

# GALNT14 expression in renal clear cell carcinoma and its effect on prognosis

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## Research Article

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# Abstract

**Background** The expression of GALNT14 in kidney renal clear cell carcinoma (KIRC) and its clinical significance remains unknown.

**Methods** The KIRC data expressed by GALNT14 was downloaded from The Cancer Genome Atlas (TCGA) database. The expression of GALNT14 was analyzed by R software, Perl software and online analysis database. The relationship between GALNT14 expression and clinicopathological features in KIRC was analyzed by univariate, multivariate Cox regression and some databases. Gene Expression Profiling Interactive Analysis (GEPIA), Starbase v3.0, UALCAN, and Kaplan-Meier were used to analyze the relationship between GALNT14 expression and overall survival (OS) in KIRC. UALCAN detects the expression of GALNT14 methylation in KIRC. Linkedomics and Genemania were used to analyze the gene co-expression of GALNT14. Gene Set Enrichment Analysis (GSEA) was performed to search for potential regulatory pathways.

**Results** We found that GALNT14 was overexpressed in KIRC ( $p=1.433e-25$ ). Patients with high GALNT14 expression in KIRC had a better prognosis than patients with low GALNT14 expression ( $p=0.008$ ). In addition, high GALNT14 expression in KIRC was significantly associated with low T stage and positive OS ( $p<0.05$ ). Univariate Cox analysis showed that GALNT14 was positively correlated with OS ( $p<0.001$ ). Multivariate Cox analysis showed that GALNT14 was associated with OS ( $p<0.001$ ), age ( $p=0.01$ ) and histological grade ( $p=0.02$ ). GALNT14 methylation is low expressed in KIRC ( $p<0.001$ ). GSEA analysis showed that GALNT14 was enriched in histidine metabolism, peroxisome, and renin-angiotensin system pathways.

**Conclusion** GALNT14 can be used as an independent prognostic factor for renal clear cell carcinoma and a potential target for clinical diagnosis and treatment of KIRC.

## 1 Introduction

RCC is one of the most common malignancies in the world[1]. The most common histologic subtype is KIRC, which accounts for 80–90% of patients with RCC[2]. Currently, the clinical diagnosis of RCCs is achieved by non-invasive radiological techniques such as dynamic enhanced CT, US and MRI, but the specificity of these methods in the diagnosis of RCCs is limited[3–5]. Indeed, many biomarkers for the diagnosis and prognosis of KIRC have been investigated, such as CRB3, SFRP1, P4Hb and so on[6, 7]. However, the study population for these indicators was mainly Caucasian, and the results were verified only in the TCGA cohort[8, 9]. Namely, although there are no effective biomarkers for the diagnosis and treatment of KIRC, there is insufficient evidence that these targets can be used clinically. Thus, there is an urgent need for new biomarkers to diagnose KIRC early and to personalize treatment for patients.

Recent studies have demonstrated that GALNT14 is involved in various biological functions. Muhammad et al. indicated that GALNT14 can not only inhibit cell migration and change cell morphology, but also lead to insufficient or excessive proliferation during embryogenesis, thus causing growth defects[8].

Besides, GALNT14 has been identified as a destructive Mendelian mutation that led to the death of embryos, suggesting that GALNT14 plays an irreplaceable role in human development[9]. Otherwise, GALNT14 is abnormally expressed in multiple cancers. It has been reported that GALNT14 can change the catalytic efficiency of apoptosis signal and tissue invasiveness, regulate the migration of cancer cells, etc., and play a great role in malignant tumors of the breast, lung, gastrointestinal tract and ovary[10, 11]. Together, we know that GALNT14 plays a unique role in the progression of malignant tumors. Given the above, most published studies have focused on the role of GALNT14 in cancers of the respiratory system and digestive system[2, 4, 5]. However, to date, no studies have examined the effect of GALNT14 expression in KIRC.

GALNTs have 20 members in total: GALNT1 through 14, GANLTL1 through L6, and GALNT14 is one of them[10, 12]. GALNT is an inducer of mucin O-glycation, and its expression can alter many biological behaviors of tumor cells, including formation, proliferation, migration and drug resistance[10, 13]. Nevertheless, other members of the GALNTs also play a role in cancer[14]. For example, Muhammad et al. [8] proposed that the abnormal glycation caused by the mutation of GALNT1 was associated with the occurrence of bladder cancer, and GALNT3 was highly expressed in RCC and prostate cancer in patients with high malignant tumors. In addition, GALNT4 is a positive prognostic factor in patients with DFS KIRC, and high GALNT10 expression is associated with TNM stage progression in KIRC[8, 15]. In summary, we speculate that GALNTs are involved in the occurrence, development, and metastasis of cancers in various systems. However, no comprehensive bioinformatics analysis has been reported to investigate the expression and clinical significance of GALNT14 in KIRC. Here, we demonstrate for the first time that high GALNT14 expression in KIRC is associated with lower T stage and higher OS. Our study not only provides a new independent prognostic factor for KIRC, but also reveals the pathway by which GALNT14 regulates the occurrence and development of KIRC.

## 2 Materials And Methods

### 2.1 The cancer genome atlas (TCGA) database

We downloaded a total of 611 primary KIRC tumor tissue samples with detailed GALNT14 expression data and 537 clinical data from KIRC tumor patients from TCGA (<https://portal.gdc.cancer.gov/>).

### 2.2 Differential expression analysis

We used R software (<https://www.r-project.org/>) with "limma", "beeswarm" packages and Perl software (<https://www.perl.org/>) for comparison. We also compared the expression of GALNT14 in GEPIA (<http://gepia.cancer-pku.cn/>), Starbase v3.0 (<http://starbase.sysu.edu.cn/>) and UALCAN (<http://ualcan.path.uab.edu/>) to test whether different methods would affect the results. We also verified the above results against the CCLE(<https://portals.broadinstitute.org/ccle/about>).

### 2.3 Clinical correlation analysis—Kolmogorov-Smirnov test

First, we examined the correlation between GALNT14 and the clinical characteristics of patients with KIRC in Perl software. GEPIA and UALCAN were used to investigate the expression characteristics of GALNT14.

Logistic regression analysis was used to investigate the clinical significance of GALNT14 in KIRC. Cox analysis was performed using the “Survival” and “SurvMiner” software packages of R software.

## **2.4 Survival analysis**

We combined Perl software, survival analysis and Kaplan-Meier prognostic analysis, and used R software to calculate *p*-value by log-rank test. In addition, Kaplan-Meier overall survival curves and log-rank were used to examine the OS differences between GALNT14 expression groups. Meanwhile, GEPIA, Starbase v3.0, UALCAN and Kaplan-Meier Plotter (<http://kmplot.com/analysis/>) were used to detect GALNT14 expression and prognosis (OS or DFS) in KIRC, KIRP and KICH.

## **2.5 Methylation analysis**

We used UALCAN to detect the expression of GALNT14 methylation in KIRC and its relationship with clinical features.

## **2.6 Analysis of gene co-expression**

In order to explore the genes associated with GALNT14 in different molecular functions and biological pathways, we used Linkedomics (<http://www.linkedomics.org/>) and Genemania (<http://genemania.org>) to analyze gene co-expression. The proteins interacting with GALNT14 were obtained from the STRING database (<https://www.string-db.org/>).

## **2.7 Gene Set Enrichment Analysis**

KEGG (<http://www.genome.jp/kegg/>) was used to analyze the enrichment of GALNT14-related Genes in KIRC. Each GSEA analysis performed 1000 genome sequences.

# **3 Results**

## **3.1 Characteristics of the study population**

Clinical data of 537 patients were downloaded from TCGA in September 2020. According to the age classification standards of the United Nations WHO, the age groups are divided into young people from 18 to 65 years old, middle-aged people from 66 to 79 years old and old people from 80 to 99 years old (Table 1).

