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MicroRNA-140 regulates cell growth and invasion in pancreatic duct adenocarcinoma by targeting iASPP

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Abstract

MicroRNAs are ~22 nucleotide RNAs processed from RNA hairpin structures that play important roles in regulating protein expression level via binding to mRNA, either suppressing its translation or speeding up its degradation. In humans, they regulate most protein-coding genes, including genes important in cancer and other diseases. In this study, the expression of microRNA-140 (miR-140) was demonstrated to be significantly suppressed in pancreatic duct adenocarcinoma specimens and cell lines, compared with their adjacent normal tissues. With the help of bioinformatics analysis, inhibitor of apoptosis-stimulating protein of p53 (iASPP) was identified to be a direct target of miR-140, and luciferase reporter experiment confirmed this discovery. Overexpression of miR-140 decreases the protein expressions of iASPP, ΔNp63, MMP2, and MMP9. Growth and invasion of PANC-1 cells were attenuated by overexpression of miR-140 *in vitro*. The suppressive effect of miR-140 on PANC-1 cell line could be partly balanced out by manual overexpression of iASPP. Above all, these findings provided insights into the functional mechanism of miR-140, suggested that the miR-140/iASPP axis may interfere with the proliferative and invasive property of pancreatic duct adenocarcinoma cells, and indicated that miR-140 could be a potential therapeutic target for pancreatic duct adenocarcinoma.

Key words: miR-140, pancreatic duct adenocarcinoma, cell proliferation, cell invasion, iASPP

Introduction

Pancreatic duct adenocarcinoma (PDAC) is of high malignance, known for its progressive growth, early-stage metastasis, and poor outcome responding to both radiotherapy and chemotherapy. In the past 10 years, despite newly approved therapeutic methods and large evolutions in nursing care, no revolutionary progress has been made in improving the survival rate of PDAC patients [1,2]. Therefore, exploring specific biomarkers that enable earlier diagnosis and support personalized managements for treating patients at high risk of PDAC has become more and more urgent [1].

Almost in all malignancy studies, microRNAs (miRNAs) are found to be extremely critical because they can be the results of chromosome lesions, modulated by classic cell signaling, and they themselves can act as both oncogenes and tumor suppressors [3,4]. Recently, a close association between miRNAs and PDAC tumorigenesis has been elucidated [5,6]. Various miRNAs, such as miR-200 [7], miR-146a [8], miR-486 [9], and let-7 family [10] have been confirmed to be tumor suppressors. Meanwhile, PDAC malignancy was found to be positively associated with miR-196a [11], miR-212 [12], and miR-31 [13]. We chose to focus on miR-140 because it is closely related to many different malignancies, such as mastocarcinoma [14], colorectal carcinoma [15], liver cancer [16], and osteosarcoma [17]. Our findings suggest that miR-140 could play tumor-inhibitive role in the above cancers. However, as far as we know, its potential functional mechanisms remain uncertain and need further exploration in PDAC.

In this study, miR-140, known as tumor-suppressive miRNA, was confirmed to have regulatory relationship with an oncogene, inhibitor of apoptosis-stimulating protein of p53 (iASPP). We confirmed that miR-140 inhibited PDAC cell proliferation and invasion, partly by choosing iASPP as its binding site. The iASPP level was downregulated by manual overexpression of miR-140, which also reduced the protein expressions of ΔNp63, MMP2, and MMP9. The effects of miR-140 might be neutralized when iASPP was overexpressed by transfection with iASPP ORF-expressing vector. These findings provide novel knowledge about miR-140's character and its functional mechanism regarding the pathobiology of PDAC. Besides, a potential therapeutic blueprint for treating PDAC might be developed accordingly in the future.

Materials and Methods

Tissue samples, cell lines, and cell transfection

A total of 23 pairs of surgical tissue samples of PDAC were collected, each including the adjacent normal tissues. All samples were obtained from the Surgical Department at Xiangya Hospital of Central South University (Changsha, China). Once resected, the tissues were instantly frozen by soaking in liquid nitrogen, and then stored at −80°C. The experiment protocol was approved by the Ethics Committee of Xiangya Hospital of Central South University.

Human PDAC cell lines, including HEPD6-C7, CFPAC-1, PANC-1, and BxPC3, were purchased from the American Type Culture Collection (ATCC, Manassas, USA). All cells were cultured in RPMI-1640 medium (Invitrogen, Carlsbad, USA) containing 10% fetal bovine serum (FBS; Gibco, Carlsbad, USA) at 37°C in a humidified atmosphere with 5% CO_2. Intervention of miR-140 expression was conducted by transfection with miR-140 mimics or miR-140 inhibitor (Genepharma, Shanghai, China) with the help of Lipofectamine 2000 (Invitrogen). An iASPP ORF-expressing clone (GeneCopoecia, Guangzhou, China) was used to achieve overexpression of iASPP. Cells were seeded in 96-well plates or 6-well plates, transfected, and incubated for a certain period of time before further managements like RNA/protein extraction.

RNA extraction and quantitative polymerase chain reaction analysis

TRIzol reagent (Invitrogen) was used to extract total RNA from cells. Hairpin-it™ miRNAs qPCR kit (Genepharma) was used to detect the mature miR-218 expression level in cells. Expression of RNU6B served as an endogenous control. SYBR green qPCR assay (Takara, Dalian, China) was used to measure and calculate the expressional level. The $2^{-\Delta\Delta CT}$ method was applied in data processing.

3-(4,5-Dimethyldiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

A modified 3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to evaluate cell viability. Cells were transfected with the intended agent then seeded into the 96-well culture plates at a density of 2×10^3 cells/well. Cell viability of PANC-1 was then assessed at five different time points (24, 48, 72, 96, and 120 h). Theoretically, the enzymatic change of MTT (Sigma-Aldrich, St Louis, USA), which results in a colored formazan product, was seen as the indicator of mitochondrial dehydrogenase activity. MTT (10 μl, 10 mg/ml) was applied to the cells. After 4 h of incubation, the supernatant was removed to stop the reaction. Then, to dissolve the formazan product, 100 μl dimethyl sulfoxide was added to each well. After 0.5 h, the optical density at 570 nm of each well was detected with a microplate reader (ELx808; Bio-Tek Instruments, Hercules, USA).

