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ORIGINAL RESEARCH

GABRP is a Promising Prognostic Biomarker and Associated with Immune Cell Infiltration in Lung Squamous Cell Carcinoma

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Background: GABRP has been reported to play an oncogenic role in various carcinomas. However, no report has been found for its involvement in lung squamous cell carcinoma (LUSC) development yet. We aimed to explore the expression and prognostic roles of GABRP and assessment of its association with tumor microenvironment in LUSC.

Methods: The GABRP expression in LUSC was analyzed using TCGA, GEO, and HPA databases. The Kaplan-Meier, Cox regression analysis, and receiver operating characteristic (ROC) curve were applied to assess the prognostic and diagnostic values of GABRP in LUSC. We also performed ESTIMATE and ssGSEA to explore the association between GABRP expression and immune cell infiltrations. GABRP was highly expressed in LUSC patients, and up-regulation of GABRP was associated with shorter overall survival (OS). Cox regression analysis indicated that GABRP was an independent prognostic factor for LUSC patients. KEGG analysis revealed that GABRP may play an important role in starch and sucrose metabolism and nicotine addiction. Specifically, GABRP expression showed significant positive correlations with the infiltration levels of most types of immune cells, as well as immune checkpoint molecules expression.

Conclusion: Up-regulation of GABRP in LUSC could be severed as a prognostic marker and a potential target for immunotherapy in LUSC.

Keywords: lung cancer, immune infiltrates, TCGA, prognosis, bioinformatics

Introduction

Lung cancer is the major cause of cancer-associated deaths and the second most diagnosed tumor in developed country.[1] Nearly 1.8 million people are diagnosed with lung cancer, and 1.6 million people died from lung tumor each year.[2,3] Lung squamous cell carcinoma (LUSC), a subtype of non-small cell carcinoma, accounts for about 40% of lung cancer patients and is associated with exposure to tobacco or age.[4] Although great progress has been made in the prevention, diagnosis, and targeted therapy of lung cancer, its clinical efficacy is still unsatisfactory. Besides, the five-year survival rate for LUSC patients is about 17%, and over half of patients die with a year after diagnosis. Lung cancer diagnosis is a challenge due to often not diagnosed until the cancer is advanced.[5] Therefore, diagnosing and screening for lung cancer is critical, especially when screening high-risk groups. Furthermore, identifying new sensitive biomarkers to promote the prognosis of LUSC and discovery of clinical candidate drugs.

Gamma-aminobutyric acid (GABA) is an important inhibitory neurotransmitter in the mammalian central nervous system, and it acts as a main regulator of synaptic suppression.[6] $(GABA)_A$ receptor π subunit (GABRP) is mostly located on the plasma membrane, and dysregulation of GABRP expression plays an important role in cancer growth and

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migration. For example, GABRP promoted tumor metastasis, growth and correlated with macrophage infiltration in pancreatic cancer.[7] GABRP may act as a CD44s downstream target to inhibit gemcitabine resistance in pancreatic cancer.[8] GABRP has been reported to promote breast cancer cells invasion via activation of ERK1/2 pathway.[9] It has been reported that aberrant expression of GABRP related to aggressive phenotype of ovarian cancer cells.[10] Furthermore, increased GABRP expression associated with poor prognosis in pancreatic cancer.[11] All these studies revealed that GABRP is closely associated with the tumorigenesis. However, the functional role of GABRP in LUSC and its prognostic value have not been investigated, which deserves further investigation.

In this study, we used The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) databases to comprehensively explore the association between GABRP expression and the prognostic value of LUSC patients. Besides, the GABRP-associated co-expression genes and the correlations between GABRP expression and immune cell infiltrations, as well as the immune checkpoint genes, would be analyzed, which would investigate the potential mechanism of GABRP implicated in the occurrence and progression of LUSC.

Methods

Collection of Data

The mRNA sequencing data and clinical data of LUSC were downloaded from GEO and TCGA databases. A total of 502 tumor tissues and 49 normal tissues obtained from TCGA-LUSC database, and the data was converted to as log2 Transcripts Per Million (TPM). Besides, the GABRP expression profiles of lung cancer from the GEO databases were used to validate the GABRP expression, which consisted of GSE1037 dataset (19 normal tissues and 12 tumor tissues) and GSE43458 dataset (30 normal tissues and 80 tumor tissues).

Analysis of GABRP Protein Expression in Lung Cancer

The human protein atlas (HPA) (https://www.proteinatlas.org) was used to compare the protein expression of GABRP between normal adjacent tissue and tumor tissue.

Survival Analysis and Construction of Nomogram

The samples were divided into low- and high-GABRP groups based on the GABRP expression. We used the Kaplan-Meier analysis to visualize the overall survival (OS) of LUSC patients. Furthermore, we also performed univariate and multivariate Cox regression analyses to obtain death hazard ratios of GABRP expression and pathological features, and investigate the independent prognostic value for LUSC patients. A nomogram was constructed to predict the OS of LUSC patients based on the multivariate Cox proportional hazards analysis results. The "ggplot2" and survival R package was applied to generate nomograms and plot calibration curves.

Identification and Functional Enrichment Analysis of Differentially Expressed Genes (DEGs) Between the Low- and High-GABRP LUSC Groups

We used DESeq2 package of R to identify the DEGs between the low- and high-GABRP patients from TCGA-LUSC dataset. The screening criteria were as follows: an absolute FC larger than 1 and an adjusted p value < 0.05. The ggplot2 was applied to generate the volcano plots and co-expression heatmaps. Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed using the ClusterProfiler R package, and an adjusted $p < 0.05$ was considered significant.

Immune Infiltration Analysis

We compared the proportion of 24 immune cell types between the low- and high-GABRP expression groups by using the GSVA package of R. The Estimation of STromal and Immune cells in MAlignant Tumours using Expression (ESTIMATE) Score was a scoring tool that used gene expression profiles to infer the score of the stromal cells and immune cells in the tumor sample. "Immune Score" which was designed to represent the level of immune cell infiltration in tumor tissue. "Stromal Score", which was designed to calculate the relative abundance of stromal cells in the tumor

tissue.[12] ESTIMATE Score, Immune Score, and Stromal Score were also analyzed between the two groups by using ESTIMATE software. The Spearman correlation analysis was performed using the ggplot2 R package.

Cell Culture

The lung cancer cell lines (NCI-H1299, NCI-H1650, A549, NCI-H520, H1703, and SK-MES-1) and human normal bronchial epithelial cell line (16HBE) were purchased from the Cell Bank of Chinese Academy of Science (Shanghai, China). The cell lines were grown in RPMI-1640 medium (Gibco, China). All medium were supplemented with 1% complex of streptomycin and penicillin and 10% fetal bovine serum (Gibco, China) in a 5% CO2 humidified atmosphere at 37°C.

