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ORIGINAL RESEARCH

Pan-Cancer Analysis of GALNT6 with Potential Implications for Prognosis and Tumor Microenvironment in Human Cancer Based on Bioinformatics and qPCR Verification

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Purpose: We explored the expression and prognostic value of GALNT6 and the tumor microenvironment of pan-cancer in humans.

Methods: In this study, we explored the expression pattern of GALNT6 pan-cancer across multiple databases. The prognostic value of GALNT6 was evaluated using the Kaplan–Meier method. The types and numbers of GALNT6 gene alterations were exhibited using the cBio Cancer Genomics Portal. The correlations between GALNT6 expression and immune infiltration in cancers were analyzed using the database Tumor Immune Estimation Resource 2. We also used the Kyoto Encyclopedia of Genes and Genomes pathway and Gene Ontology analysis to investigate the molecular mechanisms of the GALNT6 gene in tumorigenesis. The expression of GALNT6 was also further verified by qPCR in lung adenocarcinoma tissues.

Results: In general, compared with normal tissue, tumor tissue had a higher expression level of GALNT6. GALNT6 showed a protective effect in colon carcinoma and other cancers; however, a high expression level of GALNT6 was detrimental to survival in bladder cancer and in pheochromocytoma and paraganglioma. Mutation, amplification, and deep deletion were the three main types of GALNT6 mutations in tumors. There was a significant positive correlation between GALNT6 expression and immune infiltration of CD8+ T-cells in skin cutaneous melanoma metastasis, based on most of the algorithms used. Moreover, protein processing- and glycoprotein metabolic-associated functions were involved in the functional mechanisms of GALNT6.

Conclusion: This first pan-cancer study offers a relatively comprehensive understanding of the oncogenic roles of GALNT6 across different cancer types.

Keywords: GALNT6, pan-cancer, survival analysis, mutation, immune infiltration, functional mechanisms

Introduction

Cancer is a major public health problem worldwide[1,2] and the dominant cause of premature death and decreased life expectancy in many countries.[3] The hallmarks of cancer were proposed as a set of functional capabilities acquired by human cells as they make their way from normalcy to forming malignant tumors. The hallmarks currently comprise sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing and accessing vasculature, activating invasion and metastasis, reprogramming cellular metabolism, and avoiding immune destruction.[4] Given the complexity of cancer phenotypes and genotypes, it is important to conduct a pan-cancer expression analysis of genes of interest and assess their correlation with clinical prognosis and potential molecular mechanisms. Polypeptide N-acetylgalactosaminyltransferase 6 (GALNT6), also known as GALNAC-T6 or GalNAcT6, is located on chromosome 12q13, and was first identified as an enzyme in the initiation of O-glycosylation.[5] Glycosylation is a dynamic process of glycoconjugate synthesis that can modulate inflammatory responses, enable viral immune escape, and regulate apoptosis or promote cancer cell metastasis.[6] Mucin-type O-linked protein glycosylation can be initiated by

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GALNT family members that transfer GalNAc to serine or threonine residues on the target protein.[7] This modification occurs in the Golgi complex.[8] Biochemical and biological function analyses of GALNT6 have been conducted from the perspective of physiology across different species.[9–11] Previous studies have explored the effects of GALNT6 in tumors, suggesting that GALNT6 is of great importance in cancer development.

The role of GALNT6 in tumors was first reported for breast cancers, and overexpression of GALNT6 may contribute to mammary carcinogenesis through aberrant glycosylation and stabilization of MUC1.[12] Overexpression of GALNT6 in serous ovarian carcinomas was found using the independently derived Affymetrix GeneChip Human Genome U133 Plus 2.0 array. Moreover, GALNT6 expression was correlated with a higher risk of disease progression and poor prognosis in serous epithelial ovarian cancer patients.[13] Further studies are needed to more completely elucidate, in vitro and in vivo, the role of the GALNT6 gene in ovarian tumorigenesis. A previous study analyzed the molecular differences between B-cell precursor acute lymphoblastic leukemia and T-lineage acute lymphoblastic leukemia and identified the multi-omics signatures using Boruta and Monte Carlo feature selection methods. There were seven expression signature genes (CD3D, VPREB3, HLA-DRA, PAX5, BLNK, GALNT6, and SLC4A8) and 168 methylation sites corresponding to 175 methylation signature genes.[14] However, owing to a lack of external validation, a larger acute lymphoblastic leukemia cohort is required to further validate the conclusions. However, the potential implications of GALNT6 in the prognosis and tumor microenvironment in human cancers have not been extensively explored.

In this study, we comprehensively explored the expression profile and prognostic value of GALNT6 in human cancer, based on an analysis of multiple databases. Our study, for the first time, conducted a pan-cancer analysis of GALNT6 with potential implications in prognosis and tumor microenvironment, aiding in understanding the role of GALNT6 in tumorigenesis from the perspective of clinical tumor samples.

Materials and Methods

GALNT6 Expression in Human Cancers

We observed the difference in expression of GALNT6 between tumor and adjacent normal tissues for different tumors or specific tumor subtypes in the Cancer Genome Atlas (TCGA)[15] project using the Tumor Immune Estimation Resource, version 2 (http://timer.cistrome.org/),[16] and abbreviations for all types of cancer were on display, as shown in Table 1. For certain tumors without normal or with highly limited normal tissues (eg, UCS, and LAML), we obtained box plots of the

Table 1 33 Types of Human Cancers Employed in Our Research

Abbreviation	Full name
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

(*Continued*)

Table 1 (Continued).

Abbreviation	Full name
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

difference in expression between these tumor tissues and the corresponding normal tissues in the Genotype-Tissue Expression (GTEx)[17] database using the Expression Analysis–Box Plots module of the Gene Expression Profiling Interactive Analysis, version 2 (GEPIA2; http://gepia2.cancer-pku.cn/#analysis).[18] Thresholds were set at a P-value < 0.05, and log2FC (fold change) cutoff > 1.0.We used the UALCAN[19] portal (http://ualcan.path.uab.edu/analysis-prot.html), an interactive web resource for analyzing cancer omics data, to conduct protein expression analysis of the Clinical Proteomic Tumor Analysis Consortium (CPTAC)[20] dataset. Herein, we explored differences in the expression level of the total protein of GALNT6 (NP_009141.2) between primary tumor and normal tissues. The available datasets of six tumors were selected, namely, BRCA, OV, COAD, KIRC, UCEC, and LUAD. In addition, we obtained violin plots of GALNT6 expression at different pathological stages of all TCGA tumors using the Pathological Stage Plot module of HEPIA2. The log2 [transcripts per million (TPM) + 1] transformed expression data were applied for the box and violin plots.

GEPIA2 Survival Analysis

The correlation between GALNT6 expression and survival pan-cancer was analyzed using PrognoScan[21] (http://dna00.bio.kyutech.ac.jp/PrognoScan/index.html), Kaplan–Meier Plotter[22] (https://kmplot.com/analysis/), and GEPIA2. Specifically, GALNT6 expression levels were searched in all available microarray datasets of PrognoScan to determine their relationship with prognosis, including overall survival (OS) and disease-free survival (DFS). The threshold was set as Cox P-value < 0.05. GEPIA2 is an interactive online platform with tumor sample information obtained from the TCGA, and normal sample information from the TCGA and GTEx projects. We explored the effect of GALNT6 expression on OS and DFS for each available cancer type (total number = 34). Kaplan–Meier Plotter is a powerful online tool that can be used to assess the effect of 54,000 genes on survival in 21 cancer types. We analyzed the relationship of GALNT6 expression with OS and relapse-free survival (RFS) in BRCA, CESC, ECSA, LIHC, OV, PAAD, PCPG, READ, STAD, and UCEC. Hazard ratios (HRs) with 95% confidence intervals (CIs) and log-rank P-values were calculated.