5-Bromo-2′-deoxyuridine incorporation assay

5-Bromo-2′-deoxyuridine (BrdU) incorporation is the direct indicator of DNA synthesis accompanied by cell proliferation, and we performed BrdU assay on PANC-1 cells. After being transfected, cells were seeded into 96-well culture plates at a density of 2×10^3 cells/well, and cultured for 24 or 48 h, then soaked in 10 μM BrdU (BD Pharmingen, San Diego, USA) for 2–24 h. After incubation, the upper liquid was discarded, and the cells were fixed for 30 min at room temperature (RT). The fixed cells were incubated with peroxidase-conjugated anti-BrdU antibody (Sigma-Aldrich) for 60 min at RT, washed three times with phosphate buffered saline, and incubated with peroxidase substrate (tetramethylbenzidine) for another 30 min. Finally, the absorbance values at 450 nm were measured with the above-mentioned microplate reader. Cells not exposed to BrdU but stained with the BrdU antibody were also detected as the background BrdU immunofluorescence.

Cell invasion assay

Evaluation of invasive and migratory potential of cells was performed with Transwell inserts with 8 μm pores (Corning Co., Coring, USA). For invasion assay, each upper insert was seeded with 3×10^5 cells suspended in serum-free medium at 24 h after transfection. The upper inserts were precoated with matrigel matrix (BD, Bedford, USA). The lower chambers were each filled with 500 μl medium containing 10% FBS. After 48 h, a cotton swab was used to remove the noninvaded cells from the upper surface of the transwell membrane. Then, on the lower membrane surface, the invaded cells were fixed with methanol and stained with 0.1% crystal violet. Photographs were taken and cell numbers were counted under a microscope. For migration assay, similar procedures were conducted, except that 2×10^5 cells were applied and the inserts were not precoated by matrix gel. Six random fields were counted at ×100 magnification for each insert. The experiment was conducted three times separately.

Western blot analysis

The expressions of iASPP, ΔNp63, MMP2, and MMP9 in PDAC cells were detected by immunoblotting. RIPA buffer containing 1% phenylmethanesulfonyl fluoride was used to lyse the cells. Proteins were then loaded onto a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis minigel and transferred onto polyvinylidene fluoride membrane under an electric field. Then the blots were probed with 1 : 1000 diluted rabbit polyclonal antibodies against iASPP, ΔNp63, MMP2, and MMP9 (Abcam, Cambridge, USA) at 4°C overnight. After that, the blots were incubated with horseradish peroxidase-conjugated secondary antibody (1 : 5000). Enhanced chemiluminescence substrates were used to visualize signals (Millipore, Billerica, USA). For normalization, the endogenous β-actin was also tested.

Luciferase reporter assay

After seeded into a 24-well plate, PANC-1 cells were cultured overnight, then cotransfected with the wild-type/mutated iASPP 3′ untranslated regions (UTR) luciferase reporter vector and pRL-TK plasmids, or transfected with miR-140 mimics/miR-140 inhibitor. Forty-eight hours after transfection, the luciferase assays were performed with the help of the Dual Luciferase Reporter Assay System (Promega, Madison, USA).