Quantitative Reverse Real-Time PCR (qRT-PCR)

Total RNA was extracted from the 16HBE and six kinds of lung cancer cells (NCI-H1299, NCI-H1650, A549, NCI-H520, H1703, and SK-MES-1) using TRIpure RNA Extraction Reagent (ELK Biotechnology, Wuhan, China) based on the manufacturer's protocol. 2 μg of purified RNA was used to synthesize cDNA using the M-MLV Reverse Transcriptase (ELK Biotechnology, Wuhan, China) according to the manufacturer's instruction. Then, the qRT-PCR was performed in the StepOne^{TM} PCR System (Life technologies) using SYBR GREEN I reagent. The $2^{-\Delta\Delta Ct}$ method was applied to quantify the GABRP expression. Experiments were repeated three times. The primer sequences were showed in Table S1.

Statistical Analysis

The R software (version 3.6.3) was used to perform the statistical data derived from the TCGA and GEO databases. The differences between tumor samples and adjacent noncancer samples were compared by Wilcoxon signed-rank test and Mann–Whitney *U*-test. The correlation analysis between GABRP expression and pathological features was performed by the Wilcoxon rank-sum test. The independent prognostic value of GABRP expression was assessed by the combination of univariate Cox regression analysis and multivariate Cox regression analysis. The receiver operating characteristic (ROC) analysis of GABRP was performed using the pROC package. $P < 0.05$ was regarded as statistically significant.

Results

Profiles of GABRP Expression in Different Types of Human Cancers

Based on the TCGA RNA-seq data, the expression levels of GABRP in pan-cancer were analyzed. As shown in Figure 1A, our results showed that relative to normal tissues, GABRP expression was decreased in adrenocortical carcinoma (ACC), bladder urothelial carcinoma (BRCA), head and neck squamous cell carcinoma (HNSC), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), liver hepatocellular carcinoma (LIHC), prostate adenocarcinoma (PRAD), and skin cutaneous melanoma (SKCM). However, the cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), ovarian serous cystadenocarcinoma (OV), pancreatic adenocarcinoma (PAAD), rectum adenocarcinoma (READ), stomach adenocarcinoma (STAD), testicular germ cell tumors (TGCT), thymoma (THYM), uterine corpus endometrial carcinoma (UCEC), and uterine carcinosarcoma (UCS) exhibited higher expression. Analysis of the GABRP expression between lung cancer samples and normal samples, results showed that GABRP exhibited higher expression level in lung cancer (LUSC and LUAD) (Figure 1B–D). Besides, the findings were further confirmed by GSE1037 and GSE43458 datasets ($p < 0.001$; $p < 0.05$, respectively) (Figure 1E and F). Furthermore, the immunohistochemistry from HAP indicated that GABRP was up-regulated in lung cancer tissues (Figure 1G and H).

Assessment of Correlation Between the Pathological Features and GABRP Expression

LUSC patients were divided into the low- and high-GABRP groups based on the median expression level of GABRP. The pathological features were presented in Table 1. GABRP expression was significantly associated with T stage ($p <$

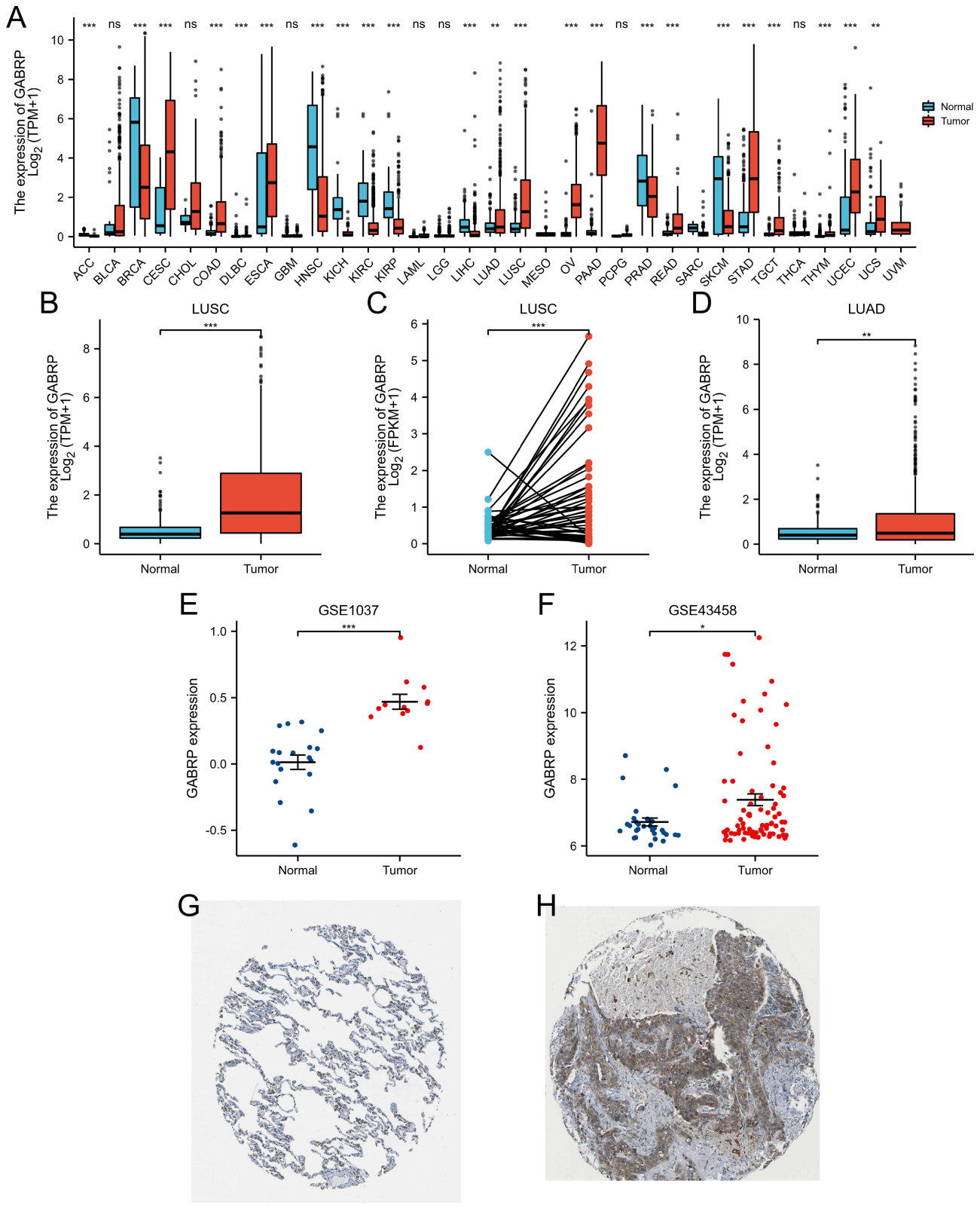

Figure 1 GABRP expression levels in human cancers. (**A**) Abnormal expression of GABRP in human cancers based on TCGA databases. (**B**) The expression of GABRP in LUSC (**B** and **C**) and LUAD (**D**). Abnormal expression of GABRP in lung cancer based on GSE1037 (**E**) and GSE43458 (**F**). Light blue indicates normal group, red indicates tumor group. (**G**) Protein levels of GABRP in normal lung tissue: https://www.proteinatlas.org/ENSG00000094755-GABRP/tissue/lung#img. Staining: low, intensity: moderate, quantity: < 25%. (**H**) Protein levels of GABRP in tumor tissue: https://www.proteinatlas.org/ENSG00000094755-GABRP/pathology/lung+cancer#img, Staining: medium, intensity: moderate, quantity: > 75%. *p < 0.05, **p < 0.01 and ***p < 0.001; ns indicates no significant difference.

