KCNH2 Regulates the Growth and Metastasis of Pancreatic Cancer

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Research Article

Keywords: Pancreatic cancer, Potassium voltage-gated channel subfamily H member 2 (KCNH2), Proliferation, Epithelial-mesenchymal transition, Metastasis

Posted Date: December 9th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1097988/v1

Abstract

The mortality rate of pancreatic cancer (PC) remains high due to late diagnosis, early metastasis, and difficulty of complete resection. The online databases showed that potassium voltage-gated channel subfamily H member 2 (KCNH2) was highly expressed in pancreatic tumor tissues and was closely related to the poor survival of patients with PC. However, the mechanism of action of KCNH2 in PC is still unclear. In the present study, for the first time, we explored the regulatory effect of KCNH2 in PC. The results showed that KCNH2 was upregulated in PC compared with normal pancreatic tissues. High KCNH2 expression was associated with low tissue differentiation, high malignancy, and poor prognosis of PC. Moreover, knockdown of KCNH2 inhibited the proliferation and apoptosis of PC cells, as well as the epithelial-mesenchymal transition process, thereby promoting PC cell migration and invasion. In addition, KCNH2 knockdown inhibited the progression and metastasis of PC in a mouse xenograft model. In conclusion, these findings highlighted the potential of KCNH2 as a targeted molecule in the treatment of PC.

Introduction

Pancreatic cancer (PC) is a lethal malignancy characterized by an insidious onset of symptoms, late diagnosis, and early metastasis[1]. Multiple factors have been identified as risk factors for PC, including smoking history, obesity, alcohol consumption, dietary intake, family history, intestinal flora, etc[2]. The prevalence of PC in China has been gradually increasing in recent years, and the numbers of diagnoses and deaths each year has exceeded those in the United States [3]. The prognosis of PC remains extremely unfavorable (5-year survival < 10%) because patients are often diagnosed at an advanced stage and it is difficult to completely remove the tumor by surgery [4]. Surgical resection is the only treatment that provides a potential cure for patients with PC; however, the recurrence rate is inevitably high with poor long-term survival [2]. Chemotherapy agents, such as gemcitabine, have also been considered first-line drugs for PC, but the clinical outcomes remain unsatisfactory due to the acquisition of resistance within a few weeks after treatment [5]. Thus, it is of great clinical significance to identify novel targeted molecules for the treatment of PC.

The human ether-a-go-go-related gene, also known as potassium voltage-gated channel subfamily H member 2 (*KCNH2*), is located on chromosome 7q36.1 and encodes the alpha unit of the Kv11.1 potassium voltage-gated channel [6]. The KCNH2 proteins form functional homo- or hetero-tetramers and regulate voltage-gated channels [7, 8]. The channels can be found in three states: closed, open, and inactivated [4], which mediate various physiological and pathological processes, such as angiogenesis, cell proliferation, migration, and apoptosis. KCNH2 is expressed in many tissues, including the heart and the brain. Previous evidence has demonstrated that KCNH2 plays an important role in the repolarization of the heart [9]. Recent findings have also revealed that KCNH2 is also overexpressed in ductal, lobular, and invasive breast carcinomas [10].

Our previous studies have shown that KCNH2 is closely related to the metastasis of PC [11]. However, the regulatory mechanisms of KCNH2 in PC remain elusive. Therefore, in the present study, we investigated the effects of KCNH2 in both PC cell lines and mouse xenografts. Our results provided a scientific basis for further exploration of KCNH2 in the treatment of PC.

Materials And Methods

Cell culture

Human PC cell lines Panc-1, BxPC-3, AsPC-1, and SW1900 were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and cultured in RPMI-1640 medium (GIBCO, New York, USA) containing 10% fetal bovine serum (FBS) and 100 U/mL penicillin/streptomycin (Invitrogen, Carlsbad, USA). All cells were maintained in a humidified atmosphere containing 5% CO_2 in air at 37°C.

Western blot

After washed with phosphate-buffered saline (PBS), PC cells were lysed on ice with RIPA buffer containing 1 mM phenylmethylsulfonyl fluoride (PMSF) (PMSF:RIPA = 1:100) for 15 min. After centrifugation, the supernatant was collected. The BCA kit was used to measure the total protein concentration. Equal amounts of protein samples were separated by 10% SDS-PAGE and transferred to the polyvinylidene fluoride membrane. After blocked with 10% skimmed milk in Tris-buffered saline Tween-20 at room temperature for 2 h, the membrane was incubated with the following primary antibodies (dilution 1:1000; Santa Cruz Biotechnology, Santa Cruz, USA) overnight at 4°C: anti-MAPK, anti-pMAPK, anti-p53, anti-cyclin D1, anti-Cyclin-dependent kinase 2 (CDK2), anti-HSP70, anti-KCNH2, anti-E-cadherin, anti-Vimentin, anti-MMP9, anti-RhoA, anti-GAPDH, anti-β-actin. After washed with PBS, the membrane was incubated with horseradish peroxidase-linked secondary antibody (dilution: 1:5000) at room temperature for 2 h. The signal was detected using the ECL detection reagent (Beyotime, Haimen, China).

RNA extraction and real-time quantitative PCR (qRT-PCR)

Total RNA was extracted from cells with Trizol reagent (Invitrogen, Shanghai, China) and converted into cDNA using the MMLV Reverse Transcriptase Kit (Promega, USA). The cDNA aliquots were then analyzed by qRT-PCR using the Step One Plus Real-Time PCR System (Applied Biosystems, USA). The mRNA level was normalized to the level of GAPDH or β-actin. The primers used in this experiment were as follows: KCNH2 (157 bp), forward: 5′-CAATGGCGACCGTCACTC-3′; reverse: 5′-TCCTCTGCCCGCTTCTTC-3′; GAPDH (121 bp), forward: 5′-TGACTTCAACAGCGACACCCA-3′; reverse: 5′-CACCCTGTTGCTGTAGCCAAAC-3′. β-actin forward: 5′-CCCA TCTATGAGGGTTACGC-3′ and reverse: 5′-TTTAATGT CACGCACGATTTC-3′. The 2-ΔΔCt method was used to analyze the data.