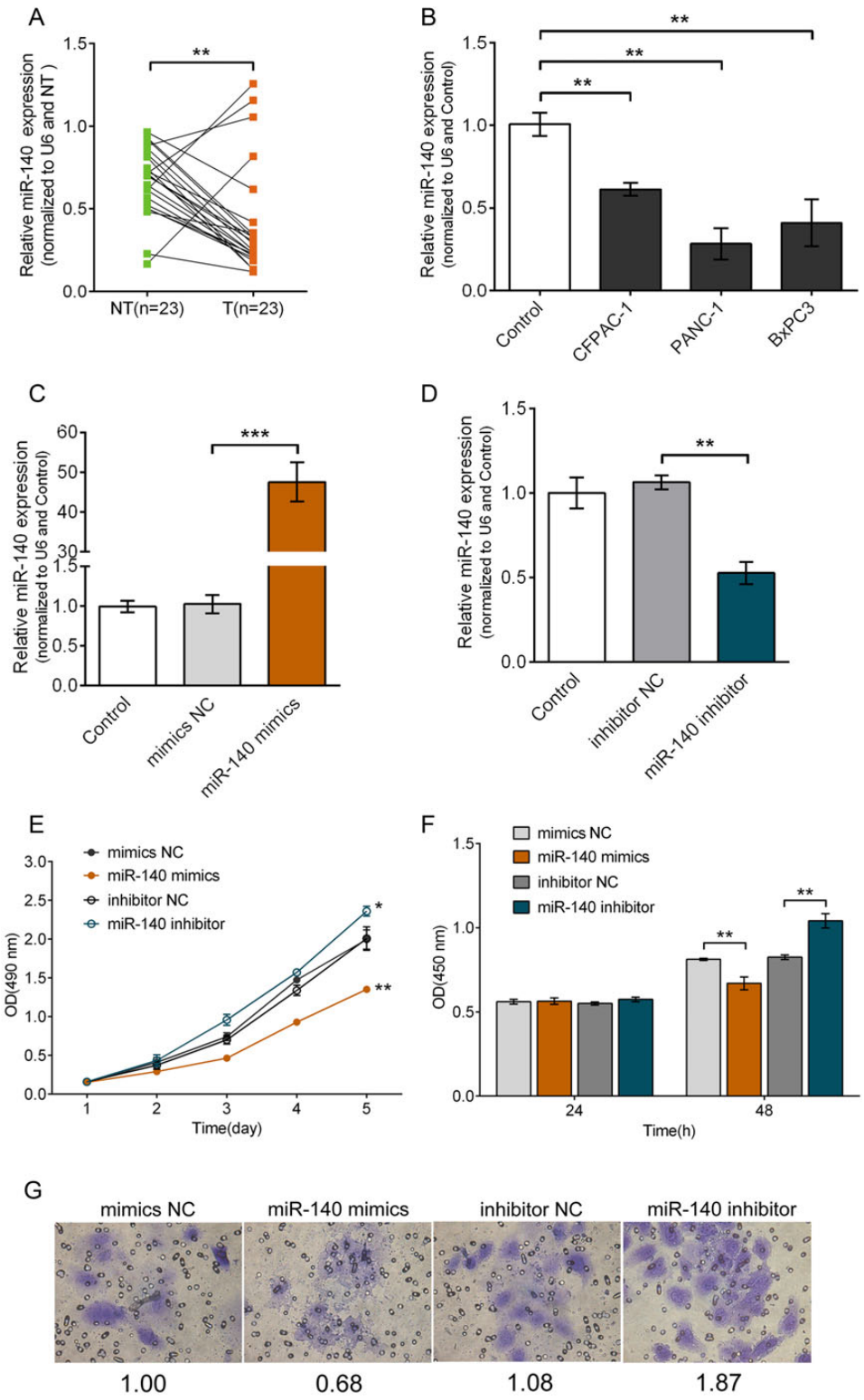

Figure 1. miR-140 is downregulated in both primary PDAC tissues and PDAC cell lines and inhibits the proliferation and invasion of PANC-1 cell line (A) Decreased expression of miR-140 was observed in PDAC tissues compared with the adjacent normal tissues in a panel of matched tissues from 23 PDAC patients. (B) miR-140 expression was at significantly lower levels in three PDAC cell lines compared with human normal pancreatic ductal epithelium cells. PANC-1 cell line was transfected with miR-140 mimics (C) or miR-140 inhibitor (D), and the expression of miR-140 was analyzed by real-time PCR. The endogenous miR-140 in PANC-1 cell line was induced with miR-140 mimics and silenced with miR-140 inhibitor, and the effects of miR-140 on cell proliferation and invasion were determined using MTT assay (E), BrdU assay (F), and transwell assay with matrigel (G), respectively. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

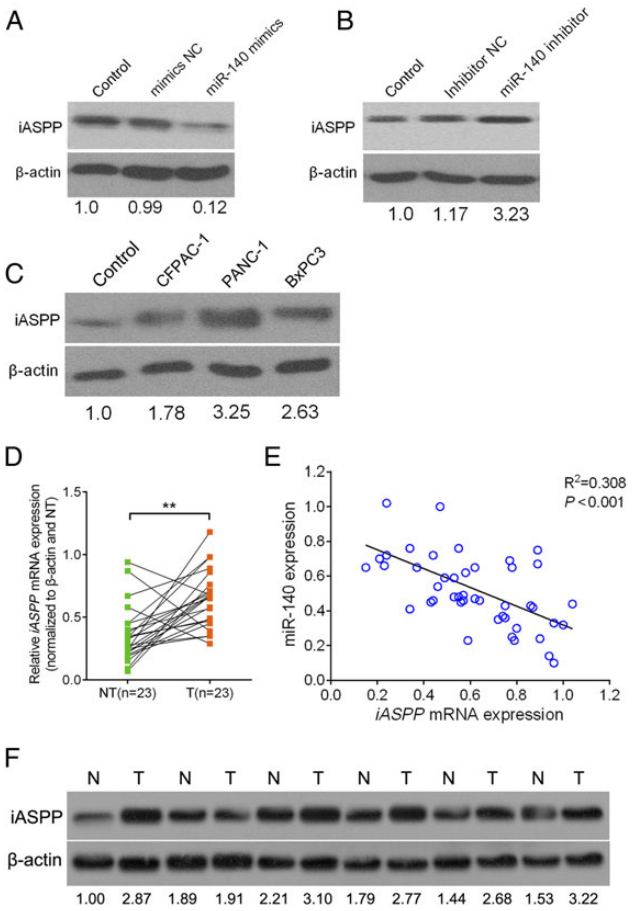

Figure 2. miR-140 inhibits iASPP expression and the miR-140 expression is negatively correlated with the iASPP expression The expression of iASPP in PANC-1 cell line 48 h after transfection with miR-140 mimics or miR-140 inhibitor was measured by western blotting. (A) Overexpression of miR-140 decreased the iASPP protein level. (B) Downregulation of miR-140 increased the iASPP protein level. (C) iASPP was expressed at significantly higher levels in three PDAC cell lines in comparison with human normal pancreatic ductal epithelium cells. (D) Increased expression of *iASPP* mRNA was observed in PDAC tissues compared with the adjacent normal tissues in a panel of matched tissues from 23 PDAC patients. (E) A statistically inverse correlation between miR-140 and iASPP mRNA levels in 46 PDAC tumor tissues by correlation analysis. (F) Upregulated expression of iASPP was observed in PDAC tissues compared with the adjacent normal tissues in a panel of matched tissues from six PDAC patients. $^{**}P < 0.01$.

Statistical analysis

Data were processed using SPSS 17.0 statistical software (SPSS, Chicago, USA), and presented as the mean ± SD of three independent experiments. By using Wilcoxon's paired test, the expression of miR-140 in PDAC surgical biopsy tissues was compared with that in the adjacent normal tissues. One-way analysis of variance was used to analyze the differences between groups in terms of migration and invasion. *P* values of <0.05 were considered to be of statistical significance.

Results

miR-140 is largely downregulated in both PDAC tissues and cell lines and hinders the proliferation and invasion of PANC-1 cells

To quantify the expression level of miR-140 in PDAC tissues and cell lines, SYBR green quantitative polymerase chain reaction (PCR) analysis was performed. Among a large group of 23 case pairs of primary PDAC tissues and their adjacent normal tissues, 19 of 23 (82.61%) showed decreased miR-140 level, when compared with the paired adjacent normal tissues (**Fig. 1A**). Besides, compared with HEPD6-C7 (Control), the miR-140 expression level was significantly lower in all three human PDAC cell lines (**Fig. 1B**).

