Observational Study

Medicine

OPEN

Biological and clinical significance of the *glypican-3* gene in human lung adenocarcinoma

An *in silico* analysis

Raihan Rahman Imon, BSc[a,b], Sharmin Aktar, BSc[a,c], Niaz Morshed, BSc[a,d], Suza Mohammad Nur, PhD[e], Rumana Mahtarin, BSc[a,f], Farazi Abinash Rahman, BSc[a,b], Md. Enamul Kabir Talukder, BSc[a,b], Rahat Alam, BSc[a,b], Tomasz M. Karpiński, PhD[g], Foysal Ahammad, MSc[a,h,*], Mazin A. Zamzami, PhD[e,i], Shing Cheng Tan, PhD[j]

Abstract

Glypican-3 (GPC3), a membrane-bound heparan sulfate proteoglycan, has long been found to be dysregulated in human lung adenocarcinomas (LUADs). Nevertheless, the function, mutational profile, epigenetic regulation, co-expression profile, and clinicopathological significance of the *GPC3* gene in LUAD progression are not well understood. In this study, we analyzed cancer microarray datasets from publicly available databases using bioinformatics tools to elucidate the above parameters. We observed significant downregulation of *GPC3* in LUAD tissues compared to their normal counterparts, and this downregulation was associated with shorter overall survival (OS) and relapse-free survival (RFS). Nevertheless, no significant differences in the methylation pattern of *GPC3* were observed between LUAD and normal tissues, although lower promoter methylation was observed in male patients. *GPC3* expression was also found to correlate significantly with infiltration of B cells, CD8+, CD4+, macrophages, neutrophils, and dendritic cells in LUAD. In addition, a total of 11 missense mutations were identified in LUAD patients, and ~1.4% to 2.2% of LUAD patients had copy number amplifications in *GPC3*. Seventeen genes, mainly involved in dopamine receptor-mediated signaling pathways, were frequently co-expressed with *GPC3*. We also found 11 TFs and 7 miRNAs interacting with GPC3 and contributing to disease progression. Finally, we identified 3 potential inhibitors of GPC3 in human LUAD, namely heparitin, gemcitabine and arbutin. In conclusion, GPC3 may play an important role in the development of LUAD and could serve as a promising biomarker in LUAD.

Abbreviations: CNAs = copy number alterations, GEPIA2 = Gene Expression Profiling Interactive Analysis 2, GPC3 = Glypican-3, LUAD = lung adenocarcinoma, miRNAs = MicroRNAs, NSCLC = non-small cell lung cancer, OS = overall survival, RFS = relapse-free survival, TCGA = The Cancer Genome Atlas, TFs = transcription factors, Wnt = Canonical Wnt signaling pathway.

Keywords: bioinformatics, biomarker, gene expression, Glypican-3, lung adenocarcinoma

1. Introduction

Lung cancer is one of the most common malignancies and is the leading cause of cancer-related deaths worldwide.[1–4] Non-small cell lung cancer (NSCLC), which accounts for approximately 84% of all lung cancer cases,[5,6] is divided into 3 histological subtypes: adenocarcinoma, squamous cell carcinoma, and large cell carcinoma.[3] Among these subtypes, lung adenocarcinoma (LUAD) is the most commonly diagnosed subtype,[7] accounting for more than 50% of all NSCLC and approximately 40% of all lung cancer cases, with a dismal 5-year overall survival (OS) rate of 4% to 17%.[8–11] Despite extensive research and improved

RRI, SA, and NM contributed equally to this work.

This research was funded by the Research University Grant of Universiti Kebangsaan Malaysia (No. GUP-2020-076). The APC was funded by King AbdulAziz University.

The authors have no conflicts of interest to disclose.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Supplemental Digital Content is available for this article.

[a] Laboratory of Computational Biology, Biological Solution Centre (BioSol Centre), Jashore, Bangladesh, [b] Department of Genetic Engineering and Biotechnology, Faculty of Biological Science and Technology, Jashore University of Science and Technology, Jashore, Bangladesh, [c] Department of Microbiology, Faculty of Biological Science, University of Dhaka, Dhaka, Bangladesh, [d] Department of Pharmacy, Faculty of Biological Science, University of Dhaka, Dhaka, Bangladesh, [e] Biochemistry Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia, [f] Department of Biochemistry and Molecular Biology, Shahjalal University of Science and Technology, Sylhet, Bangladesh, [g] Chair and Department of Medical Microbiology, Poznań University of Medical Sciences, Wieniawskiego, Poland, [h] Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia, [i] Centre of Artificial Intelligence for Precision Medicines, King Abdulaziz University, Jeddah, Saudi Arabia, [j] UKM Medical Molecular Biology Institute, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia.

**Correspondence: Shing Cheng Tan, UKM Medical Molecular Biology Institute, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia (email: sctan@ukm.edu.my).*

How to cite this article: Imon RR, Aktar S, Morshed N, Nur SM, Mahtarin R, Rahman FA, Talukder MEK, Alam R, Karpiński TM, Ahammad F, Zamzami MA, Tan SC. Biological and clinical significance of the glypican-3 gene in human lung adenocarcinoma: An in silico analysis. Medicine 2023;102:45(e35347).

Received: 14 June 2023 / Received in final form: 12 August 2023 / Accepted: 1 September 2023

http://dx.doi.org/10.1097/MD.0000000000035347

therapeutic approaches, LUAD is often diagnosed at a late stage with lymph node or distant metastases.[8,12] Therefore, there is an urgent need to search for potential biomarkers with accurate specificity and high sensitivity for effective early diagnosis and personalized treatment of LUAD.

Glypican-3 (GPC3) belongs to a family of membrane-bound, heparan sulfate proteoglycans. This protein, also known as GTR2-2, SGBS1, DGSX, SGB, OCI-5, MXR7, SDYS, and SGBS, was first discovered in 1996 in patients with Simpson-Golabi-Behmel syndrome.[13,14] It remains attached to the outer surface of the cell membrane via a glycosyl-phosphatidylinositol lipid anchor[15] and modulates the signaling of heparin-binding growth factors, such as Wnts, Hedgehog proteins, fibroblast growth factors, and bone morphogenic proteins,[16–19] thereby regulating cell proliferation, differentiation, adhesion, migration and apoptosis.[20–24] Depending on the cellular context, GPC3 can activate or inhibit these signaling pathways. In general, upregulation of GPC3 suppresses cell proliferation in tissues where Hh signaling predominantly modulates proliferation. In contrast, overexpression of GPC3 triggers cell proliferation in tissues where proliferation is controlled by the canonical Wnt signaling pathway.[25,26] In addition, GPC3 expression also varies according to tissue type. High expression of GPC3 is reported in cancer tissues where it is normally suppressed, while low expression is observed in tumors derived from tissues where it is normally expressed.[27,28] This dual role, in which GPC3 can both inhibit and promote cell proliferation depending on the context, highlights its complex regulatory dynamics. The expression of GPC3 also differs according to lung cancer subtypes. It has been reported that the expression of GPC3 is increased in lung squamous cell carcinoma but decreased in LUAD.[20,24] The protein has also been recognized as a tumor suppressor[29] and its overexpression is defined as a promising predictor of prolonged survival in patients with LUAD.[30] However, despite these intriguing observations, a comprehensive characterization of *GPC3* expression in LUAD using advanced data mining tools is lacking. Such tools can provide unprecedented insights into gene expression patterns, uncover associations, and generate hypotheses that traditional studies may overlook.

In addition, while numerous studies, including a comprehensive review by Abbasian et al,[31] have highlighted genomic and proteomic biomarkers for the early detection and treatment of NSCLC, the role and importance of *GPC3* in LUAD remain underexplored. Therefore, in the current study, we sought to analyze publicly available cancer microarray datasets to investigate the mRNA expression, methylation pattern, and mutational profile (including copy number alterations; CNAs) of *GPC3* in LUAD, as well as the relationship among these variables. We also evaluated the role of *GPC3* expression in immunological infiltration in LUAD and investigated the potential prognostic value of *GPC3* in predicting survival of LUAD patients. Besides, we analyzed the co-expression profile of *GPC3* and performed gene ontology and pathway enrichment analysis to reveal the role of *GPC3*-associated pathways in LUAD carcinogenesis. MicroRNAs (miRNAs) and transcription factors (TFs) associated with *GPC3* were also analyzed, and drugs that could target this potential tumor suppressor gene were identified.

2. Materials and methods

2.1. Ethics statement

Ethical approval was not required for this study as it is an *in silico*/data mining analysis that did not involve obtaining data from humans or patients.

2.2. Analysis of GPC3 gene expression

Datasets were extracted from the Oncomine (https://www.oncomine.org/resource/login.html),[32,33] Gene Expression Profiling Interactive Analysis 2 (GEPIA2) (http://gepia2.cancer-pku.cn/#index),[34] and UALCAN (http://ualcan.path.uab.edu/index.html)[35] databases in January 2022 and used for analysis of the *GPC3* mRNA expression in different cancer types and the corresponding healthy tissues. The threshold for analysis in Oncomine was set as follows: *P* value: 1e-4, fold change: 2, gene ranking: 10%. In GEPIA2 and UALCAN databases, the expression patterns of *GPC3* in *33* different cancers included in The Cancer Genome Atlas (TCGA) data were compared with paired normal tissues. *GPC3* expression analysis was performed using default settings in these databases. Further, the mRNA expression level of *GPC3* in different subtypes of lung cancer was compared to that of normal lung tissues using the same threshold settings.

2.3. Evaluation of the correlation between GPC3 Expression and Patient Survival

We performed patient survival analysis to evaluate the clinical significance of *GPC3* expression in LUAD Multivariate survival analysis by Cox regression using PrognoScan[36] was performed to evaluate OS and relapse-free survival (RFS) in LUAD patients with high and low *GPC3* expression. A P value < .05 was considered statistically significant. In addition, an integrated meta-analysis was performed to combine data from different cancer microarray datasets, to evaluate the translational relevance of *GPC3* expression with survival in LUAD patients. GraphPad Prism 5.0 was then used to generate the forest plots of the meta-analysis.

2.4. Analysis of GPC3 methylation

The promoter methylation of *GPC3* in LUAD patients was assessed in the TCGA datasets using the UALCAN database with default settings.[35] The effect of *GPC3* promoter methylation on its transcript expression in LUAD was then analyzed according to the different clinicopathological characteristics of the patients, including the sample types (normal vs. tumor), individual cancer stages, race, sex, age, and smoking habits.

2.5. Correlation of GPC3 expression with immunological infiltration

The correlation between *GPC3* expression and immunological infiltration was investigated using TIMER (https://cistrome.shinyapps.io/timer/),[37] which provides information on the infiltration of B-cells, T-cells (CD4+ and CD8+), neutrophils, macrophages, and dendritic cells in various types of tumor.