Table 1  
The characteristics of KIRC patients

Clinical characteristics		n	%
Age(years)	26~	352	65.5%
	66~	160	29.8%
	80 ~ 90	25	4.7%
	average	61	
Gender	Female	191	35.6%
	Male	346	64.4%
Histological grade	G1	14	2.6%
	G2	230	42.8%
	G3	207	38.5%
	G4	78	14.5%
	GX	5	0.9%
	Not available	3	0.6%
Stage	I	269	50.1%
	II	57	10.6%
	III	125	23.3%
	IV	83	15.5%
	Not available	3	0.6%
T classification	T1	275	51.2%
	T2	69	12.8%
	T3	182	33.9%
	T4	11	2.0%
M classification	M0	426	79.3%
	M1	79	14.7%
	MX	30	5.6%
	Not available	2	0.4%
N classification	N0	240	44.75
	N1	17	3.2%

Clinical characteristics		n	%
	NX	280	52.1%
Survival time(years)	< 1	118	22.0%
	1 ~ 5	303	56.4%
	5 ~ 10	117	21.8%
Survival status	Death	170	31.7%
	Survival	367	68.3%

### 3.2 High GALNT14 expression in KIRC

First, we studied the expression of GALNT14 in KIRC by differential expression analysis. GALNT14 expression in KIRC and normal tissues were compared in Fig. 1A, and the results indicated that GALNT14 expression was elevated in KIRC ( $p = 1.433e-25$ ). Similarly, paired differential analysis showed that GALNT14 was highly expressed in KIRC tissues ( $p = 1.752e-18$ ) (Fig. 1B). Additionally, the results of GEPIA, Starbase v3.0 and UALCAN all showed that GALNT14 was increasingly expressed in KIRC (Fig. 1C, F, G). Accordingly, all the results illustrated high expression of GALNT14 in tumor samples. Moreover, we found high GALNT14 expression in human renal carcinoma cell line A498 and ACHN through CCLE database. To determine whether the high expression of GALNT14 in KIRC is specific, we also analyzed the expression of KIRP and KICH by GEPIA, and found that GALNT14 was low and high in KICH and KIRP respectively, and the results were statistically significant. (Fig. 1D, E).

### 3.3 Related clinicopathologic variables

To investigate whether GALNT14 is involved in the clinical progression of KIRC, we downloaded data from a total of 537 clinical samples of KIRC with GALNT14 expression from TCGA. As shown in Fig. 2, differences in GALNT14 expression were observed according to clinical stage ( $p = 0.009$ ), T classification ( $p = 4.104e-05$ ), M classification ( $p = 0.005$ ) and distant metastasis ( $p = 3.988e-05$ ). Univariate logistic regression analysis showed that GALNT14 was associated with clinicopathologic features with a favorable prognosis (Table 2). Subgroup analysis indicated that high GALNT14 expression in KIRC was significantly associated with low T stage and high OS ( $p < 0.05$ ). Likewise, UALCAN results demonstrated that GALNT14 was upregulated in stage, grade, and lymph node metastasis (Fig. 3A-E). Namely, the higher level of the grade, the less the expression of GALNT14 ( $p < 0.05$ ) (Table 3). Besides, the expression of GALNT14 in KIRC manifested a significant gender difference ( $p = 2.21E-02$ ) (Table 3). Similarly, to determine whether GALNT14 is involved in the clinical process of KICH and KIRP, the results of GEPIA analysis of the clinical stage of KICH and KIRP showed no difference in GALNT14 expression in KICH, while there was a difference in GALNT14 expression in KIRP (Fig. 3F, G).

Table 2  
 GALNT14 expression associated with clinical pathological characteristics (logistic regression)

<b>Clinical characteristics</b>	<b>Total(N)</b>	<b>Odds ratio</b>	<b><i>p</i>-value</b>
T (T4 vs. T1)	286	0.09(0.00-0.48)	0.02
T (T4 vs. T2)	80	0.09(0.00-0.52)	0.03
T (T4 vs. T3)	193	0.11(0.01–0.58)	0.04
Survival status (Yes vs. No)	537	1.64(1.13–2.38)	0.01

Table 3  
GALNT14 expression associated with clinical data and pathological characteristics by UALCAN

Clinical characteristics	Comparison	Statistical significance
Stage	Normal-vs-Stage1	1.62E-12
	Normal-vs-Stage2	9.89E-08
	Normal-vs-Stage3	1.62E-12
	Normal-vs-Stage4	1.20E-10
	Stage 1-vs-Stage 4	5.98E-04
	Stage 3-vs-Stage 4	4.43E-02
Grade	Normal-vs-Grade1	3.85E-06
	Normal-vs-Grade2	1.62E-12
	Normal-vs-Grade3	1.62E-12
	Normal-vs-Grade4	5.41E-08
	Grade 1-vs-Grade 4	1.91E-02
	Grade 2-vs-Grade 4	1.17E-04
	Grade 3-vs-Grade 4	1.98E-03
Age	Normal-vs-Age(21-40Yrs)	1.05E-04
	Normal-vs-Age(41-60Yrs)	1.62E-12
	Normal-vs-Age(61-80Yrs)	< 1E-12
	Normal-vs-Age(81-100Yrs)	5.90E-07
Gender	Normal-vs-Male	< 1E-12
	Normal-vs-Female	1.62E-12
	Male-vs-Female	2.21E-02
Metastasis status	Normal-vs-N0	< 1E-12
	Normal-vs-N1	4.13E-02

### 3.4 Survival outcomes and Cox regression analyses

#### 3.4.1 The prognostic values of GALNT14 for OS in KIRC Patients

Firstly, we analyzed the relationship between GALNT14 expression and OS in KIRC patients using GEPIA, Starbase v3.0, UALCAN, and Kaplan-Meier. The results showed that the high expression of GALNT14 in

GEPIA, Starbase v3.0, UALCAN and Kaplan-Meier Plotter were all associated with high OS in KIRC patients, and the results were statistically significant, as shown in Fig. 4A-D. Moreover, the results of Kaplan-Meier in Fig. 4E indicated that KIRC patients with high GALNT14 expression had a higher OS ( $p = 0.008$ ). Besides, the GEPIA results showed that GALNT14 was not statistically significant in OS in KICH, but higher GALNT14 expression was associated with higher OS in KIRP ( $p = 0.012$ ), which was consistent with the result obtained by Kaplan-Meier Plotter ( $p = 0.023$ ) (Fig. 4F-H). Finally, the forest diagram showed the correlation between GALNT14 and OS (Fig. 4I). In all, GALNT14 was found to be significantly associated with patient OS in KIRC.

### 3.4.2 Cox regression analyses

Moreover, combining with the results of the clinicopathological characteristics of survival status, the univariate Cox analysis revealed that GALNT14-high correlated significantly with a positive OS in KIRC ( $p < 0.001$ ). Other clinicopathologic variables associated with better OS included clinical stage, lymph nodes, and distant metastasis (Table 4). Similarly, multivariate Cox analysis showed that GALNT14 was associated with OS ( $p < 0.001$ ), along with histological grade ( $p = 0.02$ ) and age ( $p = 0.01$ ). To summarize, Cox regression analyses showed that GALNT14 was an independent prognostic factor as compared with other confounders.

### 3.5 The prognostic values of GALNT14 for DFS in KIRC patients

Equally, GEPIA and Kaplan-Meier Plotter were used to analyze the relationship between the expression of GALNT14 and patient DFS in KIRC. Data from GEPIA showed that high expression of GALNT14 predicted poor DFS of patients with KIRC (Fig. 5A). Kaplan-Meier Plotter results also showed that high GALNT14 expression was associated with low DFS in patients with KIRC ( $p = 0.0091$ ) (Fig. 5B). Thereby, we can know that the up-regulation of GALNT14 was significantly associated with poor DFS in KIRC patients. In addition, the GEPIA results showed no statistically significant difference in the expression of GALNT14 in DFS in KICH patients (Fig. 5C), while high expression of GALNT14 ( $p = 0.033$ ) predicted higher DFS of patients with KIRP (Fig. 5D). But there was no statistical difference in the expression of GALNT14 in DFS of KIRP by Kaplan-Meier Plotter (Fig. 5E).

### 3.6 Methylation of GALNT14 in UALCAN

Correspondingly, we used UALCAN to determine the differential expression of GALNT14 methylation and its relationship to clinical features in KIRC. The results demonstrated that GALNT14 methylation levels in KIRC were lower than those in normal tissues ( $p < 0.001$ ) (Table 5). Moreover, specific patterns of DNA methylation differed in indicators including tumor grade and clinical stage, as shown in Fig. 3H-M and Table 5.

### 3.7 GALNT14 functions as a protection factor in KIRC

Given that GALNT14 was significantly up-regulated in KIRC and closely correlated with patient prognosis including both OS and DFS, we speculated that GALNT14 might act as a protective factor in KIRC. Furthermore, LinkedOmics and GeneMANIA were used to conduct gene co-expression analysis on GALNT14. In this way, we haven't ignored the high correlation between genes[16]. The result of gene co-

expression network analysis of GALNT14 in KIRC is shown in (Fig. 5F). Meanwhile, in the STRING database, we obtained 10 proteins that interact with GALNT14 (Fig. 5G). The overall closely co-expressed genes of GALNT14 in KIRC are shown as a volcano plot (Fig. 5H). The top-50 positively and negatively co-expressed genes of GALNT14 in KIRC are shown as heat maps (Fig. 5I, J), respectively.