Then we explored the function of miR-140 in PDAC cell lines. PANC-1 cell line was transfected with miR-140 mimics and miR-140 inhibitor, respectively. Overexpression or downregulation of miR-140 in PANC-1 cell line was validated by reverse transcriptase (RT)-PCR (**Fig. 1C,D**). MTT and BrdU assays revealed that overexpression of miR-140 inhibited the proliferation of PANC-1 cell line, while downregulation of miR-140 promoted the proliferation of PANC-1 cell line (**Fig. 1E,F**). Moreover, transwell assays (**Fig. 1G**) showed that cell invasion ability was significantly inhibited by ectopic expression of miR-140 in PANC-1 cells. By comparison, when miR-140 inhibitor silenced endogenous miR-140, the cell invasion was enhanced (**Fig. 1G**). These results indicate that miR-140 appeared to be tumor suppressive in PDAC-1 cell line *in vitro*.

miR-140 inhibits the expression of iASPP

To clarify the molecular mechanism how miR-140 inhibits the growth of PANC-1 cell line, the TargetScan, miRanda, and miRWalk online tools were used to predict the potential targets of miR-140. Among the gene candidates found with the above bioinformatics, iASPP was chosen in this study, since it is involved in the pathogenic process of many human malignancies. PANC-1 cells were transfected with miR-140 mimics/miR-140 inhibitor to testify whether miR-140 was able to inhibit the expression of iASPP. At 48 h after transfection, western blot analysis results showed that increased miR-140 level in PANC-1 cell line significantly repressed iASPP protein expression, while knockdown of miR-140 increased the protein level of iASPP when compared with negative control (NC) (**Fig. 2A,B**).

In addition, human normal pancreatic ductal epithelium cells (Control) and three human PDAC cell lines were also tested. It was found that all three cell lines showed remarkable upregulation of iASPP protein expression compared with the control (**Fig. 2C**). The test was extended to detect the levels of *iASPP* mRNA in PDAC tissues. Among 23 pairs of PDAC tissues and their adjacent normal tissues, *iASPP* mRNA level was found to be higher in 20 (86.96%) PDAC tissues compared with that in the corresponding adjacent normal tissues (**Fig. 2D**). **Figure 2E** revealed a notable inverse correlation of iASPP mRNA expression with miR-140 by Spearman's correlation test, $R^2 = 0.308$ ($P < 0.001$), which supported that the level of miR-140 was conversely associated with the level of iASPP mRNA in paired PDAC specimens. Furthermore, the iASPP protein level was determined with western blot analysis. The results showed that the expression of iASPP protein in PDAC tissues was upregulated compared with the paired adjacent normal tissues (**Fig. 2F**).

miR-140 may directly bind to *iASPP* mRNA

It was predicted that there existed an affinity between miR-140 and the *iASPP* 3′ UTR by using TargetScan, miRanda, and miRWalk [18–20]. A wild-type *iASPP* 3′ UTR luciferase reporter vector (wt-iASPP) was constructed to confirm this prediction, accompanied by a mutated *iASPP* 3′ UTR luciferase reporter vector (mut-iASPP) by manually mutating the sequence of the putative 8 bp miR-140-binding site in the 3′ UTR region of iASPP (**Fig. 3A**). The miR-140 mimics/inhibitor and wt-iASPP vector were cotransfected into PANC-1 cell line. Compared with the control cells, in miR-140 mimics-transfected

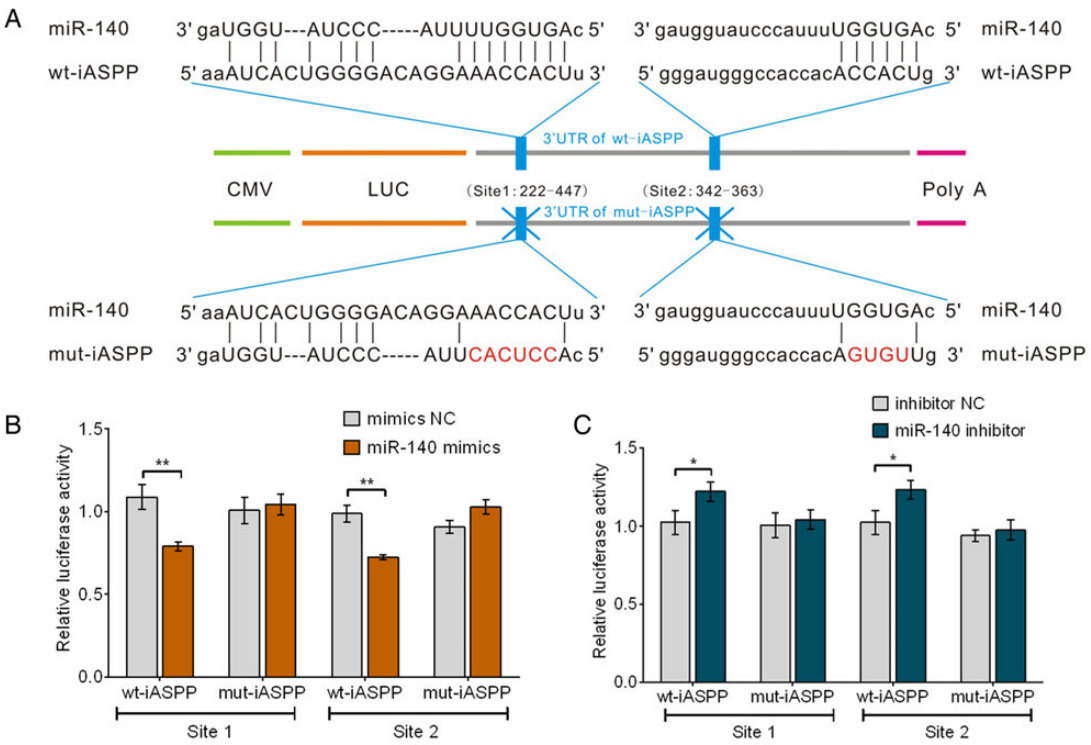

Figure 3. miR-140 directly targets iASPP by binding to its 3′ UTR (A) The predicted miR-140 binding site within the 3′ UTR of *iASPP* and the mutated version generated by site mutagenesis are shown. (B,C) Repression of wild-type iASPP 3′ UTR luciferase reporter gene activity by miR-140 mimics (B) and promotion of that by miR-140 inhibitor (C). miR-140 had no effect on the luciferase activity of the mutated iASPP 3′ UTR reporter vector when compared with control PANC-1 cell line or cells transfected with mimics/inhibitor NC. $*P < 0.05$, $**P < 0.01$.

