European Review for Medical and Pharmacological Sciences 2019; 23: 10729-10739

MicroRNA-383 suppresses pancreatic carcinoma development via inhibition of GAB1 expression

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Abstract. – OBJECTIVE: Pancreatic carcinoma (PC) is a serious malignancy associated with high morbidity and mortality rates. Previous studies have identified various microRNAs (miRNAs) involved in the development of PC; however, the role of miR-383 still remains unclear. This study investigates the role of miR-383 in the malignant transformation of PC.

MATERIALS AND METHODS: Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was used to quantify miR-383 and Grb2 associated binding protein 1 (GAB1) RNA levels, and Western blot analysis was performed to measure protein expression. The ability of miR-383 to bind and regulate the expression of GAB1 was assessed using a Luciferase reporter assay. Cell Counting Kit-8 (CCK-8) experiments and flow cytometry analysis were used to assess cell proliferation and apoptosis, respectively.

RESULTS: Down-regulation of miR-383 was associated with adverse clinical results and poor prognosis in PC patients. Mechanistically, miR-383 inhibited cell proliferation and promoted apoptosis of PANC-1 (human pancreatic cancer cell) cells. Our results show that miR-383 can act directly on GAB1 to inhibit its expression in PC. This downregulation of GAB1 limits cell proliferation and induced apoptosis of PANC-1 cells.

CONCLUSIONS: MiR-383 suppresses tumor development and progression through the downregulation of GAB1 expression.

Key Words:
Pancreatic carcinoma, MiR-383, Proliferation, Apoptosis, GAB1.

Introduction

Pancreatic carcinoma (PC) is an incurable malignant tumor that is difficult to diagnose. In recent years, the incidence of PC has been rising[1]. PC generally develops rapidly and is prone to metastasis[2]. It has a 5-year survival rate of <1%, making it one of the most deadly malignant tumors[3]. If left untreated, PC patients can only survive about 4 months, whereas patients who undergo surgery usually survive for approximately 16 months[4]. Therefore, early diagnosis and early treatment are critical for improving the survival rate of PC patients. MicroRNAs (miRNAs) are able to affect carcinogenesis by inducing mRNA instability[5]. In PC, a group of miRNAs has been found to play many roles in tumor development. For example, miR-431 inhibits cell proliferation and induces apoptosis by targeting CDK14 in PC[6]. Similarly, miR-216b has been shown to inhibit tumor progression and promote apoptosis in PC by modulating KRAS (Kirsten ras)[7]. In contrast, miR-224 was found to promote proliferation and migration of PC cells by regulating TXNIP[8]. Another miRNA, miR-132, is able to promote the development of PC by mediating the Hedgehog signaling pathway[9]. Previous studies have shown that miR-383 plays a role in several cancers; however, its role in PC remains unclear. MiR-383 has been reported to be downregulated in medulloblastoma[10], and was found to limit cell proliferation and serve as a prognostic marker in lung adenocarcinoma[11]. Additionally, downregulation of miR-383 promoted cell invasion in glioma by targeting IGF1R[12]. However, downregulation of miR-383 showed an inhibitory effect through the regulation of CASP2 (Caspase 2) in epithelial ovarian cancer[13]. These findings demonstrate that miR-383 can have different functions in different tissues. In addition, miR-383 has been reported to be significantly reduced in PC tissues and cell lines[14]. Yet, the role of miR-383 in PC remains unclear and warrants further investigation. The Grb2-associated binder (GAB) scaffolding/adapter protein family includes three members, Grb2 associated binding protein 1 (GAB1), GAB2, and GAB3[15], which

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are involved in signal transduction of cytokines and growth factor receptors[16]. GAB1 is the most well-known protein in the GAB family, as it is associated with certain diseases and cancers. GAB1 was found to be involved in postnatal angiogenesis after ischemia[17] and the dysregulation of GAB1 has been reported to be a predictor of clinical outcomes in glioma patients[18]. Functionally, downregulation of GAB1 inhibited proliferation and migration of hilar cholangiocarcinoma cells[19]. Recent studies have shown that GAB1 may interact genetically and functionally with miRNAs to exert these effects on cancer cells. For example, miR-409-3p has been shown to suppress colorectal cancer metastasis by targeting GAB1[20]. In addition, downregulation of miR-29a-3p was found to promote glioma cell proliferation by up-regulating GAB1[21]. However, an interaction between miR-383 and GAB1 has not been reported. Therefore, understanding the mechanism by which a potential miR-383/GAB1 pathway regulates PC development can provide valuable insights into the role of miR-383 in regulating PC cell proliferation and apoptosis.

Materials and Methods

Collection of Clinical Specimens

The cancerous specimens and matched adjacent normal specimens used in this study were obtained from PC patients at Chengwu County People's Hospital from January 2015 to December 2018. Patients with PC did not receive other treatments, except for surgery. Informed consent was obtained from each PC patient. Approval to conduct this study was granted by the Institutional Ethics Committee at Chengwu County People's Hospital.

