Original Article

KIF11 is a potential prognostic biomarker and therapeutic target for adrenocortical carcinoma

Yan Zhou[1], Xiang Chen[2], Bingsheng Li[2], Yang Li[2], Bo Zhang[2]

[1]Department of Physiology, Xiangya School of Medicine, Central South University, Changsha, China; [2]Department of Urology, Xiangya Hospital, Central South University, Changsha, China

Contributions: (I) Conception and design: B Zhang, Y Zhou, X Chen; (II) Administrative support: X Chen; (III) Provision of study materials or patients: X Chen, B Li, Y Li; (IV) Collection and assembly of data: Y Zhou, B Zhang; (V) Data analysis and interpretation: B Zhang, Y Zhou; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Bo Zhang. Department of Urology, Xiangya Hospital, Central South University, Changsha, China. Email: bozhangxy@163.com.

Background: Adrenocortical carcinoma (ACC) is a rare endocrine neoplasia with poor prognosis. Emerging evidence suggests that kinesin family member 11 (KIF11) protein is overexpressed in several tumors and associated with the onset and progression of certain types of cancer; however, its biological functions and mechanisms in ACC progression have not been studied yet. Therefore, this study evaluated the clinical significance and therapeutic potential of the KIF11 protein in ACC.

Methods: The Cancer Genome Atlas (TCGA) database (n=79) and Genotype Tissue Expression (GTEx) database (n=128) were utilized to explore the expression of KIF11 in ACC and normal adrenal tissues. The TCGA datasets were then data mined and statistically analyzed. R survival analysis and univariate and multivariate Cox regression analyses were used to evaluate the effect of KIF11 expression on the survival rates, and a nomogram was used to predict its impact on prognosis. The clinical data from 30 ACC patients' from Xiangya Hospital were also analyzed. The effects of KIF11 on the proliferation and invasion of ACC NCI-H295R were further validated *in vitro*.

Results: Analytical data from the TCGA and GTEx databases showed that KIF11 expression was upregulated in ACC tissues and associated with T (primary tumor), and M (metastasis) and stages of tumor progression. Increased KIF11 expression was significantly associated with shorter overall survival, disease-specific survival, and progression-free intervals. Clinical data from Xiangya Hospital illustrated that increased KIF11 had a significantly positive correlation with shorter overall survival, T and pathological stages, and tumor recurrence risk. Monastrol, a specific inhibitor of KIF11, was further confirmed to significantly inhibit the proliferation and invasion of ACC NCI-H295R cell *in vitro*. The nomogram demonstrated KIF11 was an excellent predictive biomarker in patients with ACC.

Conclusions: The findings demonstrate that KIF11 could be a predictor of poor prognosis and thus possibly serve as a novel therapeutic target for ACC.

Keywords: Adrenocortical carcinoma (ACC); kinesin family member 11 (KIF11); prognosis; experimental validation; therapeutic target

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Introduction

Adrenocortical carcinoma (ACC) is a rare and aggressive malignant endocrine neoplasia of the adrenal cortex with an incidence rate of 0.5 to 2 cases per million population per year (1). For patients with localized ACC, complete operative resection is the preferred treatment for long-term survival (2). Unfortunately, ACC is a highly malignant tumor, with 70% to 85% of patients experiencing

recurrence after surgical resection (3,4). Initial staging of ACC is the most important factor in predicting its prognosis. The 5-year overall survival (OS) rate ranges from 60% to 80% in patients with stage I ACC and decreases to 13% in those with stage IV ACC (5).

Although several factors have been reported to be associated with the prognosis and risk of tumor recurrence, an accurate prediction of patients with ACC remains a challenge. Moreover, treatment options are limited for patients with advanced ACC. Currently, the major of adjuvant treatment consists of mitotane alone or in combination with multi-drug chemotherapeutics, such as etoposide, doxorubicin, and cisplatin, which is known as the Italian protocol (6). However, this multi-drug treatment regimen has significant toxicity potential in patients. Furthermore, mitotane, which has a specific cytotoxic effect on the steroidogenic cells of the adrenal cortex, is the only approved drug for the treatment of advanced ACC (7,8). The side effects of mitotane treatment include toxicity affecting the bone marrow, liver, skin, gastrointestinal tract, and neuromuscular junctions (8). Therefore, ACC prognosis remains poor, and novel treatments and prognostic markers are urgently required.

Kinesin family member 11 (KIF11), also known as EG5 or kinesin-5, is thought to be vital in the tetrameric microtubule cross-linkage, cell mitosis, cell cycle, and cell differentiation (9,10). Although the physiological function of KIF11 remains largely unclear, studies have suggested that high KIF11 expression levels are associated with advanced stages of cancer, invasion, metastasis, and recurrence. Recently, KIF11 is shown to be overexpressed in several tumors and associated with poor prognosis of diseases such as breast cancer (11), liver cancer (12), prostate cancer (13), bladder cancer (14), clear cell renal cell carcinoma (15), gastric cancer (16), non-small cell lung cancer (17), and meningioma (18). However, the relevant roles and mechanisms of KIF11 in ACC progression have not been studied yet. Monastrol, a cytotoxic small molecule, from dihydropyrimidinone scaffold, is an inhibitor of KIF11. The effect of Monastrol on ACC is yet to be verified. This study aimed to evaluate the clinical significance and therapeutic potential of KIF11 protein in ACC. We present the following article in accordance with the STROBE reporting checklist (available at https://tau.amegroups.com/article/view/10.21037/tau-22-706/rc).

Highlight box

Key findings

- KIF11 could be a predictor of poor prognosis and possibly serve as a novel therapeutic target for ACC

What is known and what is new?

- ACC is a rare and aggressive malignant endocrine neoplasia of the adrenal cortex, ACC prognosis remains poor, and novel treatments and prognostic markers are urgently required.
- High KIF11 expression levels are significantly associated with the progression of ACC and resulting in poor survival rates. KIF11 expression appears to be a novel and independent prognostic marker in ACC. KIF11 is a key protein involved in cell proliferation and invasion, and may thus serve as a potential novel therapeutic target for ACC.

What is the implication, and what should change now?

- Treatment options are limited for patients with advanced ACC.

Methods

Differential expression of KIF11: The Cancer Genome Atlas (TCGA) and Genotype Tissue Expression (GTEx) database analyses

TCGA database (n=79) and GTEx database (n=128) were utilized to explore the differential expression of KIF11 in ACC and normal adrenal tissues. The data is RNA-seq data in transcripts per million reads (TPM) format, which is uniformly processed by Toil process (19). KIF11 differential expression in the ACC tissues in the TCGA database was defined as KIF11-high or KIF11-low when the values were above or below the KIF11 median value, respectively. Detailed clinical information, including sex, age, TNM stages, pathologic stages, therapy outcome, and survival information, including the OS, disease-specific survival (DSS), and progress-free interval (PFI), for the ACC samples was obtained from the TCGA.

Differentially expressed genes (DEGs) analysis, correlation analysis, Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, and Gene set enrichment analysis (GSEA)

DEGs in patients with KIF11-high and KIF11-low expression in the TCGA dataset were identified using the unpaired Student's *t*-test and DESeq2 (1.26.0) package (20). Genes with an adjusted P value <0.05, and $\log_2$ fold change >2.0, were considered statistically significant. GO and KEGG pathway enrichment analyses were performed

using the clusterProfiler package in R (21). Adjusted P values <0.05 were considered to indicate statistically significant pathways. GSEA was performed using the GSEA software (22).

Ethical statement

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of the Xiangya Hospital, China (No. 202109889). And all samples and clinical information used in this study were obtained with informed consent from patients.

ACC samples and normal tissues

ACC samples (n=30) and normal tissues (n=20) were collected from patients undergoing surgery at Xiangya Hospital between January 2009 and January 2016. The ACC inclusion criteria were as follows: (I) unilateral tumor, (II) postoperative pathologic diagnosis of ACC, (III) no other systemic diseases, (IV) and no other treatment before surgery. Hematoxylin and eosin (H&E)-stained sections were reviewed by two independent pathologists for confirmation. Clinicopathological parameters, such as age, sex, clinical symptoms, tumor size, stages, metastasis status, and survival data, were extracted from the medical records, pathological reports, and patient follow-up. Follow-up was done for all patients 72 months after surgery.

H&E staining

ACC and normal tissues were fixed in 4% paraformaldehyde solution and embedded in paraffin. For H&E staining, slides were immersed in a hematoxylin solution for 3 to 5 min, differentiated with acid alcohol, and counterstained with eosin for 3 min. Images of the H&E-stained sections were acquired using a microscope (Nikon, Japan).