cells, the *iASPP* 3′ UTR's luciferase activity was significantly reduced, while it was induced in miR-140 inhibitor-transfected cells (**Fig. 3B, C**). Besides, the miR-140-mediated repression of *iASPP* 3′ UTR luciferase reporter activity could be abolished by mutating the putative miR-140-binding sequence in the *iASPP* 3′ UTR (**Fig. 3B,C**).

iASPP knockdown inhibits PANC-1 cells from proliferation and invasion

We then investigate the functional mechanism of iASPP in PDAC cells. PANC-1 cell line was transfected with iASPP-siRNA. Downregulation of iASPP in PANC-1 cell line induced by iASPP-siRNA was confirmed by RT-PCR and western blotting (**Fig. 4A,B**). MTT and BrdU assays revealed that knockdown of iASPP inhibited the proliferation of PANC-1 cell line (**Fig. 4C,D**). Moreover, matrigel transwell assays showed that cell invasion ability was significantly inhibited by silenced endogenous iASPP (**Fig. 4E**).

Manual expression of iASPP reinstates the effects of miR-140 in PANC-1 cell line

As shown above, miR-140 might be able to directly target iASPP mRNA. So, we wondered whether forced overexpression of iASPP could reverse the miR-140-suppressed proliferation and invasion of PANC-1 cell line. iASPP ORF clone was confirmed to enhance the protein expression of iASPP (**Fig. 5A**). Then, an ORF-expressing plasmid of iASPP was transfected into miR-140 mimics/mimics NC cells. The BrdU and transwell results shown in **Fig. 5B,C** suggested that inhibition of cell proliferation induced by miR-140 was largely reinstated by forced expression of iASPP. Similarly, the cotreatment of miR-140 mimics and iASPP reversed the inhibitory effect of miR-140 mimics (**Fig. 5D**).

iASPP was identified as a key player to attach to p63 and suppress its activities [21]. In addition, iASPP and ΔNp63 could enhance the oncogenic potential of pancreatic cancer cells [22]. MMP2 and MMP9 have been reported to be involved in invasion and metastasis of PDAC [23]. We extend the test to confirm the expressions of ΔNp63, MMP2, and MMP9. Manually increased expression of iASPP reinstated the iASPP, ΔNp63, MMP9, and MMP2 protein expressions suppressed by miR-140 (**Fig. 5E**).

Discussion

Abnormalities of miRNAs' expression are observed almost in all fields of malignant biology, such as cell replication, apoptosis, invasion, and/or metastasis, and they may serve as either tumor inhibitors or promoters [19]. In this study, we focused on miR-140 because of its potential suppressive function in human malignancies. Song *et al.* [15] demonstrated that cell proliferation in both colorectal carcinoma and osteogenic sarcoma cell lines were inhibited by overexpression of miR-140. Lately, it was reported that hepatocellular carcinoma tissues and cell lines displayed a significantly lowered miR-140-5p level, and by binding to fibroblast growth factor 9 and transforming growth factor β receptor 1, its overexpression decreased both cancer growth and metastasis [16]. Nevertheless, miR-140's role in PDAC tumorigenesis and the detailed functional mechanisms of how miR-140 performs still remain unclear. In our study, the real-time PCR results indicated that miR-140's expression level was obviously downregulated in both PDAC tissues and cell lines. Manually enhanced expression of