Cell Transfection and Culture

The normal pancreatic cell line hTERT-HPNE and the PC cell line PANC-1 (American Type Culture Collection, ATCC, Manassas, VA) were seeded in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA) and incubated at 37°C with 5% CO_2. A miR-383-encoding vector (cat. no., miR1003114-1-2), a pcDNA3.1-GAB1 plasmid (GenePharma, Shanghai, China), a miR-383 mimic (5'-CGA GCT CTG TGT GTG TGT GTG TGT G-3'), an inhibitor (5'-TTC CGC GGC CGC TAT GGC CGA CGT CGA CGG GAA TGG GGA AAG GGA A-3'), and GAB1 siRNA (5'-GGC GCC TCC CTT CCC CCT CCC CT-3') were used in this study. These reagents were transferred into PANC-1 cells for 30 min at 37°C, respectively. Transfections were performed using Lipofectamine 3000 (Thermo Fisher Scientific, Waltham, MA, USA). Untreated PANC-1 cells were used as controls.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Transfected cells were harvested after 48 h incubation. Total RNA was extracted from tissues or cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and cDNA was synthesized using the Rayscript cDNA Synthesis Kit (Generay, Shanghai, China). The reverse transcription reaction was heated at 37°C for 15 min, followed by a 5 s incubation at 85°C. QRT-PCR was performed using SYBR Green qPCR Master Mix (MedChemExpress, MCE, NJ, USA) according to the manufacturer's instructions. The following thermocycling conditions were used for amplification: 10 min at 95°C, followed by 40 cycles of 15 s at 95°C, then 30 s at 60°C. MiR-383 expression was normalized to U6 small nuclear RNA expression, while GAB1 was normalized to glyceraldehyde phosphate dehydrogenase (GAPDH). Relative expression was calculated using the $2^{-\Delta\Delta Cq}$ method. The primers used are listed in Table I.

Western Blot Analysis

Transfected cells were lysed in radioimmunoprecipitation assay buffer (RIPA; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Equal amounts of protein from each sample were run on a 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and then transferred to a polyvinylidene difluoride (PVDF) membrane (Beyotime Institute of Biotechnology, Haimen, China). Membranes were blocked in 5% skim milk for 2 h at room temperature, followed by overnight incubation in primary antibody at 4°C. The following primary antibodies were used: anti-GAB1 (1:1000; Abcam, Cambridge, MA, USA), anti-Bax (1:800 Abcam, Cambridge, MA, USA), anti-Bcl-2 (1:800 Abcam, Cambridge, MA, USA), and anti-GAPDH (1:500; Abcam, Cambridge, MA, USA). After washing, the membranes were incubated with goat anti-rabbit secondary antibody (HRP, 1:1000; Abcam, Cambridge, MA, USA) for 1 h at room temperature. Proteins were detected using an enhanced chemiluminescence (ECL)

Table I. Primer sequences for qRT-PCR.

Primer		Sequence
miR-383	forward	5'-GGG AGA TCA GAA GGT GAT TGT GGC T-3'
	reverse	5'-CAG TGC GTG TCG TGG AGT-3'
U6	forward	5'-CTC GCT TCG GCA GCA CA-3'
	reverse	5'-AAC GCT TCA CGA ATT TGC GT-3'
GAB1	forward	5'-TGG CAG CTC TTT ACA AGC ACC-3'
reverse		5'-TCA TGA GCA ACA GGT AGT CTT GA-3'
GAPDH	forward	5' AAGGTCGGAGTCACCGGATT 3'
	reverse	5' GCCATCACGCCACAGTTTC 3'

U6: small nuclear RNA, snRNA; GAPDH: glyceraldehyde-3-phosphate dehydrogenase

protein detection kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Luciferase Reporter Assay

A wild-type or mutant version of the GAB1 3'-UTR was inserted into the PmirGLO luciferase vector (RiboBio, Guangzhou, China). The luciferase vector and miR-383 mimics were then transfected into PANC-1 cells for 48 h. Luciferase activity was assessed using a Dual-Luciferase reporter assay kit (Promega, Madison, WI, USA). The sequences of the wild-type and mutant GAB1 3'UTR are as follows: wild-type GAB1: 5'-UGA UGU GCU AAU ACA CUG AUC AG-3'; mutant GAB1: 5'-UGA UGU GCU AAU ACA GAC UAG A-3'.

Cell Counting Kit-8 Assay (CCK-8)

The prepared PANC-1 cells were cultured in 96-well plates at a density of 2×10^4 cells/well for 24 h at 37 °C, 5% CO_2. After 24, 48, 72, or 96 h of incubation, 20 µL of CCK-8 solution [5 mg/ml in phosphate-buffered saline (PBS); Dojindo Laboratories, Kumamoto, Japan] was added to each well and the cells were incubated for an additional 4 h. After this incubation, the sample absorbance at 450 nm was measured using a spectrophotometer (Molecular Devices, Shanghai, China).

Flow Cytometric Analysis

Flow cytometry was used to detect apoptosis in PC cells. Transfected PANC-1 cells were maintained in 6-well plates at a concentration of 2×10^3 cell/well. After 48 h, PANC-1 cells were harvested using EDTA (Ethylene Diamine Tetraacetic Acid)-free trypsin (Sigma-Aldrich, St. Louis, MO, USA). Then, the collected PANC-1 cells were washed in cold PBS (Invitrogen, Carlsbad, CA, USA) and the PBS was discarded after pelleting the cells. The PANC-1 cells were stained with Annexin V-FITC (Annexin V-FITC Apoptosis Detection Kit; Biovision, K101, San Diego, CA, USA) for 15 mins, followed by the addition of Binding Buffer to a final concentration of 1x. Propidium Iodide (PI; Sigma-Aldrich, St. Louis, MO, USA) was then added to the mixture. Apoptosis was analyzed using a flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA), and PI and Annexin V-FITC signals were graphed according to the manufacturer's instructions.

Statistical Analysis

GraphPad Prism 6 (Version X; La Jolla, CA, USA) and Statistical Product and Service Solutions (SPSS) 20.0 (SPSS Inc., Chicago, IL, USA) were used for data analysis. The variance between the two groups was evaluated by Student's *t*-test, while one-way ANOVA (Analysis of Variance) was used to measure variation between three or more groups. Tukey's test was used for post-hoc analysis following ANOVA. Comparison of categorical variables was assessed by χ^2-test or Fisher's exact test. Survival differences were analyzed by Kaplan-Meier analysis using a log-rank test. A p-value < 0.05 was defined as a significant difference.