Immunohistochemical (IHC) staining and analysis

For IHC staining, the paraffin sections were deparaffinized, rehydrated, immersed in 3% hydrogen peroxide for 10 min to inactivate endogenous peroxidase, and then incubated for 30 min in blocking buffer containing 5% bovine serum albumin. After blocking, the sections were incubated with primary antibodies against KIF11 (1:100; Proteintech, China) overnight at 4 ℃, followed by incubation at room temperature with horseradish peroxidase-conjugated anti-rabbit secondary antibody (1:100; Sigma-Aldrich, USA). The IHC staining results for the KIF11 proteins were determined using a semi-quantitative analysis technique, in which samples were analyzed using a bright-field microscope (Nikon, Japan). For each sample, the protein expression intensity was determined using ImageJ software. The KIF11 values were expressed as the percentage of positive cells in each case. The cell staining intensity was divided into two categories: cases with greater than or equal to 30% positive nuclei were classified as KIF11-high group, and those with less than 30% were classified as KIF11-low group. Three independent observers inspected the specimens in a blinded manner.

Cell culture and treatment

Human ACC cell lines, NCI-H295R (hormonally active) were purchased from Procell Life Science & Technology Co., Ltd. (Wuhan, China). NCI-H295R cell were cultured in DMEM (Procell, China) supplemented with 10% fetal bovine serum (FBS; CellMax, Australia) and 1% penicillin/streptomycin in a humidified 5% CO_2 atmosphere at 37 ℃.

Cell viability assay

For the cell counting kit-8 (CCK-8) assay, cells (1×10^4 cells/well) were seeded into 96-well plates and cultured with or without mitotane or monastrol (3, 10, 30, and 100 μM) for 48 h. Subsequently, 10 μL of CCK-8 solution (Dojindo, Japan) was added to each well at different time points. After 2 h of incubation, the optical density at 450 nm was measured using a microplate reader (Thermo Scientific, USA).

Transwell assay

A cell invasion assay was performed using 8.0 μm PET Membrane 24-well Transwell chamber (Millipore, USA). For the invasion assay, the chamber was coated with Matrigel (BD Biosciences, USA), cells were seeded into the upper chamber with serum-free medium (2×10^5 cells), and DMEM with 20% FBS was added to the bottom chamber with or without mitotane or monastrol (30 μM) for 24 hours. After incubation for 24 hours, the cells were fixed in 4% paraformaldehyde and stained with 1% crystal violet. Cells that had migrated were counted under an inverted light microscope by counting the number of cells from 10 random fields at 100× magnification.

Colony formation assay

In brief, cells were plated into 6-well plates at a density of 2,000 cells per well and maintained in an incubator at 37 ℃ and 5% CO_2. The cells were allowed to grow for 7 days to allow colony formation with or without mitotane or monastrol (30 μM). When the colonies had grown to an appropriate size, 1 mL of 4% paraformaldehyde was used to fix the cells for 20 min, and crystal violet was used for staining for 10 min, followed by washing with phosphate buffered saline, drying to obtain the images, and then counting the number of colonies.

Statistical analysis

All data were expressed as means ± standard deviations. Data from multiple groups were analyzed using analysis of variance (ANOVA) followed by Student's *t*-test or Wilcoxon rank-sum test for comparisons between two groups. Correlations between clinical characteristics and KIF11 expression were analyzed using logistic regression. Univariate and multivariate Cox regression analyses were performed to determine the relationship between the KIF11 levels and clinical parameters. Receiver operating characteristic (ROC) curves for survival were plotted using the Kaplan-Meier survival ROC package. Nomograms and calibration plots were constructed with the "rms" package. Survival analyses and c-index calculations were performed using the Kaplan-Meier "survival" package. The above analyses were performed using R software (3.6.3) and GraphPad Prism (6.0). Differences were considered statistically significant at P<0.05.

Results

KIF11 expression was significantly elevated in ACC tissues

To explore the relationship between KIF11 expression and cancer progression, pan-cancer analyses was applied to compare the KIF11 expression levels in multiple cancer samples from the GTEx combined with TCGA and corresponding normal samples from the TCGA database. KIF11 was found to be highly expressed in most malignant tumors, including ACC, bladder urothelial carcinoma, breast infiltrating carcinoma, cervical squamous cell carcinoma, and adenocarcinoma (*Figure 1A*). The expression of KIF11 was then compared between 128 normal samples and 79 ACC samples from the TCGA_GTEx dataset. The KIF11 expression levels in the ACC tissues were found to be significantly higher than those in the normal adrenal tissues (P<0.001) (*Figure 1B*). Clinical information was collected from 30 patients with ACC who underwent surgery at Xiangya Hospital between January 2009 and January 2016. A typical enhanced computed tomography image of an ACC sample is shown in *Figure 2A*. Normal adrenal cortex tissues are neatly arranged into zona glomerulosa, zona fasciculata, and zona reticularis. In contrast, in the ACC tissues, the nucleus is large and deeply stained, the arrangement is disordered, and heterogeneity is evident. Furthermore, IHC staining to detect KIF11 expression showed that it was significantly higher in ACC samples than in normal adrenal tissues (*Figure 2B*,*2C*). The staining intensity was divided into 'KIF11-high' (greater than or equal to 30% positive nuclei) and 'KIF11 low' (less than 30% positive nuclei) groups, for comparison with clinical severity (given below). H&E staining of normal adrenal tissues and an ACC sample is shown in *Figure 2D*. The resulting data indicate that KIF11 expression is significantly elevated in ACC tissues, and that KIF11 may be an important marker of ACC.

Identification of genes corelated with KIF11 in ACC

The ACC samples from the TCGA database were divided into two groups according to their KIF11 expression, and the 40 samples from the KIF11-high group were compared with 39 samples from the KIF11-low group used as a control. A total of 941 DEGs (395 downregulated and 546 upregulated) showed statistically significant differences between the two cohorts (adjusted P<0.05, $|\text{Log}_2$ fold change$|$ >2.0) (*Figure 3A*). The relative expression levels of the top 20 DEGs between the two cohorts are shown in *Figure 3B*, including 10 up-regulated and 10 down-regulated genes. Metascape was then used for GO and KEGG analyses of the DEGs. The top 12 GO enrichment terms were identified (*Figure 3C*), which included receptor-ligand activity, pattern specification process, DNA-binding transcription activator activity, RNA polymerase II-specific, and collagen-containing extracellular matrix. The correlations among the top 12 enriched terms from the GO analysis are shown as a network in *Figure 3D*. GSEA was conducted to identify the KIF11-related signaling pathways in ACC using the TCGA data. Several pathways and biological processes were found to be differentially enriched in the KIF11-high ACC group, including the activated KEGG-p53, PID-PLK1, Notch, Rho-GTPase, KEGG-TGFβ, and Wnt signaling pathways (*Figure 3E-3J*).

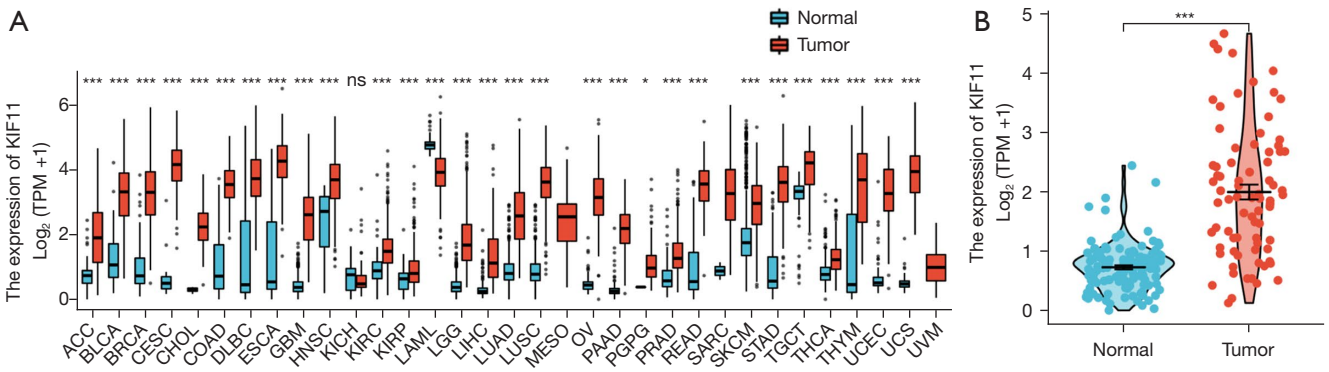


Figure 1 KIF11 expression is significantly elevated in ACC samples compared with normal tissues (from TCGA and GTEx database). (A) Expression level of KIF11 in different types of human cancers in the TCGA and GTEx database. (B) Expression level of KIF11 in unpaired normal and ACC samples. ns, no significant; *, P<0.05; ***, P<0.001. TPM, transcripts per million reads; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangio carcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM uveal melanoma; KIF11, kinesin family member 11; TCGA, The Cancer Genome Atlas; GTEx, Genotype Tissue Expression.