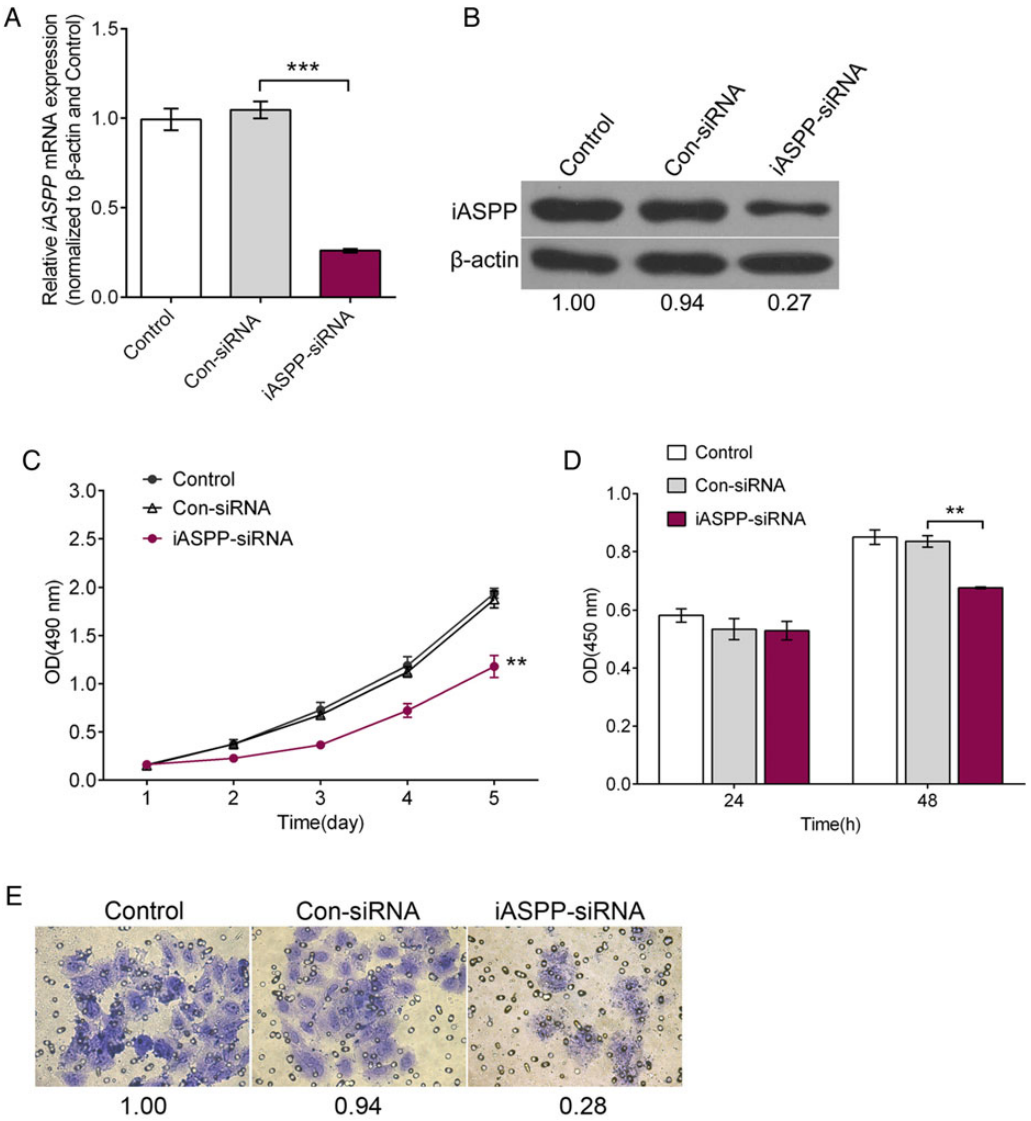

Figure 4. Knockdown of iASPP inhibits the proliferation and invasion of PANC-1 cell line The endogenous iASPP mRNA (A) and protein (B) expression in PANC-1 cell line was determined by real-time PCR and western blotting. Knockdown of iASPP suppressed the expression of iASPP. The effects of iASPP-siRNA on cell proliferation and invasion were determined using MTT assay (C), BrdU assay (D), and transwell assay with matrigel (E), respectively. Silenced iASPP inhibited the proliferation of PANC-1 cell line. $^{**}P < 0.01$, $^{***}P < 0.001$.

miR-140 significantly inhibited the proliferation and invasion of PDAC cell. Conversely, knocking-down of miR-140 might accelerate cell growth and invasion. The above results suggested that miR-140 could potentially be tumor suppressive in PDAC.

Since the effect of certain miRNAs on carcinogenesis depends on their downstream targets [24,25], various predictive algorithms were used to calculate target sequences for miR-140 in order to clarify the corresponding reaction chains involved in the inhibition of PDAC's proliferation and metastasis by induction of miR-140. The iASPP oncogene was verified as an important downstream target of miR-140 because of its frequent overexpression in many types of cancer and serves as an essential modulator of cell replication, survival, and migration [26–29]. Overexpression of miR-140 could downregulate the protein level of iASPP, while knockdown of miR-140 could up-regulate the expression of iASPP. The luciferase reporter assay confirmed the inhibitory effect on the translation of miR-140's putative binding in the iASPP 3′ UTR region in PANC-1 cells. Western blot analysis also supported that iASPP was downregulated by miR-140 upregulation, and upregulated by miR-140 downregulation. Moreover, in surgical PDAC samples, the expression levels of miR-140 and iASPP are significantly inversely correlated. To further confirm the role of iASPP in the PANC-1 cell line, we revealed that iASPP knockdown could inhibit the proliferation and invasion of PANC-1 cell line. Concerned with the importance of miR-140/iASPP axis in PDAC, it is reasonable to believe that miR-140 might be potentially valuable as a therapeutic agent.

As the oldest member of ASPP protein family, iASPP, which is evolutionarily conserved, also can inhibit p53 [30]. iASPP plays a key role in epithelial subtratification, a feature achieved by binding and inhibiting p63's activities [21]. For p63, there are six transcription variants originated from two independent promoters and alternative cropping. A full-length trans-activation domain can be seen within isoforms derived from P1 (TAp63a, b, and c). N-terminally truncated variants ensue following P2 (ΔNp63a, b, and c). In plenty of human cancers,

