Research Article

IQ motif containing GTPase activating protein 3 is associated with cancer stemness and survival in pancreatic ductal adenocarcinoma

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Short title: IQGAP3 in PDAC stemness and survival

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Abstract

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal types of malignancy, with poor prognosis and rising incidence. IQ motif-containing GTPase-activating protein 3 (IQGAP3) is a member of the IQGAPs family of scaffolding proteins that govern multiple cellular activities like cytoskeletal remodeling and cellular signal transduction. This study aimed to analyze the expression and biological function of IQGAP3 in PDAC.

Methods: We analyzed IQGAP3 expression in 81 PDAC samples by immunohistochemistry. RNA interference was used to inhibit IQGAP3 expression in PDAC cell lines.

Results: Immunohistochemical analysis of IQGAP3 showed that 54.3% of PDACs were positive for cytoplasmic expression of IQGAP3, with no expression found in non-neoplastic tissue. Furthermore, IQGAP3 expression was an independent poor prognostic factor in our immunostaining-based studies and analyses of public databases. Our cohort and The Cancer Genome Atlas database indicated that IQGAP3 is co-localized with kinesin family member C1 (KIFC1), which we previously reported as a cancer stem cell-associated protein. *IQGAP3* siRNA treatment decreased PDAC cell proliferation and spheroid colony formation via ERK and AKT pathways.

Discussion/Conclusion: These results suggest that IQGAP3, a transmembrane protein, is involved in survival and stemness and may be a promising new therapeutic target for PDAC.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the most common and aggressive malignancy of the pancreas, and patients with PDAC often have poor outcomes. The proportion of patients with resectable PDAC at diagnosis is only 10-20%[1]. Furthermore, neoadjuvant and adjuvant chemotherapies have only minimal improvement in survival rates[2]. PDAC is anticipated to become the second leading cause of cancer-related deaths by 2030[3]. Despite recent progress in the elucidation of PDAC tumor biology and the development of novel treatments, it has an average 5-year survival rate of only 11%[4]. Therefore, it is necessary to identify novel molecular targets for PDAC treatment strategies.

The process of selecting precision candidate molecules for targeted molecular therapy is a critical aspect of drug discovery, with focusing on cancer stem cells (CSCs) being one of the approaches. The CSCs possess the ability for self-renewal, proliferation, and generation of downstream progenitor cells to promote tumor growth, progression, metastasis, and resistance to therapy[5,6]. Therefore, investigating the characteristics of CSCs and discovering CSC-specific treatment options can be important for improving PDAC prognosis. Several surface markers, such as CD44 and CD133 (prominin-1), have been reported as CSC markers for PDAC[7-9]. The formation of multicellular spheroidal cell aggregates, or spheroids, is a conspicuous characteristic of CSCs. Thus, these spheroids contain abundant CSCs and can be cultured in non-attachment dishes with serum-free media for the formation of spheroid colonies[10]. We have previously examined the gene expression profile of spheroid colonies using microarray analysis and found numerous CSC-related molecules, such as IQ motif containing GTPase-activating protein 3 (IQGAP3)[11], and kinesin family member C1 (KIFC1)[12] in gastric cancer. However, the molecular mechanisms underlying CSCs in PDAC remain largely unexplored.

IQGAP3 is a member of the IQGAP family, which is a class of scaffolding proteins that govern multiple cellular activities by facilitating cytoskeletal remodeling and cellular signal transduction. The name IQGAP originates from the multiple functional domains of these molecules that harbor four IQ motifs and have a Ras-GAP-related domain[13]. The multidomain structure of IQGAPs facilitates the formation of protein complexes necessary for cellular functions[14]. IQGAP1 has been suggested to function in cell migration and regulation of the cytoskeleton[15], and it may play a role in cancer cell motility and invasion [16], while IQGAP2 seems to act as a tumor suppressor[14]. *IQGAP3* has been shown to be necessary and sufficient for cell proliferation[17]. IQGAP3 protein has recently been reported as a new prognostic biomarker for some cancers, including hepatocellular carcinoma[18], breast cancer[19], and lung cancer[20]. We have previously shown that IQGAP3 is expressed at high levels in gastric cancer and is involved in cancer stemness[11]. Recently, *IQGAP3* mRNA expression

has been shown to be increased in PDAC and associated with tumor progression[21]. However, to the best of our knowledge, no studies have reported immunohistochemical analysis of IQGAP3 protein in PDAC tumor tissues and the relationship between IQGAP3 and CSC markers in PDAC. This study aimed to demonstrate the association between IQGAP3 and its clinicopathological significance in PDAC, investigate IQGAP3 protein expression in patients with PDAC using immunohistochemistry (IHC), and explore the biological function of IQGAP3 in PDAC-derived cell lines using RNA interference.

Materials and Methods

Tissue samples

A consecutive cohort of 81 histopathologically confirmed patients with pancreatic cancer who underwent surgical resection at the Kure Medical Center and Chugoku Cancer Center (Hiroshima, Japan) between April 1, 2015, and March 31, 2020, was included in the study, as described previously[22,23]. Archived formalin-fixed and paraffin-embedded (FFPE) tumor tissues obtained from the resected specimens were used for immunohistochemical analyses. One representative tumor block from each specimen was assessed using IHC. Histological classifications were determined according to the World Health Organization guidelines[24]. Tumor stage was determined according to the criteria defined in the Union for International Cancer Control tumor-node-metastasis (TNM) classification guide[25]. Informed consent was obtained from all the patients. This study was approved by the Ethical Committee for Human Genome Research of Kure Medical Center and Chugoku Cancer Center (2019-91) and the Ethical Committee for Human Genome Research of Hiroshima University, Hiroshima, Japan (E2001-9923).