2.6. Analysis of GPC3 mutations and CNAs

The mutations and CNAs of the *GPC3* gene in LUAD were analyzed using the cBioPortal platform (http://www.cbioportal.org/).[38,39] The location, frequency, and the pattern of mutations were assessed in 3 TCGA datasets that contain information on LUAD. CNAs derived from RNA-seq data were plotted with the mRNA expression data using cBioPortal. Correlation between *GPC3* mRNA expression and CNAs was measured using one-way ANOVA in GraphPad Prism 5.0 version.

2.7. Correlation between GPC3 and other common biomarkers

The Oncomine database was used to identify genes co-expressed with *GPC3* in LUAD. The correlation between the expression of *GPC3* and the top 10 genes in LUAD patients was examined

using TCGA data through the Lung Cancer Explorer server.[40] The correlation between top 10 genes and tumor purity was investigated using TIMER to confirm whether these genes are potentially correlated biomarkers with GPC3.

2.8. Profiling of GPC3 pathways and GO

The PANTHER web portal is a widely used comprehensive enrichment analysis server that can comparing multiplegenomics datasets. The common signaling pathways and GO of *GPC3* and the co-expressed genes were analyzed using PANTHER (http://pantherdb.org/)[41] and the results were presented as a series of pie charts using the Microsoft Excel. For both cases, we considered the cox *P* value less than .05 as statistically significant.

2.9. Identification of miRNAs and TFs interacting with GPC3

The miRNAs and TFs interacting with *GPC3* were identified using NetworkAnalyst v3.0.[42] The constructed miRNAs and TFs interaction network was redesigned using Cytoscape v3.7.[43] The expression of *GPC3*-related miRNAs in LUAD was then analyzed in TCGA datasets using UALCAN with default settings.

2.10. Identification of GPC3-targeting drugs

Drugs that can target the GPC3 protein were identified in the DSigDB database via Enrichr.[44] A cox *P* value of < .05 was used to indicate statistical significance.

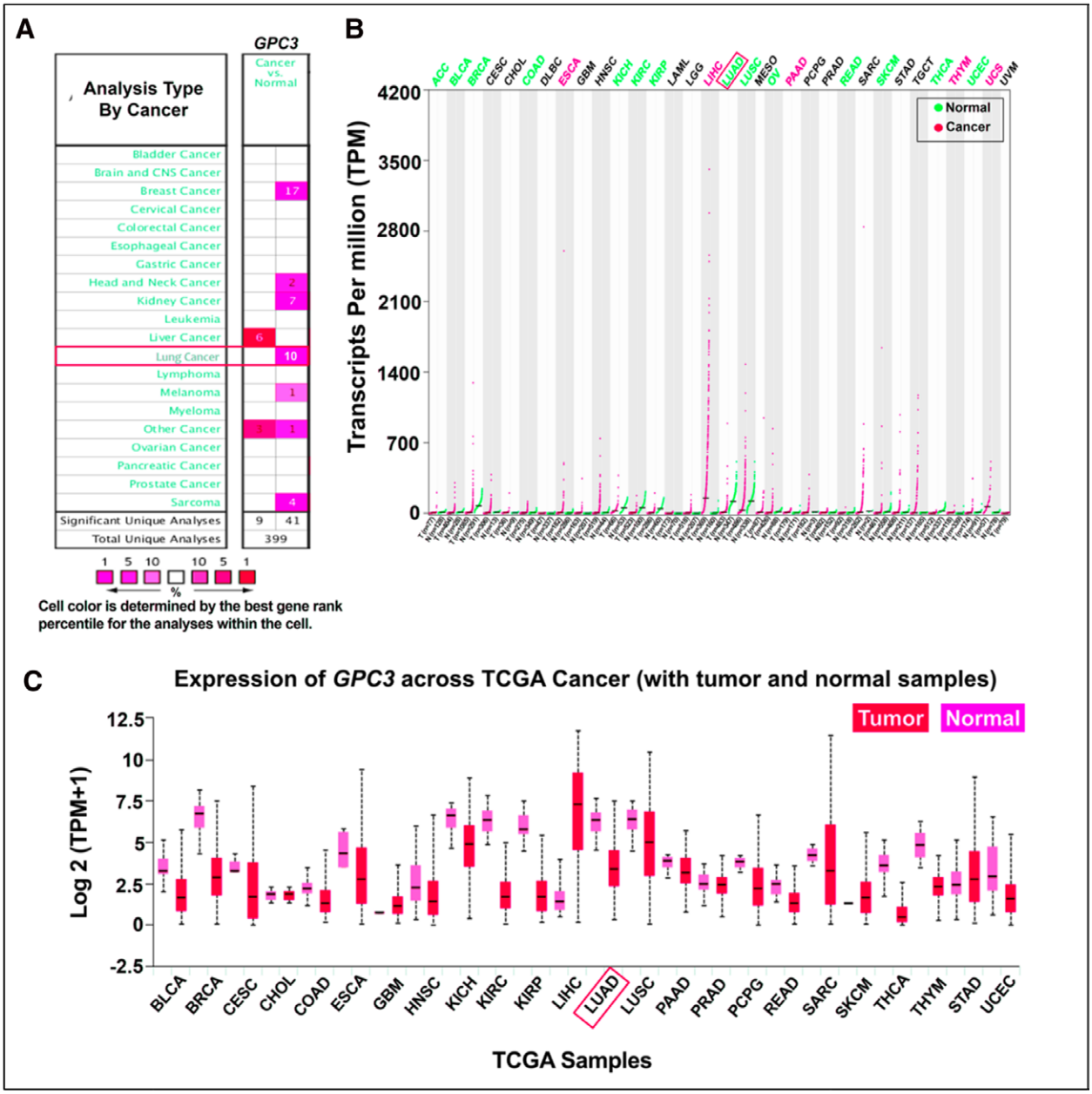

Figure 1. *GPC3* transcription and expression levels in different types of cancer. The expression of *GPC3* was ostensibly lower in lung cancer tissues compared to the normal counterparts. (A) The graphical representation generated from Oncomine shows the comparison between *GPC3* mRNA expression in cancer tissues (left column) and normal tissues (right column). Overexpression was indicated with red color and underexpression with blue, and the numbers indicate statistically significant datasets. (B) Data retrieved from the Cancer Genome Atlas via GEPIA2 show the *GPC3* gene expression profile in 33 types of human cancer and the paired normal tissues. (C) *GPC3* mRNA expression in different cancer types derived from the UALCAN database. Boxes represent median and 25th and 75th percentiles. Boxplots represent outliers. Blue boxes represent normal tissues and red boxes represent tumor tissues.

3. Results

3.1. Expression of GPC3 in different cancers

Data on the expression pattern of *GPC3* in different cancers were mined using 3 different bioinformatics databases, namely Oncomine, GEPIA2, and UALCAN. In all 3 databases, we found discrepancies in the mRNA expression of *GPC3* between cancer tissues and their healthy counterparts. In Oncomine, using the threshold parameters of fold-change > 2, *P* value < 1E-4 and top 10% gene ranking, we found approximately fifty significant datasets that revealed alterations in *GPC3* expression in various cancer tissues compared to their normal counterparts. These

Table 1

Comparison of *GPC3* mRNA expression levels in different lung cancer subtypes with their non-cancerous counterparts using Oncomine datasets.

Dataset	Cancer type (vs normal)	Reporter Id	*P* value	Fold change
Beer Lung	LUAD	L47125_s_at	8.33E-20	–34.866
Bhattacharjee Lung	LUAD	39350_at	6.62E-09	–18.064
Bhattacharjee Lung	Lung carcinoid tumor	39350_at	2.39E-12	–59.581
Su Lung	LUAD	209220_at	3.48E-13	–4.352
Selamat Lung	LUAD	ILMN_2051972	8.82E-33	–5.006
Stearman Lung	LUAD	39350_at	7.80E-09	–6.549
Landi Lung	LUAD	209220_at	5.22E-22	–3.785
Okayama Lung	LUAD	209220_at	6.70E-16	–3.738
Garber Lung	LUAD	IMAGE:878564	5.97E-05	–3.315
Hou Lung	LUAD	209220_at	4.29E-15	–4.581

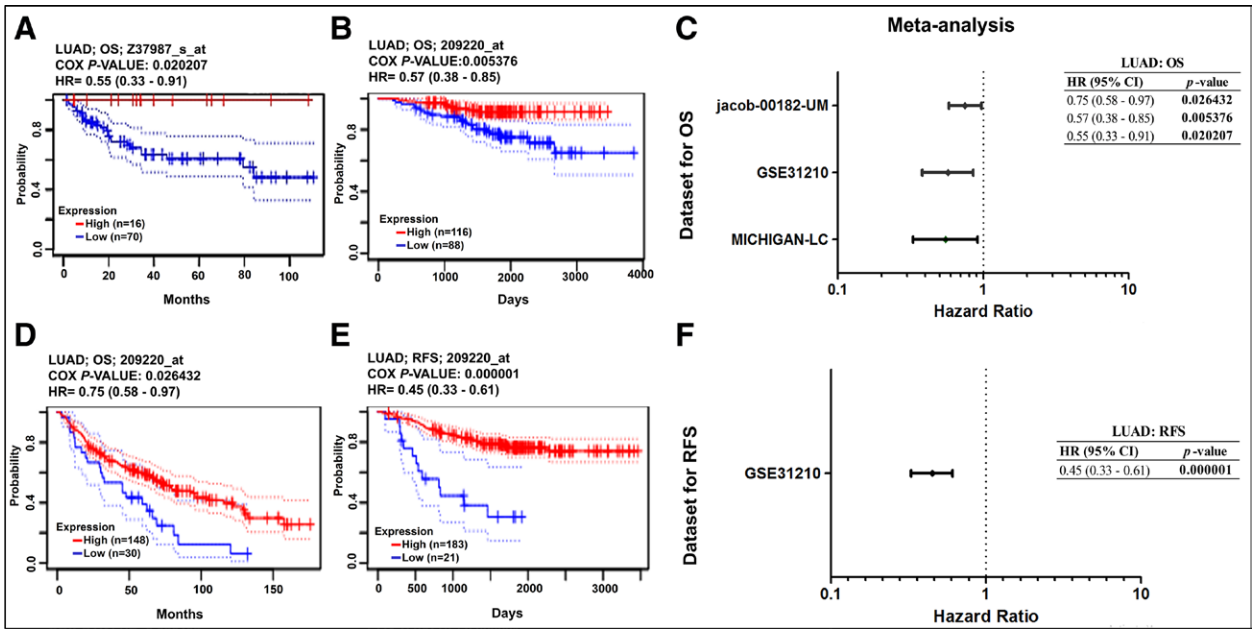

Figure 2. Correlation between *GPC3* expression and survival of LUAD patients. (A, B, and D) Survival curves for OS, and (E) survival curves for RFS. High and low GPC3 expression are indicated by red and blue, respectively. (C and F) Meta-analysis of the correlation between mRNA expression level of *GPC3* gene and OS and RFS in LUAD patients.