Genetic Alteration Analysis in the cBioPortal Tool

The cBio Cancer Genomics Portal (https://www.cbioportal.org/) is an open-access resource for interactive exploration of multidimensional cancer genomics data sets.[23] We observed the genetic alteration characteristics of GALNT6 in the "TCGA Pan Cancer Atlas Studies". Alteration frequency, mutation type, and copy number alteration (CNA) across all TCGA tumors were observed in the Cancer Types Summary module. We also used the Comparison module to obtain data

on OS, DFS, and PFS differences for the TCGA cancer cases with or without the GALNT6 genetic alteration. Kaplan–Meier plots with log-rank P-value were also generated.

Immune Infiltration Analysis in TIMER2

We used the Immune-Gene module of the TIMER2 web server to explore the association between GALNT6 expression and immune infiltrates across all TCGA tumors. The immune cells CD8+ T-cells and cancer-associated fibroblasts were selected. The TIMER, CIBERSORT, CIBERSORT-ABS, QUANTISEQ, XCELL, naive_ XCELL, central memory_ XCELL, effector memory_ XCELL, MCPCOUNTER, and EPIC algorithms were applied for immune infiltration estimations. The P-values and partial correlation (cor) values were obtained via the purity-adjusted Spearman's rank correlation test. The data were visualized as a heatmap and a scatter plot.

GALNT6-Related Gene Enrichment Analysis

We first searched the STRING website (https://string-db.org/)[24] using the query of a single protein name ("GALNT6") and organism ("Homo sapiens"). Subsequently, we set the following main parameters: minimum required interaction score ["Low confidence (0.150)"], meaning of network edges ("evidence"), maximum number of interactors to show ("no more than 50 interactors" in 1st shell), and active interaction sources ("experiments"). Finally, the available experimentally determined GALNT6-binding proteins were obtained.

We used the Similar Gene Detection module of GEPIA2 to obtain the top 100 GALNT6-correlated targeting genes based on the datasets of all TCGA tumor and normal tissues. We also applied the Correlation Analysis module of GEPIA2 to perform a pairwise-gene Pearson correlation analysis of GALNT6 and selected genes. The log2 TPM was applied for the dot plot. The P-value and the correlation coefficient (R) were generated. Moreover, we used the Gene_Corr module of TIMER2 to supply the heatmap data of the selected genes, which contained the partial correlation (cor) and P-value in the purity-adjusted Spearman's rank correlation test.

We combined the GALNT6-binding and interacting genes to perform Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. In brief, we uploaded the gene lists to the Database for Annotation, Visualization, and Integrated Discovery (DAVID) with settings for selected identifier ("OFFICIAL_GENE_SYMBOL") and species ("Homo sapiens"), and obtained functional annotation chart data.

Quantitative Real-Time Polymerase Chain Reaction

Lung adenocarcinoma tissues and normal tissues were collected from 6 patients. The study was approved by the Ethics Committee of the Affiliated Zhongshan Hospital of Dalian University and all individuals provided written informed consents. Total RNA was extracted from tissues using RNA Easy Fast Tissue/Cell Kit [TIANGEN BIOTECH (BEIJING) CO., LTD., Beijing, China]and reverse-transcribed into cDNA using Fasting cDNA Dispelling RT SuperMix [TIANGEN BIOTECH (BEIJING) CO., LTD., Beijing, China]. Q-PCR was performed using Fastfire qPCR PreMix (SYBR Green [TIANGEN BIOTECH (BEIJING) CO., LTD., Beijing, China]. The relative expression of GALNT6 was calculated using the $2^{-\Delta\Delta Ct}$ method. PCR primers used are listed in Table 2.

Statistical Analysis

We investigated GALNT6 expression levels across human cancers using TCGA database analyses with P-values determined by *t*-tests, z-values, fold changes, and gene ranks. The Kaplan–Meier method was used to analyze the relationship between GALNT6 transcription and patient survival. To compare survival curves, we used the Log rank test to calculate HRs and log-rank P-values using Kaplan–Meier Plotter. A univariate Cox regression model was used to calculate HR and Cox P-values in

Table 2 PCR Primers

GALNT6	Forward (5′-3′)	CTCTGGAACTTGGAGGGTTGT
	Reverse (5′-3′)	TGAGCCCAACCCTGGAGATA

PrognoScan. Spearman correlation was used to evaluate the gene expression correlation. R language software (R 3.6.3, 64-bit; https://www.r-project.org/) was used in KEGG pathway analysis. Differences were analyzed by *t*-test. P<0.05 was considered statistically significant.

Results

Gene Expression Analysis Data

In this study, we aimed to explore the oncogenic role of human GALNT6 (NM_007210 for mRNA and NP_009141.2 for protein). We applied the TIMER2 approach to analyze the expression status of GALNT6 across various TCGA cancer types. As shown in Figure 1A, the expression level of GALNT6 in the tumor tissues of BLCA, BRCA, CHOL, COAD, HNSC, LUAD, LUSC, READ, and UCEC (all P < 0.001), and of PCPG (P < 0.01) was higher than in the corresponding control tissues. After including values for normal tissue from the GTEx dataset as controls, we further evaluated the difference in GALNT6 expression between the normal tissues and the tumor tissues of UCS, LAML, OV, TGCT, and THYM (Figure 1B; P<0.05). However, we did not obtain a significant result for other tumors, including SKCM, LGG, ACC, and SARC, as shown in Figure S1A–D.

The results of the CPTAC dataset showed higher expression of GALNT6 total protein in the primary tissues of LUAD, COAD, BRCA, and UCEC (P<0.001; Figure 2A–D) than in normal tissues. However, expression of GALNT6