Immunohistochemistry (IHC)

For IHC, 81 representative FFPE slides were sliced into 4 μm-thin sections, deparaffinized, and rehydrated. Immunohistochemical analysis was performed using a Dako EnVision+ Peroxidase Detection System (Dako Cytomation; Carpinteria, CA, USA). Antigen retrieval was performed by microwave heating the sections in citrate buffer (pH 6.0) for 30 min. Peroxidase activity was blocked with 3% H_2O_2-methanol for 10 min, and non-specific antibody binding sites were blocked by incubating the sections with normal goat serum (Dako Cytomation) for 20 min. The sections were incubated with rabbit polyclonal anti-IQGAP3 antibody (1:100; Abcam), rabbit polyclonal anti-KIFC1 antibody (1:100; Abnova, Tapei, Taiwan), mouse monoclonal anti-CD44 antibody (1:100; clone DF1485; Novocastra, Newcastle upon Tyne, UK), mouse monoclonal anti-CD133 antibody (1:100; clone AC133; Miltenyi Biotec, Auburn, CA, USA) or mouse monoclonal anti-Ki-67 antibody (1:100;

clone MIB-1; Dako, Carpinteria, CA, USA) for 1 h at room temperature, followed by incubation with EnVision+ peroxidase-conjugated anti-rabbit or anti-mouse secondary antibodies for 1 h. For the color reaction, the sections were incubated with DAB substrate-chromogen solution (Dako Cytomation) for 5 min, followed by counterstaining with 0.1% hematoxylin. IQGAP3 expression was evaluated as either positive or negative across all slides. Islets of Langerhans were used as positive controls. When >50% of the tumor cell cytoplasmic were stained, the section was considered positive for IQGAP3 expression. Two surgical pathologists (A.K. and A.I.) followed this classification and independently reviewed the immunoreactivity of each specimen.

Kaplan–Meier analysis

Kaplan–Meier analysis was performed for IQGAP3 using Kaplan–Meier Plotter software from a database of public Pan-cancer RNA-seq datasets (http://kmplot.com/analysis)[26]. Data from 177 PDAC patients were collected from this database. To analyze the prognostic value, the samples were assigned to two groups based on the cut-off value stipulated by the automated software program. Hazard ratios (HRs) and *p* values (log-rank *p*) were determined for each survival analysis.

In silico analysis

UCSC Xena (https://xena.ucsc.edu/)[27] was used to determine *IQGAP3* expression in GDC (Genomic Data Commons Data Portal) and The Cancer Genome Atlas (TCGA) pancreatic cancer (PAAD) dataset. Using this platform, the expression of IQGAP3 in PDAC was analyzed and evaluated to correlate with prognosis, clinicopathological characteristics, and cancer stem cell markers.

Cell lines

The human PDAC cell lines PANC-1, PK-1, KP-4, and T3M-4 were purchased from the Japanese Collection of Research Bioresources Cell Bank (Osaka, Japan). All cell lines were maintained at 37 °C in Dulbecco's modified Eagle's medium (DMEM) medium (Thermo Fisher Scientific, Waltham, MA, USA) containing 10% fetal bovine serum (Corning, Corning, NY, USA) in a humidified atmosphere with 5% CO_2.

RNA interference

Small interfering RNA (siRNA) targeting *IQGAP3* and negative control oligonucleotides were purchased from Invitrogen (Carlsbad, CA). Three independent *IQGAP3*-specific siRNA oligonucleotide sequences were used. Transfections of PDAC cell lines were performed using Lipofectamine RNAiMAX (Invitrogen), as previously described[28]. Briefly, 60 pmol of siRNA and 10 µL of

Lipofectamine RNAiMAX were mixed in 1 mL of DMEM (final siRNA concentration, 10 nmol/L). After 20 min of incubation, the mixture was added to the cells, which were then plated in culture dishes. Forty-eight hours after transfection, PDAC cells were analyzed as described below.

Western blot analysis

Cells were lysed as previously described[23], and the lysates (30 μg) were solubilized in Laemmli sample buffer by boiling and subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Following electrophoresis, proteins were electrotransferred to nitrocellulose membranes and incubated with primary antibodies for 1 hour. Anti-AKT, anti-phospho-AKT, p44/p42 (extracellular signal-regulated kinase [ERK] 1/2), and anti-phospho-p44/p42 (ERK1/2) monoclonal antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). Anti-IQGAP3 polyclonal antibody was purchased from Abcam (Cambridge, MA, USA). Peroxidase-conjugated anti-rabbit IgG was used as a secondary antibody. Immunocomplexes were visualized using the ECL Western Blot Detection system (Amersham Biosciences Corp., Piscataway, NJ, USA). Anti-β-actin (Sigma-Aldrich, St. Louis, MO, USA) was used as the loading control.

Cell growth assay

A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed to examine cell growth, as previously described[12]. PDAC cells were seeded at a density of 4,000 cells/well in 96-well plates. MTT (1mg/ml) was added and incubated for 1 h at 37 °C. 50 μl DMSO was added and mixed to obtain the O.D.595 value. Cell growth was monitored after 1, 2, and 4 days. Three separate MTT experiments were performed, and the mean ± standard deviation (SD) was calculated.