Table 2

Hazard ratio of the correlation between *GPC3* mRNA expression and LUAD patient survival in different datasets.

				Multivariate analysis	
Dataset	**Survival type**	**Probe ID**	**N**	**Hazard ratio**	***P* value**
jacob-00182-CANDF	OS	209220_at	82	0.97 [0.67–1.39]	.865794
HARVARD-LC	OS	39350_at	84	0.78 [0.55–1.10]	.161652
jacob-00182-HLM	OS	209220_at	79	0.67 [0.44–1.02]	.065004
MICHIGAN-LC	OS	Z37987_s_at	86	0.55 [0.33–0.91]	.020207
jacob-00182-MSK	OS	209220_at	104	0.70 [0.47–1.04]	.075571
GSE13213	OS	A_24_P57631	117	0.95 [0.79–1.13]	.561613
GSE31210	OS	209220_at	204	0.57 [0.38–0.85]	.005376
GSE31210	RFS	209220_at	204	0.45 [0.33–0.61]	.000001
jacob-00182-UM	OS	209220_at	178	0.75 [0.58–0.97]	.026432

analyses revealed that *GPC3* transcript expression was comparatively downregulated in lung, breast, head and neck, and kidney cancers, as well as in melanoma and sarcoma (Fig. 1A). Of note, the decrease in *GPC3* expression was greater in lung cancer than in all other cancers (Fig. 1A).

We further examined *GPC3* transcript expression in 33 types of human cancer and their corresponding normal tissues using GEPIA2 (Fig. 1B). We found that most cancer types (N = 23), including lung cancer, had lower *GPC3* expression compared to their normal counterparts. Subsequent analysis using the UALCAN database also showed that *GPC3* was underexpressed in several cancer types, including cancers of the lung, bladder, breast, colon, esophageal, head and neck, kidney, and thyroid (Fig. 1C).

Taken together, all of the above data suggest an apparent decreased expression of *GPC3* in various cancers. Moreover, the expression of *GPC3* transcripts was ostensibly lower in lung cancer in all 3 databases compared to normal lung tissues, suggesting the tumor-suppressive function of the *GPC3* gene in normal lung tissue and that its loss of function may lead to lung tumorigenesis.

3.2. GPC3 expression in different subtypes of lung cancer

To examine the mRNA expression levels of *GPC3* in the different lung cancer subtypes, each dataset in the Oncomine database was analyzed individually (Figure S1A–F, Supplemental Digital Content, http://links.lww.com/MD/K465). Of 10 significant datasets, 9 showed lower *GPC3* expression in LUAD (with the remaining dataset showed *GPC3* underexpression in lung carcinoid tumors). The detailed information of these datasets with the corresponding statistical significance and fold change information are shown in Table 1. In view of this result, we focused our subsequent analyses on LUAD only.

Table 3

Correlation between the mRNA expression and promoter methylation level of *GPC3* and the clinicopathological parameters of LUAD.

Parameters	mRNA expression	N	*P* value	Promoter methylation	N	*P* value
Sample type						
Normal	↑	59		↓	32	
Primary tumor	↓	515	<1E-12	↑	473	3.51E-01
Cancer stage						
Normal	↑	59		↓	32	
Stage 1	↓	277	1.89E-15	↑	260	1.54E-01
Stage 2	↓	125	<1E-12	↑	115	9.01E-01
Stage 3	↓	85	<1E-12	↑	73	2.53E-01
Stage 4	↓	28	3.01E-03	↑	20	5.89E-01
Ethnicity						
Normal	↑	59		↓	32	
Caucasian	↓	387	<1E-12	↑	366	2.59E-01
African-American	↓	51	1.62E-12	↑	52	6.38E-01
Asian	↓	8	3.17E-04	↑	6	9.54E-01
Sex						
Normal	↑	59		↓	32	
Male	↓	238	<1E-12	↑	219	5.45E-04
Female	↓	276	1.11E-16	↑	254	1.62E-05
Age						
Normal	↑	59		↓	32	
21–40 yr	↓	12	1.62E-12	↑	5	2.47E-01
41–60 yr	↓	90	2.14E-12	↑	149	4.82E-01
61–80 yr	↓	149	<1E-12	↑	276	3.20E-01
81–100 yr	↓	32	9.64E-09	↑	24	9.42E-01
Smoking habit						
Normal	↑	59		↓	32	
Nonsmoker	↓	75	5.69E-12	↑	68	1.33E-02
Smoker	↓	118	1.62E-12	↑	108	5.69E-01
Reformed smoker (<15 years)	↓	135	1.03E-08	↑	152	1.26E-01
Reformed smoker (>15 years)	↓	168	<1E-12	↑	127	6.12E-01
Tumor histology						
Normal	↑	59		↓	32	
Lung adenocarcinoma-not otherwise specified	↓	320	<1E-12	↑	309	2.92E-01
Lung adenocarcinoma mixed subtype	↓	107	<1E-12	↑	84	9.35E-01
Lung clear cell adenocarcinoma	↓	2	1.28E-02	↑	65	3.83E-01
Lung solid pattern predominant adenocarcinoma	↓	5	5.61E-11			
Lung acinar adenocarcinoma	↓	18	1.80 E-05			
Lung bronchioloalveolar carcinoma mucinous	↓	5	3.38E-11			
Mucinous carcinoma	↓	10	1.62E-12			
Lung papillary adenocarcinoma	↓	23	1.68E-10			
Lung mucinous adenocarcinoma	↑	2	6.99E-01			
Lung micropapillary adenocarcinoma	↓	3	1.63E-12			
Nodal metastasis status						
Normal	↑	59				
No regional lymph node metastasis	↓	331	1.62E-12			
Metastases in 1 to 3 axillary lymph nodes (N1)	↓	96	<1E-12			
Metastases in 4 to 9 axillary lymph nodes (N2)	↓	74	<1E-12			
Metastases in 10 or axillary lymph nodes (N3)	↓	2	1.18E-02			

Datasets from GEPIA2 (involving 483 tumors and 347 controls) and UALCAN (involving 515 tumors and 59 controls) were then used to further evaluate the expression of the *GPC3* transcript in LUAD. We obtained similar results showing underexpression of *GPC3* in LUAD (Figure S1G and H, Supplemental Digital Content, http://links.lww.com/MD/K465, respectively). Overall, mining of the gene expression data in these databases revealed that *GPC3* is downregulated in LUAD compared to healthy lung tissues.

3.3. Correlation between GPC3 expression and luad patient survival

To gain deeper insight into the correlation between *GPC3* mRNA expression and patient survival in LUAD, the PrognoScan online platform was used. Survival curves obtained from this database showed that patients with low expression of *GPC3* had worse OS and RFS than patients with high expression of this gene (Figure 2A, B, D, and E). In other words, OS and RFS were positively correlated with GPC3 expression in LUAD patients. We performed meta-analysis to combine the results from different datasets to estimate the prognostic value of *GPC3* expression (Figs. 2C and 2F). All hazard ratios (HRs) were less than 1.0, and statistical significance was observed for OS in 3 datasets (MICHIGAN-LC, GSE31210, and jacob-00182-UM) and for RFS in GSE31210 (Table 2), indicating that lower GPC3 expression is correlated with worse clinical outcome. Overall, these analyses confirmed that decreased expression of *GPC3* could confer a poor prognosis in LUAD patients.

3.4. Correlation of GPC3 expression and methylation with clinicopathological parameters of LUAD patients

The correlation between *GPC3* expression level and clinicopathological parameters of LUAD was analyzed using The Cancer Genome Atlas (TCGA) data via the UALCAN database. Data from 515 tumors and 59 controls revealed that *GPC3* was significantly downregulated in LUAD compared to the normal lung tissue, regardless of the cancer stage, ethnicity, sex, age, and smoking habits of the patients (Figure S2, Supplemental Digital Content, http://links.lww.com/MD/K466). A summary of clinicopathological parameters associated with GPC3 mRNA expression in LUAD is provided in Table 3. The analysis also showed no significant change in DNA methylation level in LUAD compared to normal counterparts (Figure S3A, Supplemental Digital Content, http://links.lww.com/MD/K467). The *GPC3* promoter was also neither hypermethylated nor hypomethylated, regardless of the cancer stage, ethnicity, and age of the patients (Figure S3B, C, and E, Supplemental Digital Content, http://links.lww.com/MD/K467). Remarkably, promoter methylation of *GPC3* was found to be lower in male patients (Figure S3D, Supplemental Digital Content, http://links.lww.com/MD/K467,).

3.5. Correlation of GPC3 expression with immunological infiltration

We investigated whether *GPC3* mRNA transcript was correlated with immunological infiltration in LUAD using TIMER.