Abbreviations: GABRP, Gamma-aminobutyric acid A receptor π subunit; TCGA, The Cancer Genome Atlas; LUSC, lung squamous cell carcinoma; LUAD, lung adenocarcinoma.

Table 1 Correlation Analysis Between GABRP Expression and Pathological Features of LUSC Patients

Characteristic	Low Expression of GABRP	High Expression of GABRP	p
n	251	251	
T stage, n (%)			< 0.001
T1	45 (9%)	69 (13.7%)	
T2	152 (30.3%)	142 (28.3%)	
T3	47 (9.4%)	24 (4.8%)	
T4	7 (1.4%)	16 (3.2%)	
N stage, n (%)			0.492
N0	166 (33.5%)	154 (31%)	
N1	63 (12.7%)	68 (13.7%)	
N2	19 (3.8%)	21 (4.2%)	
N3	1 (0.2%)	4 (0.8%)	
M stage, n (%)			0.123
M0	202 (48.2%)	210 (50.1%)	
M1	1 (0.2%)	6 (1.4%)	
Pathologic stage, n (%)			0.328
Stage I	121 (24.3%)	124 (24.9%)	
Stage II	83 (16.7%)	79 (15.9%)	
Stage III	42 (8.4%)	42 (8.4%)	
Stage IV	1 (0.2%)	6 (1.2%)	
Primary therapy outcome, n (%)			0.549
PD	17 (4.7%)	14 (3.9%)	
SD	8 (2.2%)	9 (2.5%)	
PR	4 (1.1%)	1 (0.3%)	
CR	151 (41.8%)	157 (43.5%)	
Gender, n (%)			0.223
Female	72 (14.3%)	59 (11.8%)	
Male	179 (35.7%)	192 (38.2%)	
Race, n (%)			0.431
Asian	4 (1%)	5 (1.3%)	
Black or African American	18 (4.6%)	12 (3.1%)	
White	170 (43.7%)	180 (46.3%)	
Age, n (%)			0.738
<=65	98 (19.9%)	93 (18.9%)	
>65	149 (30.2%)	153 (31%)	
Residual tumor, n (%)			0.410
R0	196 (47.2%)	203 (48.9%)	
R1	8 (1.9%)	4 (1%)	
R2	2 (0.5%)	2 (0.5%)	
Anatomic neoplasm subdivision, n (%)			0.440
Left	103 (21.8%)	111 (23.5%)	
Right	135 (28.5%)	124 (26.2%)	
Anatomic neoplasm subdivision2, n (%)			0.897
Central Lung	67 (27.9%)	80 (33.3%)	
Peripheral Lung	44 (18.3%)	49 (20.4%)	
Number_pack_years_smoked, n (%)			0.304

(*Continued*)

Table 1 (Continued).

Characteristic	Low Expression of GABRP	High Expression of GABRP	p
<40	71 (16.7%)	63 (14.8%)	
≥40	137 (32.2%)	154 (36.2%)	
Smoker, n (%)			0.482
No	11 (2.2%)	7 (1.4%)	
Yes	235 (48%)	237 (48.4%)	
OS event, n (%)			0.087
Alive	153 (30.5%)	133 (26.5%)	
Dead	98 (19.5%)	118 (23.5%)	
DSS event, n (%)			1.000
Alive	178 (39.6%)	183 (40.7%)	
Dead	44 (9.8%)	45 (10%)	
PFI event, n (%)			0.378
Alive	172 (34.3%)	182 (36.3%)	
Dead	79 (15.7%)	69 (13.7%)	
Age, median (IQR)	68 (60, 73)	69 (63, 74)	0.107

0.001). Furthermore, we performed univariate and multivariate analyses to assess the role of GABRP in LUSC prognosis (Table 2). Multivariate analysis indicated that high GABRP expression was an independent prognostic factor.

Prognostic and Diagnostic Values of GABRP in LUSC Patients

We also analyzed the correlation between GABRP expression and the OS of LUSC patients from TCGA and GEPIA by Kaplan-Meier analysis. As shown in Figure 2A and B, higher GABRP expression was significantly associated with shorter OS

Table 2 Univariate and Multivariate Analyses Were Used to Explore the Prognostic Value of GABRP in LUSC Patients

Characteristics	Total (N)	Univariate Analysis		Multivariate Analysis	
		Hazard Ratio (95% CI)	P value	Hazard Ratio (95% CI)	P value
T stage	496				
T1	114	Reference			
T2	289	1.237 (0.872–1.753)	0.233	1.204 (0.824–1.759)	0.337
T3&T4	93	1.931 (1.277–2.920)	0.002	1.777 (1.117–2.828)	0.015
N stage	490				
N0	317	Reference			
N1	128	1.076 (0.786–1.473)	0.647		
N2&N3	45	1.383 (0.887–2.158)	0.152		
M stage	415				
M0	408	Reference			
M1	7	3.112 (1.272–7.616)	0.013	2.179 (0.879–5.403)	0.093
Gender	496				
Female	130	Reference			
Male	366	1.211 (0.879–1.669)	0.241		
Age	490				
<=65	190	Reference			
>65	300	1.279 (0.960–1.704)	0.093	1.511 (1.098–2.079)	0.011
GABRP	496				
Low	247	Reference			
High	249	1.442 (1.097–1.895)	0.009	1.779 (1.307–2.421)	<0.001