Construction of lentiviral vectors

To construct lentiviral vectors expressing short hairpin RNA (shRNA) targeting KCNH2, the RNA interference (RNA1) sequence of human KCNH2 (CTC CCA ATA TCC ATC TGC T) was designed using the RNAi Designer Program (GeneChem, Shanghai, China). The negative control vector (control RNAi) was constructed using a scrambled sequence (TTC TCC GAA CGT GTC ACG T) with no homology to the human genome. The DNA oligonucleotide containing the target sequence was synthesized and inserted into the vector by double digestion with Age I and EcoR I, and subsequent ligation with T4 DNA ligase. The constructed plasmids were then transformed into E. coli DH5α cells. DNA sequencing analysis was performed using restriction endonucleases. The sequence was cloned then cloned into the lentiviral vector pGCSIL-Green fluorescent protein (GFP; GeneChem, Shanghai, China) to generate a lentiviral vector expressing short hairpin RNA (shRNA) targeting KCNH2 (pGCSIL-KCNH2-shRNA-LV) or a control vector (pGCSIL-neg-shRNA-LV). The vectors were then transfected into 293 T cells using Lipofectamine 2000. After 48 h, the supernatant containing the lentiviral vectors was harvested. After purified by ultracentrifugation, and the titer of the lentiviral vector was determined.

Cell transfection

PC cells in the logarithmic growth phase were seeded in a 96-well plate (5×10^3/well) and cultured overnight. The lentiviral vectors were diluted in 0.2 mL complete culture medium containing 10 μg/mL of polybrene and incubated at 37°C for 12 h. Next, vector-containing medium was replaced with fresh culture medium. A fluorescence microscope (TE2000, Nikon, Tokyo) was used to detect the percentage of GFP-positive cells, which indicated transfection efficiency. Five days after infection, the expression of KCNH2, proliferation, migration, and invasion of transfected cells were analyzed.

Transwell migration and invasion assay

The migration and invasion assessment was performed using a Boyden chamber composed of Transwell membrane filters (#3422, Corning Costar, Cambridge, USA). PC cells (5×10^4/well) were plated to 24-well Transwell plates (pore size 8 μm) for migration assay or to the Matrigel-coated plates for invasion assay. Plates were filled with complete culture medium containing 10% FBS. The periods of migration and invasion assay were 24 h and 48 h, respectively. At the end of the experiment, cells that had not penetrated the filter membrane were wiped off. Cells on the lower surface of the filter membrane were stained with 0.4% crystal violet. The number of migrating or invading cells in a single chamber was counted from five fields under an optical microscope. This experiment was performed in triplicate and the average values were shown as mean ± standard error.

MMT assay

MMT assay was performed to detect the proliferation rate of cells in the Scrambled, shKCNH2_6, and shKCNH2_7 groups, each in triplicate. Cells were seeded in 96-well plates (1×10^4 cells/well). At 1, 2, and 3 days of culture, cells were incubated with 2 μL of MTT reagent (5 mg/mL; Sigma, St. Louis, USA) for 4 h. Then, the original medium was aspirated and 150 mL of DMSO was added to cells. Finally, the absorbance of each sample was measured at 492 nm by a microplate spectrophotometer (Thermo, Spectronic, Madison, USA).

Colony formation assay

The colony formation assay was performed to investigate the effect of KCNH2 silencing on the colony formation ability of PANC-1 cells. In this assay, 8×10^2 cells were seeded in a 6-cm petri dish and cultured in RPMI-1640 medium supplemented with 10% FBS. Cells were maintained in an atmosphere of 5% CO_2, 95% humidity, and 37°C for 2 weeks. Then, cell colonies were washed twice with PBS and fixed with 4% paraformaldehyde for 15 min. The fixed colonies were subsequently stained with Giemsa for 20 min and washed twice with ddH_2O. The colonies consisting of ≥ 50 cells were counted.

Flow cytometry

PC cells were transfected with designated sequences for 48 h. Then, cells were harvested, centrifuged, and resuspended with 500 μL of 1X binding buffer (BD Biosciences, Franklin Lakes, NJ, USA). After incubation with 5 μL V-FITC and 5 μL propidium iodide (BD Biosciences, Franklin Lakes, NJ, USA) for 5 min at room temperature in the dark, the apoptosis rate was analyzed by flow cytometry using a Cytomics FC 500 flow cytometer system (Beckman Coulter, Brea, CA, USA) as previously described[12].

PC tumor growth and metastasis *in vivo*

Six-week-old male BALB/c nude mice were purchased from the Shanghai Institute of Biological Sciences (Shanghai, China). Mice were anesthetized by inhaling a 1:1 mixture of isoflurane gas and oxygen. shKCNH2-PANC-1 cells were injected into the tail of the pancreas of nude mice as previously described[13, 14]. Eight weeks after inoculation, mice were sacrificed, the pancreatic tumors were removed and weighed, and metastatic liver nodules were counted. All animal experiments were performed in compliance with the Guidelines of the Institutional Animal Care and Use Committee at the Affiliated Hospital of Qingdao University. This study was reported in accordance with ARRIVE guidelines.

Patients' samples and immunohistochemistry

The use of clinical samples was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University. All patients provided written informed consent. Tissue samples were obtained from 83 patients who were diagnosed with PC between November 2015 and December 2017 in our hospital. Samples were fixed with 4% formalin, embedded with paraffin, sectioned, and stained with hematoxylin and eosin. The 5-μm-thick tissue sections were incubated with 3% H_2O_2 in methanol for 20 min. After incubation with blocking buffer, the sections were then stained with primary anti-KCNH2 antibody (Abcam) for four nights, followed by incubation with a secondary antibody (Santa Cruz Biotechnology) for 30 min at room temperature and then with the DAB (DAB) kit (Gene Tech). Tissue sections were washed three times with TBS for more than 10 min after each incubation. The staining results were evaluated according to the following criteria: (1) Percentage of positively-stained tumor cells: 0 (0–10%), 1 (11–25%), 2 (26–50%), 3 (51–75%), 4 (76–100%); (2) Signal intensity: 0 (no signal), 1 (weak), 2 (medium), 3 (strong). The immunoreactivity score (range 0–12) was calculated by multiplying the score

of positive cells by the intensity score. The final score was as follows: – (0 point), + (1–4 points), ++ (5–8 points), +++ (9–12 points). In this study, – and + were considered as low expression, while ++ and +++ were high expression.

Ethics statement

Written informed consent was obtained from all patients whose tissues were used in this study. The study was approved by the Institutional Review Board of the Affiliated Hospital of Qingdao University, Qingdao, China. All methods were performed in accordance with the relevant guidelines and regulations.

Statistical analysis

Pearson's Chi-squared test was used to evaluate the relationship between KCNH2 expression and clinicopathological characteristics. Overall survival (OS) was defined as the time from surgery to death. The Kaplan-Meier method was used to evaluate OS and the log-rank test was used for comparison. A Cox regression model was used to perform multivariate analysis on all significant parameters in univariate analysis. A two-sided *p*-value of less than 0.05 was considered statistically significant.