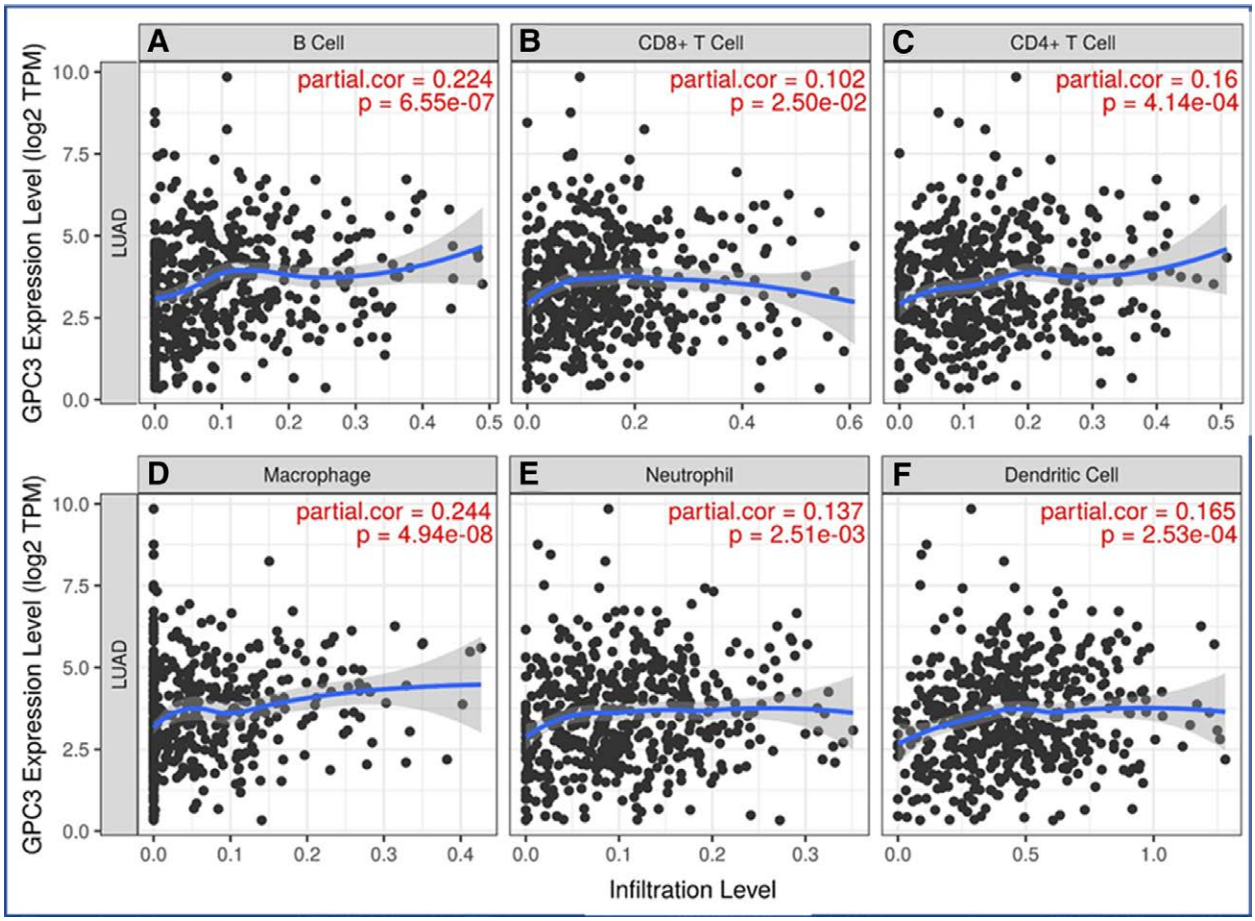

Figure 3. Correlation between *GPC3* mRNA expression and immunological infiltration in LUAD. (A) B cells, (B) CD8+, (C) CD4+, (D) macrophages, (E) neutrophils, and (F) dendritic cells.

The data showed that GPC3 was positively and significantly correlated with the infiltration of B cells, CD8+, CD4+, macrophages, neutrophils, and dendritic cells in human LUAD (Fig. 3).

3.6. CNAs and mutations of GPC3 in LUAD

To evaluate the frequency of genetic mutations and CNAs of *GPC3* in LUAD, the cBioPortal database was used. In 3 TCGA datasets reporting LUAD (N = 1382 samples), *GPC3* mutations were found in 2% of the samples, with a somatic mutation frequency of 0.8%. A total of 11 mutations (missense type only) were identified between codons 0 and 580 (Fig. 4A). These missense mutations mainly affect the glypican domain of the GPC3 protein. Approximately 1% of clinical LUAD cases had mutations in all 3 datasets (Fig. 4B). In addition,~ 1.4% to 2.2% of LUAD patients had CNAs in *GPC3*. All CNAs were copy number amplifications (Fig. 4B). The mRNA expression (RNA Seq V2) of the *GPC3* gene was also analyzed using the cBioportal web tool (Fig. 4C). No significant correlation was found between CNAs and mRNA expression of *GPC3* in LUAD patients in all 3 TCGA datasets (one-way ANOVA analysis, $P > .05$). Overall, these results suggest that the underexpression of *GPC3* in LUAD may not be related to mutations or CNAs, although these alterations were clearly affect the GPC3 protein.

3.7. Co-expression profile of GPC3 and tumor purity of correlated genes in LUAD

Next, we analyzed the Stearman dataset using Oncomine to identify genes that are co-expressed with *GPC3* in LUAD. As shown in Figures 5A, 17 genes were frequently co-expressed with *GPC3*. Among them, *FHL1* was the most prominently co-expressed gene, while *CD3* was the least. To validate this result, we further analyzed the top 10 correlated genes (*ABCA8, FHL1, FBLN5, ANGPT1, CHRDL1, ERG, SVEP1, SLIT2, MAOB,* and *AHNAK*) with GPC3 using TCGA data through the Lung Cancer Explorer platform. Here, *FHL1* and *AHNAK* emerged as the highest and lowest correlated genes, respectively, highlighting them as potentially significant correlated biomarkers (Fig. 5B). In addition, analysis using the TIMER browser

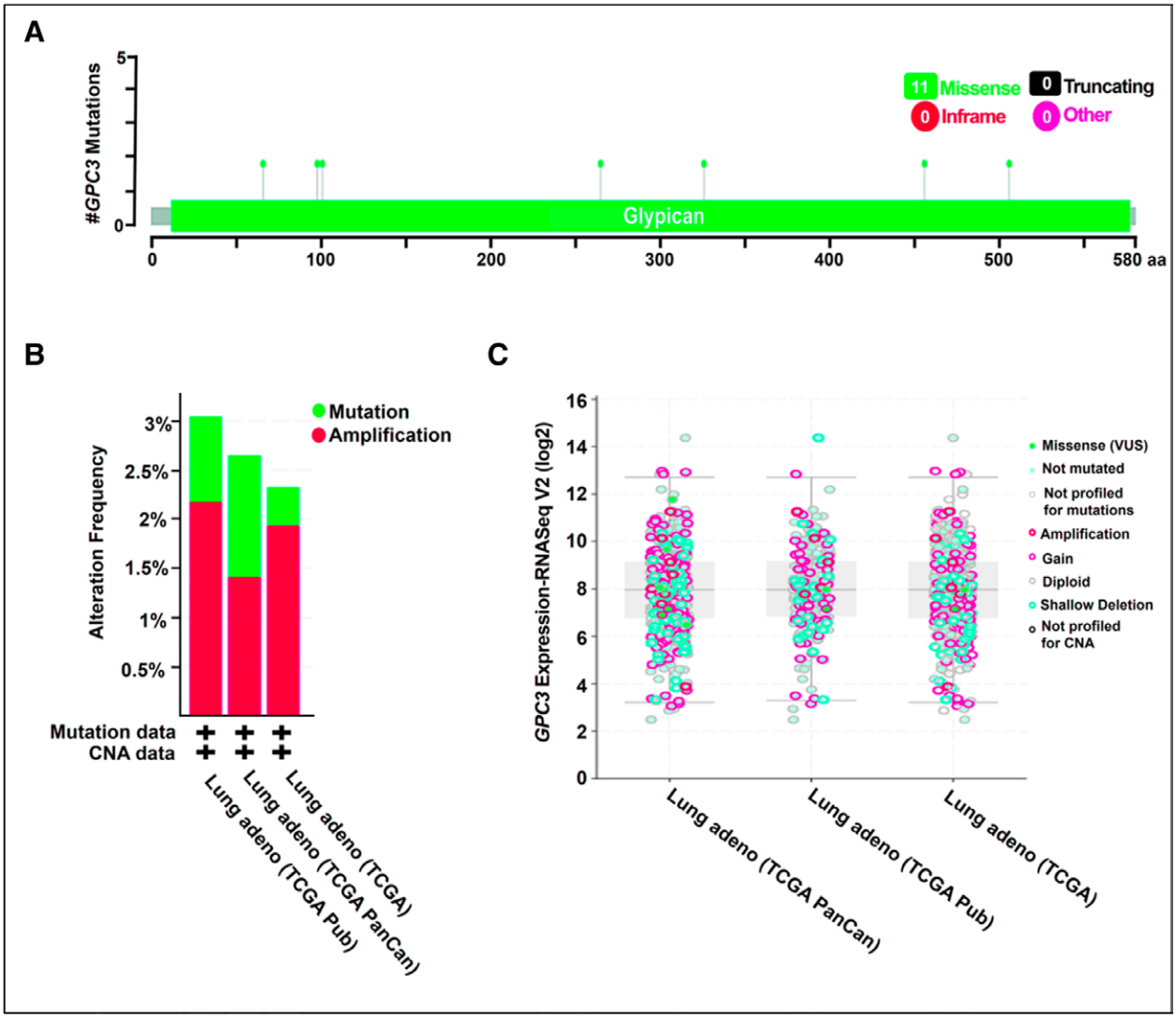

Figure 4. Frequency of mutations, CNAs, and gene expression of *GPC3* in LUAD. (A) A total of 11 mutations (missense) were located between codons 0 to 580 of *GPC3*. Lollipop plots show the type and location of mutations. (B) Frequencies of *GPC3* mutations (green bar) and CNAs (red bar) in LUAD cases. (C) The graph shows the correlation between mRNA expression of *GPC3* (RNA Seq V2) and mutations and CNAs in LUAD. Expression frequencies show missense mutations (green), no mutations (blue), no profiled mutations (white), and different types of CNAs, including amplification, gain, diploid, shallow deletion, and non-profiled CNAs.

revealed that *GPC3* was highly co-expressed with *FHL1*, while its co-expression with AHNAK was low, desoute achieving significant *P* value (Table S1, Supplemental Digital Content, http://links.lww.com/MD/K464). Finally, we checked tumor purity as a validation metric for biomarkers using the TIMER browser. The data showed that all the genes were positively and significantly correlated with the infiltration level of tumor purity in human LUAD (Fig. 5C). These observations suggest that the co-expression of the top 10 genes serves as closely correlated biomarkers, and their collective influence may have an impact on specific signaling pathways or biological functions in LUAD.

3.8. Pathway and gene ontology analyses of GPC3 and its co-expressed genes

To understand the biological role and possible signaling pathways for the 17 genes positively co-expressed with *GPC3* in LUAD, gene ontology (GO) and pathway enrichment analyses were performed using the PANTHER database. Pathway analysis showed that *GPC3*, together with the cluster of co-expressed genes, affected 14 cellular signaling pathways and was mainly involved in dopamine receptor-mediated signaling pathways (Fig. 6). Besides, GO analysis showed that the biological processes of genes co-expressed with *GPC3* were associated with cellular processes and biological regulation (Fig. 7A). Analysis of the GO molecular function revealed that the binding and catalytic activities were mainly associated with these correlated genes (Fig. 7B). In addition, analysis of GO cellular components showed that the *GPC3* gene clusters were mainly localized on cells, cell parts, and cell membranes (Fig. 7C).

3.9. Interactions of GPC3 with miRNAs and TFs

Using NetworkAnalyst v3.0, we found 11 TFs and 7 miRNAs with degree centrality ≥ 1 and betweenness centrality ≥ 100 that could affect the expression pattern of *GPC3* and contribute to disease progression (Figs. 8A and 8B). The miRNAs and TFs are hsa-mir-140-5p, hsa-mir-15a-5p, hsa-mir-15b-5p, hsa-mir-16-5p, hsa-mir-34a-5p, hsa-mir-29a-3p, and hsa-mir-19a-3p and NKX2-5, NKX3-2, FOXC1, GATA2, PPARG, TFAP2A, NFKB1, TP53, TFAP2C, ELF5, and NFYB (Figs. 8A and 8B).