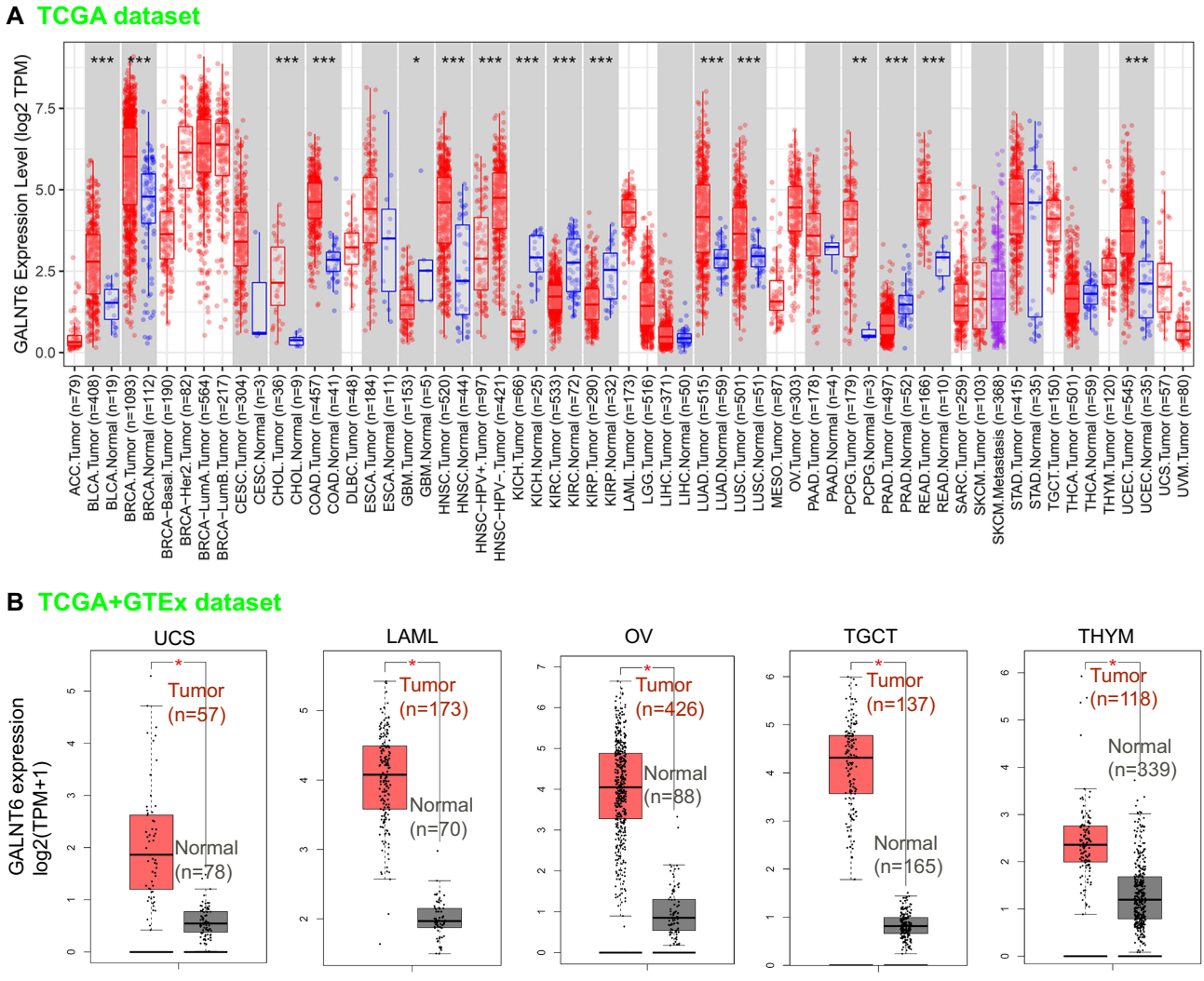

Figure 1 GALNT6 expression levels in normal tissues and tumors. (**A**) The expression status of the GALNT6 gene in different cancers or specific cancer subtypes from the TCGA database. *P< 0.05, **P < 0.01, ***P < 0.001. (**B**) GALNT6 expression in 5 cancer types from the GTEx database and TCGA database. *P< 0.05.

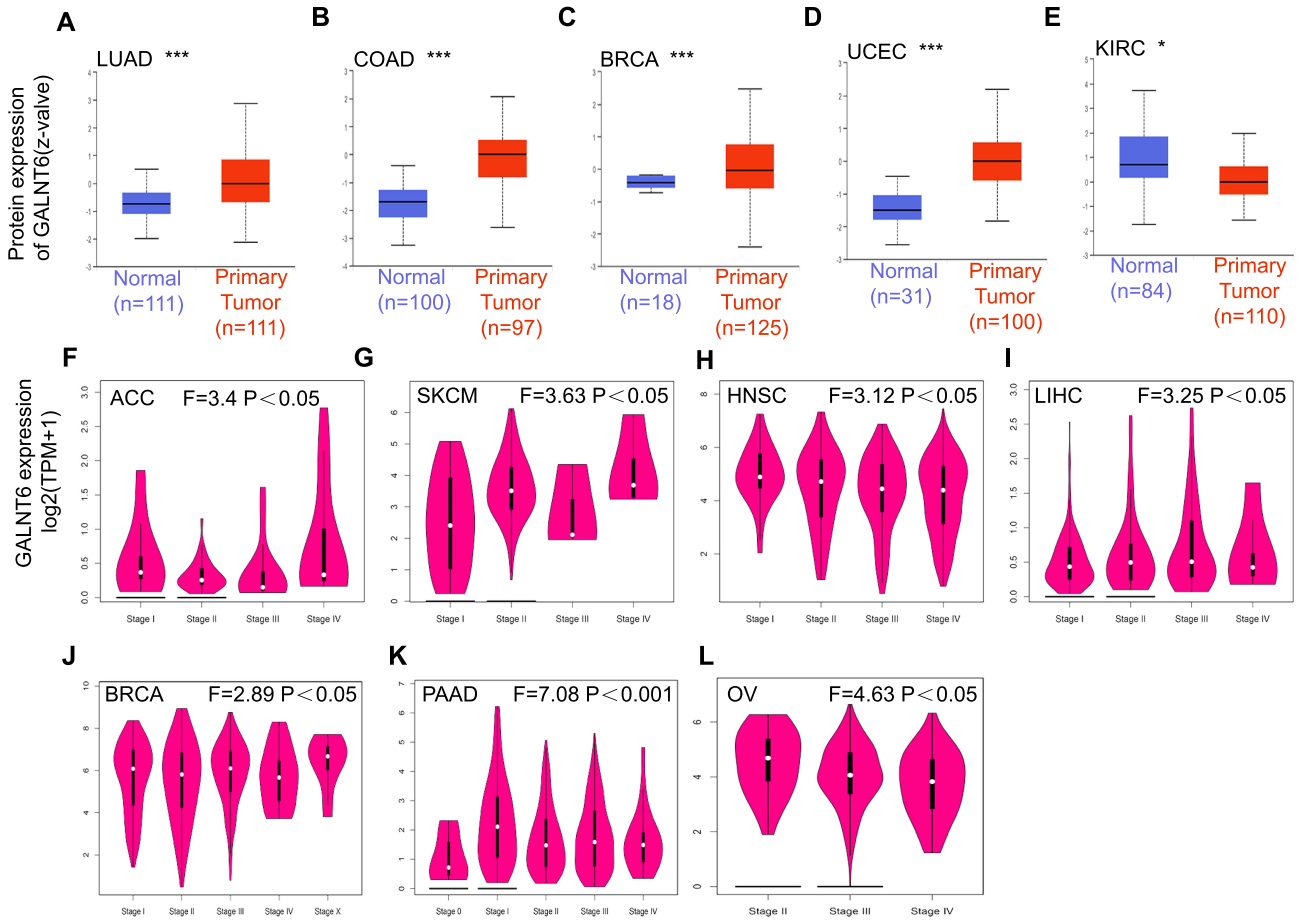

Figure 2 GALNT6 expression levels in different tumors and pathological stages. (**A**–**E**) Total protein of expression of GALNT6 in 5 cancer types from the CPTAC database. *P < 0.05, *** P<0.001. (**F**–**L**) GALNT6 expression in different pathological stages in 7 cancer types from the TCGA database.

total protein was higher in the normal tissues of KIRC (P < 0.01; Figure 2E) than in primary tissues. In addition, using the Pathological Stage Plot module of HEPIA2 we observed a correlation between GALNT6 expression and the pathological stages of cancers including ACC, SKCM, HNSC, LIHC, BRCA, PAAD, and OV (Figure 2F–L, all P < 0.05), but did not make the same observation in the other cancer types (Figure S1E–U).

