic drugs based on the pRRophetic algorithm. Interestingly, our results indicated that LSCC patients with high expression levels of *ITGA5* tended to be more sensitive to docetaxel and gemcitabine, which supported more clues for the reasonable choice of a chemotherapeutic drug for patients with LSCC in the clinic.

However, there are some limitations in our study, and additional research is required to be carried out in the future. First, our findings

are based entirely on the public databases using bioinformatics analysis. Although we also used the GEO database to verify the reliability of our results, further studies with a large sample size across different populations are needed to validate the current results. Second, we used the GO, KEGG, and GSEA analyses to detect the biological functions of *ITGA5* in LSCC. Functional experiments are needed to verify these results. Finally, the association between *ITGA5* and chemotherapy still needs to be validated in clinical trials.

In conclusion, our research was the first to identify *ITGA5* as an independent potential of OS and an RFS predictor for patients with LSCC. We also revealed the potential biological functions and pathways of *ITGA5* in LSCC. Additionally, LSCC patients with high expression levels of *ITGA5* might benefit from chemotherapy. The comprehensive analysis of *ITGA5* in patients with LSCC provided the possibility to improve the prognostic prediction and risk stratification for patients with LSCC and provided novel insights into the development of individual treatment strategies.

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CONFLICT OF INTEREST

The authors declare that there are no financial or other conflicts of interest associated with this study.

AUTHOR CONTRIBUTIONS

The research was designed by CZ and HD. The data analysis from the public database was made by CZ, ZW, MT, JZ, and YS. The data were analyzed by CZ, ZS, and YS. CZ supervised the writing of the draft. The manuscript was reviewed by all the authors.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available from The Cancer Genome Atlas database (<https://portal.gdc.cancer.gov/>) and the Gene Expression Omnibus database (<https://www.ncbi.nlm.nih.gov/geo>).

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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