3.10. Expression analysis of GPC3-associated miRNAs in LUAD

The expression of miRNAs associated with GPC3 in LUAD was examined in the UALCAN database. The hsa-mir-34a-5p,

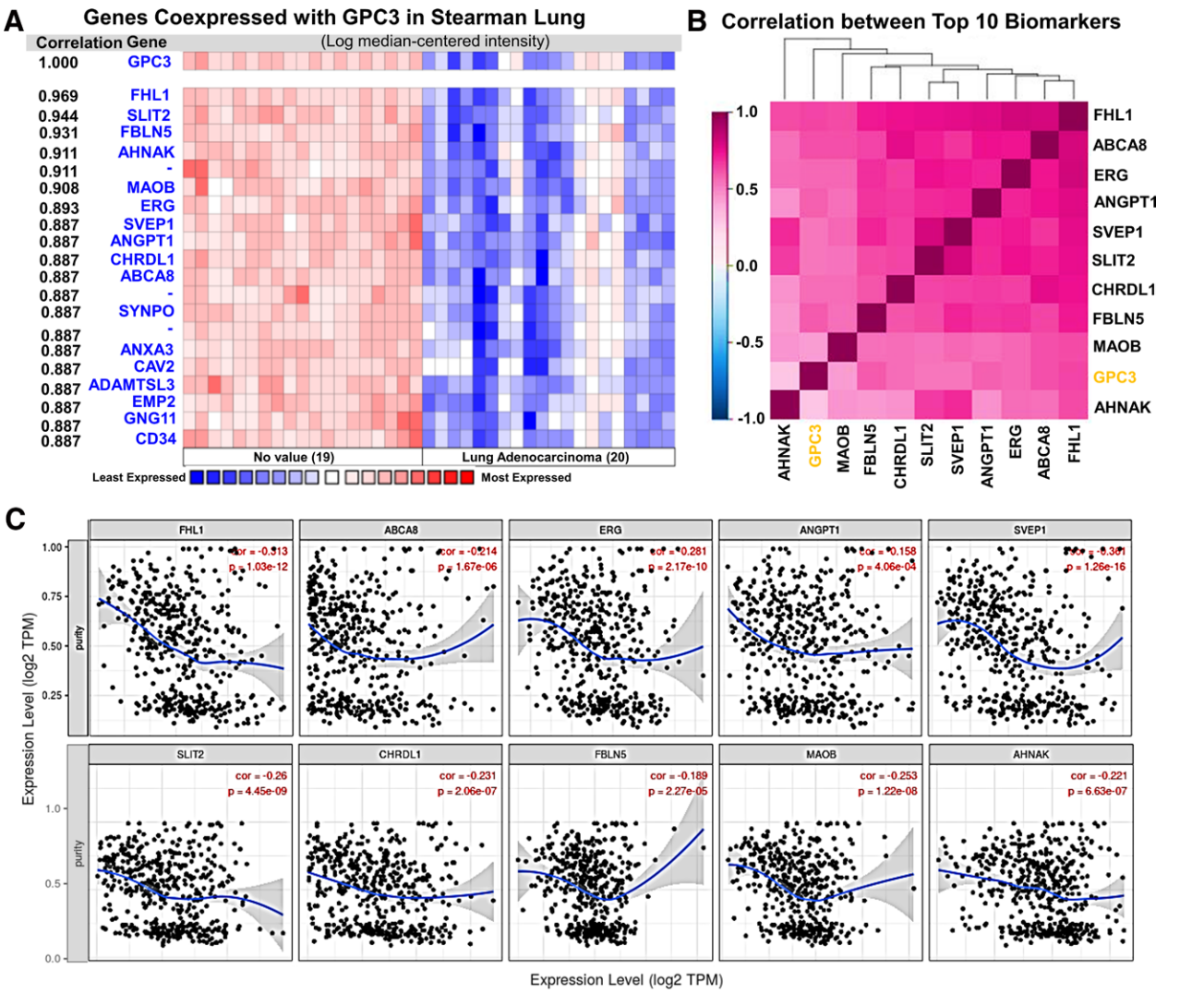

Figure 5. Co-expression profile of *GPC3* in LUAD. (A) The co-expression gene profile of *GPC3* derived from the Oncomine database. (B) Co-expression analysis of transcript levels of *GPC3* and *FHL1* in LUAD (performed with GEPIA2 based on TCGA data). (C) Heat map of *GPC3* and *FHL1* mRNA expression for LUAD in the TCGA database generated using UCSC Xena. (D) Co-expression analysis between *GPC3* and *FHL1* mRNA expression in LUAD using UCSC Xena web.

hsa-mir-19a-3p, hsa-mir-15a-5p, and hsa-mir-16-2p showed high expression in LUAD compared to healthy tissues. In contrast, the expression of hsa-mir-15b-5p was low in LUAD compared to normal tissues (Fig. 9).

3.11. Identification of GPC3-targeting drugs

The DSigDB database was used to identify drugs that may target GPC3. A total of 3 compounds were found to be potential inhibitors of GPC3 in human LUAD, namely heparitin, gemcitabine, and arbutin (Table 4).

4. Discussion

In this study, we demonstrated that *GPC3* expression was significantly downregulated in LUAD and was positively correlated to patient survival. To our knowledge, this is the first comprehensive computational analysis using multiple databases that identified *GPC3* as a tumor suppressor gene in LUAD and could be used as a novel therapeutic target for the treatment of LUAD. Our study introduces novel insights into the role of *GPC3* in LUAD, a topic not addressed in the broader review of NSCLC biomarkers by Abbasian et al[31] The distinct co-expression profile, mutational aspects, and epigenetic considerations surrounding GPC3 highlight its potential as a critical biomarker in LUAD. This underscores the need for continued exploration of single genes that may hold promise even in well-studied areas such as NSCLC.

GPC3 is a member of the glypican heparin sulfate proteoglycan family and its expression pattern varies according to cancer type.[2,14] Several reports have demonstrated increased *GPC3* expression in hepatocellular carcinomas, nephroblastomas, Wilms' tumor, melanomas, and squamous cell carcinomas of the lung.[2,45,46] In contrast, studies in ovarian carcinomas,[47] breast carcinomas,[48–50] renal carcinomas,[51] mesotheliomas,[52] and LUADs[20] showed decreased expression of *GPC3* compared to normal tissues. Peter et al[48] showed that overexpression of GPC3 protein contributed to the inhibition of proliferation and LUAD tissues compared to normal lung tissues.

The alteration of gene expression may occur through a combination of different factors such as genetic mutations, CNAs, and epigenetic modifications in cancer tissues.[53,54] Previous studies postulated that epigenetic suppression of *GPC3* mRNA expression is caused by promoter hypermethylation.[55,56] Interestingly, our results contradict these previous reports. We used TCGA data from UALCAN to perform promoter hypermethylation analysis. However, we did not find any significant correlation between DNA methylation pattern and *GPC3* expression. The LUAD datasets from TCGA were further analyzed to investigate the role of mutations and CNAs on *GPC3* transcription. Although missense mutations and amplifications were identified in *GPC3*, no statistically significant correlation was found between the decreased expression of *GPC3* and these alterations. These results suggest that further prospective studies are needed to understand the precise molecular mechanisms responsible for the altered expression of *GPC3* in LUAD.

The present results also show that downregulation of *GPC3* in LUAD patients is significantly associated with poor OS and RFS. A multivariate analysis performed with PrognoScan datasets also showed a strong association between low *GPC3* levels and poorer prognosis. A meta-analysis was further performed to combine data from different datasets to assess the correlation between *GPC3* expression and LUAD prognosis. While previous meta-analyses[57,58] showed that overexpression of *GPC3* was strongly correlated with unfavorable OS and DFS in patients with hepatocellular carcinoma ($P < .05$ and HR > 1), our results showed that lower expression of *GPC3* was significantly associated with poor prognosis in LUAD patients ($P < .05$ and HR < 1). Interestingly, our analyses are supported by the studies of Parmigiani et al,[30] which indicated that increased *GPC3* expression predicts better survival in patients with lung adenocarcinoma. To provide a clearer conclusion on whether underexpression of *GPC3* promotes the aggressiveness of LUAD, further studies are needed and must include a large cohort of cancerous and complementary healthy lung tissues to compare *GPC3* expression.

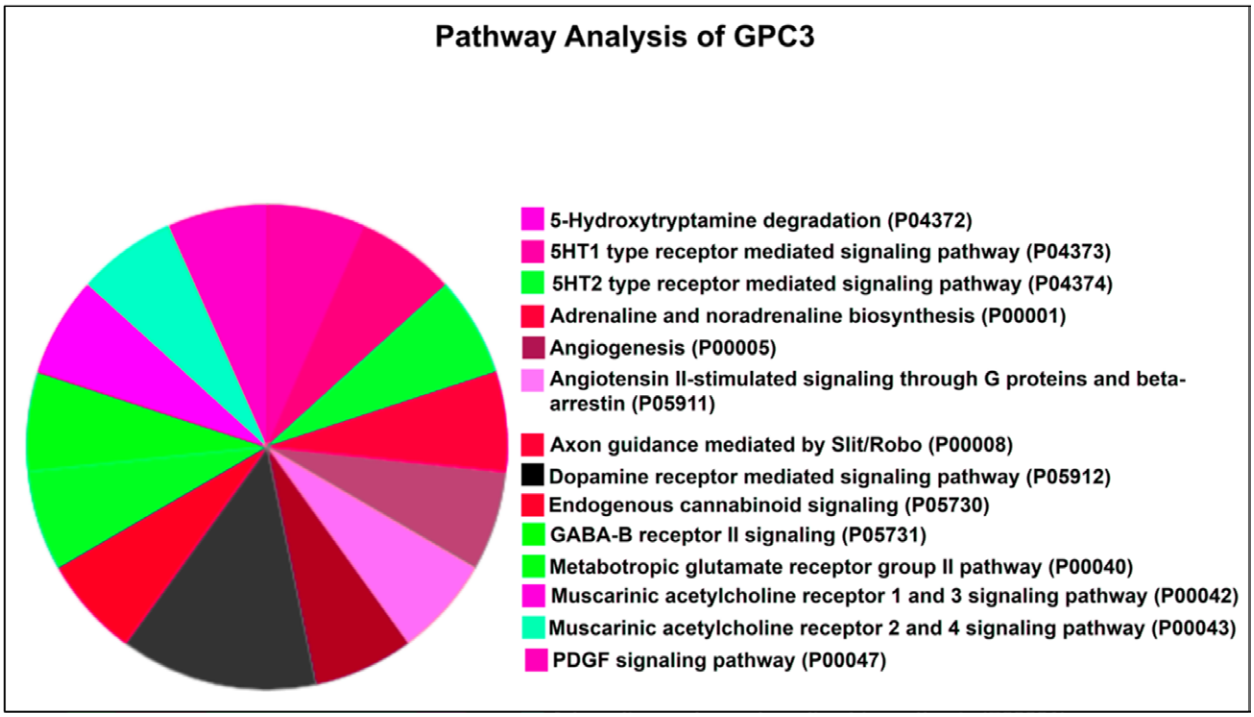

Figure 6. Pathway analysis of genes co-expressed with *GPC3* in LUAD patients.