Multifaceted Prognostic Value of GALNT6 in Cancer

Next, we investigated the prognostic value of GALNT6 among cancers in different databases. In PrognoScan, we explored the relationships between GALNT6 expression and cancer prognosis. Notably, GALNT6 expression was significantly correlated with a total of seven cancer types, including bladder, brain, eye, breast, ovarian, colorectal, and lung cancers (Figure 3). Among them, GALNT6 had a protective role in two cancer types: ovarian (OS: total number = 278, HR = 0.79, Cox P = 0.027760), and colorectal (DSS: total number = 177, HR = 0.56, Cox P = 0.048033; OS: total number = 177, HR = 0.59, Cox P = 0.041916). GALNT6 played a detrimental role in the other four cancer types: bladder (DSS: total number = 155, HR = 1.67, Cox P = 0.043439; OS: total number = 165, HR = 1.49, Cox P = 0.030522), brain (OS: total number = 74, HR = 1.50, Cox P = 0.018168), breast [OS: total number = 155, HR = 1.55, Cox P = 0.035020; DMFS (distant metastasis-free survival)]: total number = 198, HR = 1.22, Cox P = 0.047213; RFS: total number = 155, HR = 1.21, Cox P = 0.017690), and lung cancers (OS: total number = 204, HR = 1.97, Cox P = 0.000050; RFS: total number = 204, HR = 1.92, Cox P< 0.05). However, GALNT6 made no difference in the prognosis of eye cancers (DMFS: total number = 63, HR = 0.00, Cox P = 0.045169).

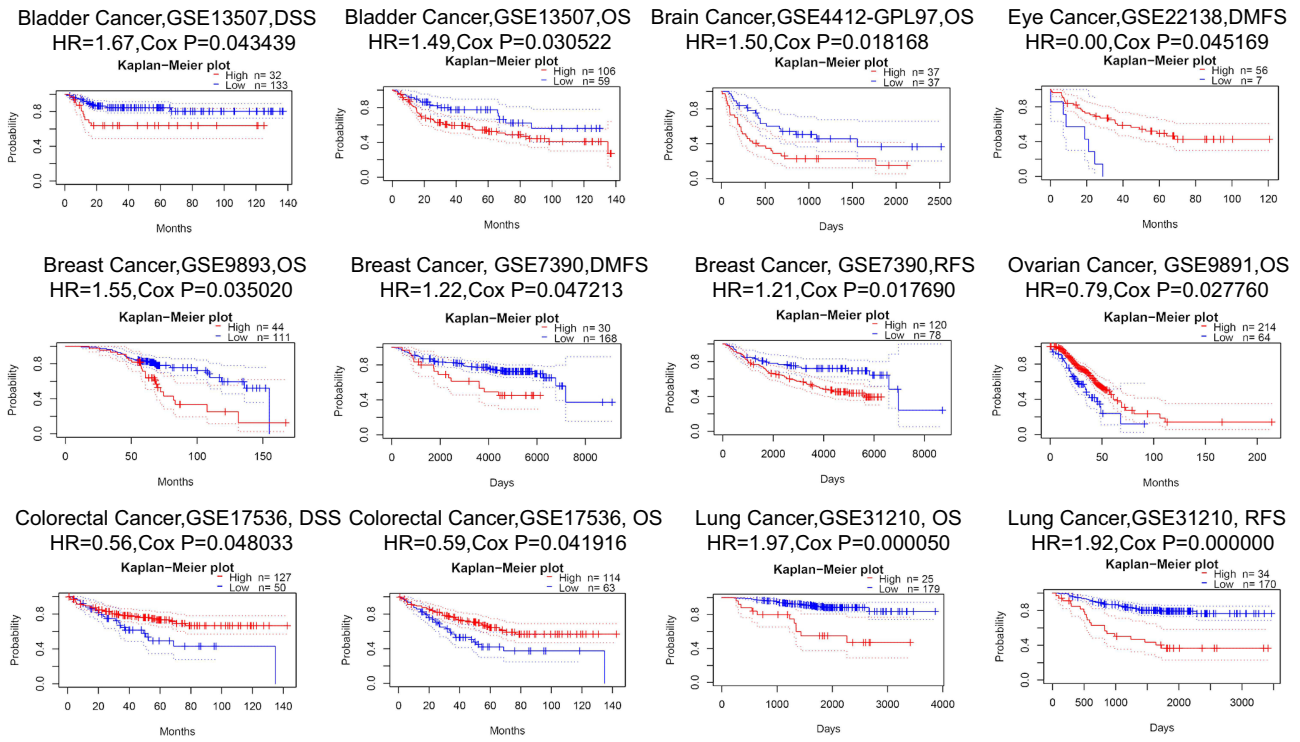

Figure 3 Kaplan-Meier survival curves comparing the high and low expression of GALNT6 gene in various cancer types.
Abbreviations: DSS, disease-specific survival; OS, overall survival; DMFS, distant metastasis free survival; RFS, relapse-free survival; HR, Hazard ratio.

Using Kaplan–Meier Plotter, which is mainly based on Affymetrix microarray information from TCGA, we further assessed GALNT6-related survival (OS and RFS), because the data in PrognoScan are mainly extracted from the Gene Expression Omnibus (GEO) database. The findings for breast cancers partially differed from the findings using PrognoScan, with a high expression of GALNT6 benefiting RFS for BRCA (RFS: HR = 0.63, 95% CI 0.41–0.96, log-rank P = 0.031; Figure 4A). The OS for ovarian cancers were in accord with the findings using PrognoScan, with GALNT6 found to have a protective effect for ovarian cancers (OS: HR = 0.65, 95% CI 0.44–0.86, log-rank P = 0.003) and RFS (HR = 0.74, 95% CI 0.52–1.06, log-rank P = 0.00019; Figure 4E and F). For all ESCA, READ, STAD, and UCEC, GALNT6 significantly influenced OS (ESCA: OS, HR = 0.4, 95% CI 0.16–0.96, log-rank P = 0.034; READ: OS, HR = 0.27, 95% CI 0.1–0.74, log-rank P = 0.0073; STAD: OS, HR = 0.62, 95% CI 0.44–0.87, log-rank P = 0.0049; UCEC: OS, HR = 0.49, 95% CI 0.32–0.76, log-rank P = 0.00099; Figure 4C, E, F, I–K). We identified GALNT6 as a detrimental prognostic factor in RFS for CESC, LIHC, PAAD, and PCPG (CESC: RFS, HR = 3.11, 95% CI 1.07–9,03, log-rank P = 0.028; LIHC: OS, HR = 1.59, 95% CI 1.13–2.25, log-rank P = 0.0079; PAAD: RFS, HR = 2.62, 95% CI 0.97–7.05, log-rank P = 0.048; PCPG: OS, HR = 5.74, 95% CI 1.04–31.71, log-rank P = 0.024; Figure 4B, D, G and H).

Mutation Features of GALNT6 in Different Tumors

We observed the genetic alteration status of GALNT6 in different tumor samples of the TCGA cohorts. As shown in Figure 5A, the highest alteration frequency of GALNT6 (> 6%) appears for patients with SKCM with "mutation" as the primary type. The "amplification" type of CNA was the only type of CNA found in the ACC cases, which showed an alteration frequency of ~4% (Figure 5A). The types, sites, and case numbers of the GALNT6 genetic alteration are further presented in Figure 5B. We found that missense mutation of GALNT6 was the main type of genetic alteration, and G518R/V alteration, which was detected in one case of LUSC, one case of STAD, and one case of HNSC (Figure 5B), was able to induce a frame-shift mutation of the GALNT6 gene, with translation from arginine (R) to valine (V) at the 518 site of the GALNT6 protein. In addition, we explored the potential association between genetic alteration of GALNT6 and the clinical survival prognosis of cases among different types of cancer.