Table 4  
Cox regression analysis results

Parameter	Univariate analysis			Multivariate analysis		
	HR	95%CI	<i>p</i>	HR	95%CI	<i>p</i>
age	1.02	1.00-1.04	<b>0.01</b>	3.32	1.42–7.72	<b>0.01</b>
grade	2.24	1.68–2.99	<b>3.61E-08</b>	1.49	1.06–2.09	<b>0.02</b>
stage	1.86	1.54–2.25	<b>1.26E-10</b>	1.53	0.92–2.54	0.10
T	1.94	1.54–2.46	<b>2.69E-08</b>	0.93	0.58–1.50	0.77
M	4.07	2.63–6.30	<b>2.76E-10</b>	1.58	0.71–3.50	0.26
N	2.93	1.52–5.67	<b>0.001</b>	1.40	0.67–2.92	0.36
GALNT14	0.75	0.66–0.86	<b>2.03E-05</b>	0.74	0.63–0.86	<b>0.0001</b>

Table 5  
Relationship between GALNT14 methylation and clinical characteristics of KIRC

Clinical characteristics	Comparison	Statistical significance
Normal-vs-Tumor	Normal-vs-Primary	< 1E-12
Normal-vs-metastasis status	Normal-vs-N0	1.87E-12
Normal-vs-Grade	Normal-vs-Grade2	2.76E-11
	Normal-vs-Grade3	2.11E-15
	Normal-vs-Grade4	7.67E-05
Normal-vs-Stage	Normal-vs-Stage1	2.53E-10
	Normal-vs-Stage2	1.50E-06
	Normal-vs-Stage3	1.48E-09
	Normal-vs-Stage4	1.12E-09
Normal-vs-Age	Normal-vs-Age(41-60Yrs)	1.63E-12
	Normal-vs-Age(61-80Yrs)	1.88E-12
	Normal-vs-Age(81-100Yrs)	1.12E-03
	Age(21-40Yrs)-vs-Age(41-60Yrs)	7.24E-03
	Age(21-40Yrs)-vs-Age(61-80Yrs)	2.13E-02
	Age(21-40Yrs)-vs-Age(81-100Yrs)	6.92E-03
Normal-vs-Gender	Normal-vs-Male	< 1E-12
	Normal-vs-Female	4.19E-09

### 3.8 GSEA identifies an GALNT14-related signaling pathway

To determine the signaling pathway by which GALNT14 regulates KIRC, we performed a GSEA analysis of GALNT14. GSEA revealed significant differences (FDR < 0.25, NOM  $p$ -value < 0.05) in the enrichment of MSigDB Collection (<http://www.gsea-msigdb.org/gsea/msigdb/collections.jsp>) and the details are provided in Table 6. The results indicated that GALNT14 was highly differentially expressed in histidine metabolism, peroxidase and renin-angiotensin system pathways (Fig. 6). Moreover, to make the results of gene enrichment analysis more obvious, we combined the high-expression phenotypic pathway of GALNT14 with the low-expression phenotypic pathway of GALNT14 (Table 6) obtained through GSEA ( $p$  < 0.05) to conduct GALNT14, and the results are shown in Fig. 6J.

Table 6  
Gene sets enriched in phenotype high and low

	NAME	NES	NOM <i>p</i> -val	FDR <i>q</i> -val
phenotype high	KEGG_HISTIDINE_METABOLISM	2.197	0.000	0.032
	KEGG_PEROXISOME	2.095	0.004	0.063
	KEGG_RENIN_ANGIOTENSIN_SYSTEM	2.007	0.000	0.095
	KEGG_FRUCTOSE_AND_MANNANOSE_METABOLISM	1.963	0.004	0.104
	KEGG_TRYPTOPHAN_METABOLISM	1.948	0.006	0.094
	KEGG_BETA_ALANINE_METABOLISM	1.945	0.006	0.081
	KEGG_ARGININE_AND_PROLINE_METABOLISM	1.939	0.006	0.073
	KEGG_GLYCOLYSIS_GLUCCONEOGENESIS	1.928	0.012	0.069
	KEGG_FATTY_ACID_METABOLISM	1.898	0.016	0.077
phenotype low	KEGG_OOCYTE_MEIOSIS	-1.707	0.036	1.000
	KEGG_CALCIIUM_SIGNALING_PATHWAY	-1.668	0.019	1.000
	KEGG_HYPERTROPHIC_CARDIOMYOPATHY_HCM	-1.625	0.047	0.920

Gene sets with NOM *p*-val < 0.05 and FDR *q*-val < 0.25 are considered as significant.

## 4 Discussion

First, differential expression analysis showed that GALNT14 was highly expressed in KIRC tissues, and this was verified in the CCLE database. The results of the clinicopathological analysis showed that GALNT14 expression differed significantly in clinical stage, T stage, distant metastasis, and survival status. In addition, the UALCAN results showed that GALNT14 methylation was under-expressed in KIRC. The OS outcome of the Kaplan-Meier curve also showed that high GALNT14 expression was associated with better outcomes in patients with KIRC. In addition, univariate and multivariate Cox analyses suggested that GALNT14 may be a biomarker for the prognosis of KIRC. Subsequently, Genegen network analysis demonstrated that GALNT14 was associated with multiple MUC genes. Finally, the results of GSEA revealed that GALNT14 overexpression regulates KIRC mainly through histidine metabolism, peroxidase, renin-angiotensin system and other pathways. In conclusion, GALNT14 may serve as a potential marker for prognostic and therapeutic targets of KIRC.

Our work is the first to suggest that GALNT14 is a KIRC protective factor. The results of differential expression analysis and Cox regression analysis showed that GALNT14 high expression was

significantly different in clinical-grade, T stage and distant metastasis. Further, the overexpression of GALNT14 is associated with a low T grade. In other words, GALNT14 upregulation inhibited the size and extent of the primary KIRC tumor. In the stage, Hillman et al. [2] proposed that GALNT14 is expressed in several cancers, including ovarian cancer, and its upregulation promotes clinical progression in high-grade serous cancer. Beyond that, most patients with KIRC are diagnosed with metastases, particularly to the lung, bone, and brain[17, 18]. We speculated that the high expression of GALNT14 in KIRC may affect the biological functions of tumor cell proliferation, invasion and metastasis.

In addition, we found that GALNT14 expression was significantly associated with survival in patients with KIRC. Correlation analysis between clinicopathologic features of GALNT14 and KIRC showed that GALNT14 was significantly associated with OS and DFS in patients with KIRC. So far, studies have reported the effect of GALNT14 on survival in patients with other cancers. Taking esophageal cancer, lung cancer and liver cancer as examples, Tsou et al. [9, 19] noted that the GALNT14 "TT + TG" genotype resulted in a longer OS in patients. Besides, Kwon et al. [20] indicated that patients with GALNT14 overexpression had poor DFS. What's more, Lin et al. [10] suggested that in patients with positive anti-HCV antibodies, the GALNT14 TT genotype was associated with longer OS. Therefore, we suggested that GALNT14 overexpression may affect the progression of cancers. Unfortunately, the prognostic value of GALNT14 expression in lung cancer and HCC is known[20, 21], but its prognostic value in other cancers, including renal cancer, remains unknown.

Our Cox analysis suggests that GALNT14 may serve as an independent good prognostic biomarker for KIRC. Indeed, GALNT14 is not the only protective factor for KIRC. Zhang et al. [22] indicated that ACE2 is one of the protective factors of KIRC, and ACE2 down-regulation is associated with poor OS and tumor progression in KIRC. Correspondingly, there are other genes that may act as protective factors for KIRC. For example, Qiu et al. [23] proposed that the high expression of PHYH improves the survival of patients with KIRC mainly by affecting peroxisomes and may serve as a potential prognostic molecular marker. In brief, there is not only one protective factor in each cancer. Absolutely, protective factors are not just present in KIRC or in a particular type of cancer, but in a wide range of cancers. For instance, Jiang et al. [24] found that overexpression of PTGD was positively correlated with early TNM, suggesting that this gene may be a protective prognostic factor for breast cancer. Moreover, the survival rate of cervical cancer patients with high KIR3DL1 transcription levels in tumor tissues is significantly higher than that of cervical cancer patients with low/moderate transcription levels, suggesting that KIR3DL1 is a protective factor of cervical cancer[25]. Collectively, protective factors exist not only in cancers of the urinary system, but also in cancers of other systems. Thus, we propose that the most intuitive manifestation of cancer affecting protective factors is to inhibit the metastasis and spread of cancer cells, thus improving the OS of cancer patients.