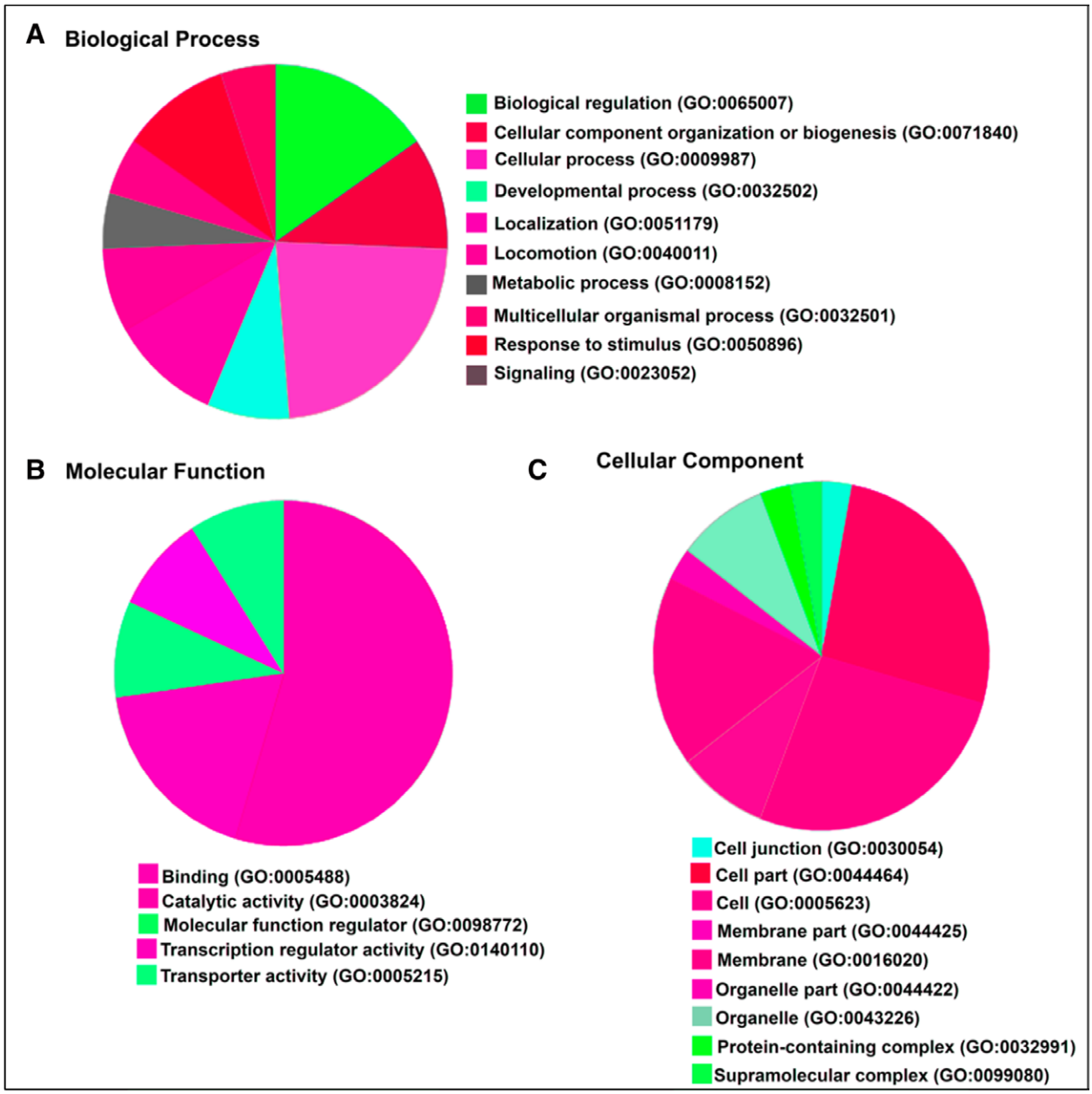

Figure 7. Gene ontology (GO) analysis of genes co-expressed with *GPC3* in LUAD. (A) biological process, (B) molecular function, and (C) cellular component.

GPC3 is known to regulate the innate immune system[59] by controlling the proteolytic activity of macrophage phagosomes and modulating its expression in macrophages and DCs.[60–62] In this study, we also found that *GPC3* expression was significantly correlated with infiltration of 6 immune cells (B cells, CD8+ T cells, CD4+ T cells, neutrophils, macrophages, and DCs) in LUAD. Immune cell infiltration can alter tumor growth and recurrence, as well as response to immunotherapy and clinical outcomes.[63,64] The significant correlation between *GPC3* expression and immunological infiltration in LUAD suggests that the protein product may play an important role in the development of LUAD and its recurrence.

The co-expression network of a gene can provide insights into the molecular mechanisms of pathogenesis.[65] Therefore, we examined the co-expression profile of *GPC3* using multiple online microarray databases to identify genes that are closely associated with *GPC3* in LUAD. Among the 17 genes found to be co-expressed with *GPC3*, *FHL1* showed the strongest co-expression correlation. *FHL1*, which belongs to the 4 and a half LIM domain (FHL) family, functions as a tumor suppressor gene, and its decreased expression is observed in a variety of human cancers, including lung, gastric, breast, kidney, and prostate cancers.[66,67] In light of these studies, our data support a synergistic downregulation of *GPC3* and *FHL1* in LUAD. The positive correlation between these 2 genes also suggests that *GPC3* functions as an anti-oncogene and its downregulation may serve as a potential prognostic biomarker for LUAD diagnosis. However, further studies are needed to test this hypothesis.

Besides, our PANTHER pathway and GO analyses showed that the dopamine receptor-mediated signaling pathway was the most enriched pathway for genes co-expressed with *GPC3*. High endothelial expression of dopamine receptor D2 (DRD2) agonists has been reported to inhibit NSCLC tumor growth by reducing vascular endothelial growth factor-dependent angiogenesis.[68,69] Moreover, overexpression of DRD2 has been reported to inhibit NF-κB signaling by attenuating protein kinase A in both lung squamous cell carcinoma and adenocarcinoma cell lines.[70] It is noteworthy that PKA-mediated

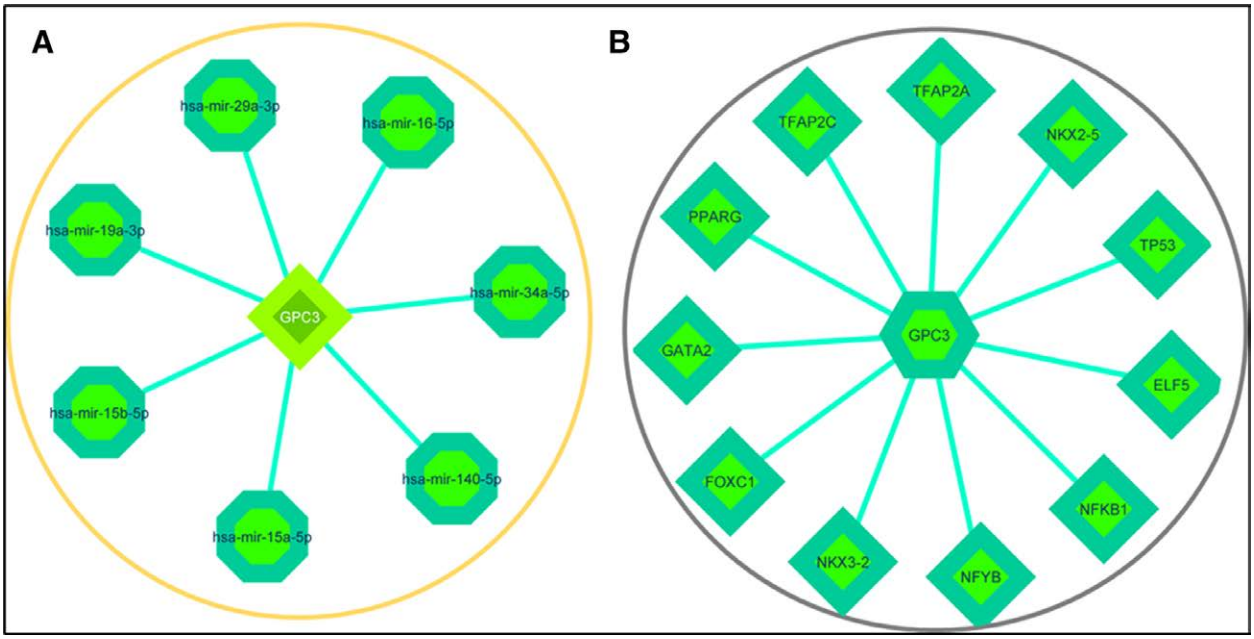

Figure 8. (A) miRNAs and (B) TFs that could interact with GPC3.

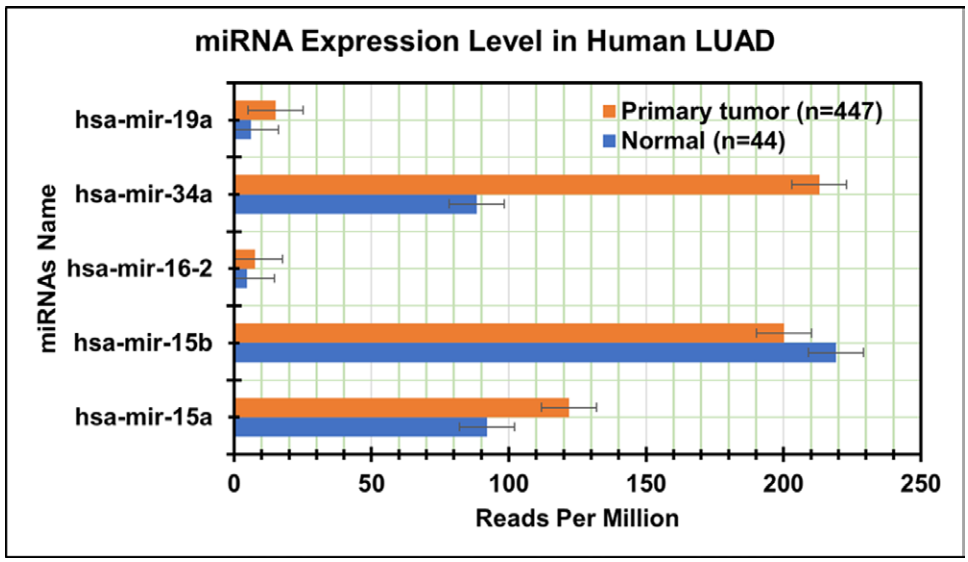

Figure 9. Expression analysis of GPC3-associated miRNAs in LUAD using UALCAN.