Results

Expression of MiR-383 is Decreased in PC

To assess the expression level of miR-383 in PC, we measured its expression in PC specimens and cell lines. As shown in Figure 1A, miR-383 expression was lower in PC samples compared to adjacent normal samples ($p < 0.01$). Furthermore, the expression of miR-383 was also downregulated in PANC-1 cells compared to hTERT-HPNE cells ($p < 0.01$, Figure 1B). Based on the expres-

sion of miR-383, PC patients were divided into two groups, a high miR-383 expression group and a low expression group, with the cut-off being 0.72. As shown in Figure 1C, PC patients with low levels of miR-383 had shorter overall survival ($p < 0.001$), suggesting that miR-383 could play an inhibitory role in PC progression. In addition, altered expression of miR-383 in PC patients was associated with the invasion of cancerous cells into adjacent organs, classification in the advanced pTNM category, and poor cell differentiation ($p < 0.05$, Table II). Overall, our results indicate that decreased miR-383 levels are associated with the progression of PC.

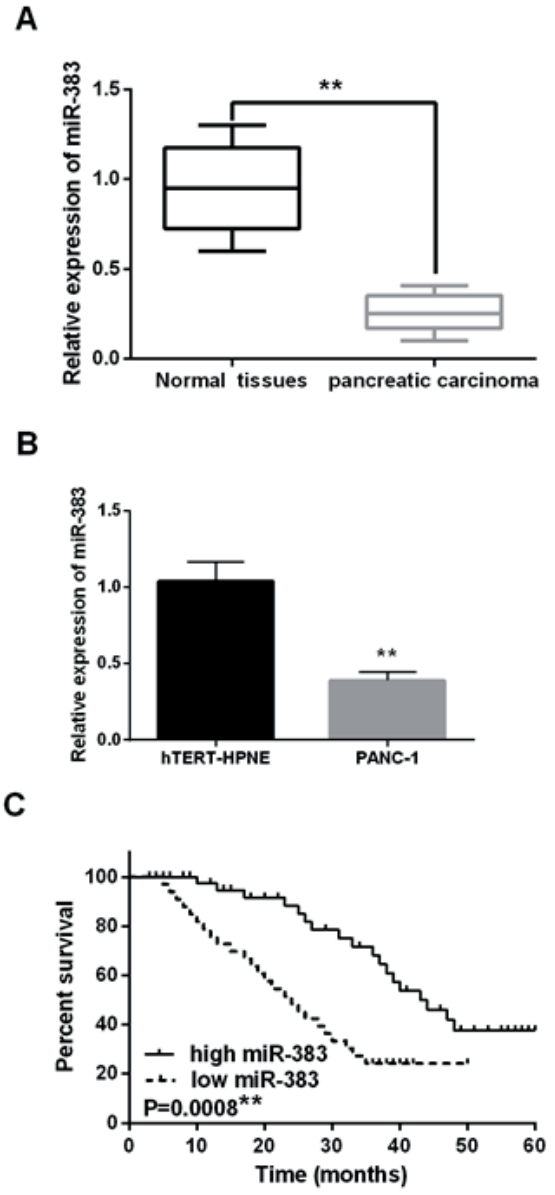

Figure 1. MiR-383 expression is downregulated in PC. **A**, MiR-383 expression in PC tissues and adjacent normal tissues. **B**, MRNA levels of miR-383 in PANC-1 and hTERT-HPNE cells. **C**, Low miR-383 expression was associated with poor prognosis in PC patients. $^{**}p < 0.01$.

MiR-383 Exhibits Tumor-Suppressive Effects on PC Development

To investigate the biological function of miR-383 in PC tumorigenesis, miR-383 mimics and inhibitors were employed to perform both gain-of-function and loss-of-function experiments. As shown in Figure 2A, the expression level of miR-383 in PANC-1 cells increased in the presence of its mimics and was reduced by the addition of its inhibitor ($p < 0.01$). Overexpression of miR-383 dramatically inhibited cell proliferation compared to control cells ($p < 0.01$, Figure 2B). Conversely, PANC-1 cells exhibited enhanced proliferative capacity when miR-383 levels were reduced by its inhibitor ($p < 0.05$, Figure 2B). Unexpectedly, miR-383 mimics were also found to induce PANC-1 cell death ($p < 0.01$), which could be blocked by the addition of the miR-383 inhibitor ($p < 0.05$, Figure 2C). Moreover, the expression of Bax and Bcl-2 in PANC-1 cells was analyzed at both high and low levels of miR-383. As shown in Figure 2D, miR-383 mimics lowered the level of the survival gene Bcl-2 and promoted expression of the apoptosis gene Bax ($p < 0.05$ and 0.01, respectively). Conversely, the miR-383 inhibitor elicited the opposite effect on the expression of Bax and Bcl-2 (Figure 2D). To further confirm the tumor-suppressive effect of miR-383 *in vivo*, PANC-1 cells expressing the miR-383 mimic or PANC-1 cells without the mimic were subcutaneously injected into nude mice, and tumor growth was measured. As shown in Figure 2E, upregulation of miR-383 significantly suppressed tumor growth compared to the control. Together, our data suggest that miR-383 inhibits the development of PC by inhibiting cell proliferation and promoting cell death, which would be why PC patients with decreased levels of miR-383 have a poor prognosis.

MiR-383 Suppresses GAB1 Expression in PC

To further investigate the mechanism by which miR-383 regulates PC development, the TargetScan database was used to predict direct targets of miR-383. As shown in Figure 3A, a miRNA-383 binding site was found in the 3'-UTR of GAB1. Additionally, miR-383 mimics were found to selectively inhibit luciferase activity of wild-type GAB1 in PANC-1 cells, but not GAB1 with a

Table II. Relationship between miR-383 expression and their clinic-pathological characteristics of pancreatic carcinoma patients.