Spheroid colony formation assay

For spheroid generation, we used the PDAC cell line T3M-4; 1,000 cells were seeded in 6-well ultra-low attachment plates (Corning). The cells were grown in mTeSR medium (STEMCELL Technologies Inc., Vancouver, BC, Canada). The plates were incubated at 37 °C in an incubator with a 5% CO_2 atmosphere for seven days. Spheroid number and size were determined using a microscope as previously described[12].

Statistical analysis

Correlations between clinicopathological parameters and IQGAP3 expression were analyzed using Fisher's exact test. Significant differences between survival curves were determined using the log-

rank test. Univariate and multivariate Cox regression analyses were used to evaluate the association between clinical covariates and overall survival (OS). HR and 95% confidence intervals (CIs) were assessed using Cox proportional hazard models. Statistical significance was set at p <0.05.

Results

Expression of IQGAP3 in PDAC patients

IHC analysis of the 81 PDAC tissue samples revealed that IQGAP3 is not expressed in non-neoplastic pancreatic ductal cells (**shown in Fig. 1A, B**). In contrast, PDAC tissues showed strong and extensive IHC staining (**shown in Fig. 1A,C-E**). IQGAP3 staining was detected in the cytoplasm and cell membranes of the tumor cells (**shown in Fig. 1E**). When >50% of tumor cells were observed to be stained, immunostaining was considered positive for IQGAP3. Based on this criteria, 44 PDAC slides (54.3%) were positive for IQGAP3. We further analyzed the relationship between IQGAP3 expression and clinicopathological characteristics (**Table 1**). However, there was no significant association between IQGAP3 expression and these features. Kaplan-Meier analysis with log-rank tests to analyze the association between IQGAP3 and patient survival indicated that IQGAP3 expression was significantly associated with poorer prognosis (p = 0.0197; **shown in Fig. 1F**). Univariate and multivariate Cox proportional hazards analyses were also performed (**Table 2**). Univariate analysis revealed two prognostic parameters: Grade (HR, 2.713; 95% CI, 1.204–6.113; p = 0.0161), pN (HR, 2.635; 95% CI, 1.076–6.453; p = 0.034) and IQGAP3 expression (HR, 2.591; 95% CI, 1.191–5.64; p = 0.0164) to be associated with poor survival. Furthermore, multivariate analysis showed that IQGAP3 expression (HR, 2.213; 95% CI, 1.005–4.873; p = 0.484) and Grade (HR, 2.637; 95% CI, 1.166–5.963; p = 0.0198) were independent prognostic factors for poor clinical outcomes in PDAC cases. These results indicate that IQGAP3 expression confers poor prognosis in patients with PDAC and is thus a prognostic marker.

In silico analysis of IQGAP3 expression in the TCGA PAAD dataset

To confirm the abovementioned results, we also accessed and analyzed a public dataset, which included 177 PDAC cases. Similar to our case cohort, IQGAP3-positive patients had significantly worse survival than IQGAP3-negative patients (p = 0.0325; **shown in Fig. 1G**). The relationship between *IQGAP3* expression and clinicopathological features was analyzed using the mRNA expression data. *IQGAP3* expression was associated with high histological grade (**Table 3;** p = 0.0126). Though there was no significant difference in the pT stage (p = 0.0976), a tendency for higher expression of IQGAP3 in advance of the stage was observed. We also performed univariate and multivariate Cox proportional hazards analyses using the TCGA PAAD dataset (**Table 4**).

Univariate analysis showed that the T grade (p = 0.0471), N grade (p = 0.0074), and IQGAP3 expression (p = 0.0341) were associated with survival. In multivariate analysis, N grade (p = 0.0136) and IQGAP3 expression (p = 0.0409) were independent prognostic predictors for survival. Therefore, the results of this *in silico* analysis were similar to those of our cohort and strongly supported our results.

Correlation between IQGAP3 expression and several molecules, including CSC markers

IQGAP3 was originally discovered while investigating its relationship with CSCs[12]. Here, we investigated its relationship with CSC markers and KIFC1 to show whether it is associated with cancer stemness. We performed immunohistochemical staining for IQGAP3 (**shown in Fig. 2A**) and CD44, CD133, and KIFC1 (**shown in Fig. 2B**) in our 81 PDAC cases. Our findings revealed that IQGAP3 co-localized with KIFC1 (**Table 5**; p = 0.0434), both previously reported as CSC-associated proteins[23]. There was no significant correlation between IQGAP3 and CD44 (p = 0.5025) or CD133 (p = 0.2776) expression. Because IQGAP3 affected cell proliferation in a previous study[11], we performed an immunohistochemical analysis of Ki67, one of the cell proliferation markers. However, there was no significant correlation between IQGAP3 and Ki67 (**Table 5**; p = 0.4964). Moreover, IQGAP3 expression was evaluated to be significantly correlated with KIFC1, CD133, and CD44 expression in the TCGA PAAD dataset (**shown in Fig. 2C**). Generally, the results suggested that IQGAP3 expression is involved in PDAC stemness.