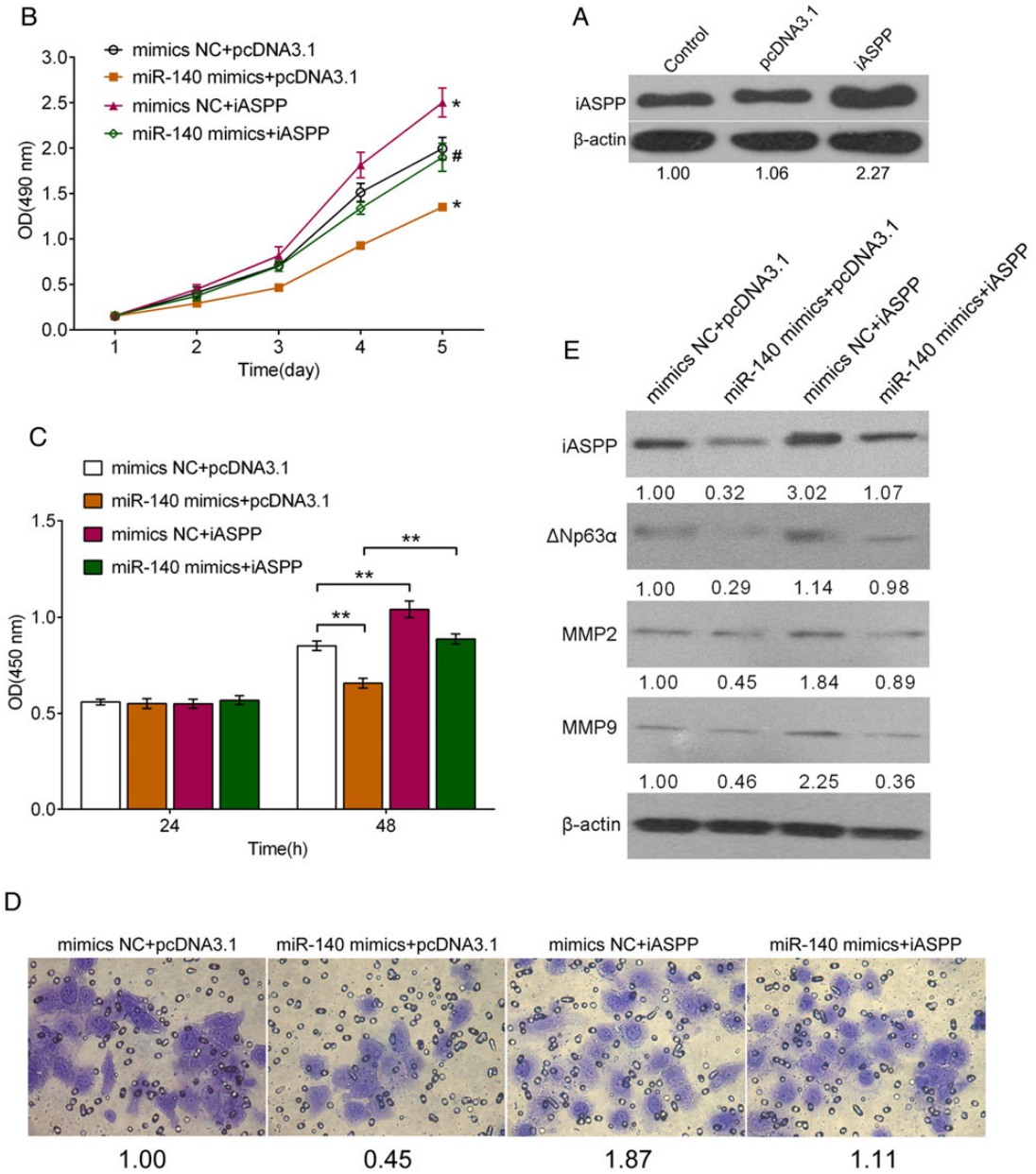

Figure 5. Force expression of iASPP restored the effects of miR-140 mimics in PANC-1 cell line and iASPP pathway PANC-1 cell line treated with miR-140 mimics was transfected with iASPP plasmid (A). After 48 h of treatment, the cell proliferation and invasion were determined using MTT assay (B), BrdU assay (C), and transwell assays with matrigel (D), respectively. Moreover, the levels of specific proteins were analyzed by western blotting (E). Upregulation of miR-140 inhibited the proliferation and invasion of the PANC-1 cell line and the protein levels of iASPP, ΔNp63, MMP2, and MMP9. Overexpression of iASPP promoted the proliferation and invasion of the PANC-1 cell line and the protein levels of iASPP, ΔNp63, MMP2, and MMP9. The cotreatment of miR-140 mimics and iASPP reversed the inhibitory effect of miR-140 mimics. #Compared to the mimics NC+pcDNA3.1 group. *$P < 0.05$, **$P < 0.01$.

such as squamous cell carcinomas neck, head, lung, bladder, and breast, the ΔNp63 variant is overexpressed [31]. In squamous cell cancer and the so-called 'triple-negative' mastocarcinoma cells, p73-related apoptosis can be suppressed by ΔNp63 and thus enhance tumor survival [32,33]. The oncogenic potential of pancreatic cancer cells could be enhanced by ΔNp63 [22]. MMP2 and MMP9 have been reported to be involved in the adhesion of cells to the peritoneum and in invasion and metastasis of PDAC [23,34,35]. They are involved in the remodeling of the tumor's environment to facilitate tumor invasion [36]. In this study, western blot analysis indicated that overexpression of miR-140 could downregulate the protein expressions of ΔNp63, MMP2, and MMP9, while cotreatment of miR-140 and iASPP reinstated the inhibition of miR-140. Therefore, these findings suggested that miR-140 may bind to the iASPP mRNA to inhibit the proliferation and invasion in PDAC cells via ΔNp63, MMP2, and MMP9 pathways.

In summary, it was identified that through targeting iASPP, PDAC cell viability, proliferation, and invasion are inhibited by miR-140. These effects were due, at least in part, to the downregulation of ΔNp63, MMP2, and MMP9. The miR-140/iASPP axis established in present study may be essential in modulating PDAC cell growth and invasion, and may serve as a new medical portal for PDAC treatment in the future.