Table 4

Compounds that are predicted to significantly inhibit GPC3 in LUAD.

Term	*P* value	Adjusted *P* value	Odds ratio	Combined score
Heparitin (CID: 53477715)	.0045	.0315	19910	107587.5
Gemcitabine (CID: 60750)	.01175	.0329	19765	87833.91
Arbutin (CID: 440936)	.0141	.0329	19718	84030.01

activation of NF-κB promotes proliferation, invasion, and metastasis of human lung cancer cells.[71–73] However, Wu et al[70] showed that DRD2 expression was decreased in NSCLC tissues compared to normal cells. In addition, the lower bioavailability of dopamine due to *DRD2* polymorphisms was found to increase the risk of developing NSCLC.[74] Based on these reports and the results of our experiment, it can be suggested that there is a positive relationship between *DRD2* expression and *GPC3* transcription, and that downregulation of both leads to progression of LUAD. GO Enrichment analyses further confirmed our findings. The underlying mechanisms of how GPC3 interacts with the DRD2-mediated signaling pathway have not been fully elucidated and further investigations are required.

Both TFs and miRNAs are involved in mRNA regulation and carcinogenesis.[75,76] It is crucial to find out how TFs, miRNAs, and genes interact within the TF-gene-miRNA regulatory network, as this will help researchers find new biomarkers and therapeutic targets for cancer.[77,78] We found several miRNAs that showed high expression in LUAD compared to healthy tissues. In addition, miR-15b-5p was significantly downregulated in human LUAD samples compared to surrounding normal tissues. This observation was similar to that of colorectal cancer and osteosarcoma, in which downregulation of miR-15b-5p has been reported and shown to inhibit cancer progression and metastasis.[79–81] Nevertheless, miR-15b-5p has been detected at increased levels in gastric cancer and prostate cancer tissues.[79,82,83] Thus, the levels and functions of miR-15b-5p appear to be dependent on tumor type. There is still no definitive study on the significance and function of miR-15b-5p in LUAD.

Considering the possible role of GPC3 in LUAD, it could potentially serve as a therapeutic target in LUAD. We searched the DSigDB database was used to identify drugs that might target GPC3, and identified heparitin, gemcitabine, and arbutin as promising candidates. Among these, gemcitabine and arbutin had a higher combined score than heparitin, thus offering good prospects as GPC3-targeting drugs. Gemcitabine is a pyrimidine nucleoside antimetabolite that has been approved for the treatment of NSCLC, pancreatic cancer, bladder cancer, and breast cancer.[84] Arbutin has been shown to be effective in treating a number of diseases, including hyperpigmentation disorders, malignancies, central nervous system disorders, osteoporosis, and diabetes, according to a review of recent research.[85] These 2 drugs may be candidates of choice if GPC3 proves to be a good therapeutic target and a drug targeting GPC3 is needed in the future.

In conclusion, our analysis provides deeper insight into the function, mutational profile, epigenetic regulation, co-expression profile, and clinicopathological significance of the *GPC3* gene in LUAD progression. The present study demonstrated that *GPC3* could be a potential tumor suppressor gene and a predictive biomarker in LUAD. Understanding the mechanism and function of *GPC3* in LUAD biology in detail may provide important insights into its prognostic and therapeutic value. Nevertheless, further in vivo, in vitro, and epidemiological studies should be performed to validate our research findings.

Acknowledgments

The authors would like to thank the Biological Solution Centre (BioSol Centre; www.biosolcentre.org) for their technical support.

Author contributions

Conceptualization: Md. Enamul Kabir Talukder, Foysal Ahammad.
Data curation: Raihan Rahman Imon, Sharmin Aktar, Niaz Morshed, Suza Mohammad Nur, Rumana Mahtarin, Farazi Abinash Rahman.
Formal analysis: Raihan Rahman Imon, Sharmin Aktar, Niaz Morshed, Suza Mohammad Nur, Rumana Mahtarin, Farazi Abinash Rahman, Md. Enamul Kabir Talukder.
Funding acquisition: Mazin A. Zamzami, Shing Cheng Tan.
Investigation: Md. Enamul Kabir Talukder, Rahat Alam, Tomasz M. Karpiński, Foysal Ahammad.
Methodology: Raihan Rahman Imon, Sharmin Aktar, Niaz Morshed, Suza Mohammad Nur, Rumana Mahtarin, Md. Enamul Kabir Talukder.
Resources: Md. Enamul Kabir Talukder, Rahat Alam, Foysal Ahammad.
Software: Raihan Rahman Imon, Md. Enamul Kabir Talukder, Rahat Alam, Tomasz M. Karpiński, Foysal Ahammad.
Supervision: Foysal Ahammad, Mazin A. Zamzami, Shing Cheng Tan.
Validation: Raihan Rahman Imon, Sharmin Aktar, Niaz Morshed, Suza Mohammad Nur, Rumana Mahtarin, Farazi Abinash Rahman, Md. Enamul Kabir Talukder.
Visualization: Raihan Rahman Imon, Sharmin Aktar, Niaz Morshed, Suza Mohammad Nur, Rumana Mahtarin, Farazi Abinash Rahman, Md. Enamul Kabir Talukder.
Writing – original draft: Raihan Rahman Imon, Sharmin Aktar, Niaz Morshed, Suza Mohammad Nur, Rumana Mahtarin, Farazi Abinash Rahman, Md. Enamul Kabir Talukder.
Writing – review & editing: Md. Enamul Kabir Talukder, Rahat Alam, Tomasz M. Karpiński, Foysal Ahammad, Mazin A. Zamzami, Shing Cheng Tan.

References

[1] Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Cancer J Clin. 2018;68:394–424.
[2] Wang D, Gao Y, Zhang Y, et al. Glypican-3 promotes cell proliferation and tumorigenesis through up-regulation of beta-catenin expression in lung squamous cell carcinoma. Biosci Rep. 2019;39:BSR20181147.
[3] Denisenko TV, Budkevich IN, Zhivotovsky B. Cell death-based treatment of lung adenocarcinoma. Cell Death Dis. 2018;9:117.
[4] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin. 2017;67:7–30.
[5] Jimenez MF, Gomez-Hernandez MT. Radical consolidative treatments a hope for patients with oligometastatic non-small cell lung cancer. J Thor Dis. 2019;11:S1986–9.
[6] Ambrogio C, Nadal E, Villanueva A, et al. KRAS-driven lung adenocarcinoma: combined DDR1/Notch inhibition as an effective therapy. ESMO Open. 2016;1:e000076.
[7] Devarakonda S, Morgensztern D, Govindan R. Genomic alterations in lung adenocarcinoma. Lancet Oncol. 2015;16:e342–51.
[8] Wang Y, Zhang Q, Gao Z, et al. A novel 4-gene signature for overall survival prediction in lung adenocarcinoma patients with lymph node metastasis. Cancer Cell Int. 2019;19:100.
[9] Yan Y, Xu Z, Qian L, et al. Identification of CAV1 and DCN as potential predictive biomarkers for lung adenocarcinoma. Am J Physiol Lung Cell Mol Physiol. 2019;316:L630–43.
[10] Pinto R, Petriella D, Lacalamita R, et al. KRAS-driven lung adenocarcinoma and B cell infiltration: novel insights for immunotherapy. Cancers. 2019;11:1145.
[11] Kumarakulasinghe NB, van Zanwijk N, Soo RA. Molecular targeted therapy in the treatment of advanced stage non-small cell lung cancer (NSCLC). Respirology. 2015;20:370–8.
[12] Nwogu CE, Groman A, Fahey D, et al. Number of lymph nodes and metastatic lymph node ratio are associated with survival in lung cancer. Ann Thorac Surg. 2012;93:1614–9; discussion 1619.
[13] Pilia G, Hughes-Benzie RM, MacKenzie A, et al. Mutations in GPC3, a glypican gene, cause the Simpson-Golabi-Behmel overgrowth syndrome. Nat Genet. 1996;12:241–7.
[14] Chen C, Huang X, Ying Z, et al. Can glypican-3 be a disease-specific biomarker? Clin Transl Med. 2017;6:18.
[15] Sun B, Huang Z, Wang B, et al. Significance of Glypican-3 (GPC3) expression in hepatocellular cancer diagnosis. Med Sci Monit. 2017;23:850–5.
[16] Capurro MI, Xiang YY, Lobe C, et al. Glypican-3 promotes the growth of hepatocellular carcinoma by stimulating canonical Wnt signaling. Cancer Res. 2005;65:6245–54.
[17] Capurro MI, Xu P, Shi W, et al. Glypican-3 inhibits Hedgehog signaling during development by competing with patched for Hedgehog binding. Dev Cell. 2008;14:700–11.
[18] Midorikawa Y, Ishikawa S, Iwanari H, et al. Glypican-3, overexpressed in hepatocellular carcinoma, modulates FGF2 and BMP-7 signaling. Int J Cancer. 2003;103:455–65.
[19] Sun CK, Chua MS, He J, et al. Suppression of glypican 3 inhibits growth of hepatocellular carcinoma cells through up-regulation of TGF-beta2. Neoplasia. 2011;13:735–47.
[20] Aviel-Ronen S, Lau SK, Pintilie M, et al. Glypican-3 is overexpressed in lung squamous cell carcinoma, but not in adenocarcinoma. Mod Pathol. 2008;21:817–25.
[21] Filmus J. The contribution of in vivo manipulation of gene expression to the understanding of the function of glypicans. Glycoconj J. 2002;19:319–23.