The correlation between KIF11 expression and immune infiltration

The correlation between the expression level of KIF11 and immune cell infiltration level quantified by ssGSEA was analyzed by Spearman correlation. The expression of KIF11 was positively correlated with the abundance of Th2 cells, and negatively correlated with the abundance of Cytotoxic cells, Mast cells, and Macrophages etc. (*Figure 4*).

Increased KIF11 was associated with shorter survival time and clinicopathological variables in patients with ACC

ROC analysis was used to analyze the ability of KIF11 to discriminate between ACC and normal adrenal tissues. The area under the curve (AUC) of KIF11 was 0.866, suggesting that KIF11 may be a potential diagnostic marker for ACC (*Figure 5A*). Patients in the KIF11-high group had poorer OS [hazard ratio (HR) =12.61, P<0.001], DSS (HR =12.08, P<0.001), and PFI (HR =5.96, P<0.001) as compared to those in the KIF11-low group (*Figure 5B-5D*). The relationship between KIF11 expression and the clinicopathological variables was then analyzed. Increased KIF11 demonstrated a significantly positive correlation with the T stages (T4 *vs.* T1, P<0.01; T4 *vs.* T2, P<0.001), M stages (M1 *vs.* M0, P<0.001), pathological stages (Stage IV *vs.* Stage I, P<0.001; Stage IV *vs.* Stage II, P<0.001; Stage IV *vs.* Stage III, P<0.05), primary therapy outcome [progressive disease (PD) and stable disease (SD) and partial response (PR) *vs.* complete response (CR), P<0.001], residual tumor (R1 and R2 *vs.* R0, P<0.001), venous invasion (present *vs.* absent, P<0.01), and invasion of the tumor capsule (present *vs.* absent, P<0.05) (*Figure 5E-5L* and *Table 1*).

Univariate logistic regression analysis showed that KIF11 expression was a categorical dependent variable associated with poor prognostic clinicopathological characteristics (*Table 2*). High expression of KIF11 in the ACC was positively associated with the T stages [odds ratio (OR) = 6.111 for T3 and T4 *vs.* T1 and T2], N stages (OR =10.133 for T3 and T4 *vs.* T1 and T2), M stages (OR =22.167 for T3 and T4 *vs.* T1 and T2), pathological stages (OR =7.837 for Stage III and IV *vs.* Stage I and II), residual tumor (OR =22.667 for R1 and R2 *vs.* R0), tumor status (OR =14.531

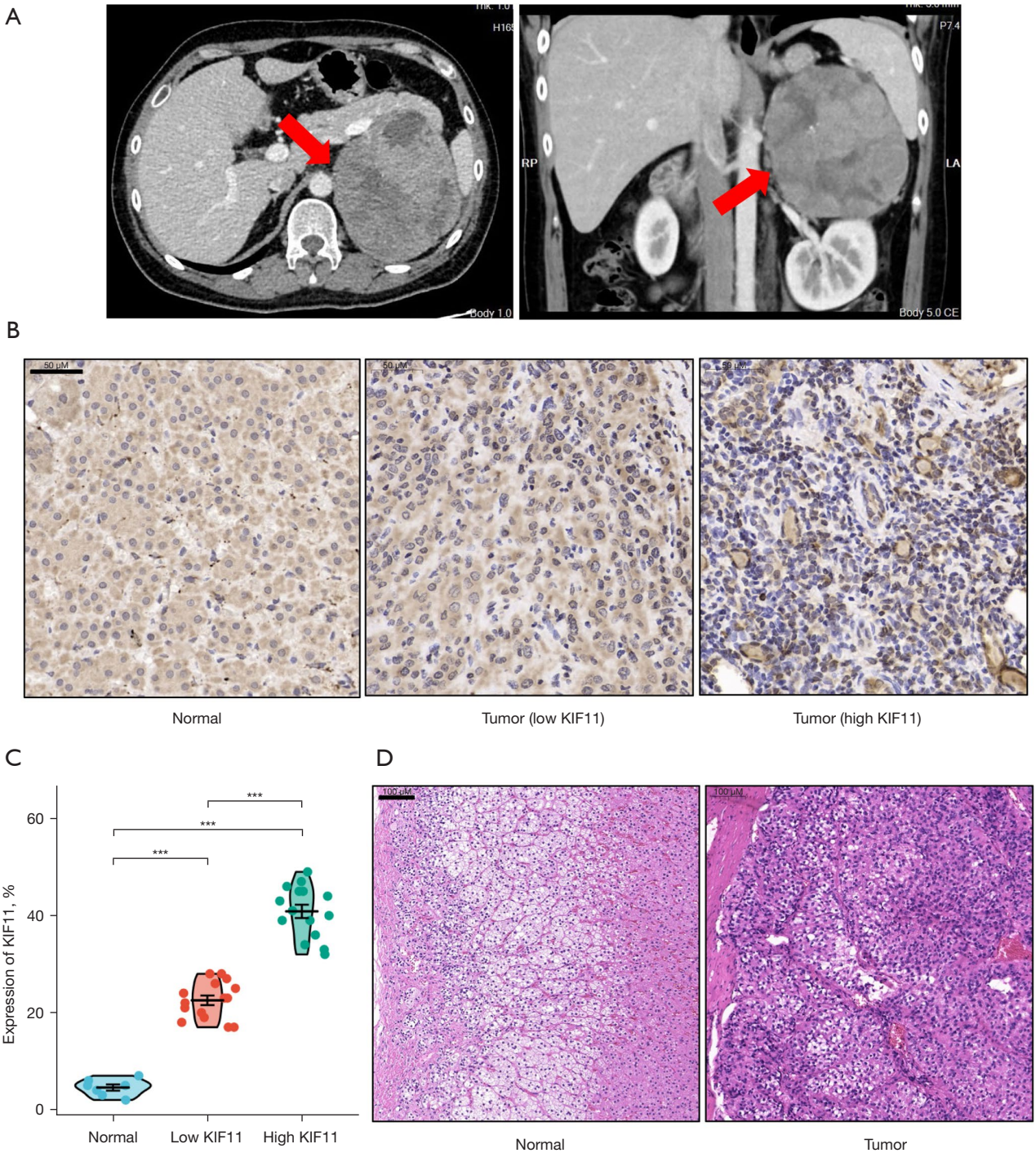


Figure 2 KIF11 expression is significantly elevated in ACC samples compared with normal tissues (from Xiangya Hospital). (A) Typical enhanced computed tomography images of ACC (red arrows). (B) Immunohistochemical staining results of KIF11 in normal adrenal tissues and ACC samples (bar = 50 μm). (C) The KIF11 immunohistochemical staining intensity. (D) HE staining of normal adrenal tissues and ACC samples (bar = 100 μm). ***, P<0.001. ACC, adrenocortical carcinoma; KIF11, kinesin family member 11.