IQGAP3 inhibition affected cell growth and spheroid formation via AKT and ERK pathways in PDAC cells

Biological studies on IQGAP3 were performed using PDAC cell lines. Western blot analysis revealed IQGAP3 expression in KP-4, PK-1, T3M-4, and PANC-1 cells (**shown in Fig. 3A**). We selected T3M-4 cells that showed high IQGAP3 expression for further experiments. IQGAP3 knockdown was confirmed using *IQGAP3*-specific siRNAs in T3M-4 cells and the same western blot analysis revealed that the levels of phosphorylated AKT (p-AKT) and ERK (p-ERK) were lower in *IQGAP3* siRNA-transfected cells than in negative control siRNA-transfected cells (**shown in Fig. 3B**). In MTT assays, cell proliferation/growth was significantly suppressed in *IQGAP3* knockdown PDAC cells compared to negative control siRNA-transfected PDAC cells (**shown in Fig. 3C**). Finally, we analyzed the effect of *IQGAP3* inhibition on the number and size of spheres in PDAC cell lines. The number of spheroids was significantly reduced in *IQGAP3* siRNA-transfected cells (**shown in Fig. 3D**) compared to negative control siRNA-transfected cells. These data suggested that IQGAP3 is associated with cancer progression and stemness via ERK and AKT signaling pathways.

Discussion/Conclusion

We have previously shown that the expression of IQGAP3 is associated with tumor progression in gastric cancer[11]. IQGAP3 has been reported to be upregulated in hepatocellular[18], clear cell renal cell[29,30] carcinomas, high-grade serous ovarian[31] and lung[20] cancers, respectively.

In the present study, our immunohistochemical analysis showed that IQGAP3 expression was detected in 44 (54.3%) of 81 PDAC cases, and high IQGAP3 expression was significantly associated with poor overall survival. IQGAP3 expression was not found in the non-neoplastic pancreatic ductal epithelium but in the cytoplasm and cell membranes of cancer cells, suggesting that its expression is relatively specific to cancer. Although elevated expression of *IQGAP3* has been previously confirmed in PDAC at the mRNA level[21,32], this report is the first to confirm IQGAP3 protein expression in PDAC using immunohistochemistry. Moreover, IQGAP3 expression was an independent poor prognostic factor in our immunostaining-based analysis and analyses of the TCGA PAAD dataset. These results suggest that IQGAP3 immunostaining is a clinically useful method for the prediction of survival in patients with PDAC.

IQGAP3 was identified as a molecule associated with cancer stemness in our previous study on gastric cancer[11]. Therefore, we examined the relationship between IQGAP3 and CSC markers, including KIFC1. In the present study, IQGAP3-positive cases showed a significant association with KIFC1 in both our cohort and the TCGA database. KIFC1 (also known as HSET), a C-type kinesin of the kinesin-14 family[33], is also related to CSC makers and has been shown to be associated with tumor progression and chemotherapy resistance[23,34-37]. Furthermore, IQGAP3 was co-expressed with other known CSC markers, such as CD44 and CD133, in the PDAC samples of the TCGA database but not in our cohort. This discrepancy may be caused by racial differences, institutions, or analytical techniques. Either way, IQGAP3 likely contributes to stemness through some pathways.

Experiments were performed with cancer cell lines to further validate our findings using clinical specimens. We have previously reported that IQGAP3 knockdown inhibits cell growth and spheroid formation in gastric cancer cell lines[11]. In this study, the results of the experiments with cell lines expressing IQGAP3 were similar to those in gastric cancer. Moreover, we have shown that the ERK and AKT pathways are affected by IQGAP3 in PDAC cell lines, again similar to a previous lung carcinoma cell study[20] and our study on gastric cancer[11]. Generally, these results suggest that IQGAP3 may contribute to the growth and stemness of carcinomas.

Our study had several limitations. First, this was a retrospective study involving a small number of clinical samples for immunohistochemical analysis. A prospective study with a larger number of patients with PDAC is needed to verify our findings. Second, we examined neither the detailed

mechanisms through which IQGAP3 contributes to the cancer stemness and progression nor the molecular interaction between IQGAP3 and KIFC1. It is reported that in the mouse stomach corpus, the IQGAP3-ERK-CD44 axis drives stem cell proliferation during homeostasis and tissue repair[38]. A previous study has shown that IQGAP3 acts as an oncogene by regulating Cdc42 expression[21], and heterogeneous nuclear ribonucleoprotein C promotes PDAC progression via IQGAP3 stabilization[32]. It would be desirable to place IQGAP3 in the context of tumor progression by examining the genes associated with IQGAP3, which can be an essential basis for increasing the potential of IQGAP3 as a precision therapeutic target.

In conclusion, we demonstrated that IQGAP3 expression increases and co-expresses with CSC markers in PDAC. We also showed that IQGAP3 knockdown inhibited cell growth and spheroid formation. These results suggest that IQGAP3 can be a promising therapeutic target for PDAC.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Statement of Ethics

All procedures were in accordance with the Ethical Committee for Human Genome Research of Hiroshima University (E2001-9923), Kure Medical Center, and Chugoku Cancer Center (2019-91), as well as the Helsinki Declaration of 1964 and later versions. Written informed consent or a substitute for it was obtained from all patients included in the study in accordance with the guideline of Ethical Committee for Clinical Research of Hiroshima University. The consent document was approved by the Ethical Committee for Human Genome Research of Kure Medical Center and Chugoku Cancer Center (2019-91) and the Ethical Committee for Human Genome Research of Hiroshima University, Hiroshima, Japan (E2001-9923).

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Author's contributions

Aya Kido and Akira Ishikawa designed this study. Akira Ishikawa, Kazuya Kuraoka, Sho Tazuma, and Takeshi Sudo collected and analyzed the patient clinical data. Aya Kido and Akira Ishikawa performed the experiments and collected and analyzed the data. Akira Ishikawa, Takafumi Fukui, Narutaka Katsuya, Kazuhiro Sentani, Masahiro Serikawa, Siro Oka, Naohide Oue, and Wataru Yasui interpreted and analyzed the results. Aya Kido and Akira Ishikawa drafted and edited the manuscript. All authors read and approved the manuscript and agreed to be accountable for all aspects of the research to ensure that the accuracy or integrity of any part of the work is appropriately investigated and resolved.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request. Data is not publicly available due to ethical reasons. Further enquiries can be directed to the corresponding author.