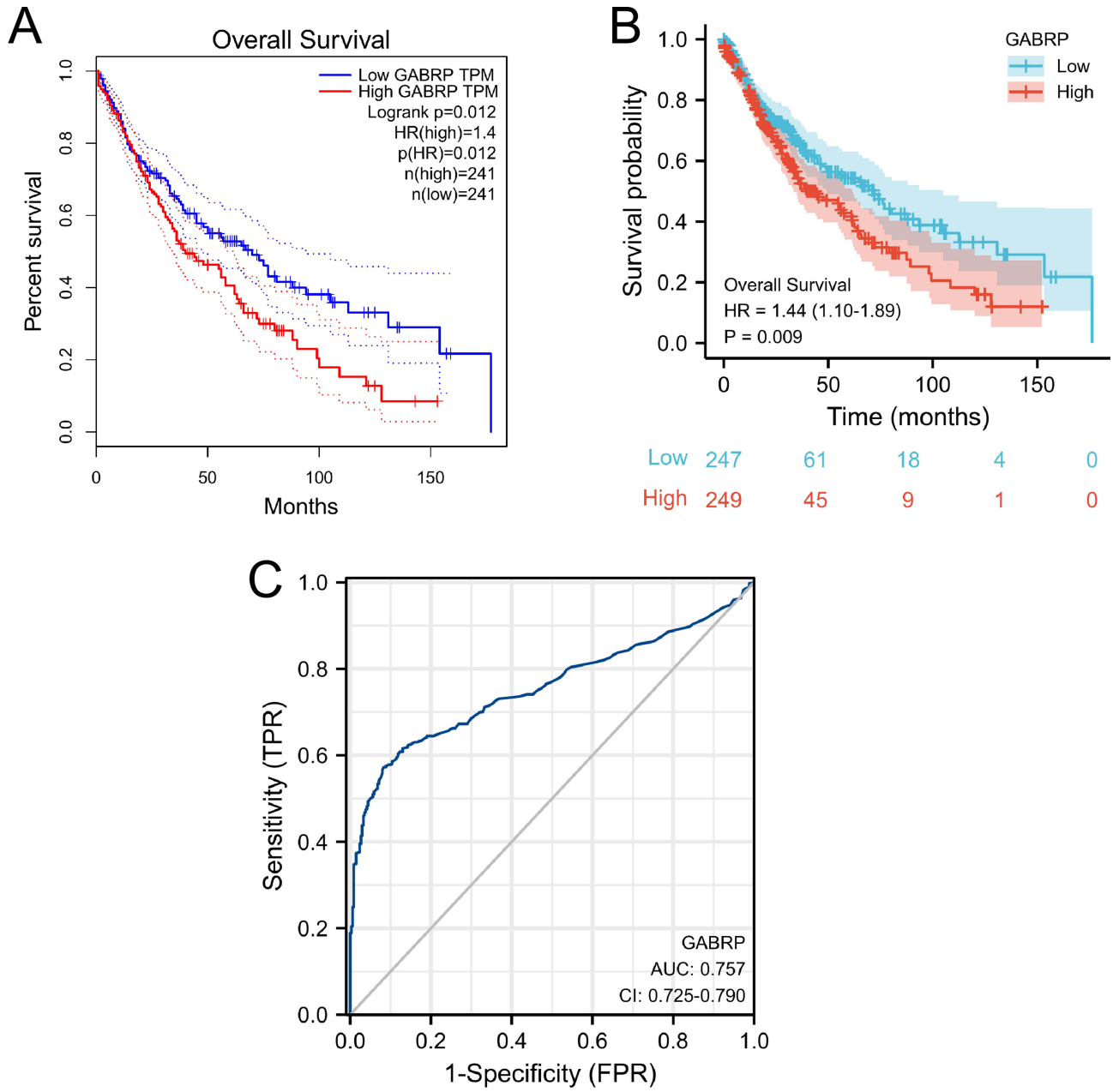

Figure 2 High GABRP expression was associated with poor OS in LUSC patients. The Kaplan-Meier survival curves of the LUSC patients based on GEPIA (**A**) and TCGA (**B**) databases. (**C**) ROC curve analysis of GABRP expression in LUSC.
Abbreviations: GABRP, Gamma-aminobutyric acid A receptor π subunit; OS, overall survival; ROC, receiver operating characteristic; AUC, area under the curve.

(HR = 1.44, P = 0.009). Furthermore, we applied receiver operating characteristic (ROC) curves to assess the diagnostic efficiency of GABRP between normal individuals and LUSC patients. As shown in Figure 2C, the area under the curve (AUC) of GABRP was 0.757, which indicating that GABRP may be a potentially biomarker for LUSC patients.

Establishment of Nomogram in LUSC

We constructed a modeled nomogram, and result indicated it could predict the 1-, 3-, and 5-year OS of LUSC patients (Figure 3A). Based on the nomogram calibration curve (Figure 3B), the prediction result of the modeled nomogram was highly consistent with the observation of LUSC patients.