Results

1. Differential expression of KCNH2 in PC tissues versus normal tissues and survival analysis

The GEPIA database (http://gepia.cancer-pku.cn/index.html) is an analysis tool for interactive analysis of gene expression profiles. In this study, we used the "single gene analysis" and "survival analysis" module in GEPIA to perform differential expression analysis of PC tissues and normal tissues, as well as KCNH2-related survival analysis of KCNH2 (Figure 1A–D). The significance threshold was set as $p = 0.05$, 2-fold change, gene ranking top 10%. The results showed that KCNH2 was significantly upregulated in PC tissues (log2(fold change) = 1.389, adjusted $p = 1.35e\text{-}18$) (Figure 1A, B). To confirm this finding, we further analyzed the expression of KCNH2 in normal pancreatic tissues and tumor tissues using the online cancer microarray database Oncomine (www.oncomine.org). Figure 1E shows the expression of KCNH2 in normal tissues (75th percentile = 0.006, median = 0.003, 25th percentile = 0.001; n = 50), pancreatic colloid carcinoma tissues (75th percentile = 0.009, median = 0.009, 25th percentile = 0.009; n = 1), PC tissues (75th percentile = 0.077, median = 0.028, 25th percentile = 0.011; n = 32), and pancreatic ductal adenocarcinoma tissues (75th percentile = 0.055, median = 0.016, 25th percentile = 0.009; n = 17). The above finding suggest KCNH2 is differentially expressed in PC tissues compared with normal pancreatic tissues.

2. The expression KCNH2 in PC tissues and PC cell lines

We further performed immunohistochemistry analysis to detect the expression of KCNH2 in tumor tissues collected from PC patients (Figure 2A-H). The representative images of tissue sections with no staining (negative; Figure 2A and B), weak staining (Figure 2C and D), medium staining (Figure 2E and F), and

strong staining (Figure 2G and H). According to the immunoreactivity scores, samples were divided into high and low expression groups. There were 34 cases (41%) with low KCNH2 expression (– and +) and 49 cases (59%) with high expression (++ and +++). The clinicopathological characteristics and KCNH2 expression of these patients are shown in Table 1. Of 83 patients, 44 were males (20 (45.45%) in the low expression group and 24 (54.55%) in the high expression group) and 39 were females (14 (35.90%) in the low expression group and 25 (64.10%) in the high expression group. No significant difference was observed ($p = 0.377$). Also, there was no significant difference in age (p = 0.741), tumor location (p = 0.184), pM stage ($p = 1.000$), and pTNM grade ($p = 0.085$). The degree of tissue differentiation of patients with low and high KCNH2 expression was significantly different ($p = 0.008$). Of the 34 patients in the low KCNH2 expression group, 12 (35.30%), 11 (32.35%), and 11 (32.35%) were poorly, moderately, and highly differentiated, respectively. The distribution was relatively even. There were 49 patients in the high KCNH2 expression group. Among them, 4 (8.16%), 20 (40.82%), and 25 (51.02%) patients were highly, moderately, and poorly differentiated, respectively, indicating that more patients with high KCNH2 expression had low and moderately differentiated tumor tissues. The pT staging ($p = 0.016$; low expression group: 40.96%, high expression group: 59.04%) and pN staging ($p = 0.030$; low expression group: 40.96%, high expression: 59.04%). The OS of PC patients with low and high expression of KCNH2 was analyzed using the Kaplan-Meier method following log-rank test (Figure 2I). The results showed that high KCNH2 expression was associated with worse OS ($p < 0.01$). Then, a Cox survival model including gender, age, tumor location, histology, T status, N status, M status, and KCNH2 expression was established. Subsequently, multivariate survival analysis of all significant parameters in the univariate analysis was performed. The results revealed that KCNH2 was an important prognostic predictor of PC (HR = 2.632, 95% CI: 1.442–4.803, $p < 0.01$, Tables 2 and 3). High expression of KCNH2 often predicts low survival. To validate this finding and investigate the effect of differentially expressed KCNH2 in the progression of PC, GEPIA was used to determine the correlations of KCNH2 with clinical outcomes and disease-free survival of PC patients (Figure 1C). The transcriptional level of KCNH2 was significantly correlated with the disease-free survival of PC patients ($p = 0.043$, n = 178). The OS of PC patients with different KCNH2 expressions was also analyzed (Figure 1D). We found that the expression of KCNH2 was significantly correlated with the OS of PC patients ($p = 0.039$, n = 360). We further used The Human Protein Atlas database (https://www.proteinatlas.org/ENSG00000055118-KCNH2/pathology/pancreatic+cancer) to confirm our results. Taken together, it could be concluded that high KCNH2 expression often predicts low survival of PC patients. The above findings suggest that high KCNH2 expression is associated with low tissue differentiation, high malignancy, and poor prognosis, but not with gender, age, tumor location, or metastasis.

Table 1
Baseline characteristics of patients with pancreatic cancer (*n*=83) and correlations with *KCNH2* expression (*p*).

Characteristic	Case	KCNH2		P
	No	- to +	++ to +++	
Total	83	34	49	
Gender				0.377
Male	44	20	24	
Female	39	14	25	
Age(years)				0.741
<65	53	21	32	
≥65	30	13	17	
Tumor Location				0.184
Head	49	23	26	
Body	34	11	23	
Histology				0.008
Poorly differentiated	36	11	25	
Moderatelydifferentiated	31	11	20	
Well differentiated	16	12	4	
pT status				0.016
T1	2	2	0	
T2	12	8	4	
T3	59	22	37	
T4	10	2	8	
pN status				0.030
N0	37	20	17	
N1	46	14	32	
pM stage				1.000
M0	73	30	43	
M1	10	4	6	

Characteristic	Case	KCNH2		P
	No	- to +	++ to +++	
pTNM stage				0.085
1	12	8	4	
2	47	20	27	
3	12	2	10	
4	12	4	8	
*Statistical significant (*P* 0.05)				