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Figure Captions

Fig. 1. Expression of IQGAP3 in PDAC tissues.

(A) Low-magnification image of IQGAP3 expression in the pancreas: Original magnification: (A) 40×; scale bars, 100 μm. (B) Immunohistochemical analysis of IQGAP3 in the non-neoplastic pancreas: Original magnification: (B) 100×; scale bars, 100 μm. (C) Immunohistochemical analysis of IQGAP3 in PDAC including in situ lesion. Original magnification: (C) 100×; scale bars, 100 μm. (D, E) Immunohistochemical analysis of IQGAP3 in PDAC, including invasive lesion. Original magnification: (D) 100× and (E) 400×; scale bars, 100 μm. (F) Kaplan–Meier survival plots for patients with PDAC by positive and negative tumor IQGAP3 expression. (G) Kaplan–Meier plot of survival for patients with PDAC in the TCGA database.

IQGAP3, IQ motif containing GTPase-activating protein 3; PDAC, pancreatic ductal adenocarcinoma; TCGA, The Cancer Genome Atlas.

Fig. 2. Immunohistochemical analysis of IQGAP3 and other CSC markers, including KIFC1.

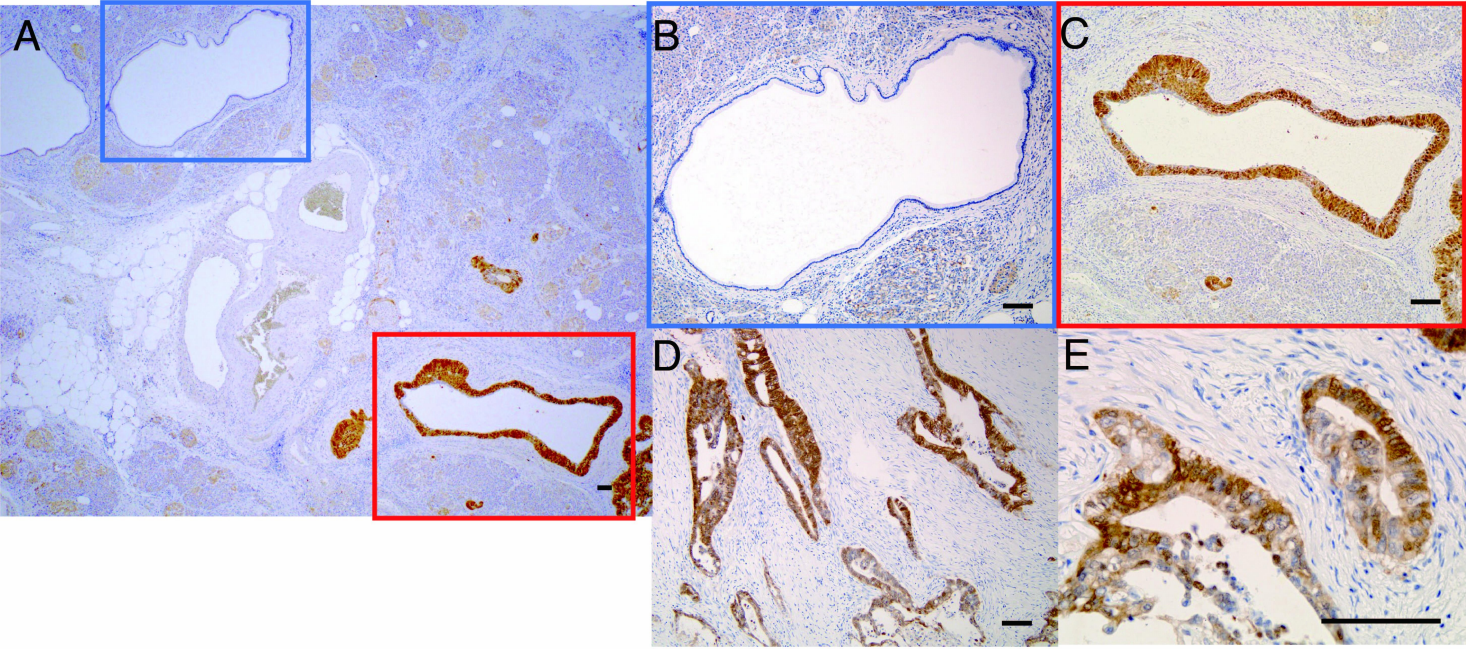
(A) IQGAP3, (B) KIFC1. Original magnification: 400×; scale bars, 100 μm. (C) Correlation between the expression of IQGAP3 and CSC markers, including KIFC1, CD133, and CD44 in TCGA PAAD dataset.

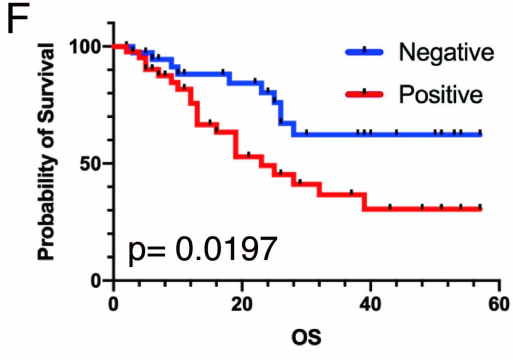
IQGAP3, IQ motif containing GTPase-activating protein 3; KIFC1, kinesin family member C1; CSC, cancer stem cell; TCGA, The Cancer Genome Atlas; PAAD, pancreatic cancer dataset.