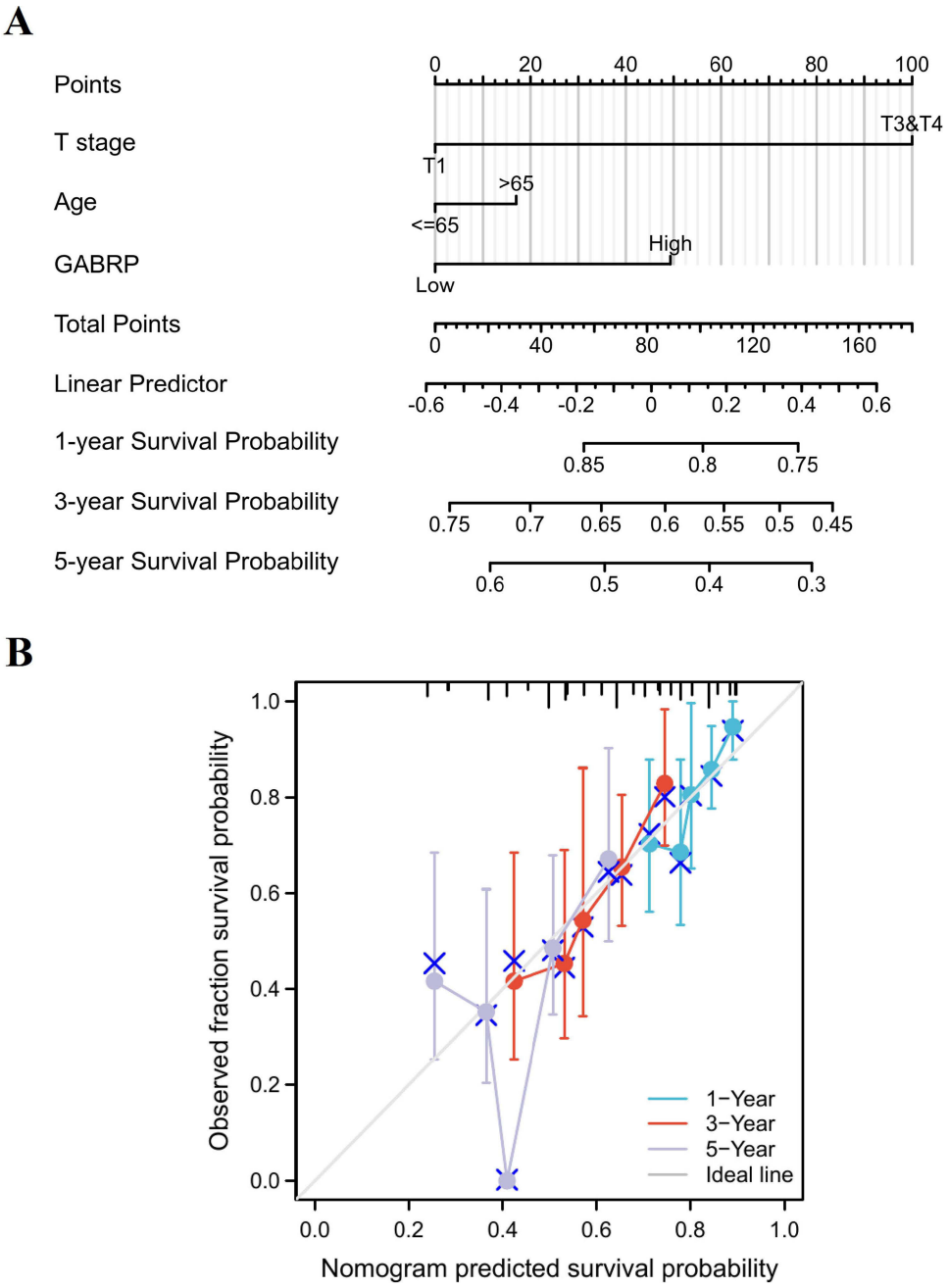

Figure 3 Establishment of a nomogram for LUSC patients. (**A**) A nomogram for assessing the survival probability of 1-year, 3-year, and 5-year for LUSC. (**B**) Calibration curve of the prognostic risk model.
Abbreviations: GABRP, Gamma-aminobutyric acid A receptor π subunit; LUSC, lung squamous cell carcinoma.

Identification and Functional Enrichment Analysis of the GABRP-Related DEGs in LUSC

As presented in Figure 4A, a total of 801 GABRP-related DEGs were identified, which including 242 down-regulated genes and 559 genes. The heatmap presented the top 50 DEGs (Figure 4B). Besides, we performed functional enrichment analysis to further investigate the function of DEGs associated with GABRP expression in lung cancer. As shown in Figure 4C, GABRP-related DEGs play roles in various cellular compositions (CC), molecular functions (MF), KEGG, such as transporter complex, transmembrane transporter complex, ion channel complex, motile cilium, substrate-specific

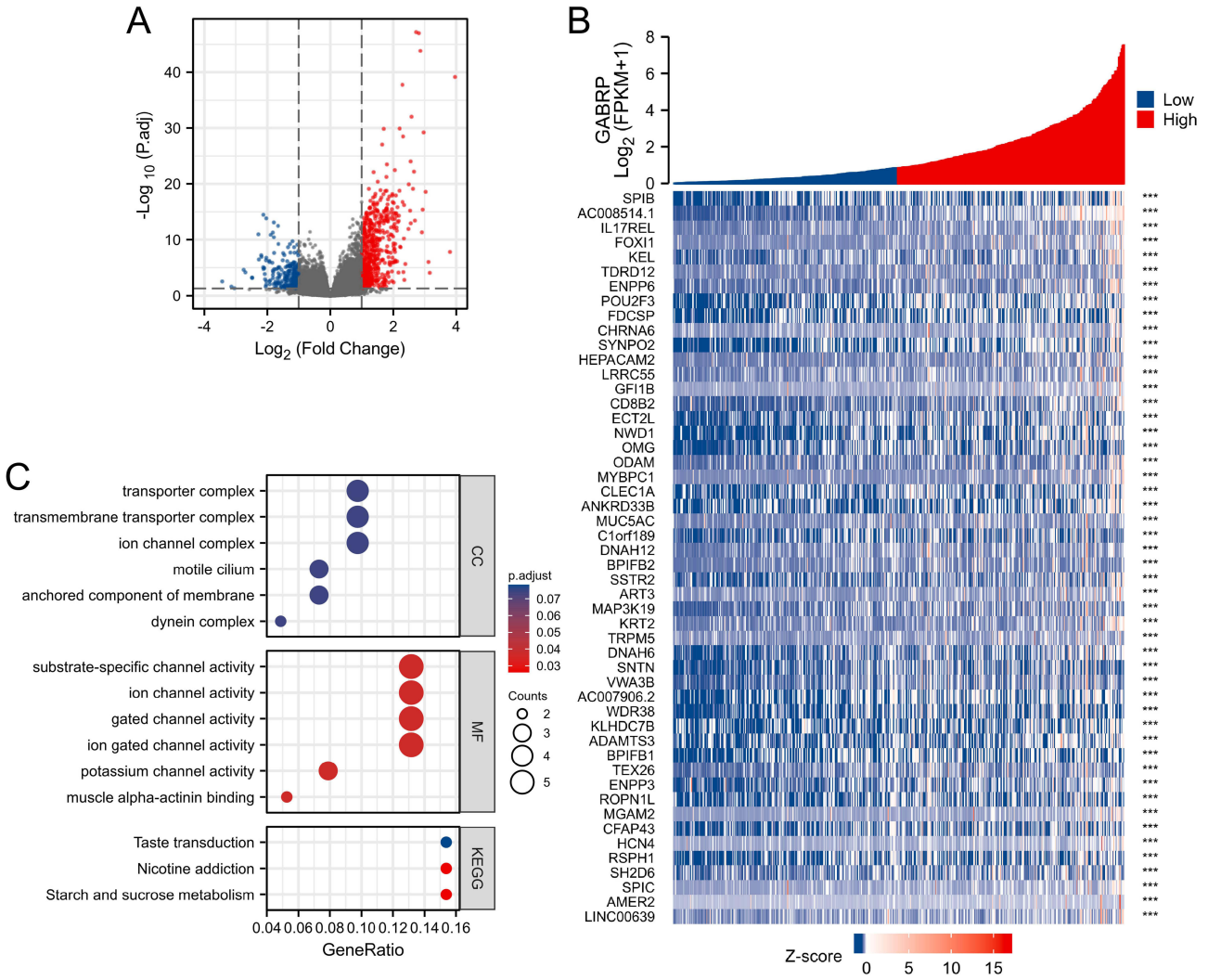

Figure 4 Identification and functional enrichment analysis of the GABRP-related DEGs in LUSC. (**A**) Volcano plots of the DEGs. (**B**) Co-expression heatmap of top DEGs. (**C**) GO and KEGG enrichment analyses of GABRP-related DEGs in LUSC. ***p < 0.001.

Abbreviations: GABRP, Gamma-aminobutyric acid A receptor π subunit; DEGs, differentially expressed genes; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; CC, cellular compositions; MF, molecular functions; LUSC, lung squamous cell carcinoma.

channel activity, ion channel activity, gated channel activity, taste transduction, nicotine addiction, and starch and sucrose metabolism.

GABRP Expression Associated with Tumor Immune Cell Microenvironment

As shown in Figure 5A, the ESTIMATE algorithm indicated that high-GABRP group exhibited higher stromal score ($p < 0.01$), immune score ($p < 0.01$), and ESTIMATE score ($p < 0.001$) than the low-GABRP group. Besides, GABRP expression was positively correlated with stromal score ($r = 0.177$, $p < 0.001$), immune score ($r = 0.185$, $p < 0.001$), and ESTIMATE score ($r = 0.194$, $p < 0.001$) (Figure 5B–D).