References

1. Kozak G, Blanco FF, Brody JR. Novel targets in pancreatic cancer research. *Semin Oncol* 2015, 42: 177–187.
2. Kongkam P, Benjasupattananun P, Taytawat P, Navicharoen P, Sriuranpong V, Vajragupta L, Klaikaew N, *et al*. Pancreatic cancer in an Asian population. *Endosc Ultrasound* 2015, 4: 56–62.
3. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004, 116: 281–297.
4. Lan H, Lu H, Wang X, Jin H. MicroRNAs as potential biomarkers in cancer: opportunities and challenges. *Biomed Res Int* 2015, 2015: 125094.
5. Lai XL, Huang YH, Li YS, Li GN, Wang LP, Sun R, Ma YS, *et al*. Differential expression profiling of microRNAs in para-carcinoma, carcinoma and relapse human pancreatic cancer. *Clin Transl Oncol* 2015, 17: 398–408.
6. Pavlakis E, Papaconstantinou I, Gazouli M, Theodosopoulos T, Karamanolis G, Genatas K, Ladas SD. MicroRNA gene polymorphisms in pancreatic cancer. *Pancreatology* 2013, 13: 273–278.
7. Yu J, Ohuchida K, Mizumoto K, Sato N, Kayashima T, Fujita H, Nakata K, *et al*. MicroRNA, hsa-miR-200c, is an independent prognostic factor in pancreatic cancer and its upregulation inhibits pancreatic cancer invasion but increases cell proliferation. *Mol Cancer* 2010, 9: 169.
8. Li Y, VandenBoom TG II, Wang Z, Kong D, Ali S, Philip PA, Sarkar FH. Up-regulation of miR-146a contributes to the inhibition of invasion of pancreatic cancer cells. *Cancer Res* 2010, 70: 5703.
9. Mees ST, Mardin WA, Sielker S, Willscher E, Senninger N, Schleicher C, Colombo-Benkmann M, *et al*. Involvement of CD40 targeting miR-224 and miR-486 on the progression of pancreatic ductal adenocarcinomas. *Ann Surg Oncol* 2009, 16: 2339–2350.
10. Li Y, VandenBoom TG II, Kong D, Wang Z, Ali S, Philip PA, Sarkar FH. Up-regulation of miR-200 and let-7 by natural agents leads to the reversal of epithelial-to-mesenchymal transition in gemcitabine-resistant pancreatic cancer cells. *Cancer Res* 2009, 69: 6704–6712.
11. Huang F, Tang J, Zhuang X, Zhuang Y, Cheng W, Chen W, Yao H, *et al*. MiR-196a promotes pancreatic cancer progression by targeting nuclear factor kappa-B-inhibitor alpha. *PLoS One* 2014, 9: e87897.
12. Ma C, Nong K, Wu B, Dong B, Bai Y, Zhu H, Wang W, *et al*. miR-212 promotes pancreatic cancer cell growth and invasion by targeting the hedgehog signaling pathway receptor patched-1. *J Exp Clin Cancer Res* 2014, 33: 54.
13. Laurila EM, Sandstrom S, Rantanen LM, Autio R, Kallioniemi A. Both inhibition and enhanced expression of miR-31 lead to reduced migration and invasion of pancreatic cancer cells. *Genes Chromosomes Cancer* 2012, 51: 557–568.
14. Li Q, Yao Y, Eades G, Liu Z, Zhang Y, Zhou Q. Downregulation of miR-140 promotes cancer stem cell formation in basal-like early stage breast cancer. *Oncogene* 2014, 33: 2589–2600.
15. Song B, Wang Y, Xi Y, Kudo K, Bruheim S, Botchkina GI, Gavin E, *et al*. Mechanism of chemoresistance mediated by miR-140 in human osteosarcoma and colon cancer cells. *Oncogene* 2009, 28: 4065–4074.
16. Yang H, Fang F, Chang R, Yang L. MicroRNA-140-5p suppresses tumor growth and metastasis by targeting transforming growth factor beta receptor 1 and fibroblast growth factor 9 in hepatocellular carcinoma. *Hepatology* 2013, 58: 205–217.
17. Hwang S, Park SK, Lee HY, Kim SW, Lee JS, Choi EK, You D, *et al*. miR-140-5p suppresses BMP2-mediated osteogenesis in undifferentiated human mesenchymal stem cells. *FEBS Lett* 2014, 588: 2957–2963.
18. Esquela-Kerscher A, Slack FJ. Oncomirs—microRNAs with a role in cancer. *Nat Rev Cancer* 2006, 6: 259–269.
19. Hwang HW, Mendell JT. MicroRNAs in cell proliferation, cell death, and tumorigenesis. *Br J Cancer* 2007, 96 (Suppl): R40–R44.
20. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 2005, 120: 15–20.
21. Notari M, Hu Y, Koch S, Lu M, Ratnayaka I, Zhong S, Baer C, *et al*. Inhibitor of apoptosis-stimulating protein of p53 (iASPP) prevents senescence and is required for epithelial stratification. *Proc Natl Acad Sci USA* 2011, 108: 16645–16650.
22. Danilov AV, Neupane D, Nagaraja AS, Feofanova EV, Humphries LA, DiRenzo J, Korc M. DeltaNp63alpha-mediated induction of epidermal growth factor receptor promotes pancreatic cancer cell growth and chemoresistance. *PLoS One* 2011, 6: e26815.
23. Guo JQ, Zheng QH, Chen H, Chen L, Xu JB, Chen MY, Lu D, *et al*. Ginsenoside Rg3 inhibition of vasculogenic mimicry in pancreatic cancer through downregulation of VEcadherin/EphA2/MMP9/MMP2 expression. *Int J Oncol* 2014, 45: 1065–1072.
24. Profumo V, Doldi V, Gandellini P, Zaffaroni N. Targeting microRNAs to withstand cancer metastasis. *Methods Mol Biol* 2015, 1218: 415–437.
25. Chen J, Sun D, Chu H, Gong Z, Zhang C, Gong B, Li Y, *et al*. Screening of differential microRNA expression in gastric signet ring cell carcinoma and gastric adenocarcinoma and target gene prediction. *Oncol Rep* 2015, 33: 2963–2971.
26. Chen J, Xie F, Zhang L, Jiang WG. iASPP is over-expressed in human non-small cell lung cancer and regulates the proliferation of lung cancer cells through a p53 associated pathway. *BMC Cancer* 2010, 10: 694.
27. Gillotin S. iASPP, a potential drug target in cancer therapy. *Leuk Res* 2009, 33: 1175–1177.
28. Liu T, Li L, Yang W, Jia H, Xu M, Bi J, Li Z, *et al*. iASPP is important for bladder cancer cell proliferation. *Oncol Res* 2011, 19: 125–130.
29. Jia Y, Peng L, Rao Q, Xing H, Huai L, Yu P, Chen Y, *et al*. Oncogene iASPP enhances self-renewal of hematopoietic stem cells and facilitates their resistance to chemotherapy and irradiation. *FASEB J* 2014, 28: 2816–2827.
30. Zhang X, Diao S, Rao Q, Xing H, Liu H, Liao X, Wang M, *et al*. Identification of a novel isoform of iASPP and its interaction with p53. *J Mol Biol* 2007, 368: 1162–1171.
31. Deyoung MP, Ellisen LW. p63 and p73 in human cancer: defining the network. *Oncogene* 2007, 26: 5169–5183.
32. Leong CO, Vidnovic N, DeYoung MP, Sgroi D, Ellisen LW. The p63/p73 network mediates chemosensitivity to cisplatin in a biologically defined subset of primary breast cancers. *J Clin Invest* 2007, 117: 1370–1380.
33. Rocco JW, Leong CO, Kuperwasser N, DeYoung MP, Ellisen LW. p63 mediates survival in squamous cell carcinoma by suppression of p73-dependent apoptosis. *Cancer Cell* 2006, 9: 45–56.
34. Zhai LL, Cai CY, Wu Y, Tang ZG. Correlation and prognostic significance of MMP-2 and TFPI-2 differential expression in pancreatic carcinoma. *Int J Clin Exp Pathol* 2015, 8: 682–691.
35. Kilian M, Gregor JI, Heukamp I, Hanel M, Ahlgrimm M, Schimke I, Kristiansen G, *et al*. Matrix metalloproteinase inhibitor RO 28–2653 decreases liver metastasis by reduction of MMP-2 and MMP-9 concentration in BOP-induced ductal pancreatic cancer in Syrian Hamsters: inhibition of matrix metalloproteinases in pancreatic cancer. *Prostaglandins Leukot Essent Fatty Acids* 2006, 75: 429–434.
36. Kessenbrock K, Plaks V, Werb Z. Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell* 2010, 141: 52–67.