Fig. 3. Effect of IQGAP3 knockdown in PDAC cells.

(A) Western blot analysis of IQGAP3 in four PDAC cell lines. (B) Western blot analysis of IQGAP3, AKT, phospho-AKT (pAKT), ERK 1/2 and phospho-ERK1/2 (pERK1/2) in T3M-4 cells transfected with the negative control or *IQGAP3* siRNA. (C) Effect of *IQGAP3* knockdown on cell growth in T3M-4 cells transfected with the negative control or *IQGAP3* siRNA. (D) Number of spheroids formed by PDAC cell lines transfected with negative control or *IQGAP3* siRNA; scale bars, 20 μm.

IQGAP3, IQ motif containing GTPase-activating protein 3; PDAC, pancreatic ductal adenocarcinoma; siRNA, small interfering RNA.


A
B
C
D
E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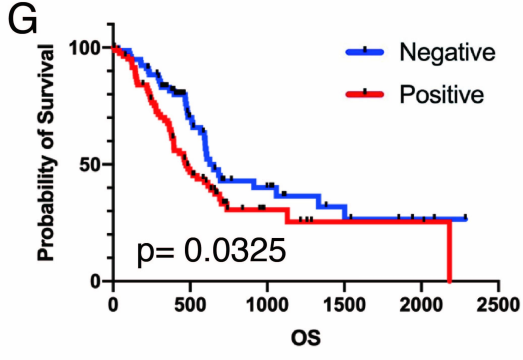
0
Negative
Positive
p= 0.0197
0
20
40
60
OS


G
Probability of Survival
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0
Negative
Positive
p= 0.0325
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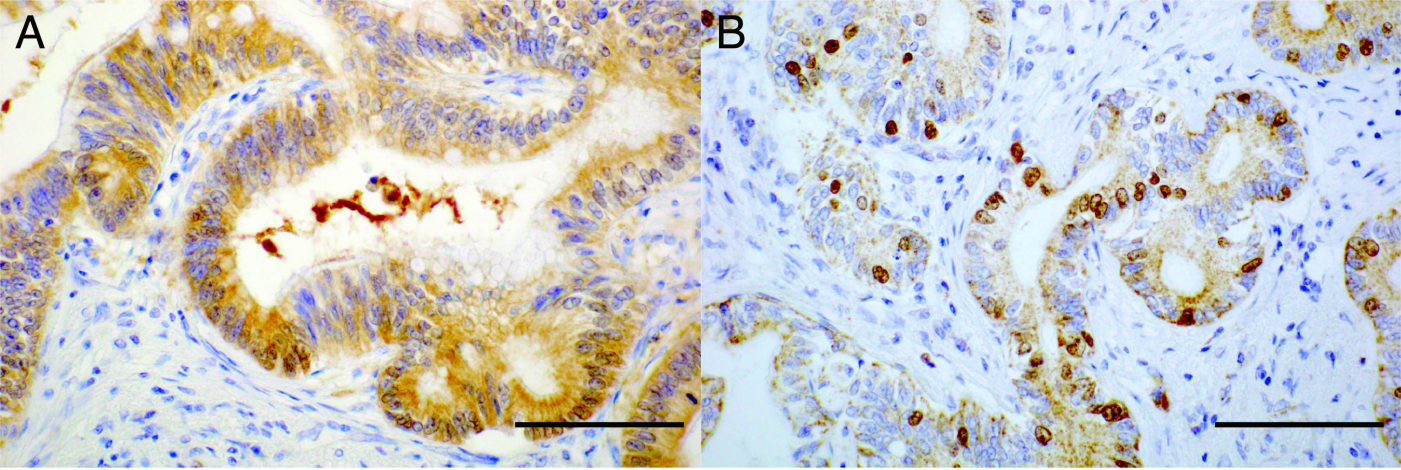
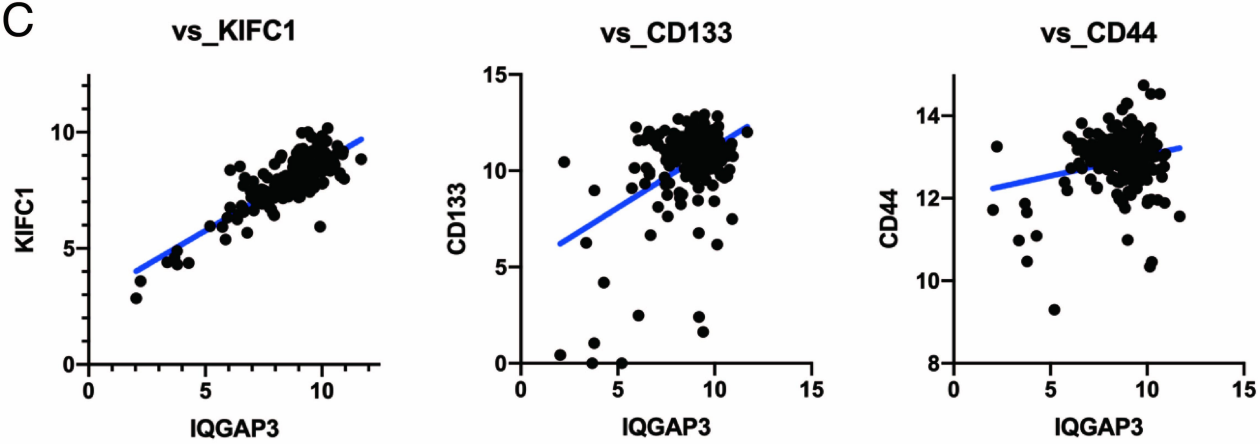
500
1000
1500
2000
2500
OS