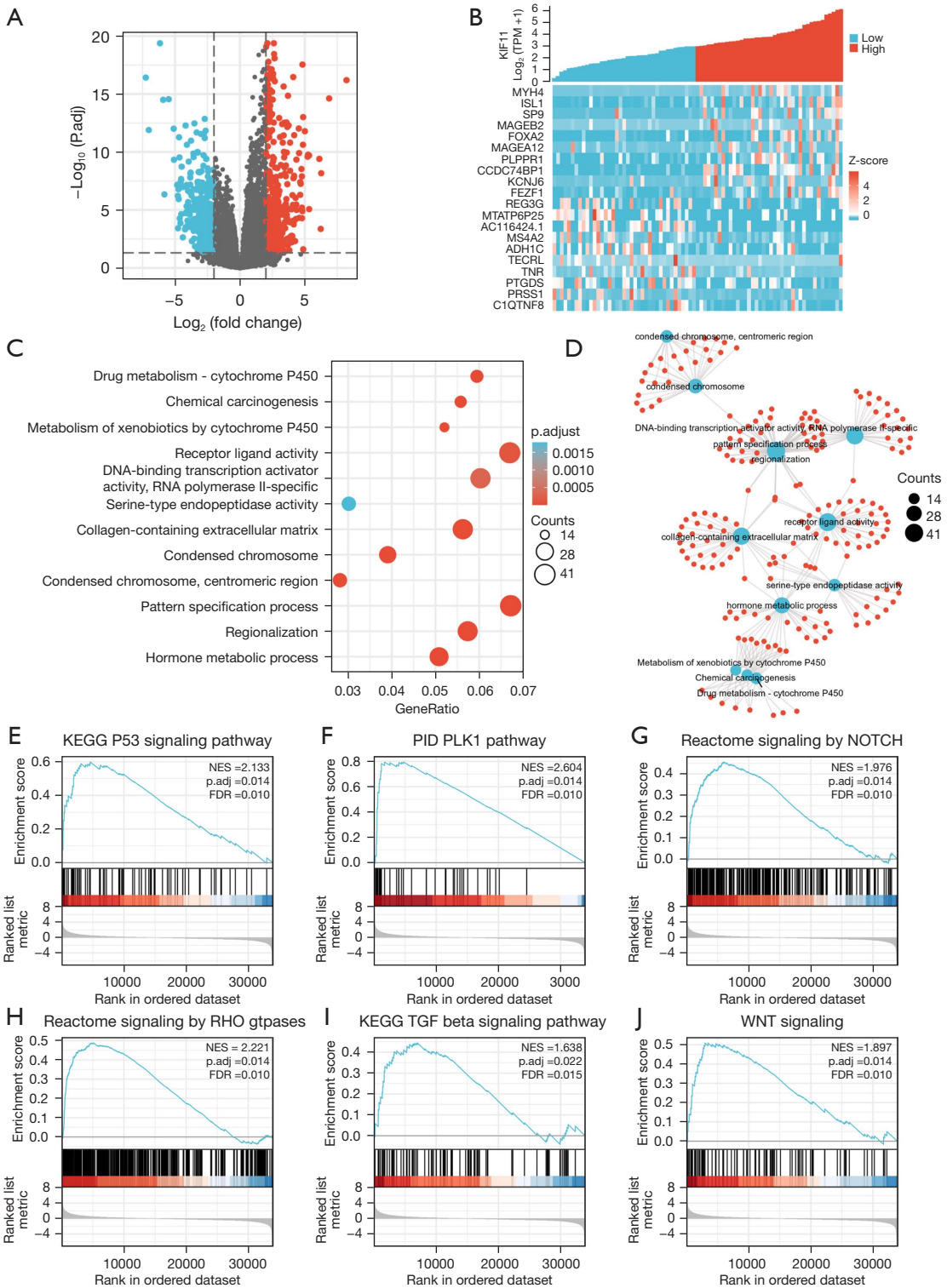


Figure 3 Genes expression correlated with KIF11 in ACC. (A) Volcano plot of differentially expressed genes. (B) Heat map of the 20 differentially expressed RNAs, including 10 up-regulated genes and 10 down-regulated genes. (C,D) Top 12 of biological process enrichment related to KIF11 related genes and network. (E-J) Several KIF11-related signaling pathways and biological processes in ACC. NES, normalized enrichment score; P.adj, adjusted P value; FDR, false discovery rate; KIF11, kinesin family member 11; ACC, adrenocortical carcinoma.

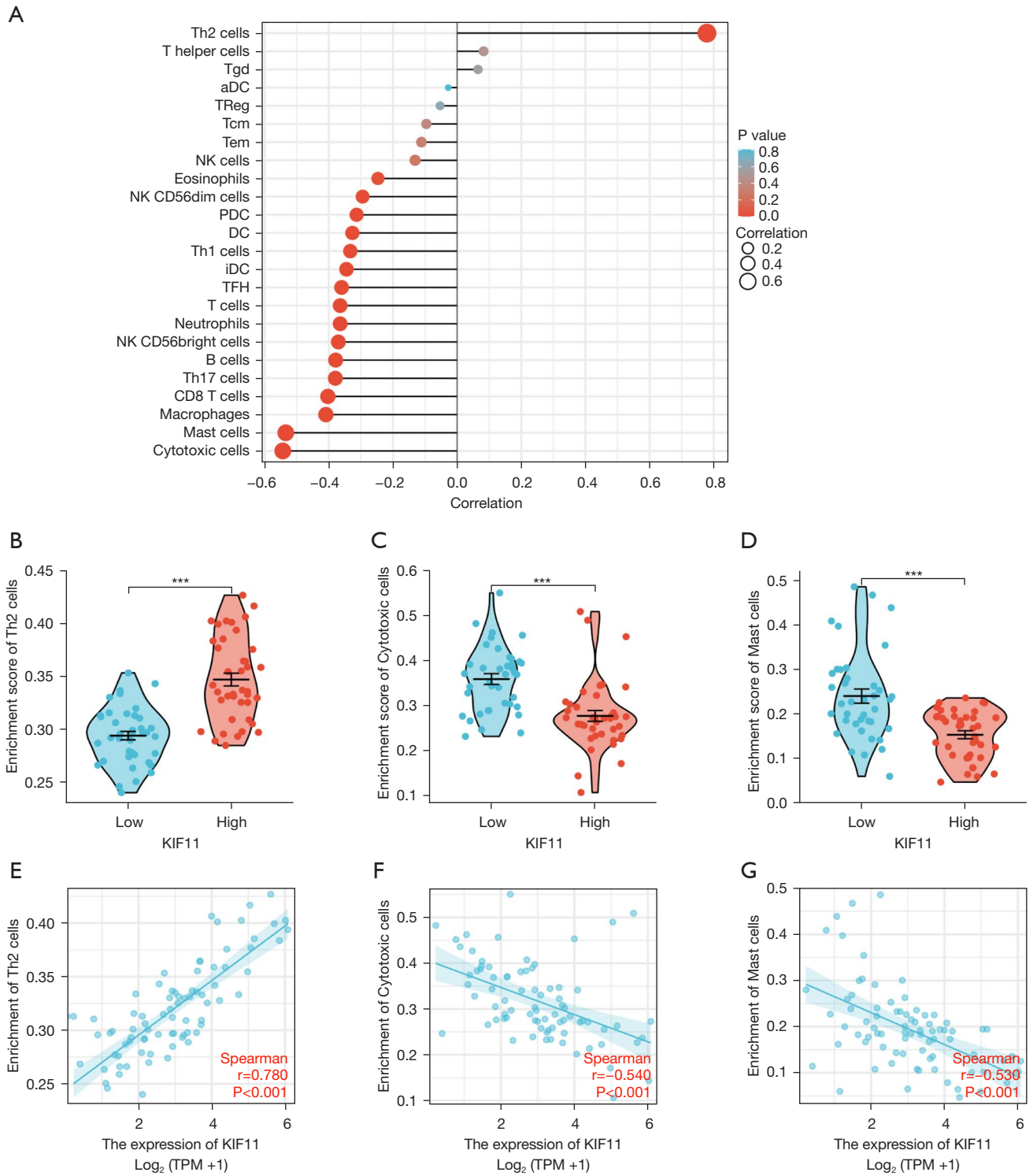
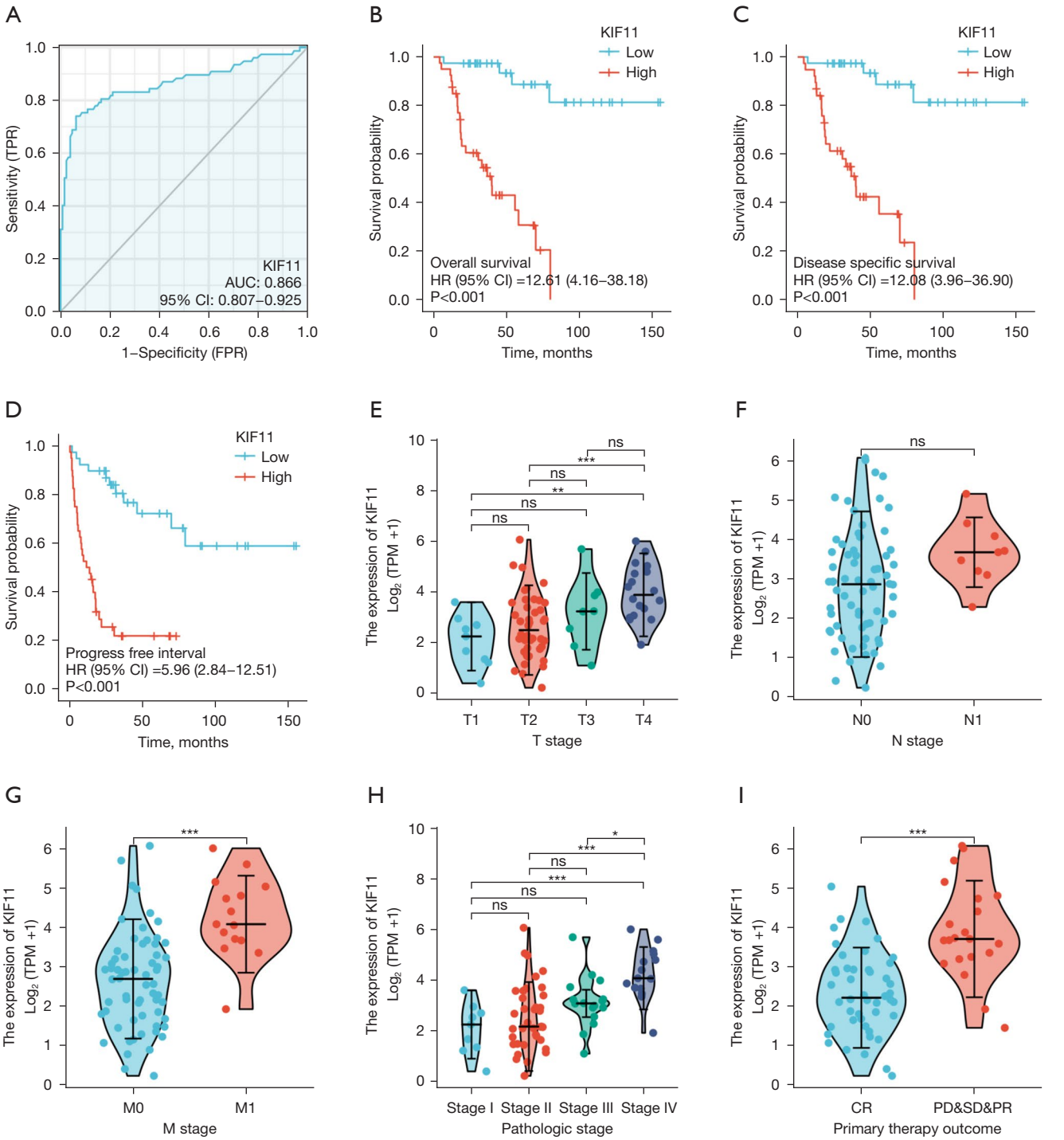