Table 2
Univariate analysis of factors affecting overall survival

Variable	HR	95%CI	P value
Sex Male vs. Female	1.172	0.662-2.075	0.586
Age <65 vs. ≥65	0.647	0.350-1.195	0.165
Tumor Location Head vs. Body and Tail	1.360	0.753-2.457	0.308
Histology Poorly vs. Moderately vs. Well differentiated	1.186	0.826-1.702	0.355
Invasive depth T_1-T_2 vs. T_3-T_4	1.161	0.577-2.339	0.675
Lymph node status N_0 vs. N_1	0.835	0.470-1.483	0.539
Distant metastasis M_0 vs. M_1	0.309	0.101-0.309	0.040
KCNH2 Low vs. High	2.607	1.429-4.757	<0.01
HR: hazard ratio			

Table 3
Multivariate analysis for overall survival

Variables	Overall survival		
	HR	95%CI	*P* value
pM stage (M_0 VS M_1)	3.392	1.096-10.506	0.034
KCNH2	2.632	1.442-4.803	<0.01
HR: hazard risk, CI: confidence interval			

Next, we detected the mRNA expression of KCNH2 in four human PC cell lines PANC-1, BXPC-3, ASPC-1, and SW1990 using qRT-PCR (Figure 2J). The results showed that KCNH2 was highly expressed in PANC-1 and ASPC-1 cells, lowly expressed in SW1990 cells, and barely expressed in BXPC-3 cells. Then, RNAi transfection technology was used to establish PANC-1 cell line that stably expresses shRNAs targeting KCNH2 at different sites. The transfection efficiency was evaluated by Western blot (Figure 2K, L). The most robust knockdown effect was observed in the shKCNH2_6 and shKCNH2_7 cell lines, and they were therefore used for subsequent experiments.

3. KCNH2 regulates PC cell proliferation and apoptosis

To explore the regulatory effect of KCNH2 on the proliferation and invasion of PC cells, lentiviral vector-mediated shRNA was used to reduce the expression of KCNH2 in PC cells. The results showed that knockdown of KCNH2 significantly decreased the growth rate (Figure 3A) of PC cells. Subsequently, we evaluated the colony-forming ability of PC cells lines (PANC-1) with KCNH2 knockdown (Figure 3B-E). The results showed that KCNH2 knockdown inhibited the proliferation of tumor cells. Consistently, KCNH2 knockdown promoted the apoptosis of PC cells (Figure 3F-L). The analysis of the MAPK pathway showed that knockdown of KCNH2 decreased the expression levels of MAPK and pMAPK also decreased (Figure 3M, N), implying that KCNH2 might be involved in MAPK-related pathways to regulate the development and metastasis of PC. The protein expressions of p53, Cyclin D1, and CDK2 did not significantly change among groups (Figure 3M, N), probably suggesting that KCNH2 did not regulate PC cell proliferation via affecting the G1/S phase of the cell cycle. These data implied that knockdown of KCNH2 inhibited the proliferation and apoptosis of PC cells.

4. KCNH2 regulates the migration and invasion of PC cells

Subsequently, we investigated the effect of KCNH2 knockdown on the migration and invasion of PC cells. Transwell assay showed that KCNH2 knockdown by shKCNH2_6 or shKCNH2_7 inhibited the migration of PC cells ($p = 2.92\times10^{-10}$, $p = 1.67\times10^{-12}$, Figure 4A-B) and the invasion of PC cells ($p = 1.93\times10^{-10}$, $p = 1.89\times10^{-13}$, Figure 4B). To explore whether KCNH2 can affect the epithelial-mesenchymal transition (EMT) process, we measured the expressions of E-cadherin, Vimentin, and MMP9 by Western blot in PC

cells. The results showed that as KCNH2 knockdown increased the expressions of E-cadherin, downregulated MMP9 and RhoA, and did not affect the expression of Vimentin (Figure 4C-D), suggesting that knockdown of KCNH2 affected the EMT process, thereby promoting tumor cell metastasis.

5. KCNH2 regulates the metastasis of pancreatic tumors *in vivo*

To further understand the impact of KCNH2 on the progression and metastasis of PC, we established a mouse xenograft model by inoculating mice with PC cells transfected with scrambled sequences or shKCNH2_6. The tumor size was observed (Figure 5A). We also injected shKCNH2_6 cells into the spleen of nude mice to establish a PC model of liver metastasis. The number of liver nodules was observed (Figure 5B). The results showed that knockdown of KCNH2 significantly reduced the size of pancreatic tumors ($p = 0.0012$) and decreased the number of liver nodules ($p = 0.0039$) in mice. These results indicate that KCNH2 knockdown inhibits the progression and metastasis of PC *in vivo*.

Discussion

At present, there is no effective screening method for PC and the major treatment approach is still surgical resection [3]. As PC is often diagnosed at late stages and cannot be completely removed by surgery, the prognosis of PC remains poor [15]. The early metastasis and late diagnosis of PC urge the development of new therapeutic approaches. With an increasing number of studies of the pathogenic mechanisms of PC in recent years, targeted therapy has become a promising clinical tool for PC treatment.

KCNH2, a member of the potassium voltage-gated channel subfamily H, has been widely studied in heart and brain diseases. KCNH2 not only regulates the excitation of myocardial cells and neurons, but also mediates the secretion of vascular endothelial growth factor and other cytokines from tumor cells [16]. Our previous study of functionally mutated genes related to the metastasis of pancreatic ductal adenocarcinoma identified 12 candidate genes, including *KCNH2*, using exon sequencing and PCR analysis [11]. Our preliminary results showed that *KCNH2* is a functionally mutated gene related to the proliferation and metastasis of PC. In this study, the analysis using the Oncomine and GEPIA online databases confirmed that KCNH2 was differentially expressed in PC tissues compared with normal pancreatic tissues, suggesting that aberrant expression of KCNH2 may promote the malignant progression of PC. We further analyzed the correlation of KCNH2 with the clinical prognosis of PC patients and found that high KCNH2 expression was closely related to low OS.