C



$R^2 = 0.6756$
$P < 0.0001$
$Y = 0.5886*X + 2.817$

$R^2 = 0.1959$
$P < 0.0001$
$Y = 0.6303*X + 4.928$

$R^2 = 0.0436$
$P = 0.034$
$Y = 0.1015*X + 12.03$

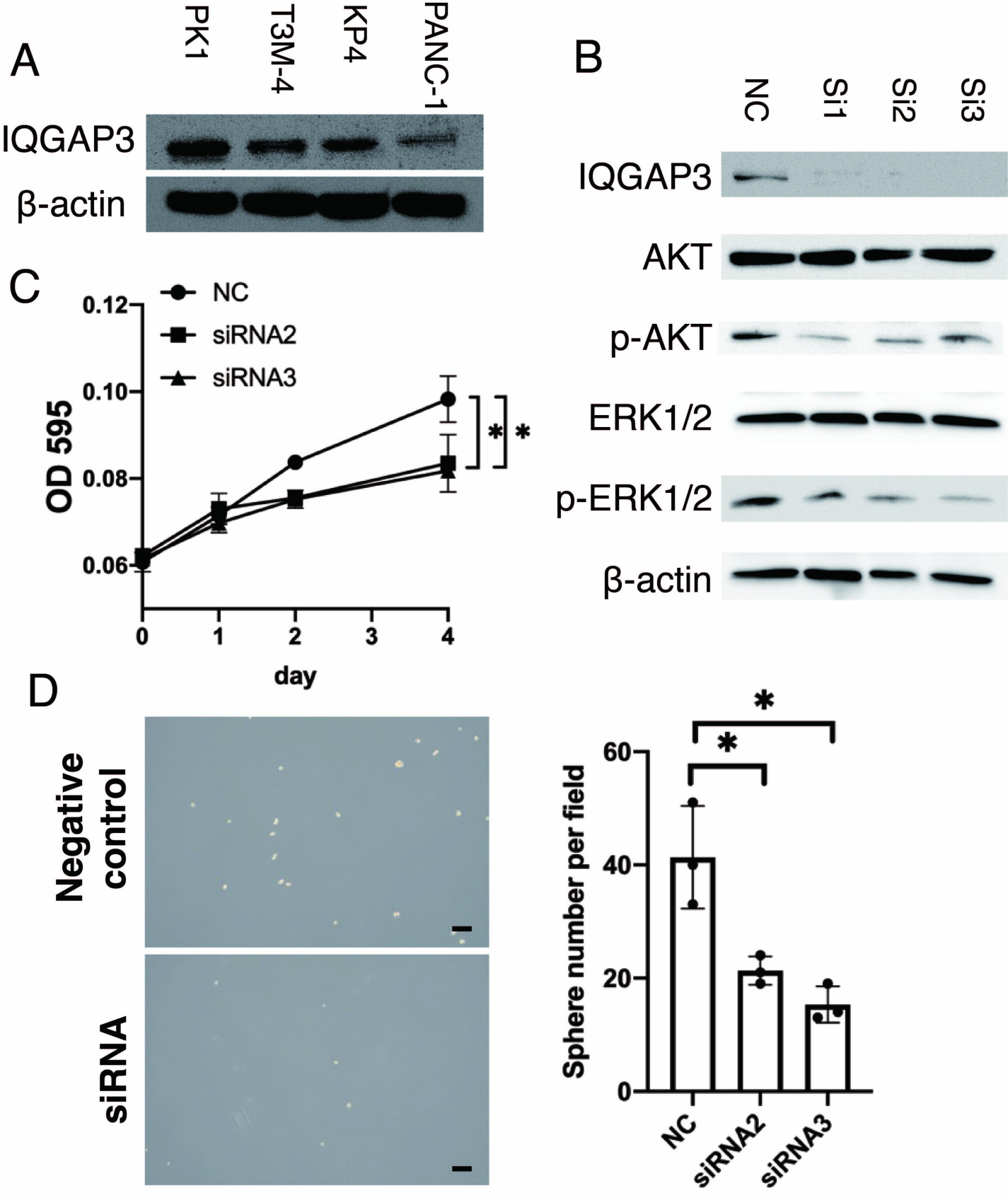

A
PK1
T3M-4
KP4
PANC-1
IQGAP3
β-actin
B
NC
Si1
Si2
Si3
IQGAP3
AKT
p-AKT
ERK1/2
p-ERK1/2
β-actin
C
NC
siRNA2
siRNA3
OD 595
0.12
0.10
0.08
0.06
0
1
2
3
4
day
*
*
D
Negative control
siRNA
Sphere number per field
60
40
20
0
*
*
NC
siRNA2
siRNA3

Table 1. The relationship between IQGAP3 expression and clinicopathological features in patients with pancreatic cancer

		IQGAP3 expression		
		Positive (%)	Negative	*p* Value
Age (years)				0.8242
	<74	22 (52)	20	
	≥74	22 (56)	17	
Sex				0.5082
	Male	25 (58)	18	
	Female	19 (50)	19	
Size (cm)				0.5049
	<2.8	21 (50)	21	
	≥2.8	23 (58)	16	
Location				0.5067
	Ph	24 (58)	17	
	Pb/Pt	20 (50)	20	
Grade (G)				0.5318
	G1/G2	36 (52)	33	
	G3/G4	8 (67)	4	
pN				0.6423
	pN0	14 (50)	14	
	pN1/2	30 (56)	23	
Venous invasion (V)				0.1748
	V0	15 (44)	19	
	V1	29 (61)	18	
Lymphatic invasion (Ly)				0.309
	Ly0	34 (57)	26	
	Ly1	10 (48)	11	
Perineural invasion (Pn)				0.8194
	Pn0	27 (53)	24	
	Pn1	17 (57)	13	

IQGAP3, IQ motif containing GTPase-activating protein 3; Ph, head of the pancreas; Pb, body of the pancreas; Pt, tail of the pancreas.