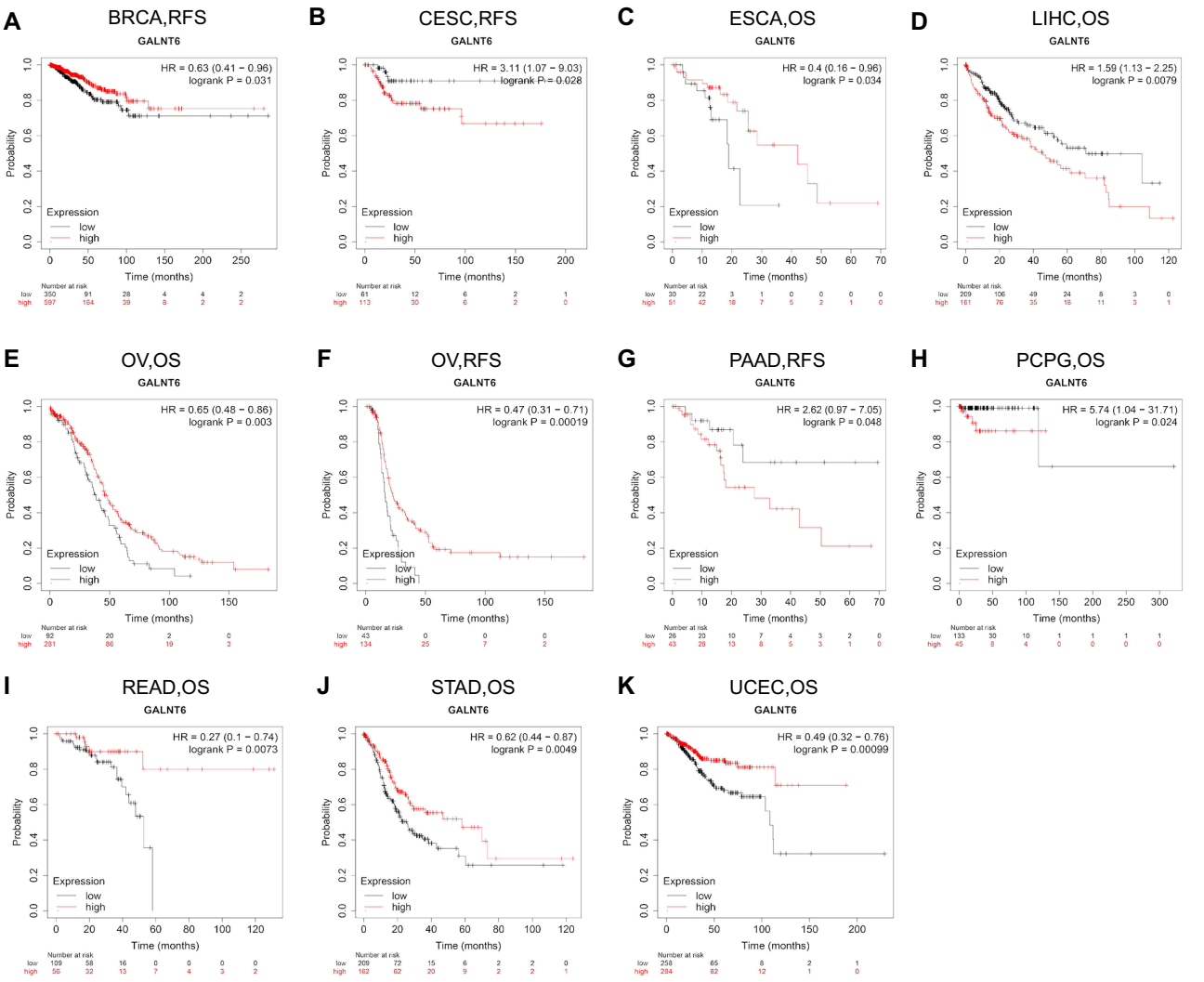

Figure 4 Correlation between GALNT6 gene expression and survival prognosis of cancers in the TCGA database. Relapse-free survival or overall survival of (**A**) BRCA, (**B**) CESC, (**C**) ESCA, (**D**) LIHC, (**E** and **F**) OV, (**G**)PAAD, (**H**) PCPG, (**I**) READ, (**J**) STAD, and (**K**) UCEC.
Abbreviations: RFS, relapse-free survival; OS, overall survival; HR, Hazard ratio.

The data in Figure 5C indicate that STAD cases without altered GALNT6 showed a better prognosis for DFS (P = 0.0119), but not DSS (P = 0.167), OS (P = 0.0986), or PFS (P = 0.0895), compared with cases with GALNT6 alteration.

Analysis of Correlation Between GALNT6 Expression and Immune Infiltration

Tumor-infiltrating immune cells, as prominent components of the tumor microenvironment, have been closely linked to the initiation, progression, and metastasis of cancer.[25,26] Cancer-associated fibroblasts and CD8+ T-cells in the stroma of the tumor microenvironment were reported to participate in modulating the function of various tumor-infiltrating immune cells.[27–29] We used the TIMER, EPIC, CIBERSORT, CIBERSORT-ABS, QUANTISEQ, XCELL, naive_XCEL, central memory_XCELL, effector memory_XCEL, MCPCOUNTER, and TIDE algorithms to investigate the potential relationship between the infiltration level of different immune cells and GALNT6 gene expression in diverse TCGA cancer types. After a series of analyses, we observed a statistically positive correlation between the immune infiltration of CD8+ T-cells and GALNT6 expression in SKCM metastasis (Figure 6A) based on all the algorithms. Moreover, we observed a statistically negative correlation between GALNT6 expression and the estimated infiltration value of cancer-associated fibroblasts for TGCTs (Figure 6B). The scatterplot data of skin cutaneous melanoma metastasis and TGCTs produced using one of the algorithms are presented in Figure 6C and D. For example, the GALNT6 expression level in TGCT is