[22] Filmus J, Capurro M. The role of glypican-3 in the regulation of body size and cancer. Cell Cycle. 2008;7:2787–90.
[23] Gonzalez AD, Kaya M, Shi W, et al. OCI-5/GPC3, a glypican encoded by a gene that is mutated in the Simpson-Golabi-Behmel overgrowth syndrome, induces apoptosis in a cell line-specific manner. J Cell Biol. 1998;141:1407–14.
[24] Yu X, Li Y, Chen SW, et al. Differential expression of glypican-3 (GPC3) in lung squamous cell carcinoma and lung adenocarcinoma and its clinical significance. Genet Mol Res. 2015;14:10185–92.
[25] Filmus J, Capurro M, Rast J. Glypicans. Genome Biol. 2008;9:224.
[26] Filmus J. Glypicans in growth control and cancer. Glycobiology. 2001;11:19R–23R.
[27] Zynger DL, Dimov ND, Luan C, et al. Glypican 3: a novel marker in testicular germ cell tumors. Am J Surg Pathol. 2006;30:1570–5.
[28] Pour AM, Masir N, Rose IM. Glypican-3 is useful but not superior to Hep Par 1 in differentiating hepatocellular carcinoma from other liver tumours. Malays J Pathol. 2016;38:229–33.
[29] Kim H, Xu GL, Borczuk AC, et al. The heparan sulfate proteoglycan GPC3 is a potential lung tumor suppressor. Am J Respir Cell Mol Biol. 2003;29:694–701.
[30] Parmigiani G, Garrett-Mayer ES, Anbazhagan R, et al. A cross-study comparison of gene expression studies for the molecular classification of lung cancer. Clin Cancer Res. 2004;10:2922–7.
[31] Abbasian MH, Ardekani AM, Sobhani N, et al. The role of genomics and proteomics in lung cancer early detection and treatment. Cancers. 2022;14:5144.
[32] Rhodes DR, Yu J, Shanker K, et al. ONCOMINE: a cancer microarray database and integrated data-mining platform. Neoplasia. 2004;6:1–6.
[33] Rhodes DR, Kalyana-Sundaram S, Mahavisno V, et al. Oncomine 30: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. Neoplasia. 2007;9:166–80.
[34] Tang Z, Kang B, Li C, et al. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. Nucleic Acids Res. 2019;47:W556–60.
[35] Chandrashekar DS, Bashel B, Balasubramanya SAH, et al. UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. Neoplasia. 2017;19:649–58.
[36] Goswami CP, Nakshatri H. PROGgeneV2: enhancements on the existing database. BMC Cancer. 2014;14:970.
[37] Li T, Fan J, Wang B, et al. TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells. Cancer Res. 2017;77:e108–10.
[38] Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signaling. 2013;6:pl1.
[39] Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012;2:401–4.
[40] Cai L, Lin S, Girard L, et al. LCE: an open web portal to explore gene expression and clinical associations in lung cancer. Oncogene. 2019;38:2551–64.
[41] Mi H, Muruganujan A, Casagrande JT, et al. Large-scale gene function analysis with the PANTHER classification system. Nat Protocols. 2013;8:1551–66.
[42] Zhou G, Soufan O, Ewald J, et al. NetworkAnalyst 30: a visual analytics platform for comprehensive gene expression profiling and meta-analysis. Nucleic Acids Res. 2019;47:W234–41.
[43] Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13:2498–504.
[44] Kuleshov MV, Jones MR, Rouillard AD, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res. 2016;44:W90–7.
[45] Wu Y, Liu H, Weng H, et al. Glypican-3 promotes epithelial-mesenchymal transition of hepatocellular carcinoma cells through ERK signaling pathway. Int J Oncol. 2015;46:1275–85.
[46] Castillo LF, Tascon RS, Joffé EBK, et al. Abstract 133: role of Glypican-3 (GPC3) on tumor progression of the human mammary gland. Cancer Res. 2014;74(19 Suppl):133–133.
[47] Liu Y, Zheng D, Liu M, et al. Downregulation of glypican-3 expression increases migration, invasion, and tumorigenicity of human ovarian cancer cells. Tumour Biol. 2015;36:7997–8006.
[48] Peters MG, Farías E, Colombo L, et al. Inhibition of invasion and metastasis by glypican-3 in a syngeneic breast cancer model. Breast Cancer Res Treat. 2003;80:221–32.
[49] Xiang YY, Ladeda V, Filmus J. Glypican-3 expression is silenced in human breast cancer. Oncogene. 2001;20:7408–12.
[50] Castillo LF, Tascón R, Lago Huvelle MA, et al. Glypican-3 induces a mesenchymal to epithelial transition in human breast cancer cells. Oncotarget. 2016;7:60133–54.
[51] Valsechi MC, Oliveira AB, Conceição AL, et al. GPC3 reduces cell proliferation in renal carcinoma cell lines. BMC Cancer. 2014;14:631.
[52] Murthy SS, Shen T, De Rienzo A, et al. Expression of GPC3, an X-linked recessive overgrowth gene, is silenced in malignant mesothelioma. Oncogene. 2000;19:410–6.
[53] Saha SK, Biswas PK, Gil M, et al. High expression of TTYH3 is related to poor clinical outcomes in human gastric cancer. J Clini Med. 2019;8:1762.
[54] Tan SC. Low penetrance genetic polymorphisms as potential biomarkers for colorectal cancer predisposition. J Gene Med. 2018;20:e3010.
[55] Hedenfalk I, Duggan D, Chen Y, et al. Gene-expression profiles in hereditary breast cancer. N Engl J Med. 2001;344:539–48.
[56] Lin H, Huber R, Schlessinger D, et al. Frequent silencing of the GPC3 gene in ovarian cancer cell lines. Cancer Res. 1999;59:807–10.
[57] Liu H, Yang C, Lu W, et al. Prognostic significance of glypican-3 expression in hepatocellular carcinoma: a meta-analysis. Medicine (Baltimore). 2018;97:e9702.
[58] Zhang J, Zhang M, Ma H, et al. Overexpression of glypican-3 is a predictor of poor prognosis in hepatocellular carcinoma: An updated meta-analysis. Medicine (Baltimore). 2018;97:e11130.
[59] Wu Q, Pi L, Le Trinh T, et al. A novel vaccine targeting glypican-3 as a treatment for hepatocellular carcinoma. Mol Ther. 2017;25:2299–308.
[60] Gururaja Rao S, Ponnalagu D, Patel NJ, et al. Three decades of chloride intracellular channel proteins: from organelle to organ physiology. Curr Protoc Pharmacol. 2018;80:11.21.1–11.21.17.
[61] Jiang L, Salao K, Li H, et al. Intracellular chloride channel protein CLIC1 regulates macrophage function through modulation of phagosomal acidification. J Cell Sci. 2012;125(Pt 22):5479–88.
[62] Ulmasov B, Bruno J, Oshima K, et al. CLIC1 null mice demonstrate a role for CLIC1 in macrophage superoxide production and tissue injury. Physiol Rep. 2017;5:e13169.
[63] Bindea G, Mlecnik B, Tosolini M, et al. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. Immunity. 2013;39:782–95.
[64] Liu X, Wu S, Yang Y, et al. The prognostic landscape of tumor-infiltrating immune cell and immunomodulators in lung cancer. Biomed Pharmacother. 2017;95:55–61.
[65] Azizan N, Hayati F, Tizen NMS, et al. Role of co-expression of estrogen receptor beta and Ki67 in prostate adenocarcinoma. Investig Clin Urol. 2018;59:232–7.
[66] Niu C, Liang C, Guo J, et al. Downregulation and growth inhibitory role of FHL1 in lung cancer. Int J Cancer. 2012;130:2549–56.
[67] Wang J, Huang F, Huang J, et al. Epigenetic analysis of FHL1 tumor suppressor gene in human liver cancer. Oncol Letters. 2017;14:6109–16.
[68] Hoeppner LH, Wang Y, Sharma A, et al. Dopamine D2 receptor agonists inhibit lung cancer progression by reducing angiogenesis and tumor infiltrating myeloid derived suppressor cells. Mol Oncol. 2015;9:270–81.
[69] Basu S, Nagy JA, Pal S, et al. The neurotransmitter dopamine inhibits angiogenesis induced by vascular permeability factor/vascular endothelial growth factor. Nat Med. 2001;7:569–74.
[70] Wu XY, Zhang CX, Deng LC, et al. Overexpressed D2 dopamine receptor inhibits non-small cell lung cancer progression through inhibiting NF-kappaB signaling pathway. Cell Physiol Biochem. 2018;48:2258–72.
[71] Chen W, Li Z, Bai L, et al. NF-kappaB in lung cancer, a carcinogenesis mediator and a prevention and therapy target. Front Biosci (Landmark Ed). 2011;16:1172–85.
[72] Zhong H, Voll RE, Ghosh S. Phosphorylation of NF-kappa B p65 by PKA stimulates transcriptional activity by promoting a novel bivalent interaction with the coactivator CBP/p300. Mol Cell. 1998;1:661–71.
[73] Jeong J, Park YU, Kim DK, et al. Cdk5 phosphorylates dopamine D2 receptor and attenuates downstream signaling. PLoS One. 2013;8:e84482.
[74] Campa D, Zienolddiny S, Lind H, et al. Polymorphisms of dopamine receptor/transporter genes and risk of non-small cell lung cancer. Lung Cancer. 2007;56:17–23.
[75] Tan SC, Lim PY, Fang J, et al. Association between MIR499A rs3746444 polymorphism and breast cancer susceptibility: a meta-analysis. Sci Rep. 2020;10:3508.
[76] Antonova SV, Haffke M, Corradini E, et al. Chaperonin CCT checkpoint function in basal transcription factor TFIID assembly. Nature Struct Mol Biol. 2018;25:1119–27.

[77] Chen B, Gao S, Ji C, et al. Integrated analysis reveals candidate genes and transcription factors in lung adenocarcinoma. Mol Med Rep. 2017;16:8371–9.
[78] Li J, Li Z, Zhao S, et al. Identification key genes, key miRNAs and key transcription factors of lung adenocarcinoma. J Thorac Dis. 2020;12:1917–33.
[79] Wu B, Liu G, Jin Y, et al. miR-15b-5p promotes growth and metastasis in breast cancer by targeting HPSE2. Front Oncol. 2020;10:108.
[80] Sun LN, Zhi Z, Chen LY, et al. SIRT1 suppresses colorectal cancer metastasis by transcriptional repression of miR-15b-5p. Cancer Lett. 2017;409:104–15.
[81] Lin S, Gregory RI. MicroRNA biogenesis pathways in cancer. Nat Rev Cancer. 2015;15:321–33.
[82] Chen R, Sheng L, Zhang HJ, et al. miR-15b-5p facilitates the tumorigenicity by targeting RECK and predicts tumour recurrence in prostate cancer. J Cell Mol Med. 2018;22:1855–63.
[83] Zhao C, Li Y, Chen G, et al. Overexpression of miR-15b-5p promotes gastric cancer metastasis by regulating PAQR3. Oncol Rep. 2017;38:352–8.
[84] Toschi L, Finocchiaro G, Bartolini S, et al. Role of gemcitabine in cancer therapy. Future Oncol. 2005;1:7–17.
[85] Saeedi M, Khezri K, Seyed Zakaryaei A, et al. A comprehensive review of the therapeutic potential of α-arbutin. Phytother Res. 2021;35:4136–54.