Figure 4 The expression level of KIF11 was associated with the immune infiltration. (A) Correlation between the relative abundances of 24 immune cells and KIF11 expression level. (B-G) Scatter plots and correlation diagrams showing the difference of Th2 cells, cytotoxic cells and Mast cells infiltration level between KIF11-high and low groups. ***, P<0.001. KIF11, kinesin family member 11; TPM, transcripts per million.



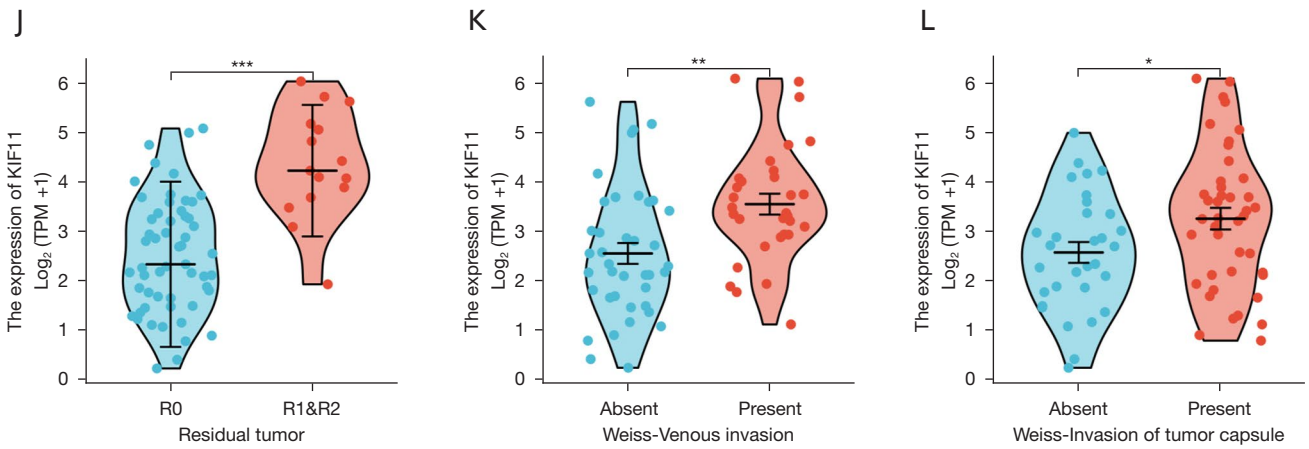


Figure 5 KIF11 expression is associated with survival time and clinicopathological characteristics in ACC patients. (A) The diagnostic efficacy of KIF11 in ACC analyzed by ROC. (B-D) Survival curves of OS, DSS, and PFI between KIF11-high and -low patients with ACC. (E-L) Association with KIF11 expression and clinicopathological characteristics, including T stages, N stages, M stages, pathologic stages, primary therapy outcome, residual tumor, venous invasion, and invasion of the tumor capsule. ns, no significant; *, P<0.05; **, P<0.01; ***, P<0.001. PD, progressive disease; SD, stable disease; PR, partial response; CR, complete response; KIF11, kinesin family member 11; ACC, adrenocortical carcinoma; ROC, receiver operating characteristic; OS, overall survival; DSS, disease-specific survival; PFI, progression-free interval.

Table 1 The association between KIF11 expression and clinicopathological variables (from TCGA database)

Characteristic	Low expression of KIF11 (n=39)	High expression of KIF11 (n=40)	P
T stage, n (%)			0.002
T1	7 (9.1)	2 (2.6)	
T2	26 (33.8)	16 (20.8)	
T3	3 (3.9)	5 (6.5)	
T4	3 (3.9)	15 (19.5)	
N stage, n (%)			0.014
N0	38 (49.4)	30 (39.0)	
N1	1 (1.3)	8 (10.4)	
M stage, n (%)			<0.001
M0	38 (49.4)	24 (31.2)	
M1	1 (1.3)	14 (18.2)	
Pathologic stage, n (%)			<0.001
Stage I	7 (9.1)	2 (2.6)	
Stage II	25 (32.5)	12 (15.6)	
Stage III	6 (7.8)	10 (13.0)	
Stage IV	1 (1.3)	14 (18.2)	

Table 1 (*continued*)

Table 1 (*continued*)

Characteristic	Low expression of KIF11 (n=39)	High expression of KIF11 (n=40)	P
Tumor status, n (%)			<0.001
Tumor free	31 (40.3)	8 (10.4)	
With tumor	8 (10.4)	30 (39.0)	
Primary therapy outcome, n (%)			<0.001
PD	1 (1.5)	17 (25.4)	
SD	1 (1.5)	1 (1.5)	
PR	1 (1.5)	0 (0.0)	
CR	34 (50.7)	12 (17.9)	
Gender, n (%)			0.928
Female	23 (29.1)	25 (31.6)	
Male	16 (20.3)	15 (19.5)	
Age, n (%)			0.571
≤50 years	22 (27.8)	19 (24.1)	
>50 years	17 (21.5)	21 (26.6)	
Age (years), median (IQR)	48 (33.5, 56.5)	52 (35.5, 61.0)	0.662
Residual tumor, n (%)			<0.001
R0	34 (48.6)	21 (30.0)	
R1	0 (0.0)	6 (8.6)	
R2	1 (1.4)	8 (11.4)	
Laterality, n (%)			0.897
Left	23 (29.1)	22 (27.8)	
Right	16 (20.3)	18 (22.8)	

KIF11, kinesin family member 11; TCGA, The Cancer Genome Atlas; PD, progressive disease; SD, stable disease; PR, partial response; CR, complete response.

Table 2 KIF11 expression association with clinical pathological characteristics (logistic regression)

Characteristics	Total (N)	OR	P
T stage (T3 & T4 *vs.* T1 & T2)	77	6.111 (2.179–19.308)	<0.001
N stage (N1 *vs.* N0)	77	10.133 (1.723–193.353)	0.033
M stage (M1 *vs.* M0)	77	22.167 (4.059–414.648)	0.004
Pathologic stage (Stage III & Stage IV *vs.* Stage I & Stage II)	77	7.837 (2.861–23.806)	<0.001
Residual tumor (R1 & R2 *vs.* R0)	70	22.667 (4.102–425.787)	0.004
Tumor status (with tumor *vs.* tumor free)	77	14.531 (5.088–46.742)	<0.001
Primary therapy outcome (PD & SD & PR *vs.* CR)	67	17.000 (4.762–82.393)	<0.001
Weiss-venous invasion (present *vs.* absent)	70	4.889 (1.810–14.161)	0.002
Weiss-invasion of tumor capsule (present *vs.* absent)	73	2.674 (1.040–7.164)	0.045

KIF11, kinesin family member 11; OR, odds ratio; PD, progressive disease; SD, stable disease; PR, partial response; CR, complete response.

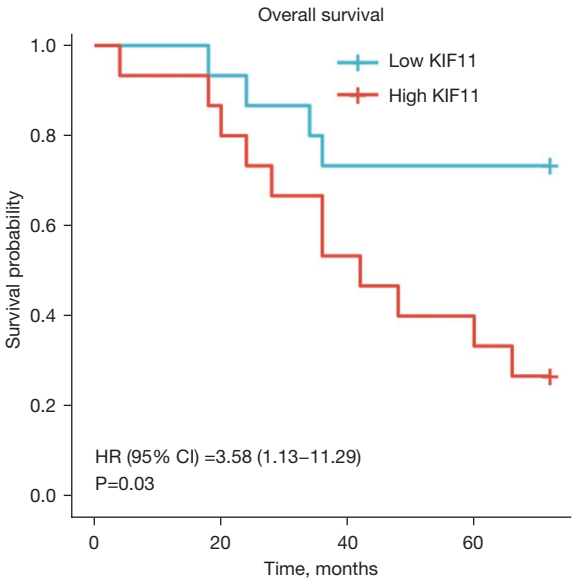


Figure 6 Survival curves of OS between KIF11-high and -low patients with ACC (from Xiangya Hospital). OS, overall survival; KIF11, kinesin family member 11; HR, hazard ratio; ACC, adrenocortical carcinoma.

for tumor *vs.* tumor free), primary therapy outcome (OR =17.000 for PD and SD and PR *vs.* CR), venous invasion (OR =4.889 for present *vs.* absent), and invasion of the tumor capsule (OR =2.674 present *vs.* absent). These results suggest that ACC in the KIF11-high group is prone to progression.