Characteristics	Cases	miR-383		p-value
		High	Low	
Age (years)				0.72
≥ 60	31	6	25	
<60	40	12	28	
Gender				0.03
Male	43	10	33	
Female	28	8	20	
Tumor diameter (cm)				0.13
≤4	32	10	22	
>4	39	8	31	
Adjacent organs invasion				0.01*
Absent	51	16	35	
Present	20	2	18	
pTNM category				0.03*
I-II	48	12	36	
III	23	6	17	
Differentiation				0.002*
Well/Moderately	52	15	37	
Poor	19	3	16	
Vessel invasion				0.72
No	38	11	27	
Yes	33	7	26	

Statistical analyses were performed by the χ^2-test. *$p < 0.05$ was considered significant.

mutant 3'UTR ($p < 0.01$, Figure 3B). GAB1 expression was analyzed in PANC-1 cells transfected with either miR-383 mimics or its inhibitor. Our results showed that miR-383 mimics reduced GAB1 expression, while the miR-383 inhibitor increased GAB1 expression ($p < 0.01$, Figure 3C). Furthermore, GAB1 was found to be upregulated in PC tissues compared to adjacent normal samples ($p < 0.01$, Figure 3D) and miR-383 levels appeared to be negatively correlated with GAB1 expression in PC tissues ($p < 0.01$, R^2=0.6824, Figure 3E). These findings suggest that miR-383 normally functions to limit GAB1 expression. When miR-383 is downregulated in PC, the expression of GAB1 could increase and exacerbate malignant transformation.

GAB1 Stimulates Tumor Growth in PC

To explore the role of GAB1 in PC development, we transfected PANC-1 cells with GAB1 siRNA and observed its effects on cell proliferation and death. As shown in Figure 3F, expression of GAB1 in PANC-1 cells was significantly reduced by siRNA ($p < 0.01$). Results from the CCK-8 assay showed that knockdown of GAB1 had a significant inhibitory effect on PANC-1 cell proliferation ($p < 0.01$, Figure 3G). Consistent with the activating effects of miR-383 on cell death, GAB1 silencing increased the level of cell death observed compared to the control cells ($p < 0.01$, Figure 3H). Furthermore, knockdown of GAB1 suppressed Bax expression and promoted Bcl-2 expression in PANC-1 cells ($p < 0.01$, Figure 3I). Based on these results, we suggest that GAB1 triggers carcinogenic activity in PC.

MiR-383 Represses GAB1 Expression to Promote Tumor Suppression

To determine whether miR-383 functions to inhibit PC by limiting GAB1 expression, we co-transfected miR-383 mimics and a GAB1 vector into PANC-1 cells. As shown in Figure 4A, transfection of the GAB1 vector attenuated miR-383-mediated suppression of GAB1. Moreover, overexpression of GAB1 also impaired the inhibitory effect of miR-383 on PANC-1 cell proliferation (Figure 4B). Similarly, the increase in apoptosis induced by miR-383 was abolished by upregulation of GAB1 (Figure 4C). In addition, ectopic expression of GAB1 counteracted the changes in expression of Bcl-2 and Bax in PANC-1 cells (Figure 4D). In conclusion, our results demonstrate that miR-383 inhibits the development of PC in a GAB1-dependent manner.