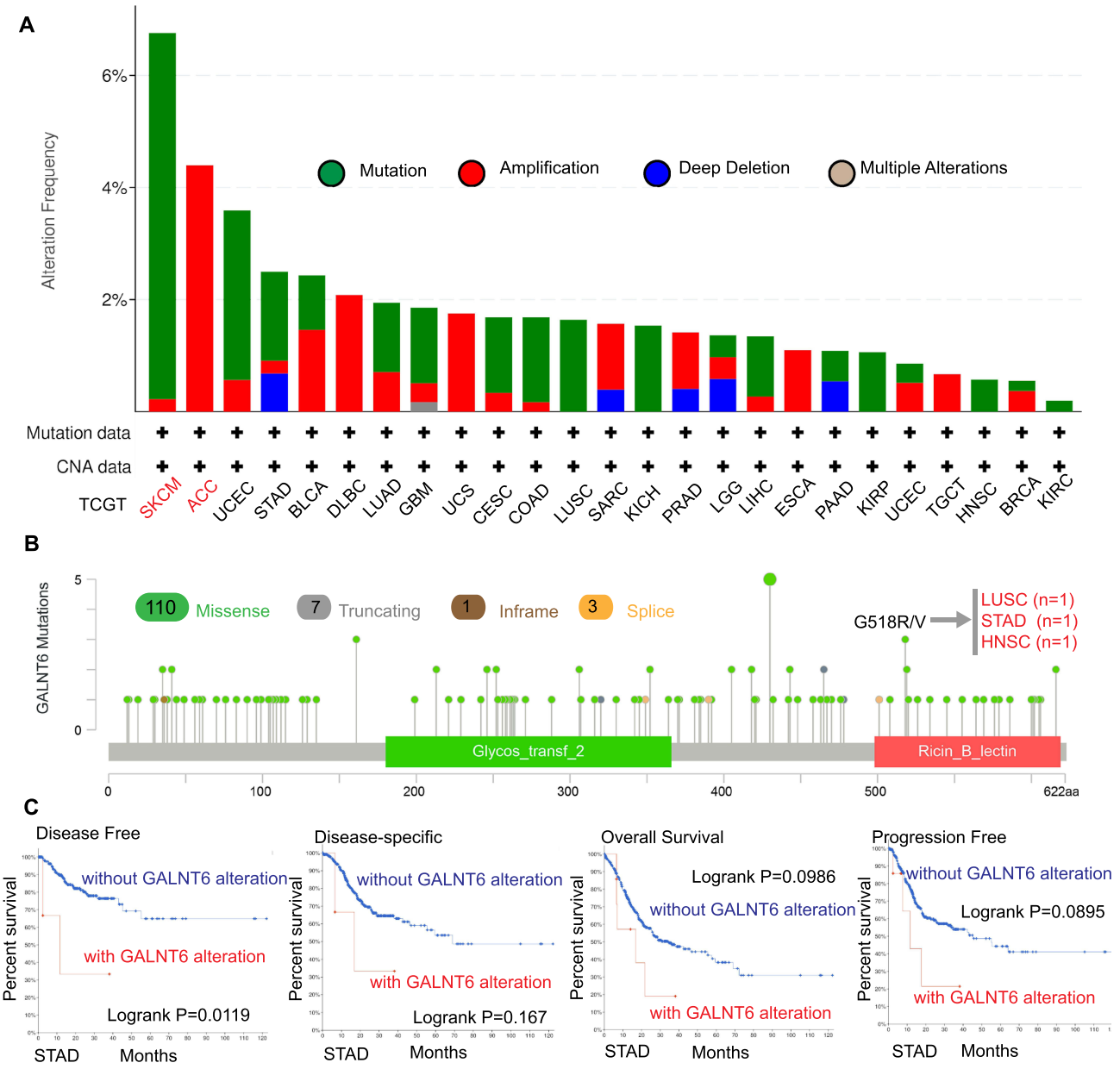

Figure 5 The genetic alteration status of GALNT6 in different tumor samples of the TCGA database (**A**). The types, sites, and case number of the GALTN6 genetic alteration (**B**). The association between genetic alteration of GALNT6 and the clinical survival prognosis of cases with stomach adenocarcinoma (STAD) (**C**).

negatively correlated with cancer-associated fibroblasts (Figure 6D, cor = −0.443, P = 1.85e−08), based on the MCPCOUNTER algorithm.

Enrichment Analysis of GALNT6-Related Partners

To further investigate the molecular mechanism of the GALNT6 gene in tumorigenesis, we attempted to screen out the targeting GALNT6-binding proteins and GALNT6 expression-correlated genes for a series of pathway enrichment analyses. Based on the STRING tool, we obtained a total of 50 GALNT6-binding proteins, supported by experimental evidence. Figure 7A shows the interaction network of these proteins. We used the GEPIA2 tool to combine all TCGA tumor expression data and obtained the top 100 genes that correlated with GALNT6 expression. As shown in Figure 7B, GALNT6 expression level was positively correlated with that of the GRIP and coiled-coil domain containing 1 (GCC1)

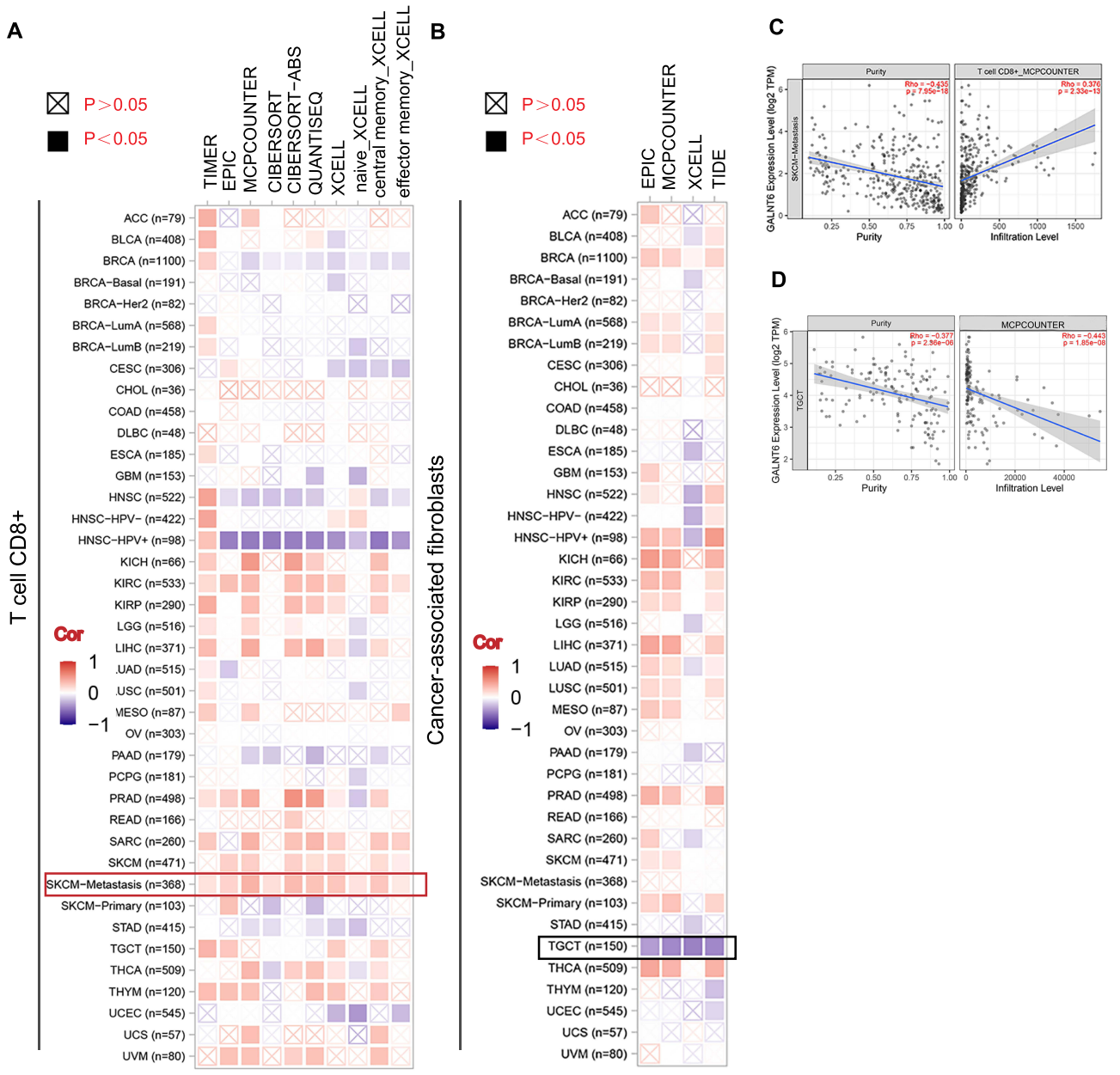

Figure 6 Correlation analysis between GALNT6 expression and immune infiltration of T-cell CD8+ (**A**) and cancer-associated fibroblasts (**B**). The scatterplot data of the GALNT6 expression level in SKCM metastasis (**C**) and TGCT (**D**).

gene (R = 0.31; P < 0.05). The corresponding heatmap data also showed almost positive correlations between GALNT6 and GCC1 genes in the majority of the detailed cancer types (Figure 7C).