In addition, we analyzed the clinical data of 30 patients with ACC who underwent surgery at Xiangya Hospital between January 2009 and January 2016 (*Figure 6*, *Table 3*). The patients were divided into two groups (KIF11-high and KIF11-low) according to the expression of KIF11 detected by IHC. The results showed that the overexpression of KIF11 had a significantly positive correlation with tumor size (7.22±0.35 *vs.* 8.35±0.36, P=0.047), T stages (T3 & T4 *vs.* T1 & T2, P=0.021), pathological stages (Stage III & IV *vs.* Stage I & II, P=0.009), and tumor recurrence rate within 2 years (P=0.025). Patients in the KIF11-high group had poorer OS (*Figure 6*, HR =3.58, P=0.03).

Blocking KIF11 inhibits NCI-H295R cell proliferation and invasion

We found that many compounds tested against the protein target KIF11 by using PubChem (Table S1). To prove that KIF11 is a druggable target, we examined the effect of the KIF11 one of the specific inhibitor monastrol on the proliferation and invasion ability of NCI-H295R cells. Mitotane was also used, as it is the standard treatment used for ACC to kill tumor cells. The CCK-8 assay showed that NCI-H295R cell treated with mitotane and monastrol (30 μM/100 μM) for 24 hours and 48 hours exhibited obvious decreases in their proliferation (*Figure 7A*,*7B*). Consequently, 30 μM of the mitotane and monastrol was then used for further experiments. The colony formation assay also showed decreased proliferation of NCI-H295R cell after treatment with 30 μM of mitotane and monastrol (*Figure 7C*). The Transwell invasion assay revealed that the mitotane and monastrol treatments significantly decreased NCI-H295R cell invasion when compared to the control group (*Figure 7D*). Overall, the results demonstrated that monastrol could inhibit NCI-H295R cell proliferation and invasion *in vitro*, similar to mitotane, which is the mainstay of adjuvant treatment for ACC.

Nomogram for predicting OS in ACC patients based on KIF11 expression

In view of the prognostic value of KIF11 in ACC, we created a nomogram for predicting the 3- and 5-year survival (*Figure 8*). The tumor status, invasion of the tumor capsule, and the expression of Ki67 have been reported to be associated with the incidence or prognosis of ACC (5,23,24). Therefore, these parameters were included in the predictive model (*Figure 8A*). We also analyzed the prediction efficiency of the nomogram, and the results indicated that the C-index of the model was 0.891, which suggests that its prediction efficiency was moderately accurate.

Discussion

To the best of our knowledge, this is the first study focusing on the potential effects of KIF11 in relation to ACC. Bioinformatics analysis using the TCGA and GTEx databases and 7 years of statistics from Xiangya Hospital demonstrated that KIF11 may be a potential prognostic biomarker for ACC. Increased KIF11 expression in ACC was found to be associated with clinical pathological characteristics, shorter survival time, and poor prognosis. Monastrol was also assessed and the results showed that KIF11 is a druggable target for limiting ACC cell proliferation and invasion. The flow chart of this study is showed in *Figure 9*.

Table 3 The association between KIF11 expression and clinicopathological variables (from Xiangya Hospital)

Characteristic	Low expression of KIF11 (n=15)	High expression of KIF11 (n=15)	P
Gender, n (%)			0.715
Female	7 (23.3)	9 (30.0)	
Male	8 (26.7)	6 (20.0)	
Age, n (%)			1.000
≤50 years	5 (16.7)	4 (13.3)	
>50 years	10 (33.3)	11 (36.7)	
Laterality, n (%)			0.272
Left	6 (20.0)	10 (33.3)	
Right	9 (30.0)	5 (16.7)	
Tumor size (cm), mean ± SD	7.2±0.4	8.4±0.4	0.037
T stage, n (%)			0.021
T1 & T2	13 (43.3)	6 (20.0)	
T3 & T4	2 (6.7)	9 (30.0)	
N stage, n (%)			0.390
N0	13 (43.3)	10 (33.3)	
N1	2 (6.7)	5 (16.7)	
M stage, n (%)			0.598
M0	14 (46.7)	12 (43.3)	
M1	1 (3.3)	3 (6.7)	
Pathologic stage, n (%)			0.009
Stage I & Stage II	12 (40.0)	4 (13.3)	
Stage III & Stage IV	3 (10.0)	11 (36.7)	
Tumor recurrence (within 2 years), n	3	10	0.025

KIF11, kinesin family member 11; SD, standard deviation.

Despite a better understanding of the pathophysiology of ACC and the gradual appearance of more treatment options, the prognosis of ACC remains poor. Etoposide, doxorubicin, cisplatin, and mitotane (EDP-M) have been established as first-line therapies for metastatic ACC. However, this treatment regimen shows significant toxicity potential in patients (6). In recent years, drugs targeting the IGF pathway, mTOR or tyrosine kinase inhibitors, and immunotherapy-targets have been extensively studied in relation to ACC (8,25). However, most of these drugs are found to have relatively low response rates and results in no significant improvements to OS rates for ACC (6,26,27).

Cell proliferation is one of the most important hallmarks of cancer development and progression. Correct alignment of the mitotic spindle during cell division is crucial for the determination of cell fate, tissue organization, and development (28). The accurate segregation of chromosomes is mediated by microtubule-based mitotic spindles and approximately 200 essential microtubule-associated proteins (29). KIF11, which is perhaps the simplest player in the mitotic spindle assembly, is a plus-end-directed motor localized to interpolar spindle microtubules and spindle poles (10). KIF11 has been reported to play an essential role in centrosome separation by cross-linking the microtubules in the mitotic spindle (30). The suppression of KIF11 increases the proportion of cells in the G2/M phase and sub-G1 phase, indicating that it has a vital role in G2/M phase transition and cell cycle