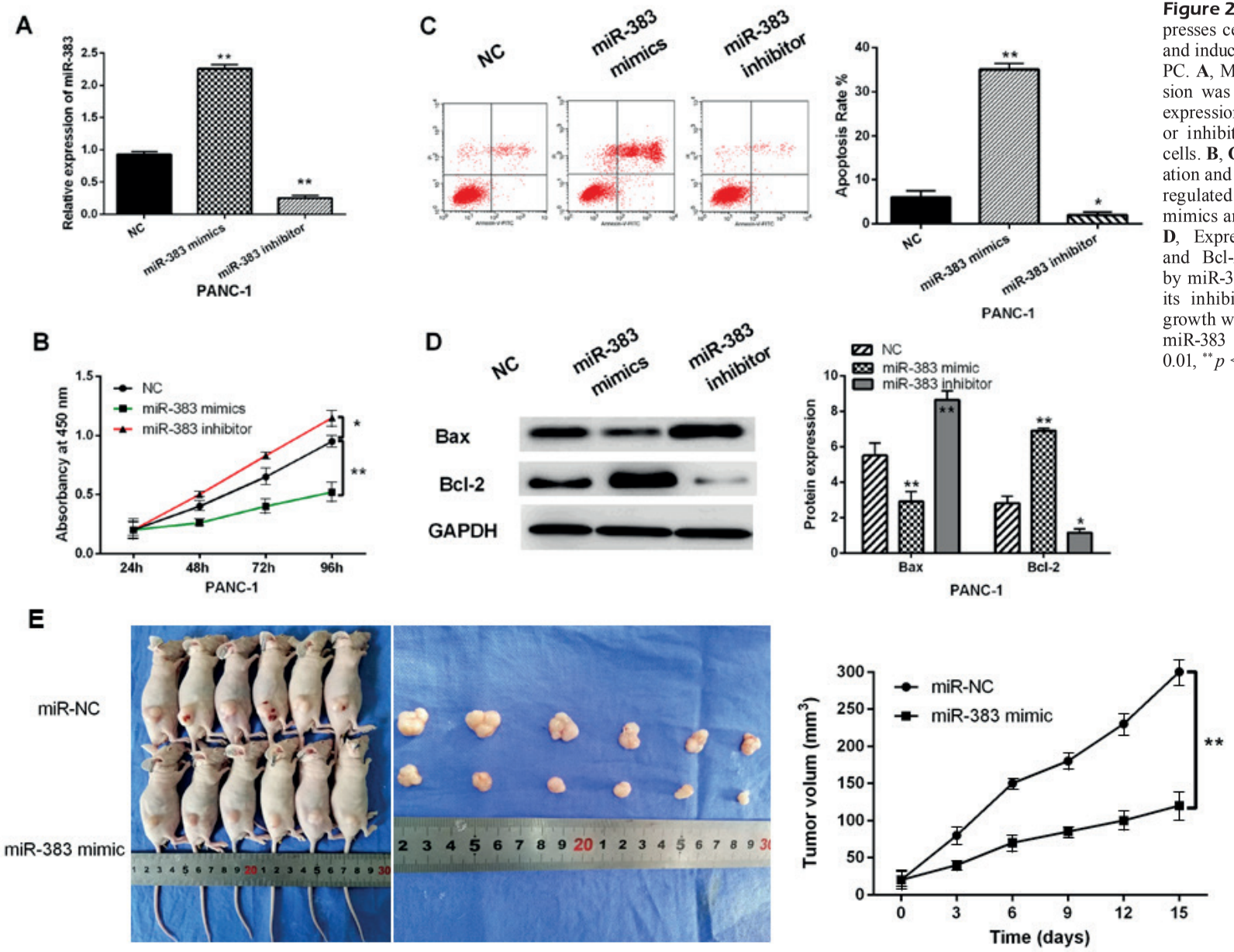

Figure 2. MiR-383 suppresses cell proliferation and induced apoptosis in PC. **A**, MiR-383 expression was altered by the expression of its mimics or inhibitor in PANC-1 cells. **B**, **C**, Cell proliferation and apoptosis were regulated by miR-383 mimics and its inhibitor. **D**, Expression of Bax and Bcl-2 was altered by miR-383 mimics and its inhibitor. **E**, Tumor growth was regulated by miR-383 in mice. $^{*}p < 0.01$, $^{**}p < 0.01$.

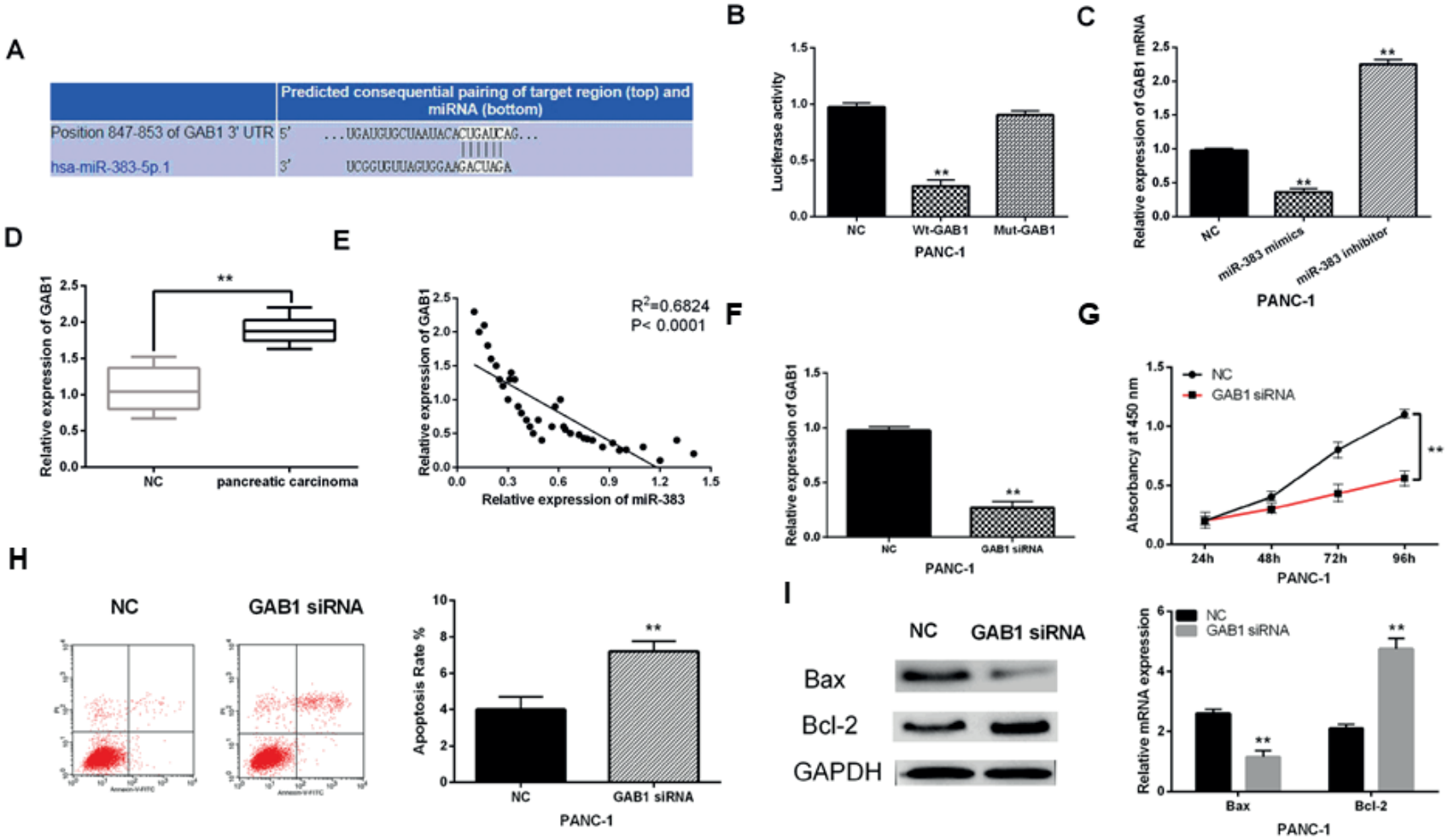

Figure 3. MiR-383 restricts GAB1 expression, which affects cell proliferation and apoptosis in PC. **A**, MiR-383 binding sites in GAB1. **B**, Luciferase reporter assay (**C**) to monitor GAB1 expression in the presence of miR-383 mimics or inhibitor. **D**, MRNA levels of GAB1 in PC tissues and adjacent normal tissues. **E**, A negative correlation was observed between miR-383 and GAB1 expression in PC tissues. **F**, GAB1 expression in PANC-1 cells transfected with GAB1 siRNA. **G**, **H**, Cell proliferation and apoptosis were affected by GAB1 siRNA. **I**, Expression of Bax and Bcl-2 was altered in GAB1 siRNA-expressing cells. $^{**}p < 0.01$.