The ssGESA algorithm was applied to compare the proportion of 24 immune cell types between low- and high-GABRP group, and our results showed that T cells, pDC, NK cells, NK CD56dim cells, NK CD56bright cells, mast cells, TReg, Th1 cells, TFH, Tem, macrophages, eosinophils, DC, cytotoxic cells, CD8 T cells, B cells, and aDC were increased in high-GABRP group compared with the low-GABRP group (Figure 6A). Besides, the correlation analysis showed that GABRP expression was positively correlated with most immune cell types, such as NK cells ($r = 0.272$, $p < 0.001$), TFH ($r = 0.212$, $p < 0.001$), aDC ($r = 0.198$, $p < 0.001$), B cells ($r = 0.179$, $p < 0.001$), pDC ($r = 0.173$, $p < 0.001$), CD8 T cells ($r = 0.172$, $p < 0.001$), T cells ($r = 0.169$, $p < 0.001$), eosinophils ($r = 0.164$, $p < 0.001$), Th1 cells ($r = 0.158$,

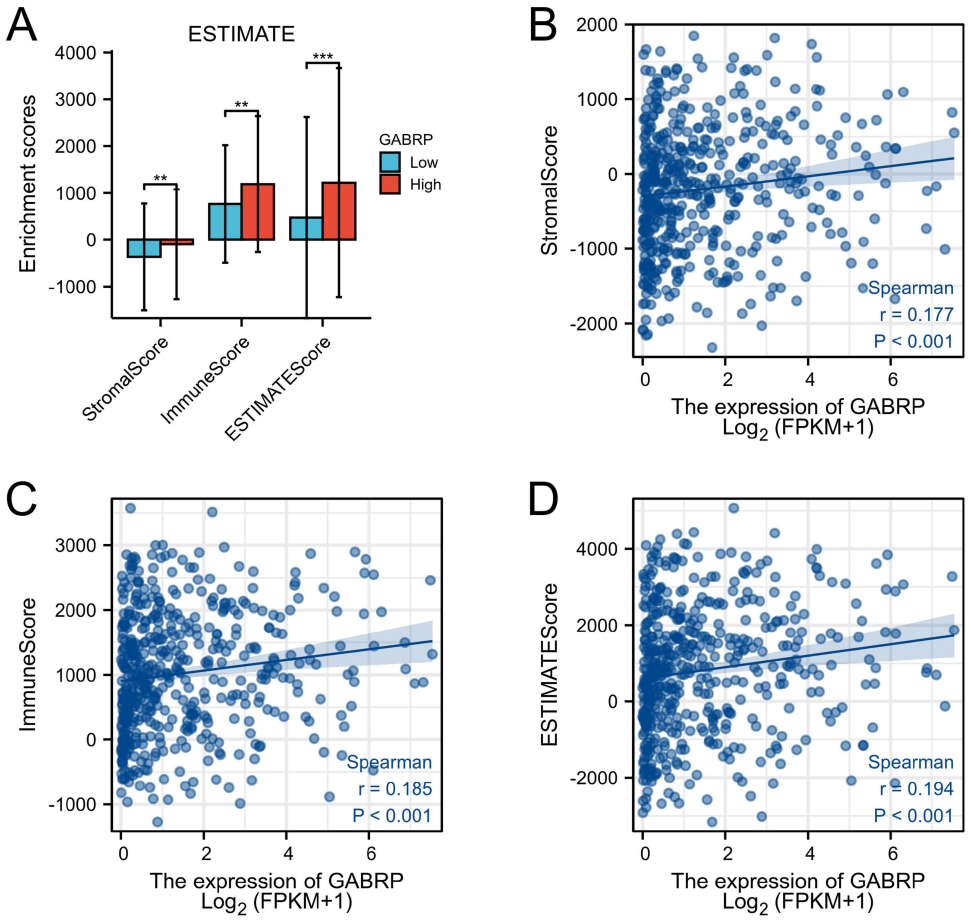

Figure 5 The relationship between GABRP expression and stromal score, immune score, and ESTIMATE score. (**A**) Box diagram presented the stromal score, immune score and ESTIMATE score in the low- and high-GABRP groups. Scatter plots presented the correlation between GABRP expression and stromal score (**B**), immune score (**C**) and ESTIMATE score (**D**) level. **p < 0.01, ***p < 0.001.
Abbreviations: GABRP, Gamma-aminobutyric acid A receptor π subunit; ESTIMATE, Estimation of STromal and Immune cells in MAlignant Tumours using Expression.

p < 0.001), mast cells (r = 0.157, p < 0.001), Tem (r = 0.157, p < 0.001), cytotoxic cells (r = 0.153, p < 0.001), macrophages (r = 0.15, p < 0.001), DC (r = 0.148, p < 0.001), NK CD56bright cells (r = 0.142, p < 0.001), TReg (r = 0.141, p < 0.01), NK CD56dim cells (r = 0.126, p < 0.01), T helper cells (r = 0.122, p < 0.01), and Th17 cells (r = 0.118, p < 0.01) (Figure 6B–E).

Finally, the association between the GABRP expression and immune checkpoint genes were analyzed. As shown in Figure 7, the GABRP expression was positively correlated with the CD247 (r = 0.162, p < 0.001), CTLA4 (r = 0.178, p < 0.001), HAVCR2 (r = 0.144, p = 0.001), TIGIT (r = 0.196, p < 0.001), LAG3 (r = 0.128, p = 0.004), and PDCD1 (r = 0.15, p < 0.001).

Analysis of GABRP mRNA Expression in Lung Cancer Cells

As shown in Figure 8, we found that GABRP expression levels were increased in lung cancer cells (NCI-H1299, NCI-H1650, A549, NCI-H520, H1703, and SK-MES-1) compared to 16HBE cells (p < 0.05). This cell experiment result was consistent with our conclusion.

Discussion

Lung cancer is now the leading cause of cancer death, which killing more people each year than pancreatic, prostate, breast, colorectal cancers combined.[13,14] Thus, it is important to identify new biomarkers as well as to understand the factors impacting the efficacy of immunotherapy for LUSC patients. Lots of studies have revealed that GABRP is