The abnormal expression of KCNH2 was also observed in other malignant diseases, such as rectal cancer [17], small cell lung cancer [18], gastric cancer [19], acute myeloid leukemia [16, 20], breast cancer [21], ovarian cancer [22], and endometrial cancer [23]. KCNH2 is also aberrantly expressed in Barrett's esophagus [24], melanoma [25], and neuroblastoma [26], and promotes the malignant progression of

these diseases. In different types of cancers, the proliferation, migration, and invasion of tumor cells may be regulated by different pathways. For example, the blocker cisapride induced apoptosis and inhibited the proliferation of gastric cancer cells by regulating cell cycle progression [19]. KCNH2 forms a complex with β1 integrin to regulate the proliferation, migration, and invasion of acute myeloid leukemia cells and melanoma cells [16, 25]. In addition, the activation of the KCNH2 channel induced the G1/S progression of acute myeloid leukemia cells, thereby regulating cell proliferation through mitosis [20]. In ovarian cancer and neuroblastoma, KCNH2 inhibitors exerted an anti-proliferative effect on tumor cells by affecting the S phase and (or) G2/M phase and G0/G1 phase, respectively [22, 26]. Consistent with previous findings, our results showed that knockdown of KCNH2 inhibited the proliferation and invasion of PC cells. It has also been found that inhibition of KCNH2 induced the apoptosis of gastric cancer cells [19]. However, it did not affect the apoptosis of ovarian cancer cells [22], which was in line with our findings in PC cells. Regarding the impact of KCNH2 on the OS of patients, abnormal expression of KCNH2 often indicates low survival of acute myeloid leukemia [16], which was consistent with our results. A previous study has also reported that KCNH2 maintains the depolarization of tumor cells, thereby accelerating cell cycle progression [18], which provided another potential mechanism of KCNH2 on the regulation of proliferation. Another study found that KCNH2 stimulated tumor cell angiogenesis by promoting the secretion of cytokines, such as vascular endothelial growth factor, in glioblastoma multiforme [27]. KCNH2 has also been reported to regulate the size of breast cancer cells [21]. Intriguingly, the outflow of potassium resulted in a decrease in the level of intracellular potassium, thereby inhibiting cell depolarization and activating the caspase-dependent apoptotic pathway [19]. The concentration of intracellular potassium directly affects the osmotic pressure of cells and thus changes the cell size. These findings further support the regulation of KCNH2 on tumor cells. The *KCNH2* gene has been identified as a biomarker for colorectal cancer [28]. In endometrial cancer, KCNH2 may be used as a biomarker to distinguish cancerous endometrial tissues from normal tissues [23]. Also, KCNH2 is closely related to the proliferation, migration, and invasion of colon cancer cells, and may be used as a new predictor for the invasion and metastasis of colon cancer [17].

The regulatory mechanism of KCNH2 in the progression of cancer remains elusive. The involvement of KCNH2 in the MAPK/c-fos signaling pathway has been reported in melanoma. Our results showed that KCNH2 knockdown decreased the expressions of MAPK and pMAPK in PC cells, suggesting that KCNH2 may be involved in the MAPK signaling pathway in PC. Further analysis revealed that KCNH2 regulated the expression of cell cycle-related proteins. CDK2 is a member of the serine/threonine protein kinase family that acts as a key regulator of G1/S phase transition and is closely related to the MAPK pathway. Additionally, under hypoxic conditions, p53 mediated cell apoptosis in a dose-dependent manner [29]. In this study, we measured the expressions of CDK2 and p53 and other related proteins. The results showed that KCNH2 did not mediate apoptosis through the p53 pathway. The effect of KCNH2 on EMT-related proteins was also explored. We found that KCNH2 knockdown increased the expressions of E-cadherin and MMP9, indicating that KCNH2 may promote the metastasis of PC by regulating the EMT process.

In conclusion, this study showed that KCNH2 was upregulated in PC tissues and regulated the growth and metastasis of pancreatic tumors. Further investigations are needed to develop novel and

personalized therapeutic strategies targeting the key molecules and signaling pathways related to the progression of PC and achieve precise treatment and personalized treatment. This study highlighted the potential of KCNH2 as a new target for the treatment of PC.

Declarations

Acknowledgements

This work was supported by the China Postdoctoral Science Foundation Funded Project (Project No.: 2019M662304). We thank all the patients and clinical investigators who were involved in this study.

Conflicts of interest

The authors declare no conflicts of interests.

Author contributions

Bin Zhou and Weidong Guo conceived and designed the experiments. Bin Zhou, Jinghao Lei, and Qiang Wang performed the experiments. Tengfei Qu analyzed the data. Bin Zhou, Lichao Cha, Hanxiang Zhan, Jianwei Xu, and Shanglong Liu wrote the manuscript. Lantian Tian, Chuandong Sun, Jingyu Cao, and Fabo Qiu provided helpful discussion, and the revision of critically important intellectual content.

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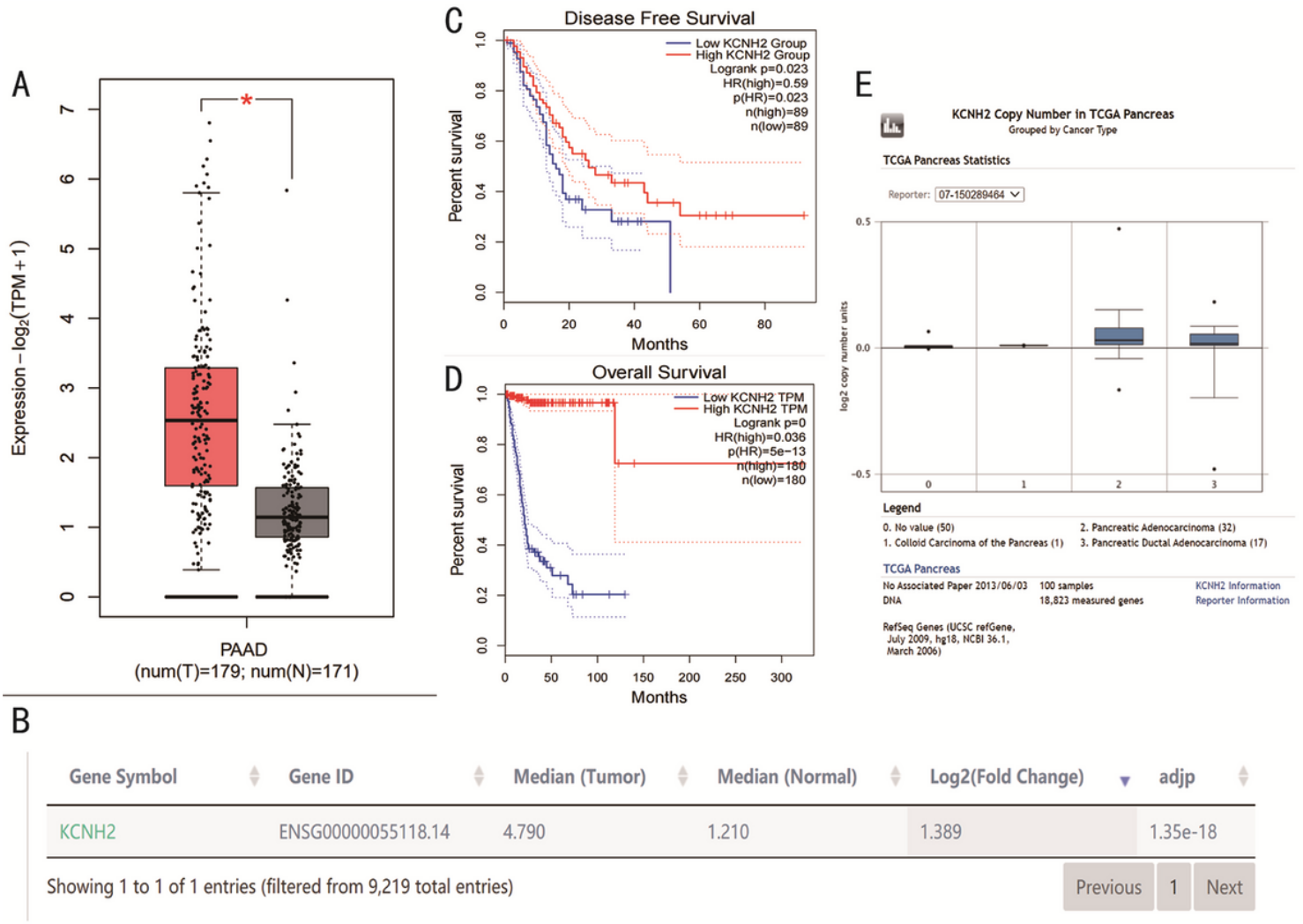
Figures