According to univariate and multivariate analysis, GALNT14 was predicted to be a novel biomarker in KIRC samples. GALNT14-mediated protein glycosylation has been reported to be associated with tumor development and malignant transformation[20, 26]. In addition, Wang et al. [27] pointed out that cancer-specific glycation changes are mainly based on the abnormal expression and activity of related

glycosyltransferases, which provides theoretical support for our belief that the glycation of KIRC is mainly based on the abnormal expression and activity of GALNT14. We know that GALNT14 encodes the glycosyltransferase GalNA-T14, which is involved in O-chain glycosylation after protein translation[26]. O-glycosylation is a type of glycosylation that plays an important role in cell growth, differentiation, transformation, adhesion and tumor immune monitoring[27–29]. In detail, DeDarya et al. [13] proposed that abnormal expression of GALNTs would lead to abnormal glycation of cancer cells, which would interfere with cell adhesion, migration and proliferation and promote tumor development. Besides, in melanoma cells, non-small cell lung cancer, and pancreatic cancer, high GALNT14 expression enhances O-glycation of DR4 and DR5, thereby reducing tumor cell sensitivity to TRAIL[30]. Hence, GALNT14 overexpression interacts with glycosylation to influence cancer progression in various systems of the body. In conclusion, we suggest that GALNT14 affects a variety of tumor cell functions, including proliferation, apoptosis, invasion, and metastasis, by altering glycation, and further affects the genesis and development of KIRC.

Moreover, by UALCAN analysis, we found that GALNT14 methylation was down-expression in KIRC, and different DNA methylation patterns differed in pathological grade, clinical stage and other clinical indicators. To our knowledge, DNA methylation has been identified as a potential target for diagnostic, prognostic and predictive biomarkers in cancer[31, 32]. Nevertheless, the PubMed database of 14 biomarkers used to measure gene methylation does not include GALNT14[31]. Thus, the correlation between GALNT14 methylation and the clinicopathological characteristics of KIRC explored in this study is the first to date, which will provide a reference for subsequent related studies. Altogether, methylation markers play an important role in the diagnosis and prediction of survival of solid tumors[33–35], which is why we explored the relationship between GALNT14 methylation and KIRC in this study. To our surprise, Chen et al. [3] proposed that the hypomethylation of oncogenes plays an indispensable role in the carcinogenesis process. In addition, Li et al. [33] proposed that the abnormal methylation contributing to cancer is common in all types of cancer. Interestingly, abnormal enhancer hypermethylation has been shown to be a predictor of poor survival in patients with KIRC[31, 36], which could fully explain the role of GALNT14 as a protective factor in KIRC. Similarly, studies have shown that the increase of hypermethylation is associated with a higher stage and grade of KIRC[3, 37], which is consistent with our finding. Therefore, we estimate that GALNT14 methylation may affect its function and thus contribute to the early diagnosis and prognosis of KIRC.

Finally, we used GSEA analysis to identify the pathway by which GALNT14 affects KIRC. The results showed that GALNT14 was highly expressed in histidine metabolism, peroxisome, renin-angiotensin system and other pathways. Our results are similar to those of Wang et al. [38], who identified three important pathways associated with KIRC, namely, arginine and proline metabolism, aldosterone-regulated sodium reabsorption, and oxidative phosphorylation. This suggests that GALNT14 plays an important role in the metabolic pathway of the KIRC enrichment pathway, which is consistent with the physiological function of the kidney. Previous studies have suggested a strong link between cancer and metabolism, with one even pointing to kidney cancer as a disorder of cell metabolism[16]. Recent research has shown that the characteristic that distinguishes KIRC from other types of tumors is its

specific metabolic changes[39]. In terms of amino acid metabolism, it has been reported that the deletion of the 3P chromosome in clear cell renal cell carcinoma leads to the inactivation of FHIT gene[40], so we speculate that this may be the reason why the histidine metabolic pathway is in the first place. Besides, Weiss et al. [41] proposed that tryptophan is catabolized by the renin pathway and that the renin metabolites have immunosuppressive effects. In addition, Liu et al. [5] have shown that tryptophan depletion leads to apoptosis through an accumulation of KP metabolites. Therefore, we speculate that GALNT14 may reduce tryptophan metabolism to maintain the immune function of patients, thereby limiting the proliferation of KIRC primary tumor. Then, in terms of fatty acid metabolism, Hillman et al. [2] showed that KIRC is associated with abnormal fatty acid metabolism. Interestingly, reduced fatty acids in KIRC were associated with increased tumor invasion and poor prognosis[41]. This suggests that the high expression of GALNT14 in KIRC may inhibit the expression of fatty acid synthase, leading to the higher OS in patients with GALNT14 upregulation. Surprisingly, there are also reports of dysregulation of carbon metabolism in KIRC[6]. This is basically consistent with our findings in KIRC enrichment analysis that GALNT14 overexpression affects glucose metabolism. KIRC has been reported to enhance uptake of glucose transporters, leading to glucose accumulation and further promoting tumor progression[39], so inhibition of gluconeogenesis will promote the development of RCC. In a word, we suggest that GALNT14 overexpression in KIRC reduces the absorptive activity of glucose transporter and accelerates glucose decomposition. Hence, we hypothesized that GALNT14 regulates the occurrence of KIRC through the above metabolism-related pathways. However, the prognosis of high GALNT14 expression is limited to KIRC I and IV stages, and further experiments are needed.

## 5 Conclusion

Consequently, GALNT14 can be served as an independent prognostic factor for KIRC and a potential target for clinical diagnosis and treatment of KIRC.

## Abbreviations

GALNTs	N-acetylgalactosaminyltransferases
GALNT1	N-acetylgalactosaminyltransferase 1
GALNT3	N-acetylgalactosaminyltransferase 3
GALNT4	N-acetylgalactosaminyltransferase 4
GALNT10	N-acetylgalactosaminyltransferase 10
GALNT14	N-acetylgalactosaminyltransferase 14
KIRC	Kidney renal clear cell carcinoma
KIRP	Kidney renal papillary cell carcinoma
KICH	Kidney chromophobe
TCGA	The Cancer Genome Atlas
OS	Overall survival
GSEA	Gene Set Enrichment Analysis
RCC	Renal cell carcinoma
CT	Computed tomography
US	Ultrasound
MRI	Magnetic resonance imaging
CRB3	Crumbs 3
SFRP1	Secreted Frizzled Related Protein 1
P4Hb	Protein Disulfide-Isomerase
DFS	Disease free survival
GEPIA	Gene Expression Profiling Interactive Analysis
CCLE	Broad Institute Cancer Cell Line Encyclopedia
KEGG	Kyoto Encyclopedia of Genes and Genomes
WHO	World Health Organization
NES	Normalized enrichment score
NOM	Nominal
FDR	False discovery rate
HCV	Hepatitis C virus
HCC	Hepatocellular carcinoma

ACE2	Angiotensin-converting enzyme 2
PTGD	Percutaneous Transhepatic Gallbladder Drainage
KIR3DL1	Recombinant Killer Cell Immunoglobulin Like Receptor 3DL1
DR4	Death receptors 4
DR5	Death receptors 5
TRAIL	Tumor necrosis factor-related apoptosis-inducing ligand
FHIT	Fragile histidine triad
KP	Tryptophan-kynurenine pathway

## Declarations

**Acknowledgements** All authors read and approved the final manuscript.

**Author contributions** WYQ, WF and ZSF carried out the information collection of database. WYQ and TYL participated in the design of the study and performed the statistical analysis. ZQN, WYQ and WQY conceived of the study, and participated in its design and coordination and helped to draft the manuscript.

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**Competing interests** The authors declare no competing interests.

**Data availability** Availability of data and supporting materials section: "Please contact author for data requests. Please contact the email: ebirdie123@126.com.