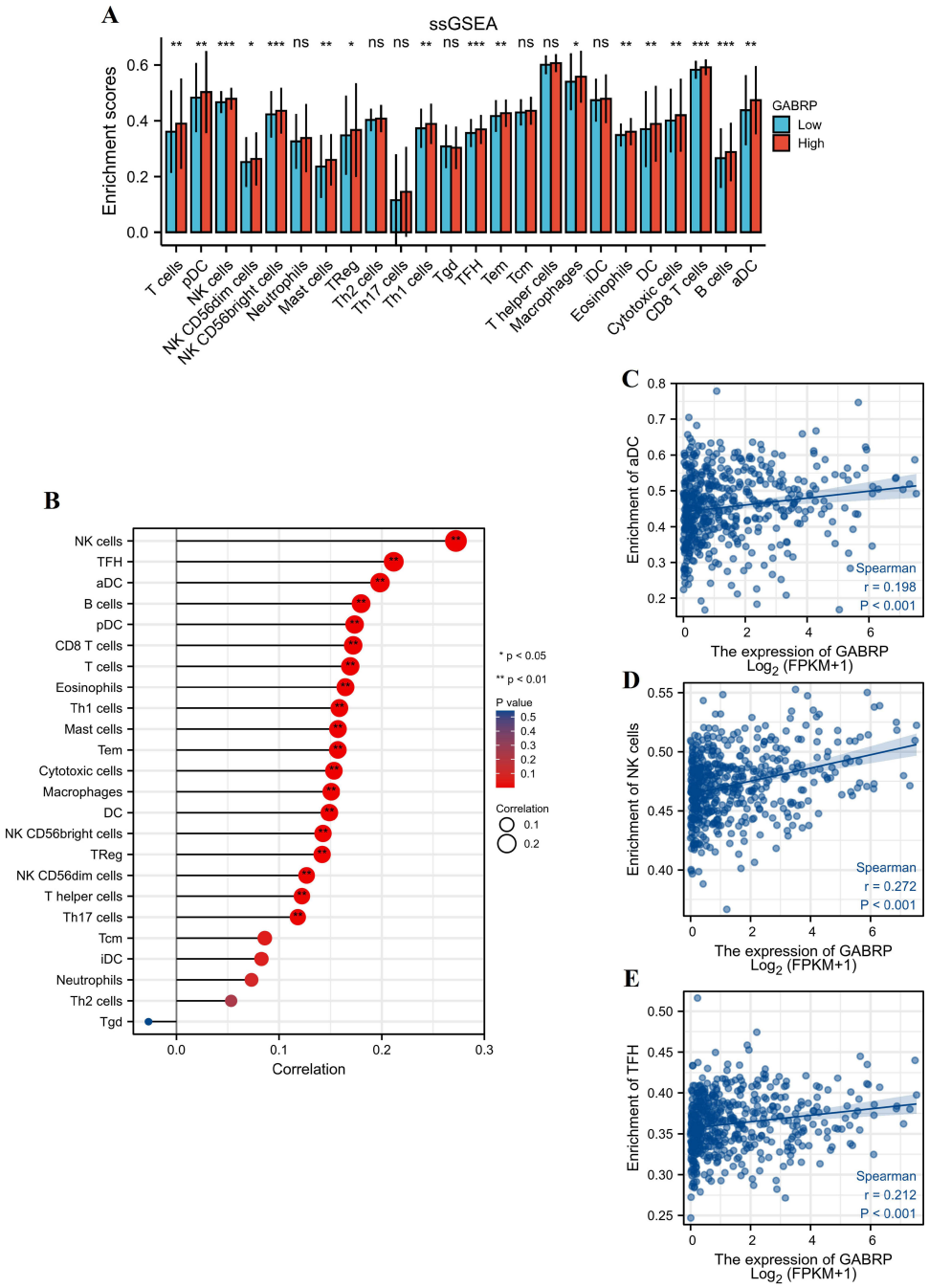

Figure 6 The relationship between GABRP expression and immune cell infiltrations. (**A**) Box diagram presented the enrichment scores of 24 immune cell types in the low- and high-GABRP groups. (**B**) Lollipop plot presented the correlation between GABRP expression and immune cells. Scatter plots presented the correlation between GABRP expression and aDC (**C**), NK cells (**D**) and TFH (**E**) level. *p < 0.05, **p < 0.01, ***p < 0.001; ns indicates no significant difference.

Abbreviations: GABRP, Gamma-aminobutyric acid A receptor π subunit; ssGSEA, single-sample gene set enrichment analysis; aDC, activated dendritic cells; NK cells, natural killer cells; TFH cells, T follicular helper cells.

implicated in the development and progression of various human cancers, such as pancreatic cancer, ovarian cancer, and breast cancer.[7,9,10] First, a pan-cancer analysis of GABRP expression was performed by using TCGA database. And our findings showed that GABRP expression up-regulated in various tumors, including CESC, COAD, ESCA, LUAD, LUSC, OV, PAAD, READ, STAD, TGCT, THYM, UCEC, and UCS. Based on the GABRP expression, LUSC patients were divided into low- and high-GABRP groups. And we found that high-GABRP group had a worse prognosis, implying this grouping could effectively differentiate the prognosis of LUSC. GABRP was up-regulated in pancreatic