B

Gene Symbol	Gene ID	Median (Tumor)	Median (Normal)	Log2(Fold Change)	adjp
KCNH2	ENSG00000055118.14	4.790	1.210	1.389	1.35e-18

Showing 1 to 1 of 1 entries (filtered from 9,219 total entries)

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Figure 1

Expression of KCNH2 in PC tissues versus normal tissues and survival analysis. (A, B) The GEPIA database showed that KCNH2 was differentially expressed in PC tissues versus normal pancreatic tissues. (C, D) The disease-free survival and OS of PC patients with different expressions of KCNH2. (E) The differential expression of KCNH2 in PC tissues versus normal tissues was analyzed by the Oncomine database.

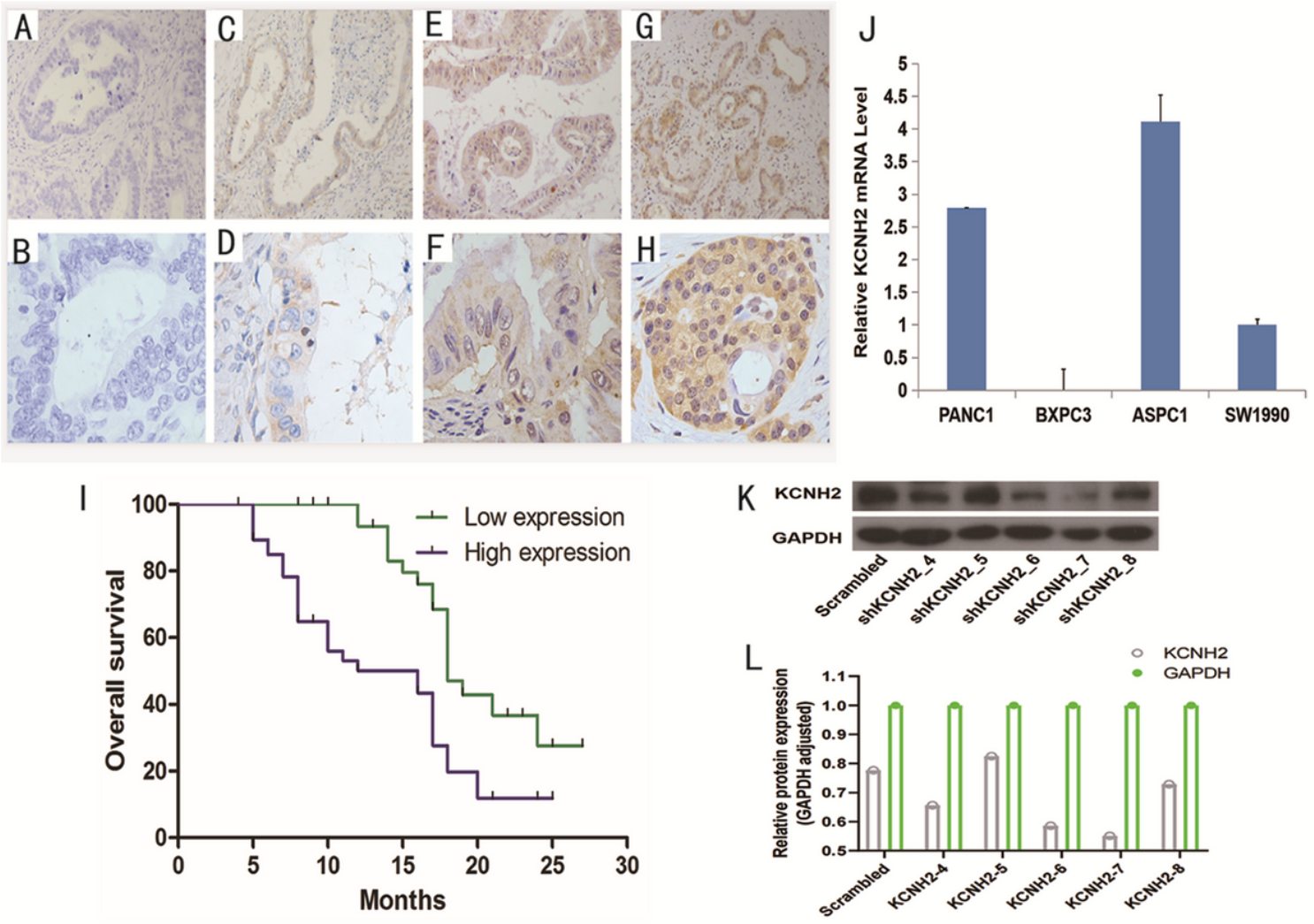

Figure 2

Immunohistochemistry staining of KCNH2 in PC tissue. (A, B) PC tissues with negative KCNH2 staining (−). (C, D) PC tissues with weak KCNH2 staining (+). (E, F) PC tissues with moderate KCNH2 staining (++). (G, H) PC tissues with strong KCNH2 staining (+++). (A, C, E, G) 40× magnification. (B, D, F, H) 200× magnification. (I) The OS of PC patients with high or low KCNH2 expression. (J) The mRNA expression of KCNH2 in different human PC cell lines. (K, L) The expression of KCNH2 in PC cells with KCNH2 knockdown at different sites. GAPDH expression was used as an internal control.