**Ethical approval** This study was approved by the Ethics Committee of Guangxi Medical University.

**Consent for publication** All authors read the final manuscript and consent for publication.

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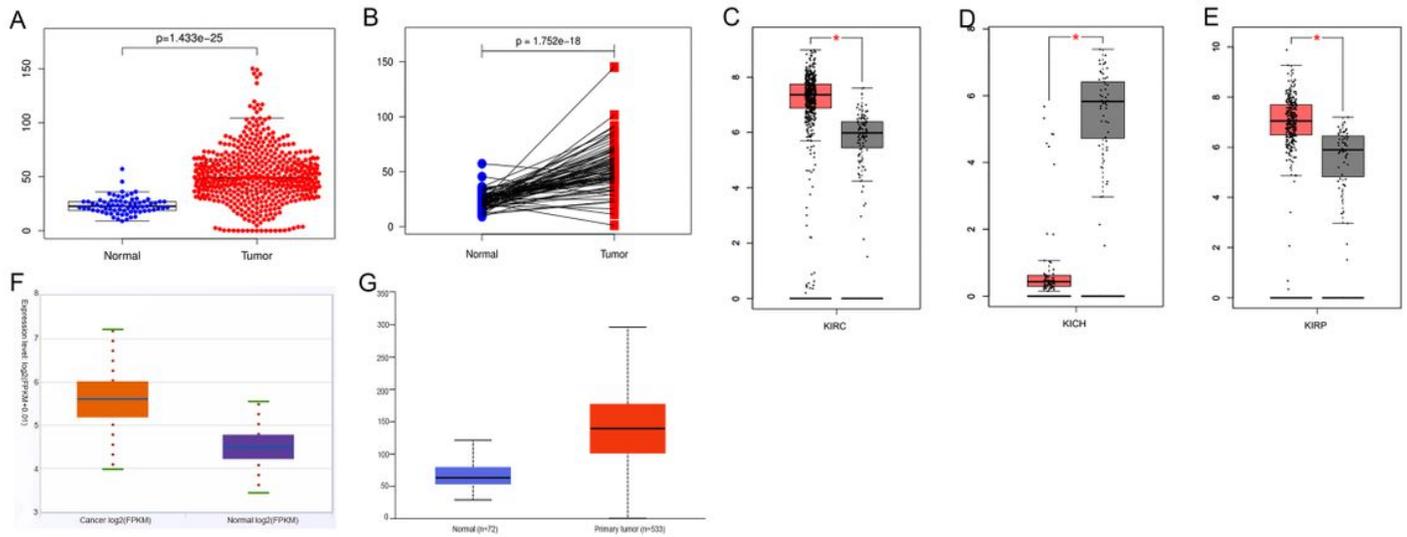
## References

1. Cui H, Shan H, Miao M Z, Jiang Z, Meng Y, Chen R, et al. Identification of the key genes and pathways involved in the tumorigenesis and prognosis of kidney renal clear cell carcinoma. *Sci Rep.* 2020;10(1):4271.
2. Hillman J C, Pugacheva E M, Barger C J, Sribenja S, Rosario S, Albahrani M, et al. BORIS Expression in Ovarian Cancer Precursor Cells Alters the CTCF Cistrome and Enhances Invasiveness through GALNT14. *Mol Cancer Res.* 2019;17(10):2051-62.
3. Chen W, Zhuang J, Wang P P, Jiang J, Lin C, Zeng P, et al. DNA methylation-based classification and identification of renal cell carcinoma prognosis-subgroups. *Cancer Cell Int.* 2019;19(1):185.
4. Escudier B, Porta C, Schmidinger M, RiouxLeclercq N, Bex A, Khoo V, et al. Renal cell carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol.* 2019;30(5):706-20.
5. Liu X, Zhang M, Liu X, Sun H, Guo Z, Tang X, et al. Urine Metabolomics for Renal Cell Carcinoma (RCC) Prediction: Tryptophan Metabolism as an Important Pathway in RCC. *Front Oncol.* 2019;9:663.
6. Hu F, Zeng W, Liu X. A Gene Signature of Survival Prediction for Kidney Renal Cell Carcinoma by Multi-Omic Data Analysis. *Int J Mol Sci.* 2019;20(22):5720.
7. Xie L, Li H, Zhang L, Ma X, Dang Y, Guo J, et al. Autophagy-related gene P4HB: a novel diagnosis and prognosis marker for kidney renal clear cell carcinoma. *Aging (Albany NY).* 2020;12(2):1828-42.
8. Hussain MRM, Hoessli D C, Fang M. N-acetylgalactosaminyltransferases in cancer. *Oncotarget.* 2016;7(33):54067-80.
9. Tsou YK, Liang KH, Tseng CK, Yeh CT, Lin WR, Chang HK. GALNT14 genotype as a response predictor for concurrent chemoradiotherapy in advanced esophageal squamous cell carcinoma. *Oncotarget.* 2017;8:29151-60.
10. Lin WR, Yeh CT. GALNT14: An Emerging Marker Capable of Predicting Therapeutic Outcomes in Multiple Cancers. *Int J Mol Sci.* 2020;21(4):1491.
11. Tian H, Zuo T, Wang X, An L, Xie Y, Wang J, et al. GALNT14 mediates tumor invasion and migration in breast cancer cell MCF-7. *Mol Carcinog.* 2015;54(10):1159-71.
12. Wu J, Chen X, Bao Q, Duan R, Jin Y, Shui Y, et al. Osterix Decreases the Chemosensitivity of Breast Cancer Cells by Upregulating GALNT14. *Cell Physiol Biochem.* 2017;44(3):998-1010.
13. Detarya M, Sawanyawisuth K, Aphivatanasiri C, Chuangchaiya S, Saranaruk P, Sukprasert L, et al. The O-GalNAcylating enzyme GALNT5 mediates carcinogenesis and progression of cholangiocarcinoma via activation of AKT/ERK signaling. *Glycobiology.* 2020;30(5):312-24.
14. Shan J, Liu Y, Wang Y, Li Y, Yu X, Wu C. GALNT14 Involves the Regulation of Multidrug Resistance in Breast Cancer Cells. *Transl Oncol.* 2018;11(3):786-93.
15. Liu L, Xiong Y, Yao J, Xu J, Xi W, Wang J, et al. Prognostic role of N-Acetylgalactosaminyltransferase 10 in metastatic renal cell carcinoma. *Oncotarget.* 2017;8(9):14995-5003.

16. Chen L, Peng T, Luo Y, Zhou F, Wang G, Qian K, et al. ACAT1 and Metabolism-Related Pathways Are Essential for the Progression of Clear Cell Renal Cell Carcinoma (ccRCC), as Determined by Co-expression Network Analysis. *Front Oncol.* 2019;9:957.
17. Meng T, Huang R, Zeng Z, Huang Z, Yin H, Jiao C, et al. Identification of Prognostic and Metastatic Alternative Splicing Signatures in Kidney Renal Clear Cell Carcinoma. *Front Bioeng Biotechnol.* 2019;7:270.
18. Yin L, Gao S, Shi H, Wang K, Yang H, Peng B. TIP-B1 promotes kidney clear cell carcinoma growth and metastasis via EGFR AKT signaling. *Aging (Albany NY).* 2019;11(18):7914-37.
19. Lin CC, Hsu CW, Chen YC, Chang ML, Liang KH, Lai MW, et al. A GALNT14 rs9679162 genotype-guided therapeutic strategy for advanced hepatocellular carcinoma: systemic or hepatic arterial infusion chemotherapy. *Pharmacogenomics J.* 2020;20(1):57-68.
20. Kwon OS, Lee H, Kong HJ, Kwon EJ, Park JE, Lee W, et al. Connectivity map-based drug repositioning of bortezomib to reverse the metastatic effect of GALNT14 in lung cancer. *Oncogene.* 2020;39(23):4567-80.
21. Liang K, Lin C, Chen S, Chiu C, Yang P, Chang M, et al. GALNT14 genotype effectively predicts the therapeutic response in unresectable hepatocellular carcinoma treated with transcatheter arterial chemoembolization. *Pharmacogenomics.* 2016;17(4):353-66.
22. Zhang Z, Li L, Li M, Wang X. The SARS-CoV-2 host cell receptor ACE2 correlates positively with immunotherapy response and is a potential protective factor for cancer progression. *Comput Struct Biotechnol J.* 2020;18:2438-44.
23. Qiu Z, Guo Z, Jiang L, Qiu H, Pan J, Ao Y. Prognostic role of PHYH for overall survival (OS) in clear cell renal cell carcinoma (ccRCC). *Eur J Med Res.* 2021;26(9):1-12.
24. Jiang J, Pan W, Xu Y, Ni C, Xue D, Chen Z, et al. Tumour-Infiltrating Immune Cell-Based Subtyping and Signature Gene Analysis in Breast Cancer Based on Gene Expression Profiles. *J Cancer.* 2020;11(6):1568-83.
25. Zhen J, Zhu L, Li W, Hu H, Deng Z, Xiong L. A protective effect conferred by KIR3DL1 and its cognate ligand against cervical cancer among ethnic Han Chinese population and its potential mechanism. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi.* 2019;36(10):1035-8.
26. Song KH, Park MS, Nandu T S, Gadad S, Kim SC, Kim MY. GALNT14 promotes lung-specific breast cancer metastasis by modulating self-renewal and interaction with the lung microenvironment. *Nat Commun.* 2016;7:13796.
27. Wang M, Zhu J, Lubman D M, Gao C. Aberrant glycosylation and cancer biomarker discovery: a promising and thorny journey. *Clin Chem Lab Med.* 2019;57(4):407-16.
28. Drake R R, McDowell C, West C, David F, Powers T W, Nowling T, et al. Defining the human kidney N-glycome in normal and cancer tissues using MALDI imaging mass spectrometry. *J Mass Spectrom.* 2020;55(4):1-10.
29. Zuo T, Shan J, Liu Y, Xie R, Yu X, Wu C. EFEMP2 Mediates GALNT14-Dependent Breast Cancer Cell Invasion. *Transl Oncol.* 2018;11(2):346-52.