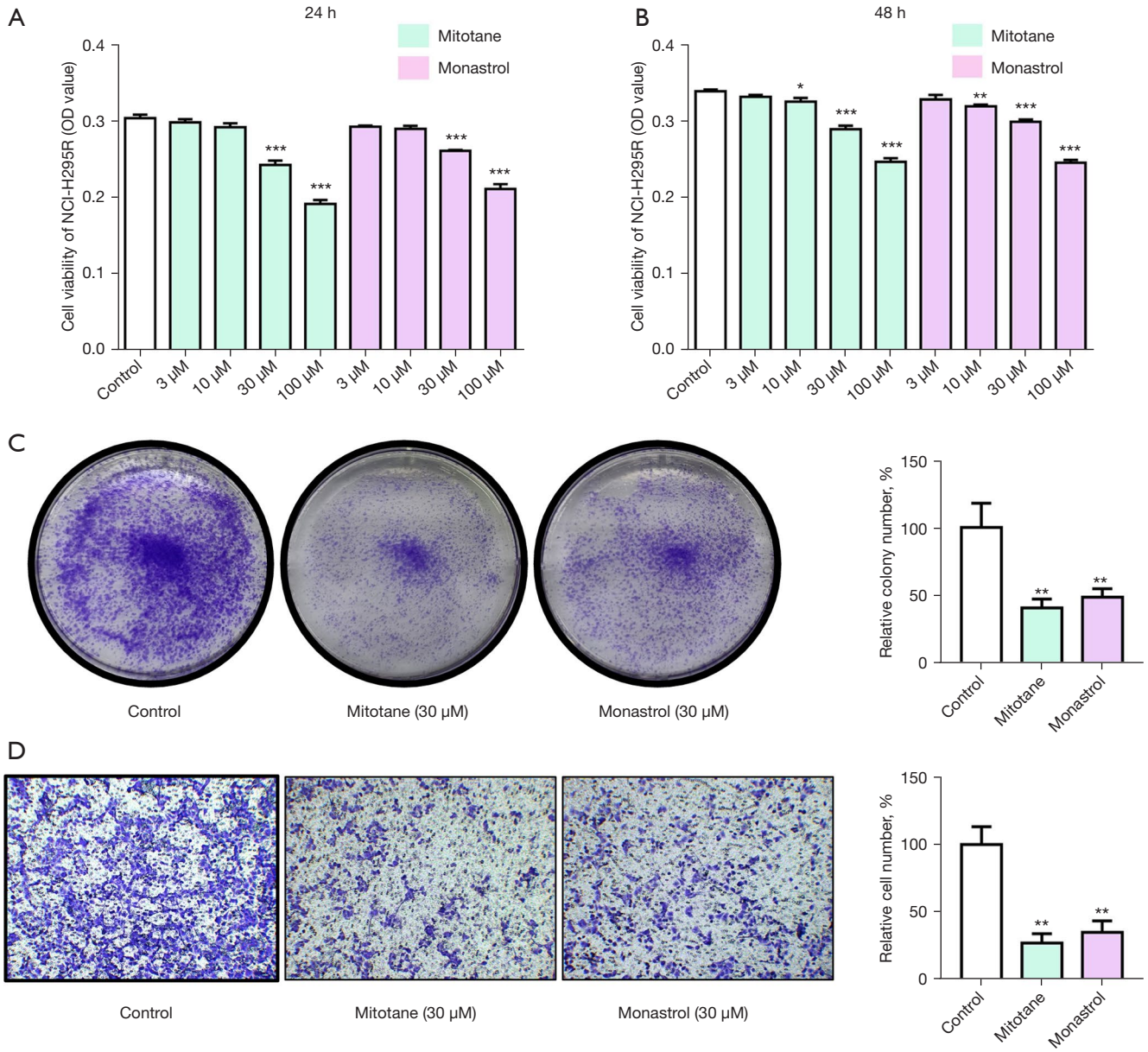


Figure 7 Blocking KIF11 with monastrol inhibits NCI-H295R cell proliferation and invasion *in vitro*. (A) Proliferation viability of NCI-H295R cell in 24 h, evaluated by the CCK-8 assay (n=5). (B) Proliferation viability of NCI-H295R cell in 48 h, evaluated by the CCK-8 assay (n=5) (C) Colony formation assay to detect the proliferation viability of NCI-H295R cell (n=3; crystal violet staining, ×4). (D) Transwell invasion assay to detect the invasion of NCI-H295R cell (n=3; crystal violet staining, ×200). *, P<0.05; **, P<0.01; ***, P<0.001. OD, optical density; KIF11, kinesin family member 11; CCK-8, cell counting kit-8.

checkpoints (31). To investigate the functions of KIF11 in ACC, GO, GSEA, and single-sample GSEA analyses with TCGA data were performed. The results revealed that the KEGG-p53, PID-PLK1, Notch, Rho-GTPase, KEGG-TGFβ, and Wnt signaling pathways were differentially enriched in the KIF11-high group. Accumulating evidence has indicated that these signaling pathways regulate many aspects of cancer biology (32-37).

In recent years, KIF11 has attracted the attention of researchers as it has been shown to have a therapeutic role in a variety of tumors, including oral cancer (31), meningioma (18), glioblastoma (38), hepatocellular

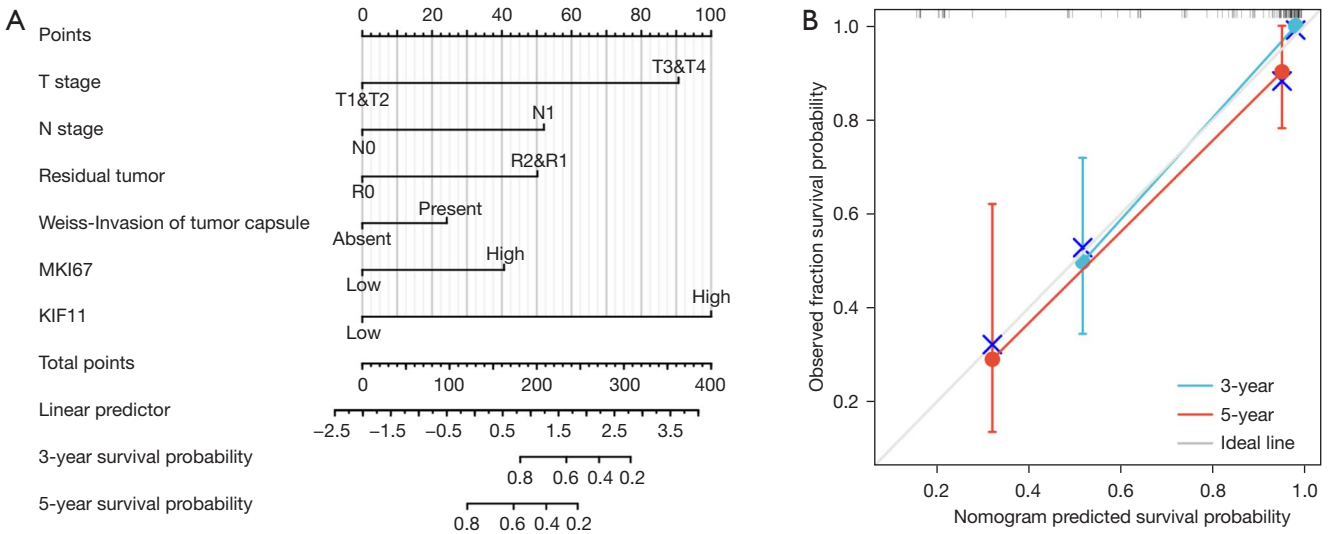


Figure 8 A prognostic predictive model of KIF11 in ACC. (A) Nomogram for predicting the probability of 3-, 5-year OS for ACC. (B) Calibration plot of the nomogram for predicting the probability of OS at 3, and 5 years. KIF11, kinesin family member 11; ACC, adrenocortical carcinoma; OS, overall survival.

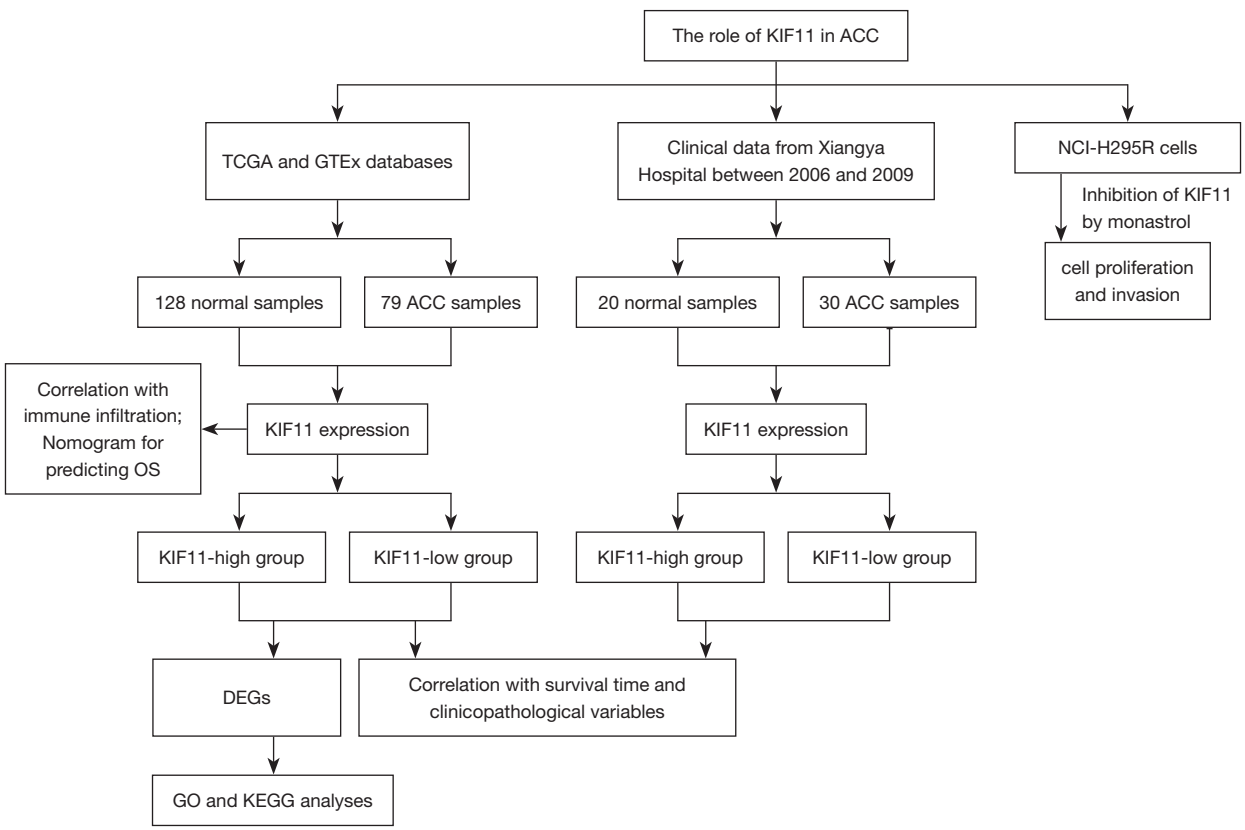


Figure 9 The flow chart of this study. ACC, adrenocortical carcinoma; TCGA, The Cancer Genome Atlas; GTEx, genotype tissue expression; OS, overall survival; KIF11, kinesin family member 11; DEGs, differentially expressed genes; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

carcinoma (12), breast cancer (39), gallbladder cancer (40), and lung cancer (41). A KIF11 inhibitor was shown to demonstrate better efficacy in hematological malignancies due to the higher proliferative rates of blood cancers and off-target activities of anti-mitotic agents against oncogenic drivers (42,43). However, clinical research studies on KIF11 inhibitor found that mitotic inhibition alone does not appear to be sufficient to achieve significant antitumor effect (44,45). Furthermore, combination therapies may be more promising due to the multiple synergistic interactions between anti-mitotic and anticancer drugs (43,46). In this study, KIF11 inhibitor monastrol was applied to treat NCI-H295R cell and cell proliferation and invasion were significantly suppressed. However, further research is required to fully understand the role and mechanism of monastrol in relation to ACC.