We combined the two datasets to perform KEGG and GO enrichment analyses. The KEGG data of Figure 7F suggest that "protein processing in endoplasmic reticulum" might be involved in the effect of GALNT6 on tumor pathogenesis. The GO enrichment analysis data further indicated that most of these genes are linked to the pathways or cellular biology of the glycoprotein metabolic process and biological oxidation (Figure 7D and E).

GALNT6 Expression in LUAD Tissues

Finally, the expression of GALNT6 was examined by qPCR in LUAD tissue samples and the corresponding normal tissue samples. As a result, qPCR showed that GALNT6 expression was significantly higher in LUAD tissues than in normal tissues (Figure 8).

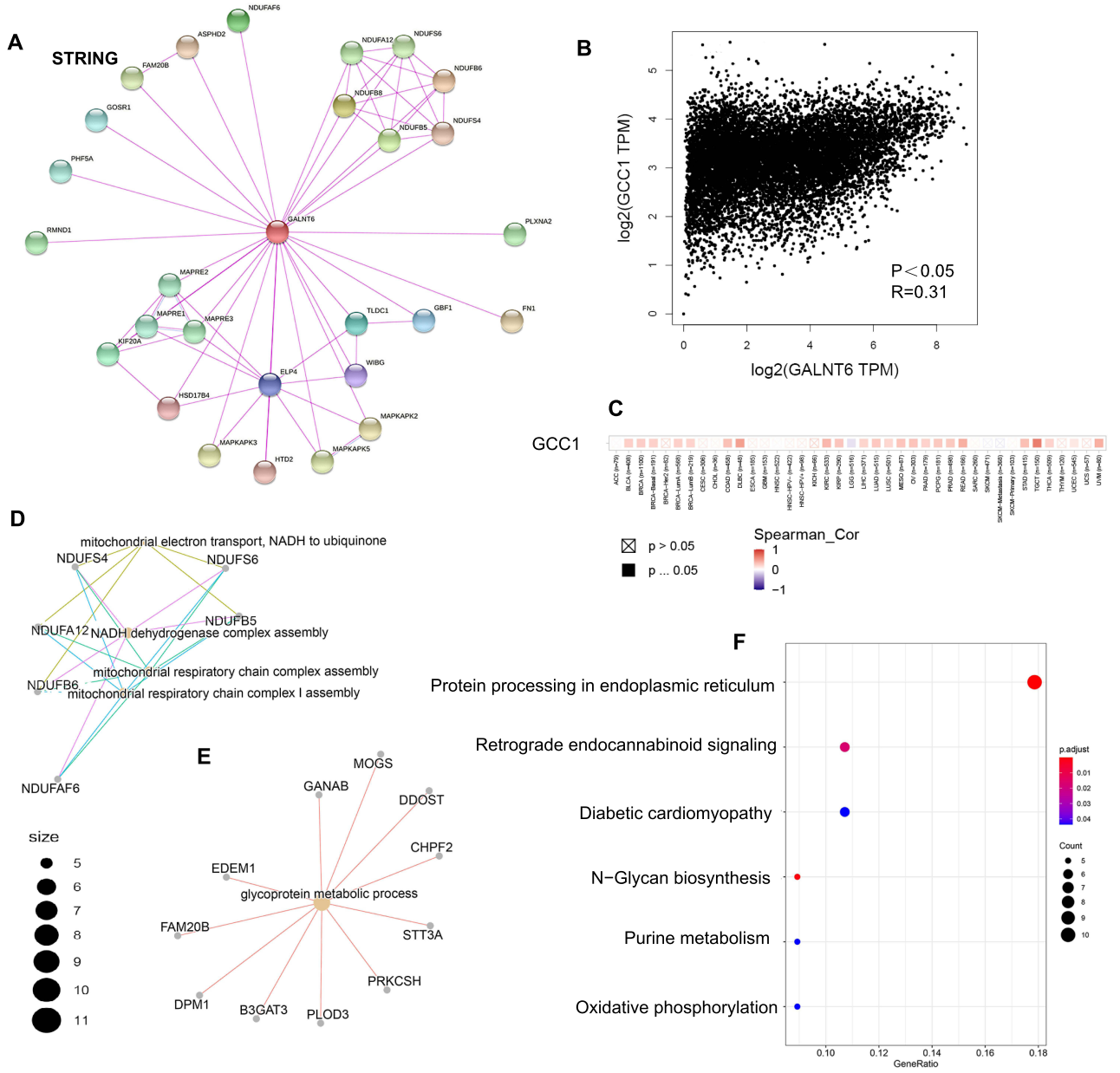

Figure 7 GALNT6-related gene enrichment analysis. (**A**) Available GALNT6-binding proteins were obtained using the STRING tool. (**B**) Expression correlation between GALNT6 and GCC1. (**C**) The corresponding heatmap data in the detailed cancer types. (**D** and **E**) The cnetplot for molecular function data in GO analysis. (**F**) KEGG pathway analysis based on GALNT6-binding and interacting genes.

Discussion

O-type glycosylation is one of a number of common modifications that have multiple functions related to the folding, stability, and targeting of various glycoproteins; O-type glycosylation is also a common post-translational protein modification observed in tumor cells.[30] O-type glycosylation is initiated by members of the GALNT family in the Golgi complex.[31] A previous study has established the important role that GALNT6 plays in O-glycosylation.[30] Multiple signaling pathways that are common in tumors are closely related to GALNT6, and the overexpression of GALNT6 is also closely related to various cancers.[2,13,32,33] It has been reported that GALNT6 silencing in SW480 cells promotes invasion, migration, and chemoresistance. GALNT6 mRNA and protein was expressed in premalignant/preinvasive lesions and decreased GALNT6 was independently associated with poor prognosis in the stage III patients in colorectal cancer.[34] GALNT6 promotes invasion and metastasis of human lung adenocarcinoma cells through O-glycosylating chaperone protein GRP78, and

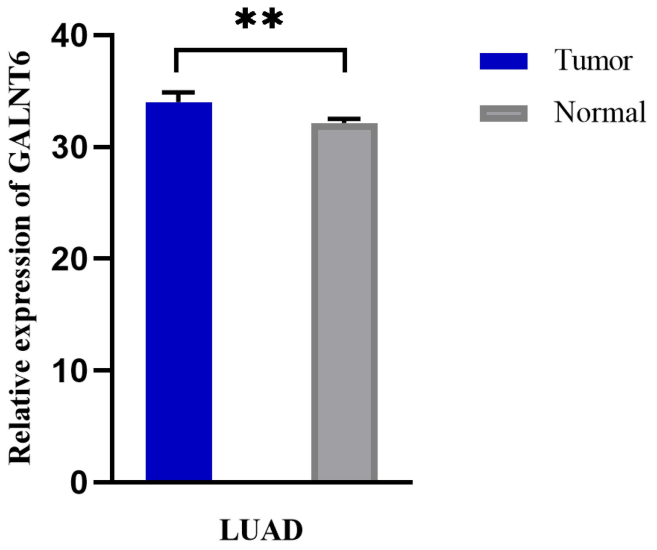

Figure 8 The mRNA relative expression of GALNT6 detected by q-PCR in LUAD and adjacent nontumor tissues. **P < 0.01.

upregulated expression of the polypeptide GALNT6 in lung adenocarcinoma is associated with lymph node metastasis and poor prognosis.[35] These findings indicate that even if there is only a small difference in the expression level of GALTN6, it may be related to the invasion, migration, and prognosis of tumors, which may also be an important finding for the therapeutics against cancer metastasis. Whether GALNT6 plays a role in the pathogenesis of different tumors through certain common molecular mechanisms remains to be answered. In our study, we examined the GALNT6 gene in different tumors based on various databases, exploring the statistical correlation of GALNT6 expression with clinical prognosis, immune cell infiltration, tumor mutational burden, and associated abnormal signaling pathways. Q-PCR was used to validated the cancer tissues and paracancer tissues differences of GALNT6 expression in LUAD.