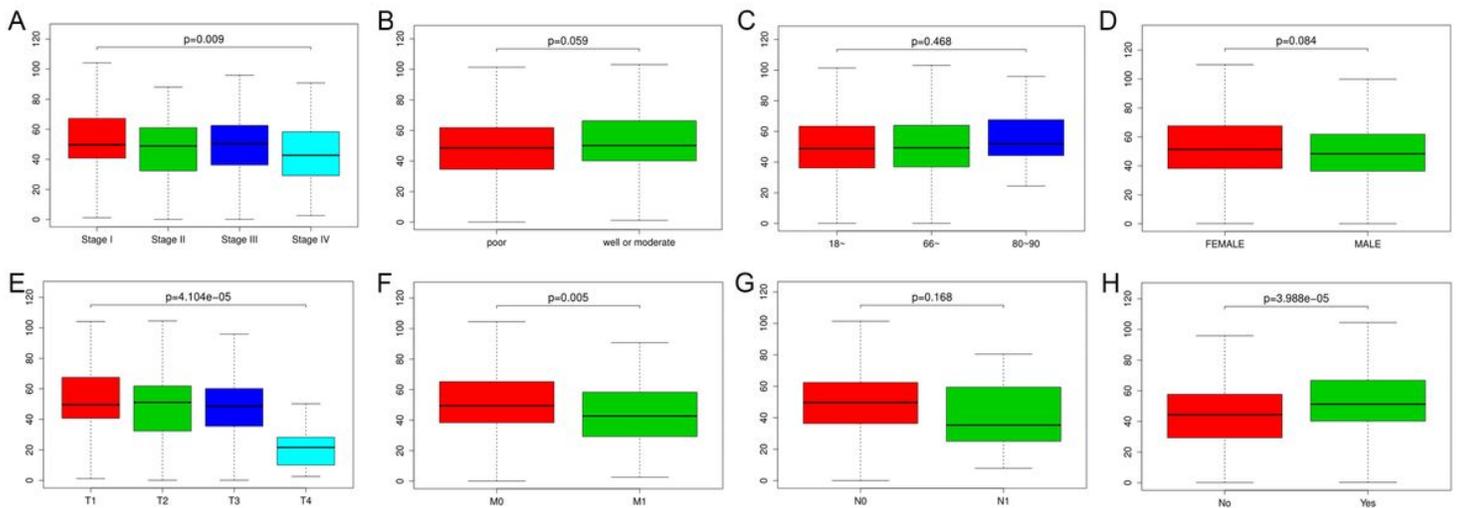
30. Jeon M Y, Seo S U, Woo S M, Min K J, Byun H S, Hur G M, et al. Oridonin enhances TRAIL-induced apoptosis through GALNT14-mediated DR5 glycosylation. *Biochimie*.2019;165:108-14.
31. Koch A, Joosten S C, Feng Z, Ruijter T C. de , Draht M X, Melotte V, et al. Analysis of DNA methylation in cancer: location revisited. *Nat Rev Clin Oncol*.2018;15(7):459-66.
32. Luo H, Zhao Q, Wei W, Zheng L, Yi S, Li G, et al. Circulating tumor DNA methylation profiles enable early diagnosis, prognosis prediction, and screening for colorectal cancer. *Sci. Transl. Med.* .2020;12(524):eaax7533.
33. Li D, Zhang L, Liu Y, Sun H, Onwuka JU, Zhao Z, et al. Specific DNA methylation markers in the diagnosis and prognosis of esophageal cancer. *AGING*.2019;11(23):11640-58.
34. Yang Y, Qi S, Shi C, Han X, Yu J, Zhang L, et al. Identification of metastasis and prognosis-associated genes for serous ovarian cancer. *Biosci Rep*.2020;40(6):BSR20194324.
35. Zhao Q, Jiang C, Gao Q, Zhang Y, Wang G, Chen X, et al. Gene expression and methylation profiles identified CXCL3 and CXCL8 as key genes for diagnosis and prognosis of colon adenocarcinoma. *J Cell Physiol*.2020;235(5):4902-12.
36. Fang J, Hu M, Sun Y, Zhou S, Li H. Expression Profile Analysis of m6A RNA Methylation Regulators Indicates They Are Immune Signature Associated and Can Predict Survival in Kidney Renal Cell Carcinoma. *DNA Cell Biol*.2020;39(12):1-18.
37. Miao Y, Cao F, Li P, Liu P. DNA methylation of HUGL-2 is a prognostic biomarker in kidney renal clear cell carcinoma. *Clin Exp Pharmacol Physiol*.2020;48(1):44-53.
38. Wang SM, Sun ZQ, Li HY, Wang J, Liu QY. Temporal Identification of Dysregulated Genes and Pathways in Clear Cell Renal Cell Carcinoma Based on Systematic Tracking of Disrupted Modules. *Comput Math Methods Med*.2015;2015:313740.
39. Poplawski P, Tohge T, Boguslawska J, Rybicka B, Tanski Z, Trevino V, et al. Integrated transcriptomic and metabolomic analysis shows that disturbances in metabolism of tumor cells contribute to poor survival of RCC patients. *Biochim Biophys Acta Mol Basis Dis*.2017;1863(3):744-52.
40. Liu X P, Yin X H, Yan X H, Zeng X T, Wang X H. The Clinical Relevance of Fragile Histidine Triad Protein (FHIT) in Patients with Bladder Cancer. *Med Sci Monit*.2018;24:3113-8.
41. Weiss R H. Metabolomics and Metabolic Reprogramming in Kidney Cancer. *Semin Nephrol*.2018;38(2):175-82.