While the results suggest that KIF11 may be a predictor of poor prognosis as well as a novel therapeutic target for ACC, some limitations exist in this study. Firstly, the overall sample size used for the RNA-seq analysis was small. The number of patients enrolled from Xiangya Hospital was also small due to the low incidence of ACC, and the follow-up time was short. Therefore, future research with a longer period of follow-up is worth investigated. Secondly, a prospective study should be performed in the future to avoid bias arising from the retrospective nature of the current study. Thirdly, the detailed mechanism of how KIF11 impacts cell proliferation and invasion in ACC should be elucidated. Finally, additional strategies to assess the role of KIF11 in ACC, including the use of KIF11 knockout cells and animal experiments, may be required in the future. These limitations need to be addressed in future studies.

Conclusions

In conclusion, this is the first study to report that high KIF11 expression levels are significantly associated with the progression of ACC and resulting in poor survival rates. Furthermore, KIF11 expression appears to be a novel and independent prognostic marker in ACC. KIF11 is a key protein involved in cell proliferation and invasion, and may thus serve as a potential novel therapeutic target for ACC.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://tau.amegroups.com/article/view/10.21037/tau-22-706/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tau.amegroups.com/article/view/10.21037/tau-22-706/coif). XC reports the funding from Xiangya Clinical Data System of Central South University. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of the Xiangya Hospital, China (No. 202109889) and all samples and clinical information used in this study were obtained with informed consent from patients.

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Supplementary

Table S1 Compounds tested against the protein target Chain A, kinesin-like protein KIF11

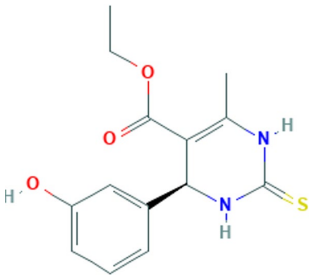
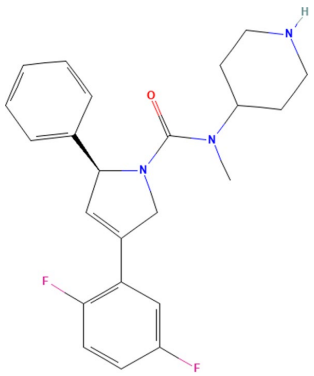
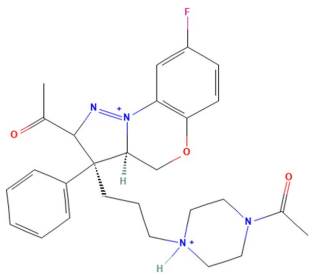
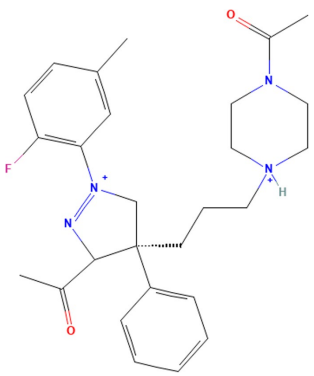
Compound CID	Name	2D structure
794323	(S)-Monastrol	
11675645	(2S)-4-(2,5-Difluorophenyl)-N-methyl-2-phenyl-N-piperidin-4-YL-2,5-dihydro-1H-pyrrole-1-carboxamide	
24916959	1-[(3S,3aR)-3-[3-(4-acetylpiperazin-1-ium-1-yl)propyl]-8-fluoro-3-phenyl-3a,4-dihydro-2H-pyrazolo[5,1-c][1,4]benzoxazin-10-ium-2-yl] ethanone	
24916961	1-[(4R)-4-[3-(4-acetylpiperazin-1-ium-1-yl) propyl]-1-(2-fluoro-5-methylphenyl)-4-phenyl-3,5-dihydropyrazol-1-ium-3-yl]ethanone	

Table S1 (*continued*)

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Table S1 (*continued*)

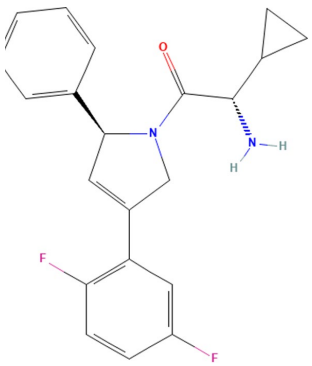
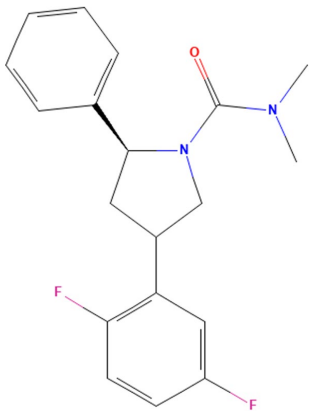
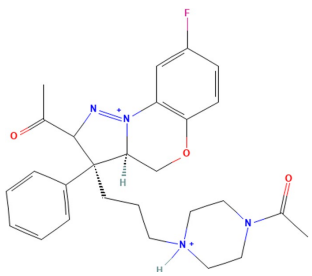
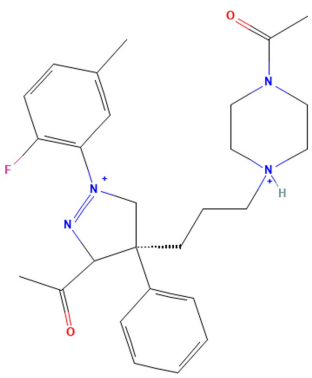
Compound CID	Name	2D structure
6102824	(1S)-1-Cyclopropyl-2-[(2S)-4-(2,5-difluorophenyl)-2-phenyl-2,5-dihydro-1H-pyrrol-1-YL]-2-oxoethanamine	
6102825	(2S)-4-(2,5-difluorophenyl)-N,N-dimethyl-2-phenylpyrrolidine-1-carboxamide	
24916959	1-[(3S,3aR)-3-[3-(4-acetylpiperazin-1-ium-1-yl)propyl]-8-fluoro-3-phenyl-3a,4-dihydro-2H-pyrazolo[5,1-c][1,4]benzoxazin-10-ium-2-yl] ethanone	
24916961	1-[(4R)-4-[3-(4-acetylpiperazin-1-ium-1-yl) propyl]-1-(2-fluoro-5-methylphenyl)-4-phenyl-3,5-dihydropyrazol-1-ium-3-yl]ethanone	

Table S1 (*continued*)

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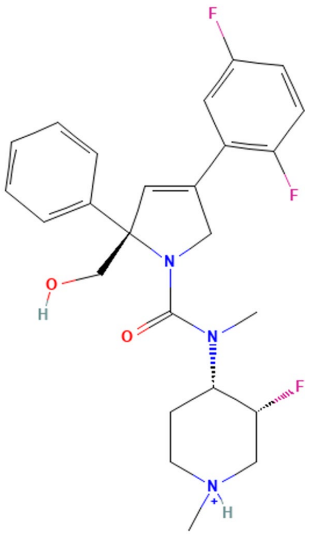
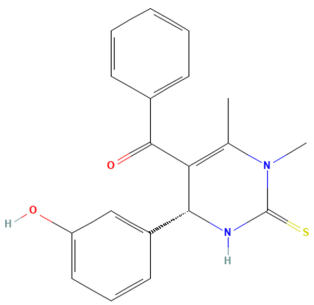
Table S1 (*continued*)

Compound CID	Name	2D structure
44629684	(5S)-3-(2,5-difluorophenyl)-N-[(3R,4S)-3-fluoro-1-methylpiperidin-1-ium-4-yl]-5-(hydroxymethyl)-N-methyl-5-phenyl-2H-pyrrole-1-carboxamide	
15942673	[(4R)-4-(3-Hydroxyphenyl)-1,6-dimethyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-YL](phenyl) methanone	

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