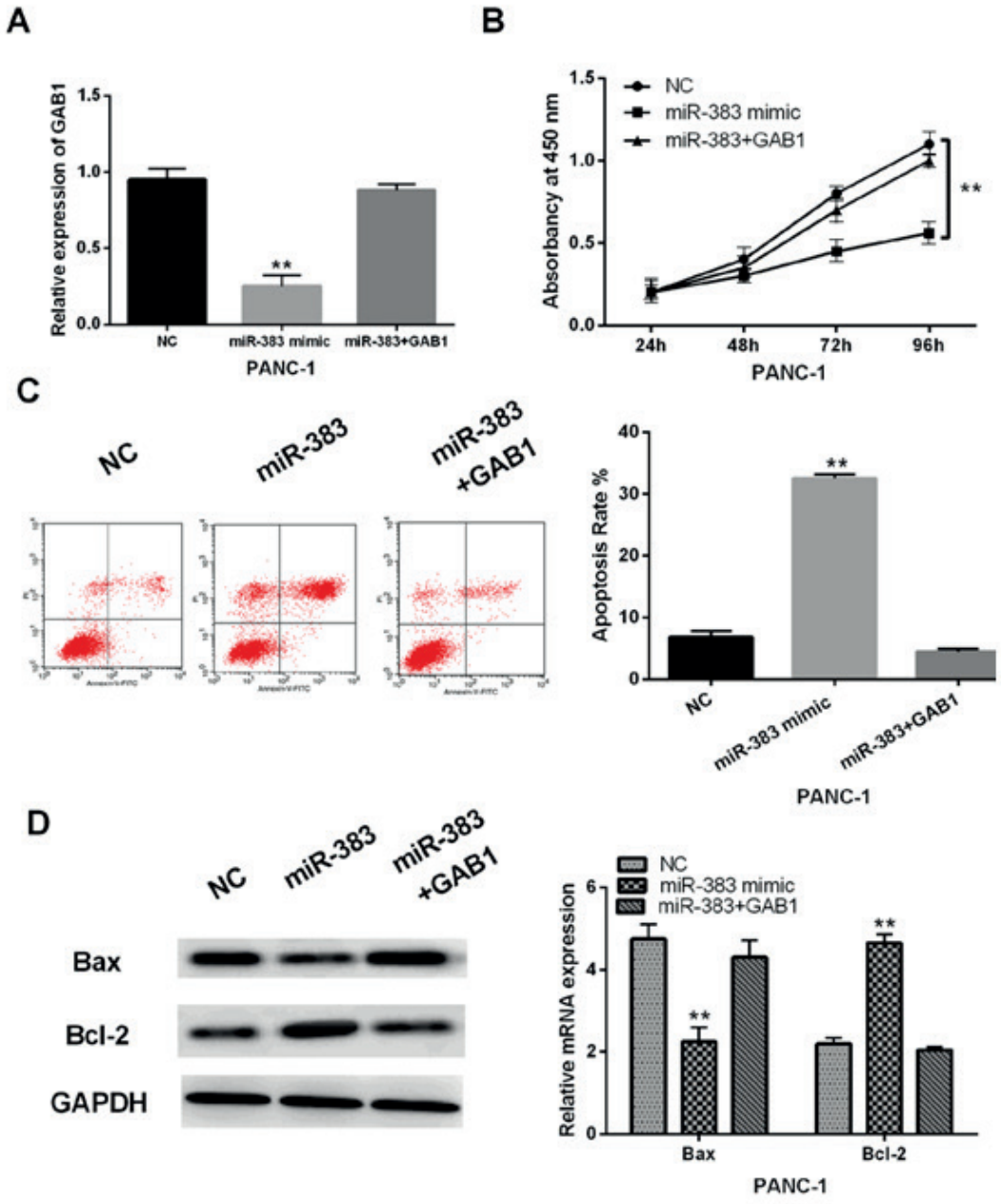

Figure 4. The interaction between GAB1 and miR-383 was identified in PC. **A**, GAB1 expression in PANC-1 cells transfected with the GAB1 vector and miR-383 mimics. **B**, **C**, Cell proliferation and apoptosis were altered in the presence of the GAB1 vector and miR-383 mimics. **D**, Expression of Bax and Bcl-2 in PANC-1 cells expressing the GAB1 vector and miR-383 mimics. ** $p < 0.01$.

Discussion

Although there have been advances in the treatment of PC in the past few years, PC patients still continue to experience a low survival rate. Therefore, there is an urgent need to discover new molecular markers to monitor the development of PC. The expression of miRNAs is stable and can be closely associated with clinical outcomes, providing significant advantages over other biomarkers. In this study, we found that miR-383 is downregulated in PC tissues and cell lines. Furthermore, low miR-383 expression is associated with more

severe clinical symptoms and poor prognosis in PC patients. MiR-383 is able to limit cell proliferation and promote apoptosis when overexpressed in PC cells, suggesting why it is so detrimental when its levels are reduced in PC patients. Finally, miR-383 regulates the development of PC by directly targeting GAB1 to reduce its expression.