## Figures



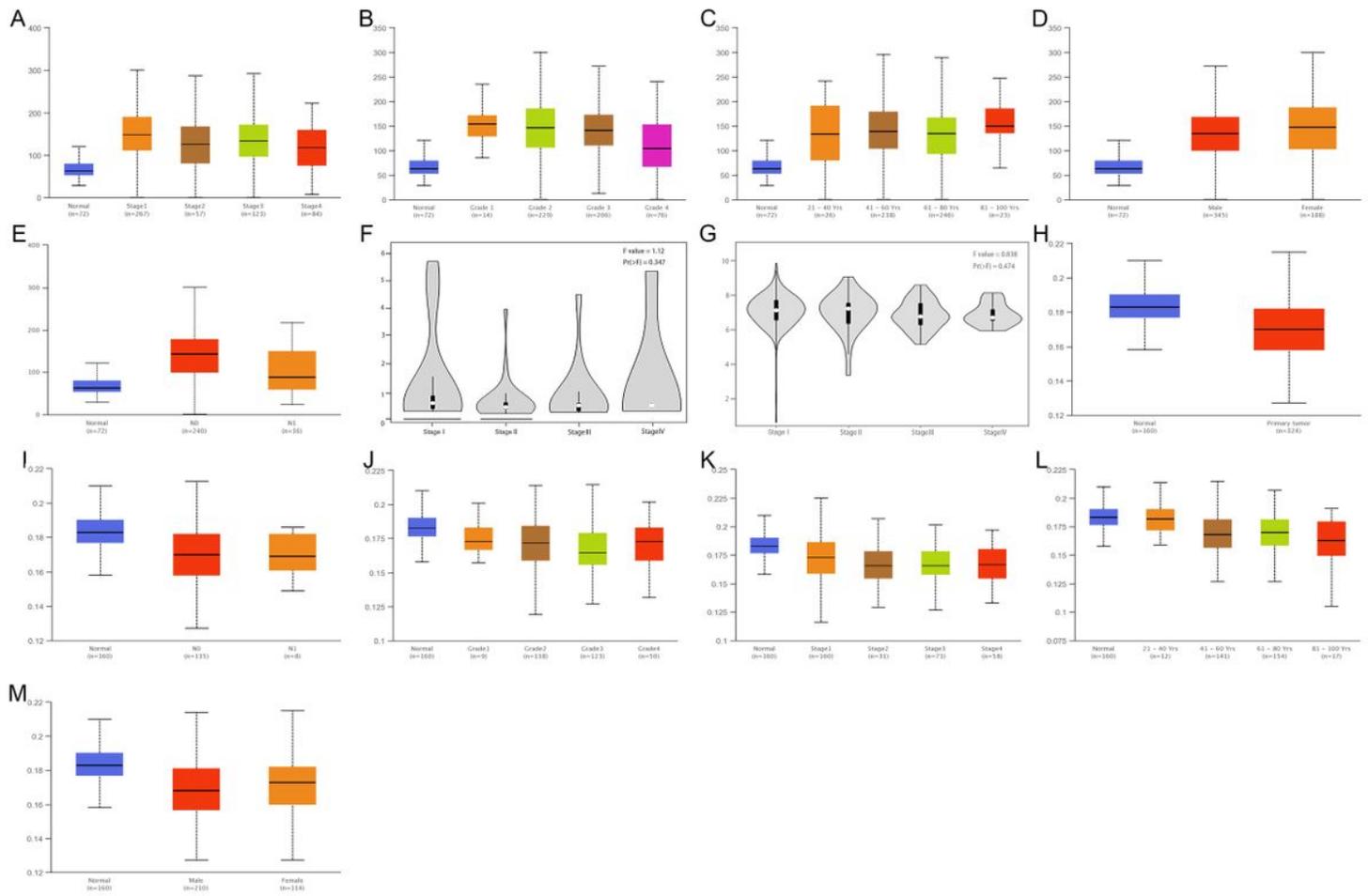
**Figure 1**

Results of differential expression of GALNT14. A GALNT14 differential expression scatter plot; B GALNT14 paired differential analysis diagram; C The expression of GALNT14 in KIRC was analyzed by GEPIA; D The expression of GALNT14 in KICH was analyzed by GEPIA; E The expression of GALNT14 in KIRP was analyzed by GEPIA; F The expression of GALNT14 in KIRC was analyzed by starBase v3.0; G The expression of GALNT14 in KIRC was analyzed by UALCAN;



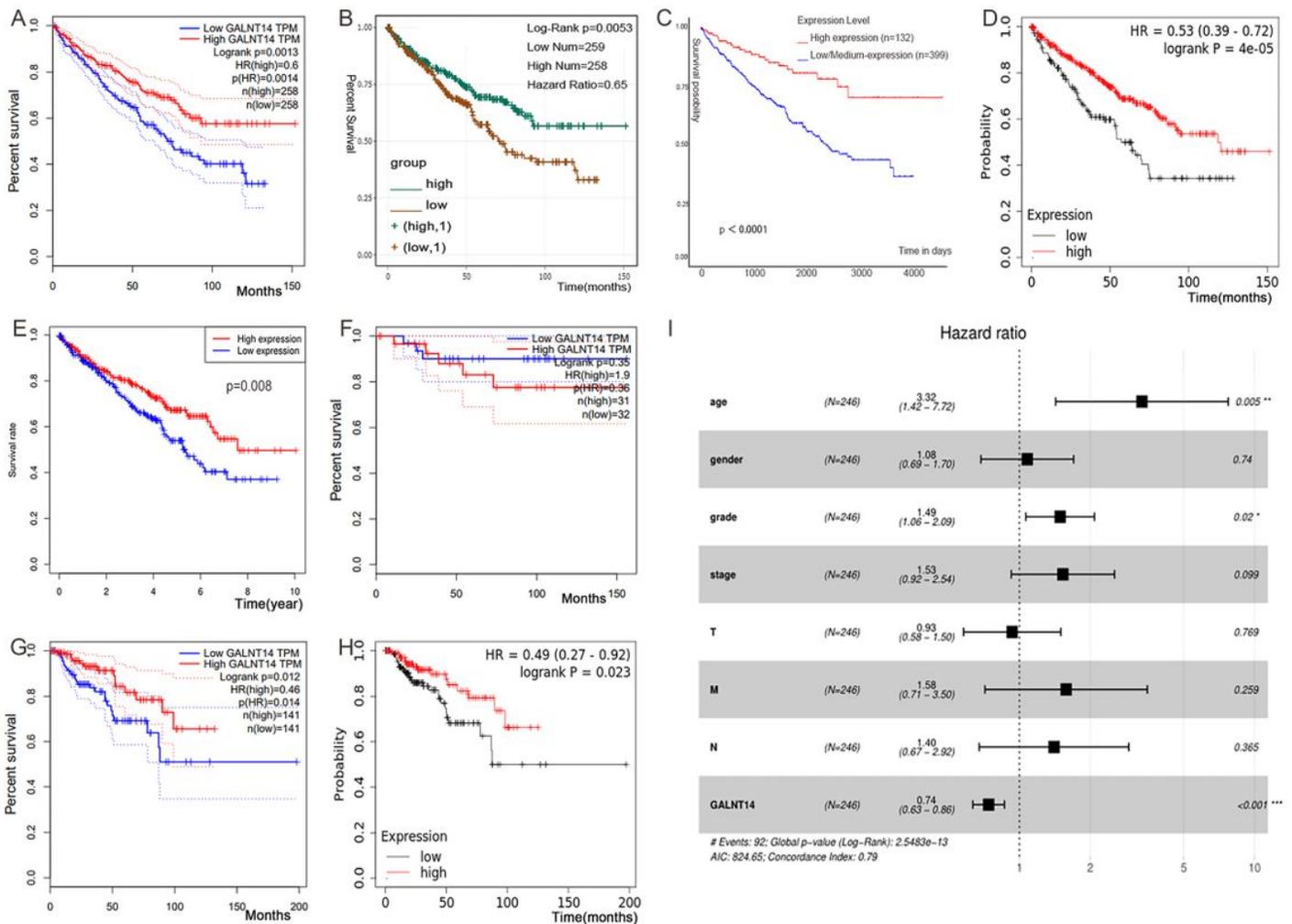
**Figure 2**

Association with GALNT14 expression and clinicopathologic characteristics. A TCGA by Histological stage; B TCGA by Histological grade; C TCGA by Histological age; D TCGA by Histological gender; E TCGA by Histological T classification; F TCGA by Histological M classification; G TCGA by Histological N classification; H TCGA by Histological metastases