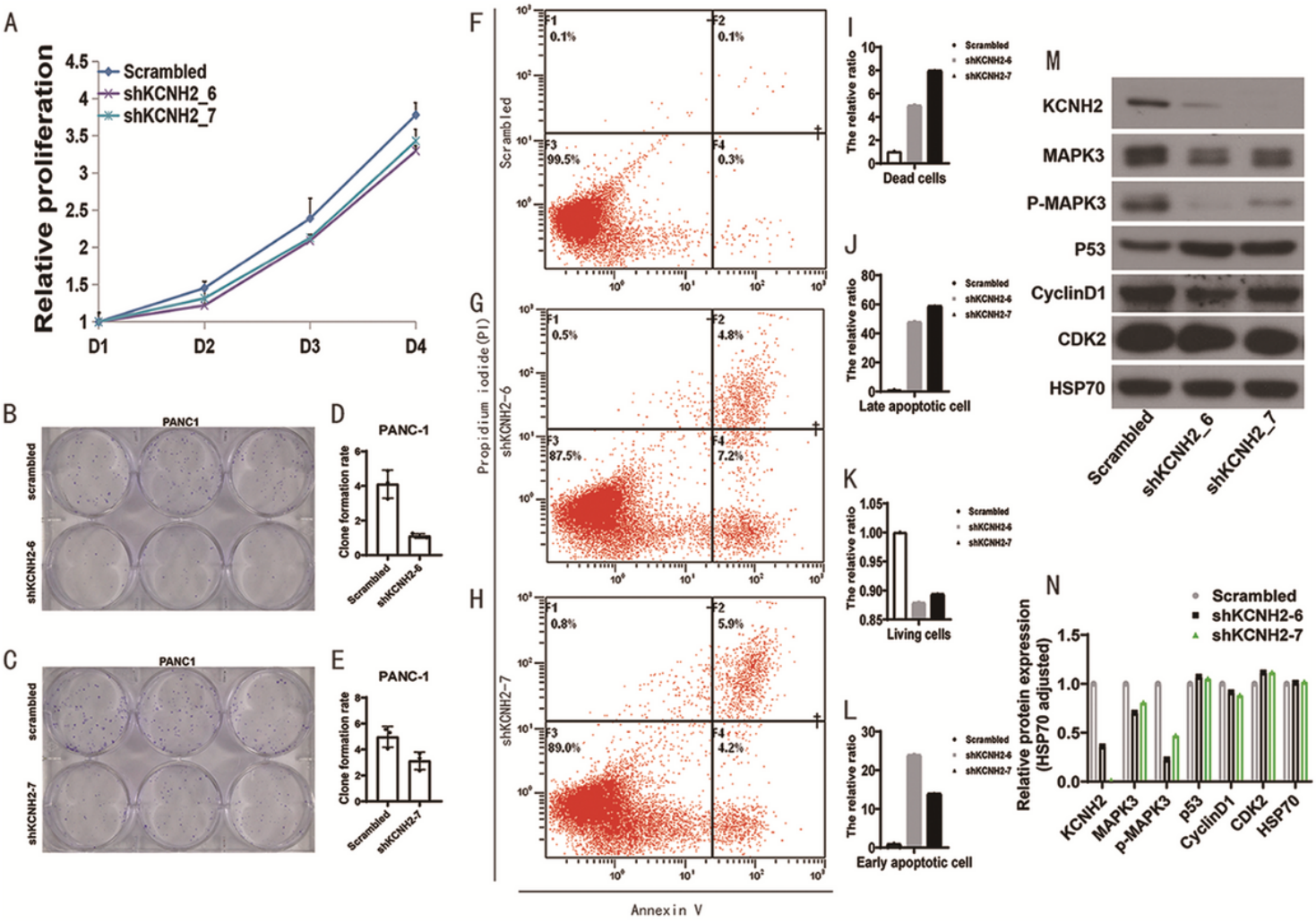

Figure 3

Effect of KCNH2 knockdown on the proliferation and apoptosis of PC cells. (A) The MTT assay was performed to determine cell viability. (B-E) The colony formation assay was used to detect colony-forming ability of different types of PC cells. (F-L) The apoptosis of PC cells was determined by flow cytometry. (M-N) The effect of KCNH2 knockdown on the MAPK pathway and the p53 pathway. The results showed that KCNH2 knockdown decreased the expressions of MAPK and pMAPK, but had no effect on the expression levels of p53, Cyclin D1 or CDK2.