Consistent with our results, downregulation of miR-383 was also observed in focal cerebral ischemia and lung cancer[22]. In addition, low miR-383 expression has been reported to be associated with poor clinical outcomes and prognosis in patients with non-small-cell lung carcinoma (NSCLC)[23]. In this study, we show that the dysregulation of miR-383 in PC patients is associated with increased cancer metastasis, more advanced pTNM classification, and decreased cell differentiation. Furthermore, low miR-383 expression predicted poor prognosis in PC patients. Gu et al[24] reported that miR-383 repressed cell survival and promoted apoptosis in NSCLC. This is in agreement with the results presented in this study, where we also demonstrate that miR-383 limits cell proliferation and increases cell death in a PC cell line. Additionally, previous studies[25,26] have shown that miR-383 can alter tumor pathogenesis by regulating downstream target genes, such as PARP2 and LDHA. In our research, we explored the downstream targets of miR-383 and found that miR-383 can directly regulate GAB1 to suppress its expression. When miR-383 levels are low in PC patients, this miR-383-mediated repression of GAB1 is affected.

Researchers[16,27] have reported a function for GAB1 in regulating cell growth and differentiation, including work on the role of GAB1 in regulating mitogenicity and morphogenesis in multipotent myeloid cells. Moreover, GAB1 has been found to be involved in tumorigenesis of human cancers, as a previous work showed that GAB1 was upregulated in melanoma[28] and colorectal cancer[29], and that downregulation of GAB1 inhibited cell migration and proliferation[19] in hilar cholangiocarcinoma. In agreement with this, our work here shows that GAB1 is also upregulated in PC. Furthermore, knockdown of GAB1 suppresses cell proliferation and induces apoptosis in PC, which is consistent with the researches in other tumors. Additionally, GAB1 has been found to act as a target of many miRNAs in human cancers, including miR-150 and miR-409[20,30]. Here, we found that GAB1 is a direct target of miR-383 and that increased levels of GAB1 abrogate the inhibitory effect of miR-383 in PC. Taken together, our results suggest that miR-383 inhibits cell proliferation and induces apoptosis in PC by reducing GAB1 expression.

Conclusions

The above data demonstrate that downregulation of miR-383 in PC is associated with poor clinical outcomes and prognosis. Furthermore, upregulation of miR-383 restricts cell proliferation and limits tumor growth by targeting GAB1. MiR-383 has potential as a possible therapeutic strategy for the treatment of PC.

Availability of Data and Materials

The datasets used and/or analyzed for the current study are available from the corresponding author upon reasonable request.

Authors' Contributions

Qingliang Su and Jijun Zhou conceived and designed the experiments. Qingliang Su, Hongjian Zhao, and Chengfang Song performed the experiments, assembled the data, performed the statistical analysis, and wrote the manuscript. Chengfang Song, Shuo Zhao, Zhishuai Tian obtained the tumor samples and tissues with clinical information where it pertained. Jijun Zhou revised the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

PC samples were collected from the Chengwu County People's Hospital (Heze, China) with written informed consent and permission from the Institutional Review Board. All patients provided written informed consent. The study protocol was approved by the Ethics Committee of the Chengwu County People's Hospital [Approval no. 20160511-2].

Conflict of Interests

The Authors declare that they have no conflict of interests.

References

1) Omura N, Goggins M. Epigenetics and epigenetic alterations in pancreatic cancer. Int J Clin Exp Pathol 2009; 2: 310-326.
2) Duffy JP, Eibl G, Reber HA, Hines OJ. Influence of hypoxia and neoangiogenesis on the growth of pancreatic cancer. Mol Cancer 2003; 2: 12.

3) Winter JM, Brennan MF, Tang LH, D'Angelica MI, Dematteo RP, Fong Y, Klimstra DS, Jarnagin WR, Allen PJ. Survival after resection of pancreatic adenocarcinoma: results from a single institution over three decades. Ann Surg Oncol 2012; 19: 169-175.

4) Fiorino S, Bacchi-Reggiani ML, Birtolo C, Acquaviva G, Visani M, Fornelli A, Masetti M, Tura A, Sbrignadello S, Grizzi F, Patrinicola F, Zanello M, Mastrangelo L, Lombardi R, Benini C, Di Tommaso L, Bondi A, Monetti F, Siopis E, Orlandi PE, Imbriani M, Fabbri C, Giovanelli S, Domanico A, Accogli E, Di Saverio S, Grifoni D, Cennamo V, Leandri P, Jovine E, de Biase D. Matricellular proteins and survival in patients with pancreatic cancer: a systematic review. Pancreatology 2018; 18: 122-132.

5) Shukla GC, Singh J, Barik S. MicroRNAs: processing, maturation, target recognition and regulatory functions. Mol Cell Pharmacol 2011; 3: 83-92.

6) Yang J, Zhu H, Jin Y, Song Y. MiR-431 inhibits cell proliferation and induces cell apoptosis by targeting CDK14 in pancreatic cancer. Eur Rev Med Pharmacol Sci 2018; 22: 4493-4499.

7) Wu X, Chen W, Cai H, Hu J, Wu B, Jiang Y, Chen X, Sun D, An Y. MiR-216b inhibits pancreatic cancer cell progression and promotes apoptosis by down-regulating KRAS. Arch Med Sci 2018; 14: 1321-1332.

8) Zhu G, Zhou L, Liu H, Shan Y, Zhang X. MicroRNA-224 promotes pancreatic cancer cell proliferation and migration by targeting the TXNIP-mediated HIF1alpha pathway. Cell Physiol Biochem 2018; 48: 1735-1746.

9) Zhao DW, Hou YS, Sun FB, Han B, Li SJ. Effects of miR-132 on proliferation and apoptosis of pancreatic cancer cells via Hedgehog signaling pathway. Eur Rev Med Pharmacol Sci 2019; 23: 1978-1985.