Table 2. Univariate and multivariate Cox regression analyses of IQGAP3 expression and survival of pancreatic cancer patients

Features		Univariate analysis		Multivariate analysis	
		HR (95%CI)	*p* Value	HR (95%CI)	*p* Value
Age			0.737		
	<74	1 (ref.)			
	≥74	1.340 (0.242-7.412)			
Sex			0.202		
	Female	1 (ref.)			
	Male	1.593 (0.779-3.259)			
Size			0.160		
	<2.8 cm	1 (ref.)			
	≥2.8 cm	1.672 (0.815-3.426)			
Location			0.646		
	Ph	1 (ref.)			
	Pb/Pt	1.180 (0.582-2.394)			
Grade (G)			0.0161		0.0198
	G1/G2	1 (ref.)		1 (ref.)	
	G3/G4	2.713 (1.204-6.113)		2.637 (1.166-5.963)	
pN			0.0340		0.0819
	pN0	1 (ref.)		1 (ref.)	
	pN1/2	2.635 (1.076-6.453)		2.236 (0.903-5.536)	
Venous invasion (V)			0.1495		
	V0	1 (ref.)			
	V1	1.716 (0.823-3.576)			
Lymphatic invasion (Ly)			0.4816		
	Ly0	1 (ref.)			
	Ly1	1.322 (0.607-2.873)			
IQGAP3 expression			0.0164		0.0484
	Negative	1 (ref.)		1 (ref.)	
	Positive	2.591 (1.191-5.64)		2.213 (1.005-4.873)	

IQGAP3, IQ motif containing GTPase-activating protein 3; HR, hazard ratio; Ph, head of the pancreas; Pb, body of the pancreas; Pt, tail of the pancreas.

Table 3. The relationship between IQGAP3 expression and clinicopathological features in patients with pancreatic cancer based on GDC TCGA Pancreatic Ductal Adenocarcinoma (PDAC) samples

		IQGAP3 expression		
		Positive (%)	Negative	*p* Value
Sex				0.2756
	Male	50 (55)	41	
	Female	34 (46)	40	
Grade (G)				0.0126
	G1/G2	50 (44)	63	
	G3/G4	34 (65)	18	
pT				0.0976
	pT1/2	10 (36)	18	
	pT3/4	74 (54)	63	
pN				0.6065
	pN0	25 (54)	21	
	pN1/2	59 (50)	60	
pM				0.7558
	pM0	37 (49)	38	
	pM1	47 (52)	43	

IQGAP3, IQ motif containing GTPase-activating protein 3; GDC, Genomic Data Commons; TCGA, The Cancer Genome Atlas.

Table 4. Univariate and multivariate Cox regression analyses of IQGAP3 expression and survival of pancreatic cancer patients based on GDC TCGA Pancreatic Ductal Adenocarcinoma (PDAC) samples

Features		Univariate analysis		Multivariate analysis	
		HR (95%CI)	*p* Value	HR (95%CI)	*p* Value
Sex			0.2653		
	Female	1 (ref.)			
	Male	1.269 (0.835-1.928)			
Grade (G)			0.0561		
	G1/G2	1 (ref.)			
	G3/G4	1.523 (0.989-2.345)			
pT			0.0471		0.3884
	pT1/2	1 (ref.)		1 (ref.)	
	pT3/4	1.957 (1.009-3.799)		1.358 (0.678-2.722)	
pN			0.0074		0.0136
	pN0	1 (ref.)		1 (ref.)	
	pN1/2	2.003 (1.209-3.418)		1.970 (1.150-3.375)	
pM			0.5077		
	pM0	1 (ref.)			
	pM1	1.153 (0.756-1.759)			
IQGAP3 expression			0.0341		0.0409
	Negative	1 (ref.)		1 (ref.)	
	Positive	1.584 (1.035-2.424)		1.576 (1.019-2.438)	

IQGAP3, IQ motif containing GTPase-activating protein 3; GDC, Genomic Data Commons; TCGA, The Cancer Genome Atlas; HR, hazard ratio.

Table 5. The relationship between IQGAP3 expression and several molecules including cancer stem cell or cell proliferation markers in patients with pancreatic cancer

		IQGAP3 expression		
		Positive (%)	Negative	*p* Value
CD44				0.5025
	Positive	22 (59)	15	
	Negative	22 (50)	22	
CD133				0.2776
	Positive	7 (41)	10	
	Negative	37 (58)	27	
KIFC1				0.0434
	Positive	25 (68)	12	
	Negative	19 (43)	25	
Ki67				0.4964
	Positive	28(58)	20	
	Negative	16(48)	17	

IQGAP3, IQ motif containing GTPase-activating protein 3; KIFC1, kinesin family member C1.