The expression profile analysis revealed that, GALNT6 was highly expressed in 15 tumors among the 20 tumors with differential GALNT6 expression. According to the literature search results, higher GALNT6 expression has been found in breast carcinoma,[11] ovarian carcinoma,[13] lung adenocarcinoma,[35] and colon adenocarcinoma,[36] which is consistent with our study. Our study is the first to demonstrate that high GALNT6 expression occurs in BLCA, CHOL, HNSC, LUSC, PCPG, READ, UCEC, UCS, LAML, TGCT and THYM. At present, we have conducted a validation experiment by qPCR, and the results show that GALNT6 is highly expressed in lung adenocarcinoma tissues, thus supporting our findings. The small difference in GALNT6 expression in cancer and paracancer tissue may be the result of experimental conditions or experimental error. Future studies focusing on the circulating levels of GALNT6 (in blood and expiration) may help in the development of a novel diagnostic marker and provide new insights into the pathological stages of these cancers.

It has been reported that GALNT6 level is related with survival in patients with early-stage colorectal cancer, breast cancer, and ovarian cancer.[11,13,36] A high GALNT6 level is an important risk factor for predicting poor OS and PFS;[11,13] however, early-stage colorectal cancer patients with GALNT6 protein loss have been found to have significantly shorter OS than those with positive GALNT6 expression.[36] In this study, we have revealed the prognostic significance of GALNT6 in multiple cancer types, including bladder cancer, brain cancer, eye cancer, cervical squamous cell carcinoma and endocervical, esophageal carcinoma, liver hepatocellular carcinoma, pheochromocytoma and paraganglioma, and uterine corpus endometrial carcinoma, which has not been previously reported. Future studies on the relationship between GALNT6 expression and patient survival will contribute to the development of GALNT6 as a clinical prognostic biomarker for these cancers.

It is worth noting that there was a confusing set of data regarding the expression and prognosis of GALNT6 in BRCA. Previously, overexpression of GALNT6 has been found to contribute to mammary carcinogenesis.[12,37] In this study, analysis of GALNT6 with PrognoScan revealed that increased GALNT6 expression was correlated with poor prognosis for OS, DMFS, and RFS in breast cancers, which is consistent with previous reports.[32,38] However, prognostic analysis with Kaplan-Meier Plotter

revealed that high expression of GALNT6 benefited RFS in breast cancers. These discrepant GALNT6 survival prognosis results for breast cancers across databases might result from heterogeneous data collection approaches; consequently, larger sample sizes are required to confirm the role of GALNT6 in the RFS prognosis of breast cancer.

Tumor mutation burden (TMB) is an important factor influencing the efficacy of immune checkpoint inhibitors in some cancers.[39,40] Higher TMB levels are indicative of a better response to immune checkpoint inhibitors.[41] It has been reported that higher TMB was associated with poorer survival, in contrast to patients treated with immune checkpoint inhibitors, in whom higher TMB was associated with longer survival.[42] Results of prior studies have suggested that high TMB of GALNT6 may be associated with poorer outcomes in hepatocellular carcinoma and non-small cell lung cancer.[43,44] In this study, we found that STAD cases with altered GALNT6 showed a poor prognosis for DFS. On the one hand, the mutation sites are randomly distributed across the whole range of the gene, and on the other hand, there is no association between the mutations and the expression levels. The poor prognosis of the cases with the mutations is likely because the cases have a large TMB. The relationship between GALNT6 expression and prognosis and TMB could be explored in the future, which may be beneficial for survival and immunotherapy.

As the key mediator of tumor progression and treatment outcome, tumor microenvironment plays an important role in clinical prognosis and response to immunotherapy.[45] The tumor Immune Microenvironment is comprised of tumor-infiltrating lymphocytes (TILs; B and T cells) and other immune cells (macrophages, neutrophils, and dendritic cells).[46] In this study, we observed a statistically negative correlation between GALNT6 expression and the immune infiltration level of cancer-associated fibroblasts in TGCT; in addition, we observed a statistically negative correlation between GALNT6 expression and the immune infiltration level of CD8+ T-cells in SKCM metastasis. Our findings are the first to suggest an association between GALNT6 expression and immune infiltration level in certain tumors. We could not clarify the effect of GALNT6 on cancers through immune infiltration. Future prospective studies focusing on GALNT6 expression and immune infiltration in a cancer population could help provide a definitive answer.

We integrated the information on GALNT6-binding components and GALNT6 expression-related genes across all tumors for a series of enrichment analyses and identified the potential impact of "protein processing in the endoplasmic reticulum", "the glycoprotein metabolic process", and "biological oxidation" in the etiology or pathogenesis of cancers. In addition to this, GALNT6 may promote the occurrence, development and poor prognosis of cancer by activating other oncogenic pathways such as EGFR signaling pathway and beta-catenin signaling pathway.[2,11] Future in-vitro experiments on GALNT6 expression and the etiology or pathogenesis of cancers.

Although our approach in the current study has improved our understanding of the oncogenic role of GALNT6, there were some limitations. First, a large proportion of the microarray and sequencing data was collected by analyzing tumor tissue information. Thus, GALNT6 total protein and correlation between GALNT6 expression and the pathological stages of cancers could only be observed in sectional tumors; however, the tumor types were incomplete in the public database. Second, in this study multiple datasets were used for analysis, which may cause unavoidable batch effects that cannot be removed. Third, the lack of complete clinical data from databases may affect the results; therefore, the statistical power may not be high. This study was based on bioinformatics and q-PCR techniques to analyze the results of LUAD patient tissues. Thus, further improvement of tumor types, sample size, sequencing data, and clinical information is essential. Further studies on GALNT6 are needed, exploring direct mechanisms at the cellular and molecular levels, and research on clinical correlation with cancers is needed.

Conclusion

Taken together, our first pan-cancer analyses of GALNT6 indicated statistical correlations between GALNT6 expression and clinical prognosis, and immune cell infiltration. It also clarified the influence of tumor mutation burden in GALNT6 on GALTN6 prognosis and identified the functional pathways that involve GALNT6.Thus, our research provides an accurate and detailed reference to better understand the role of GALNT6 in tumor genesis, prognosis and its role in future immunotherapy. The study sheds new light on the role of GALNT6 in cancer, but further research is needed to confirm these findings and reveal its specific mechanism of action. More experimental studies could be conducted to explore how GALNT6 affects the growth and differentiation of cancer cells, and how it affects immune infiltration. In addition, clinical studies may also be conducted to evaluate the potential of GALNT6 as a biomarker or therapeutic target.

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Disclosure

The authors report no conflicts of interest in this work.

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