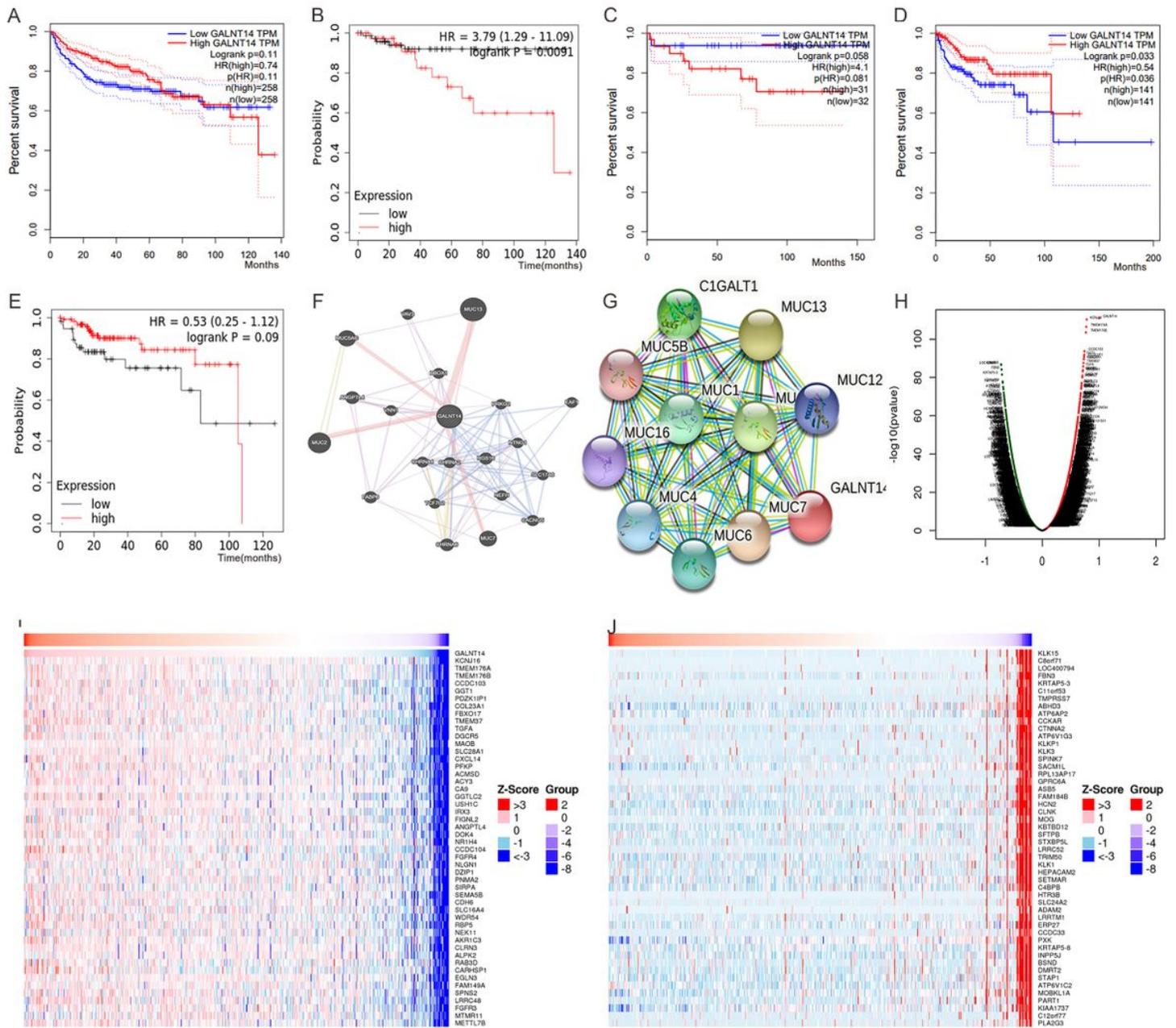
**Figure 3**

The clinicopathologic features of GALNT14 in KIRC and the relationship between GALNT14 methylation and clinical features were analyzed by UALCAN. A Expression of GALNT14 in KIRC based on cancer stages; B Expression of GALNT14 in KIRC based on tumor grade; C Expression of GALNT14 in KIRC based on patient's age; D Expression of GALNT14 in KIRC based on patient's gender; E Expression of GALNT14 in KIRC based on nodal metastasis status; F The stage of GALNT14 in KIRC; G The stage of GALNT14 in KIRP; H Differential expression of GALNT14 methylation; I Promoter methylation level of GALNT14 in KIRC on nodal metastasis status; J Promoter methylation level of GALNT14 in KIRC on grade; K Promoter methylation level of GALNT14 in KIRC on stage; L Promoter methylation level of GALNT14 in KIRC on age; M Promoter methylation level of GALNT14 in KIRC on gender



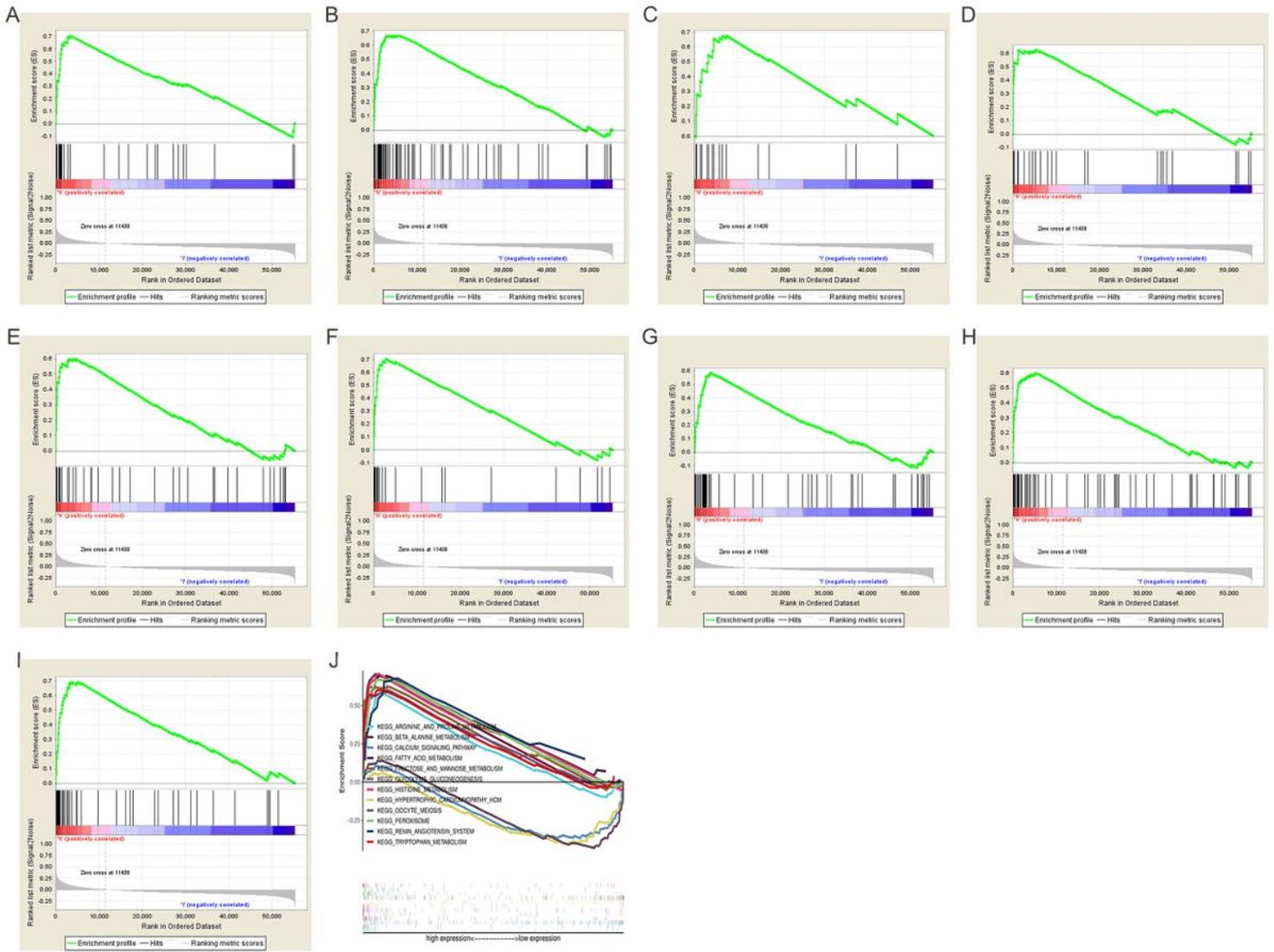
**Figure 4**

The OS prognostic value of GALNT14 in KIRC, KICH and KIRP. A The OS prognostic value of GALNT14 in KIRC was analyzed by GEPIA; B The OS prognostic value of GALNT14 in KIRC was analyzed by starBase v3.0; C The OS prognostic value of GALNT14 in KIRC was analyzed by UALCAN; D The OS prognostic value of GALNT14 in KIRC was analyzed by Kaplan–Meier Plotter; E The OS prognostic value of GALNT14 in KIRC was analyzed by Meta analysis; F The OS prognostic value of GALNT14 in KICH was analyzed by GEPIA; G The OS prognostic value of GALNT14 in KIRP was analyzed by GEPIA; H The OS prognostic value of GALNT14 in KIRP was analyzed by Kaplan–Meier Plotter; I The forest of GALNT14



**Figure 5**

The DFS prognostic value, co-expression gene and interaction network of GALNT14. A The DFS prognostic value of GALNT14 in KIRC was analyzed by GEPIA; B The DFS prognostic value of GALNT14 in KIRC was analyzed by Kaplan–Meier Plotter; C The DFS prognostic value of GALNT14 in KICH was analyzed by GEPIA; D The DFS prognostic value of GALNT14 in KIRP was analyzed by GEPIA; E The DFS prognostic value of GALNT14 in KIRP was analyzed by Kaplan–Meier Plotter; F The co-expression gene of GALNT14 was analyzed by GeneMANIA; G The interaction network of GALNT14 protein with other proteins from STRING; H The overall co-expressed genes of GALNT14 in KIRC were displayed as a volcano plot; I The positively co-expressed top-50 genes of GALNT14 in KIRC were shown as heat map; J The negatively co-expressed top-50 genes of GALNT14 in KIRC were shown as heat map



**Figure 6**

Enrichment plots from gene set enrichment analysis (GSEA). A KEGG\_histidine\_metabolism; B KEGG\_peroxisome; C KEGG\_renin\_angiotensin\_system; D KEGG\_fructose\_and\_mannose\_metabolism; E KEGG\_tryptophan\_metabolism; F KEGG\_beta\_alanine\_metabolism; G KEGG\_arginine\_and\_proline\_metabolism; H KEGG\_glycolysis\_gluconeogenesis; I KEGG\_fatty\_acid\_metabolism; J Enrichment plots from multi-enrichment analysis