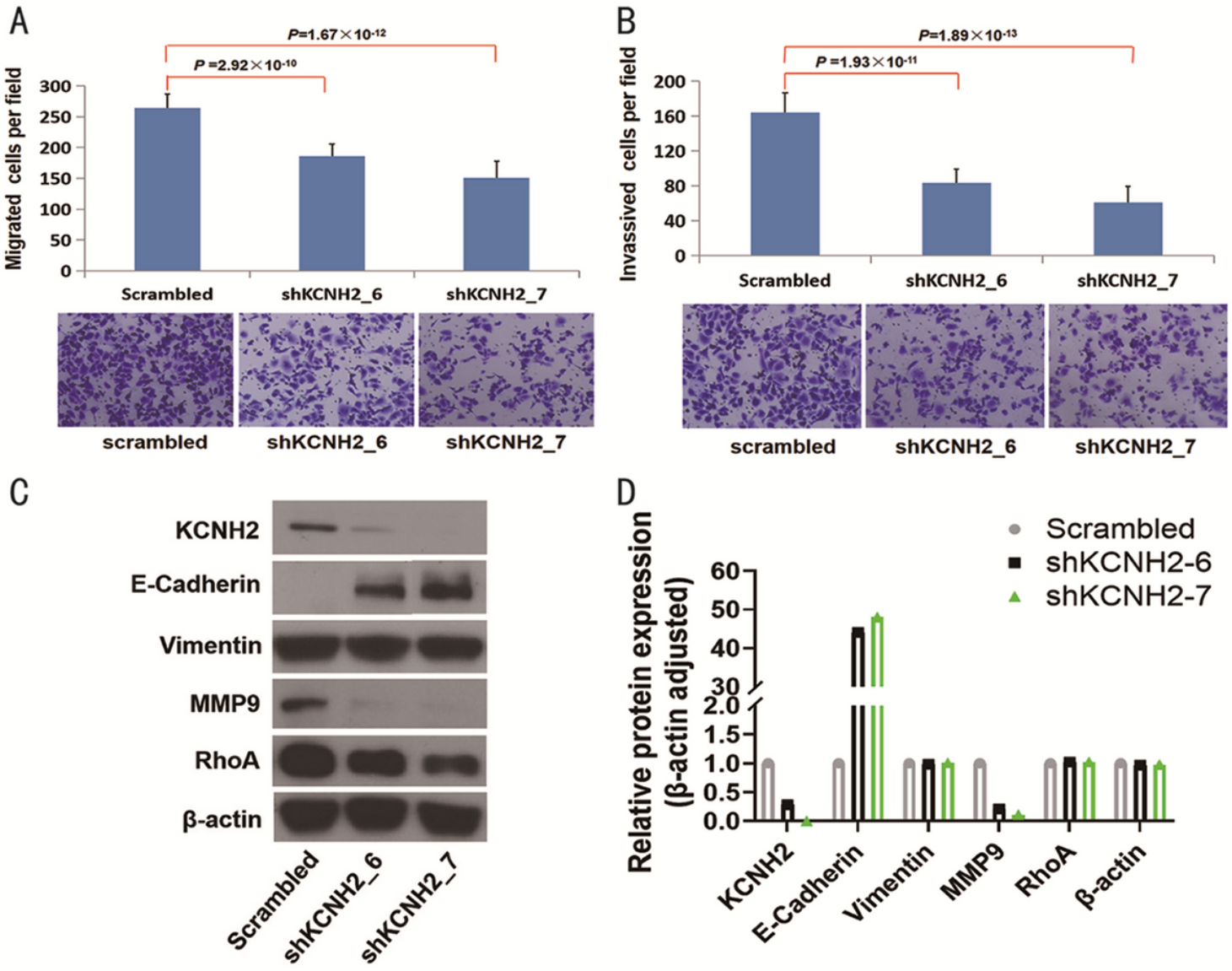

Figure 4

Effect of KCNH2 knockdown on the migration and invasion of PC cells. (A-B) Knockdown of KCNH2 reduced the migration and invasion ability of PC cells in the Transwell assay. (C-D) The effect of KCNH2 knockdown on the expression of EMT-related proteins. The results showed that with knockdown of KCNH2 increased the expression of E-cadherin, decreased the levels of MMP9 and RhoA, but did not affect the expression of Vimentin. Beta-actin expression was used as an internal control.

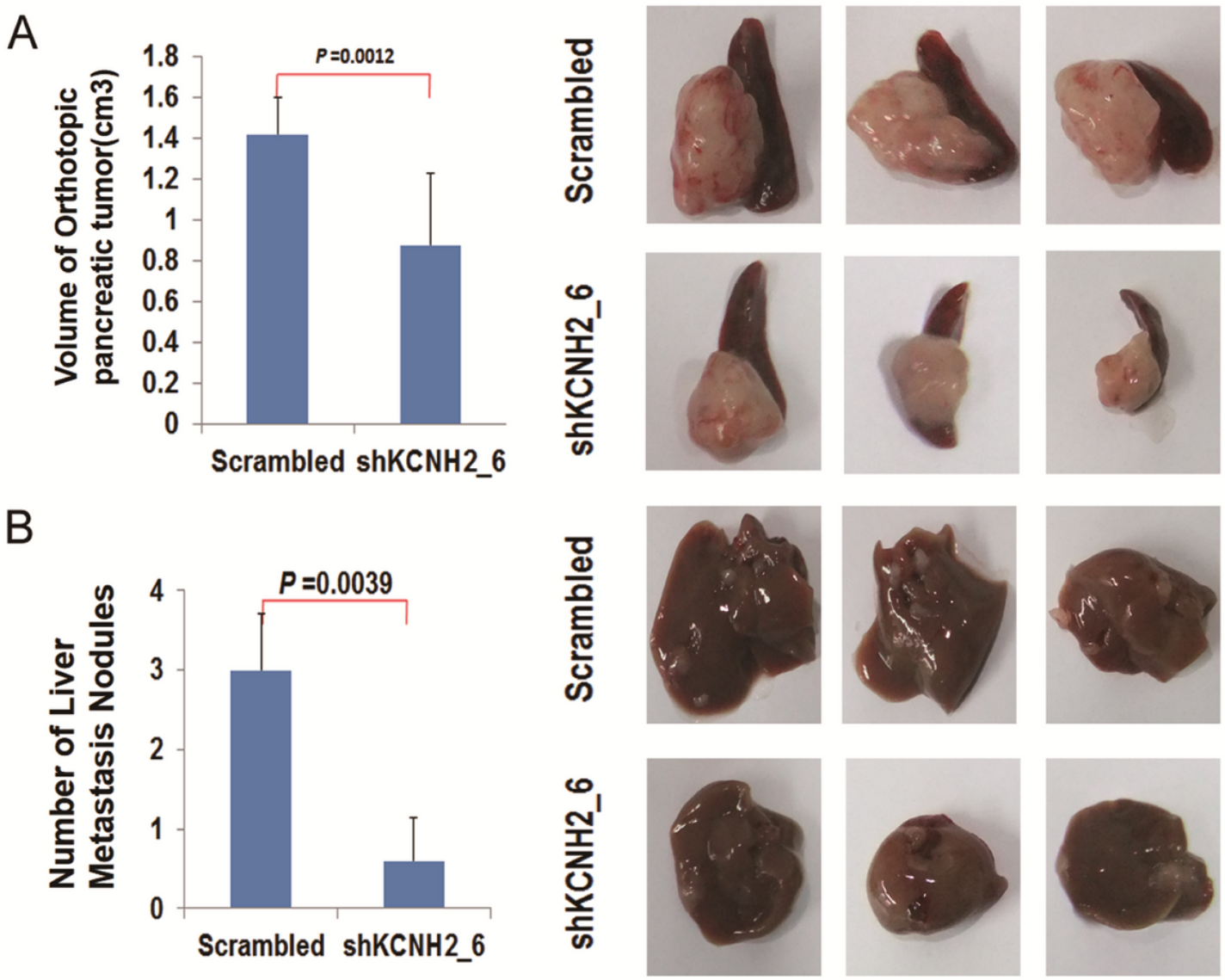

Figure 5

Effect of KCNH2 knockdown on the progression and metastasis of PC in vivo. The xenograft model was established by in-situ inoculation of PC cells. The liver metastasis model was established by injection of PC cells into the spleen. The results showed that knockdown of KCNH2 (A) reduced the volume of pancreatic tumors and (B) decreased the number of liver metastatic nodules.