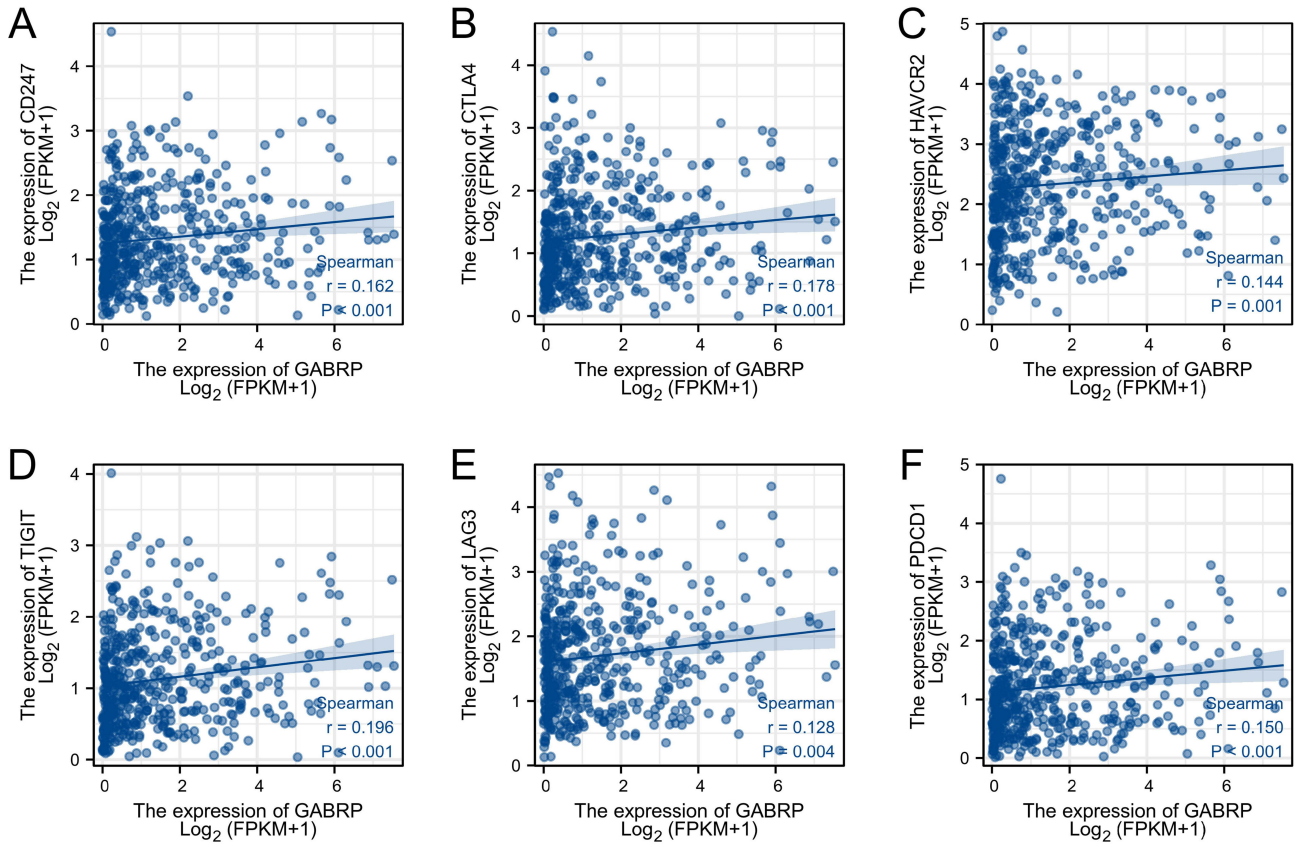

Figure 7 Correlation analysis of GABRP expression with immune checkpoint genes expression in LUSC. Scatter plots presented the correlation between GABRP expression and CD247 (**A**), CTLA4 (**B**), HAVCR2 (**C**), TIGIT (**D**), LAG3 (**E**), and PDCD1 (**F**) expression.
Abbreviations: GABRP, Gamma-aminobutyric acid A receptor π subunit; LUSC, lung squamous cell carcinoma.

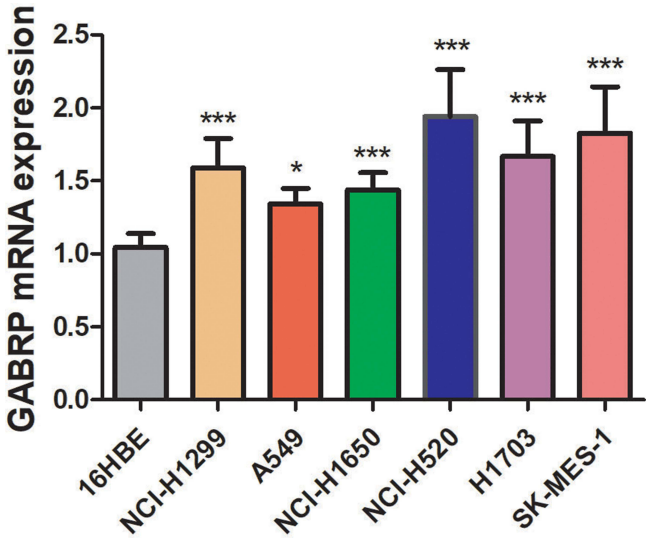

Figure 8 Expression of GABRP in lung cancer (NCI-H1299, NCI-H1650, A549, NCI-H520, H1703, and SK-MES-1) and 16HBE cell lines. The gene expression level was measured by qRT-PCR. *p < 0.05, **p < 0.01 and ***p < 0.001.
Abbreviations: GABRP, Gamma-aminobutyric acid A receptor π subunit; qRT-PCR, quantitative reverse real-time PCR.

cancer, and it was an independent prognostic biomarker for pancreatic cancer patients, this result was consistent with our study.[11]

Functional enrichment analysis was performed to further investigate the underlying biological function of GABRP-related genes in LUSC. GABRP-related genes were mainly involved in various CCs, MFs, KEGGs, such as transporter complex, transmembrane transporter complex, ion channel complex, motile cilium, substrate-specific channel activity, ion channel activity, gated channel activity, taste transduction, nicotine addiction, and starch and sucrose metabolism.

The development and progression of tumor is consistent with changes in the surrounding stroma. Tumor cells can functionally shape their microenvironment via the generation of various chemokines, cytokines, and others. This leads to reprogramming of stroma cells, allowing them to play an important role in tumor progression and survival.[15–17] Recent studies indicated that tumor-infiltrating lymphocytes were closely associated with clinical prognosis in human cancer, including LUSC.[18,19] Our findings indicated that GABRP expression may influence the immune microenvironment in LUSC. GABRP was positively associated with the abundance of immune cells, including T cells, pDC, NK cells, mast cells, TReg, Th1 cells, TFH, Tem, macrophages, eosinophils, DC, cytotoxic cells, CD8 T cells, B cells, and aDC. Thus, our hypothesis was that higher level of GABRP expression may contribute to immune cell infiltration, resulting in tumor development and poor prognosis in LUSC. This was consistent with the result of previous studies, as up-regulated GABRP showed an immunomodulatory role in pancreatic ductal adenocarcinoma.[7] GABRP was associated with tumor microenvironment and immune cell infiltration in pancreatic cancer.[11] In addition, the survival advantage of lung cancer patients may be associated with the anti-cancer effect of CD8+/IL-10+ cell phenotype.[20] Previous studies have demonstrated that NK cells could infiltrate lung tumor, implying that the infiltrations of NK cells into cancer cells may benefit the prognosis of patients.[21] A novel signature was developed based on NK cell marker genes, which had the ability to predict immunotherapy and prognosis.[22] Tumor-related macrophages are closely associated with lymphangiogenesis and angiogenesis, which promoting the non-small cell lung cancer progression.[23] Furthermore, a high level of total M2 macrophages was associated with a shorter survival time in non-small cell lung cancer patients.[24] The high infiltration of follicular helper T cells and activated mast cells was associated with poor prognosis in LUSC.[25] A recent study has revealed that high level of eosinophils, mast cells, type 1 T helper cells, memory CD8 T cells, macrophages, CD56dim NK cells, and gamma delta T cells was related to poor survival in LUSC.[26] Immune checkpoint therapy has revealed good clinical effectiveness in lung cancer.[27,28] In the present study, our findings showed that GABRP expression positively correlated with the immune checkpoint genes, including CD247, CTLA4, HAVCR2, TIGIT, LAG3, and PDCD1. In view of the association between immune cell infiltration and GABRP expression, our findings implied that up-regulation of GABRP expression may have an impact on tumor progression through promotion of immune cell infiltration, all of which has implications for the prognosis of LUSC patients.

Conclusion

The utility of GABRP in the diagnostic and prognostic prediction of LUSC was explored in this study. Up-regulation of GABRP expression was associated with poor prognosis and high immune cell infiltration for LUSC patients. Our findings showed that GABRP was a predictive marker for immunotherapy in LUSC.

Availability of Supporting Data

The data that support the findings of this study were derived from the following public databases: The Cancer Genome Atlas (http://portal.gdc.cancer.gov/) and Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/).

Ethics Approval and Consent to Participate

All experiments were approved by the Ethics Committee of Affiliated Cancer Hospital & Institute of Guangzhou Medical University. This study was performed in accordance with the Helsinki Declaration.

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Disclosure

All authors declare that they have no competing interests.

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