10) Li KK, Pang JC, Lau KM, Zhou L, Mao Y, Wang Y, Poon WS, Ng HK. MiR-383 is downregulated in medulloblastoma and targets peroxiredoxin 3 (PRDX3). Brain Pathol 2013; 23: 413-425.

11) Zhao S, Gao X, Zang S, Li Y, Feng X, Yuan X. MicroRNA-383-5p acts as a prognostic marker and inhibitor of cell proliferation in lung adenocarcinoma by cancerous inhibitor of protein phosphatase 2A. Oncol Lett 2017; 14: 3573-3579.

12) He Z, Cen D, Luo X, Li D, Li P, Liang L, Meng Z. Downregulation of miR-383 promotes glioma cell invasion by targeting insulin-like growth factor 1 receptor. Med Oncol 2013; 30: 557.

13) Liu J, Dou Y, Sheng M. Inhibition of microRNA-383 has tumor suppressive effect in human epithelial ovarian cancer through the action on caspase-2 gene. Biomed Pharmacother 2016; 83: 1286-1294.

14) Han S, Cao C, Tang T, Lu C, Xu J, Wang S, Xue L, Zhang X, Li M. ROBO3 promotes growth and metastasis of pancreatic carcinoma. Cancer Lett 2015; 366: 61-70.

15) Gu H, Neel BG. The "Gab" in signal transduction. Trends Cell Biol 2003; 13: 122-130.

16) Nishida K, Hirano T. The role of Gab family scaffolding adapter proteins in the signal transduction of cytokine and growth factor receptors. Cancer Sci 2003; 94: 1029-1033.

17) Shioyama W, Nakaoka Y, Higuchi K, Minami T, Taniyama Y, Nishida K, Kidoya H, Sonobe T, Naito H, Arita Y, Hashimoto T, Kuroda T, Fujio Y, Shirai M, Takakura N, Morishita R, Yamauchi-Takihara K, Kodama T, Hirano T, Mochizuki N, Komuro I. Docking protein Gab1 is an essential component of postnatal angiogenesis after ischemia via HGF/c-met signaling. Circ Res 2011; 108: 664-675.

18) Liu H, Li G, Zeng W, Zhang P, Fan F, Tu Y, Zhang Y. Combined detection of Gab1 and Gab2 expression predicts clinical outcome of patients with glioma. Med Oncol 2014; 31: 77.

19) Sang H, Li T, Li H, Liu J. Down-regulation of Gab1 inhibits cell proliferation and migration in hilar cholangiocarcinoma. PLoS One 2013; 8: e81347.

20) Bai R, Weng C, Dong H, Li S, Chen G, Xu Z. MicroRNA-409-3p suppresses colorectal cancer invasion and metastasis partly by targeting GAB1 expression. Int J Cancer 2015; 137: 2310-2322.

21) Shao NY, Wang DX, Wang Y, Li Y, Zhang ZQ, Jiang Q, Luo W, Cao C. MicroRNA-29a-3p sownregulation causes Gab1 upregulation to promote glioma cell proliferation. Cell Physiol Biochem 2018; 48: 450-460.

22) Pei L, Meng S, Yu W, Wang Q, Song F, Ma L. Inhibition of microRNA-383 ameliorates injury after focal cerebral ischemia via targeting PPARgamma. Cell Physiol Biochem 2016; 39: 1339-1346.

23) Shang Y, Zang A, Li J, Jia Y, Li X, Zhang L, Huo R, Yang J, Feng J, Ge K, Yang Y, Zhang Y, Jiang J. MicroRNA-383 is a tumor suppressor and potential prognostic biomarker in human non-small cell lung caner. Biomed Pharmacother 2016; 83: 1175-1181.

24) Gu B, Wang J, Song Y, Wang Q, Wu Q. MicroRNA-383 regulates cell viability and apoptosis by mediating Wnt/beta-catenin signaling pathway in non-small cell lung cancer. J Cell Biochem. 2018 Nov 13. doi: 10.1002/jcb.28069. [Epub ahead of print]

25) Teng P, Jiao Y, Hao M, Tang X. MicroRNA-383 suppresses the PI3K-AKT-MTOR signaling pathway to inhibit development of cervical cancer via down-regulating PARP2. J Cell Biochem 2018; 119: 5243-5252.

26) Han RL, Wang FP, Zhang PA, Zhou XY, Li Y. MiR-383 inhibits ovarian cancer cell proliferation, invasion and aerobic glycolysis by targeting LDHA. Neoplasma 2017; 64: 244-252.

27) Felici A, Giubellino A, Bottaro DP. Gab1 mediates hepatocyte growth factor-stimulated mitogenicity and morphogenesis in multipotent myeloid cells. J Cell Biochem 2010; 111: 310-321.

28) Oka M, Kikkawa U, Nishigori C. Protein kinase C-betaII represses hepatocyte growth factor-induced invasion by preventing the association of adapter protein Gab1 and phosphatidylinositol

3-kinase in melanoma cells. J Invest Dermatol 2008; 128: 188-195.

29) Seiden-Long I, Navab R, Shih W, Li M, Chow J, Zhu CQ, Radulovich N, Saucier C, Tsao MS. Gab1 but not Grb2 mediates tumor progression in Met overexpressing colorectal cancer cells. Carcinogenesis 2008; 29: 647-655.

30) Sun W, Zhang Z, Wang J, Shang R, Zhou L, Wang X, Duan J, Ruan B, Gao Y, Dai B, Qu S, Liu W, Ding R, Wang L, Wang D, Dou K. MicroRNA-150 suppresses cell proliferation and metastasis in hepatocellular carcinoma by inhibiting the GAB1-ERK axis. Oncotarget 2016; 7: 11595-11608.