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miR-142-5p regulates pancreatic cancer cell proliferation and apoptosis by regulation of RAP1A

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ABSTRACT

Pancreatic cancer, one of the fatal and aggressive malignancies, leads the sixth cancer-associated death in China. microRNAs are believed to exert function in the diagnosis and treatment of pancreatic cancer. In the present study, we firstly found that miR-142-5p was downregulated in pancreatic cancer tumor tissues while Ras-related protein Rap-1 A (RAP1A) was upregulated compared with para-carcinoma non-tumor tissues. Then, we found that RAP1A could be a putative target gene of miR-142-5p by bioinformatics tool TargetScan. Furthermore, we conducted luciferase reporter assay, RT-qPCR, western blot and correlation analysis to demonstrate that miR-142-5p could negatively regulate RAP1A expression by binding to its 3′UTR. In addition, cell-counting kit 8 (CCK-8) and flow cytometry assays certified that miR-142-5p overexpression may inhibit pancreatic cancer cell proliferation but promote cell apoptosis; while the variation could be reversed by co-transfected with pcDNA3.1-RAP1A. Finally, miR-142-5p overexpression downregulated p-ERK1/2, phosphate p38 mitogen-activated protein kinases (p-p38); however, the variation induced by miR-142-5p mimic could be reversed by co-transfected with pcDNA3.1-RAP1A. In conclusion, our findings indicate that targeting miR-142-5p may provide a novel strategy for the treatment of pancreatic cancer.

1. Introduction

Pancreatic cancer is one of the most lethal and aggressive malignancies, leading the sixth cause of cancer-related mortality in China [1]. It has been reported that the five-year survival of pancreatic cancer patients is ranging between 2% and 6% [2]. Although there are prominent advances in chemotherapy or radiotherapy for decades, the overall prognosis of pancreatic cancer still remains poor [3]. Therefore, novel clinical strategies for pancreatic cancer treatment are in great need.

microRNAs are small, non-coding RNA molecules (containing about 22 nucleotides) functioning in RNA silencing and post-transcriptional regulation of gene expression. Recently, a number of microRNAs were found to be dysregulated in human malignancies, including lung cancer [4], breast cancer [5], colon cancer [6] and pancreatic cancer [7]. The miR-142 hairpin give rise to the guide-strand (miR-142-3p) and the sister passenger-strand (miR-142-5p), which play crucial role in many physiological process [8]. Recently, miR-142-5p was found to be aberrantly expressed in different pathological conditions, for instance, cancers [9,10] and inflammation [11]. Previous studies have confirmed that miR-142 was downregulated in various cancers [12,13]. In pancreatic cancer, patients with high miR-142-5p had significantly longer survival times than those with low miR-142-5p, indicating that miR-142-5p may function as a predictive marker concerning the prognosis of pancreatic cancer [14]. However, the underlying mechanisms of miR-142-5p in pancreatic cancer have not yet been fully demonstrated.

To determine whether or not miR-142-5p exerts its function as a tumor suppressor gene in pancreatic cancer, we first found that Ras-related protein Rap-1 A (RAP1A) was a putative target of miR-142-5p using TargetScan. As a family member of Ras-related proteins, RAP1A has been reported to be an important oncogene in various cancers [15,16]. Furthermore, RAP1 activation was demonstrated to increase metastasis of pancreatic carcinoma cells [17]. However, the interaction between miR-142-5p and RAP1A concerning pancreatic cancer cell proliferation and apoptosis remains to be elucidated.

Here, we demonstrated that miR-142-5p could regulate the expression of RAP1A by binding to its 3′UTR. miR-142-5p was downregulated while RAP1A was upregulated in pancreatic cancer tumor tissues compared with para-carcinoma non-tumor tissues. Meanwhile, the expression of miR-142-5p was negatively correlated with RAP1A. Overexpression of miR-142-5p may inhibit pancreatic cancer cell

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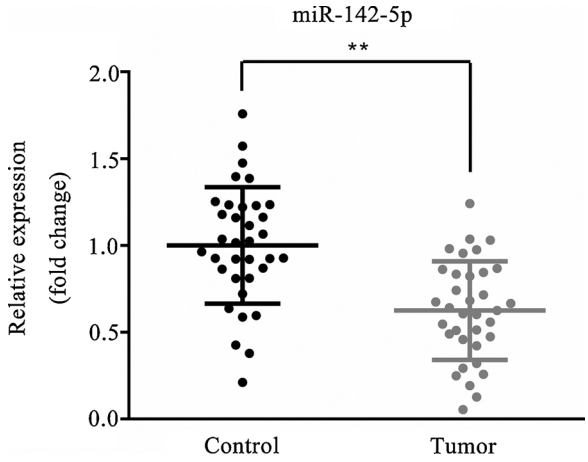

Fig. 1. miR-142-5p was downregulated in pancreatic cancer tumor tissues. The expression level of miR-142-5p in pancreatic cancer tumor and para-carcinoma non-tumor tissues.

proliferation but promote cell apoptosis by targeting RAP1A through MAPK signaling pathway, providing valuable insight into the molecular mechanisms underlying miR-142-5p-mediated biological functions in pancreatic cancer.

2. Materials and methods

2.1. Specimens and cell culture

35 paired of human pancreatic tumor tissues and para-carcinoma non-tumor tissues were obtained immediately after resection from patients in the Affiliated Huaian NO.1 People's Hospital of Nanjing Medical University who had undergone primary surgical treatment between December 2014 and April 2016. All the patients had not received any pre-operative chemotherapy, radiotherapy or immunotherapy. After surgical resection, the tissues were frozen in liquid nitrogen until use. Written consent was obtained from each patient and the protocol was approved by the Ethics Committee of the Affiliated Huaian NO.1 People's Hospital of Nanjing Medical University before our experiment.

The human pancreatic cancer cell line PANC-1 (BNCC100462) and human normal pancreatic duct epithelial cell line HPDE6-C7 (BNCC338285) were purchased from BeNa Culture Collection (Beijing, China) and maintained in Dulbecco's modified eagle's medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Thermo Fisher Scientific Scientific, Waltham, MA, USA) and 1% streptomycin-penicillin. Cell lines were cultivated and incubated in a humidified atmosphere with 5% CO_2 at 37°C.

2.2. Transfection

PANC-1 cells with the density of 1×10^4 cells/well were transfected with miR-142-5p mimic, nonrelative control (NC), pcDNA3.1 and pcDNA3.1-RAP1A using Lipofectamine®3000 reagent (Thermo Fisher Scientific, Waltham, MA, USA) under the manufacturer's instructions. miR-142-5p mimic and NC were purchased from GenePharma (Shanghai, China). In order to confirm the transfection efficiency of miR-142-5p, cells were divided into three different groups: i) control group, untransfected cells; ii) NC group, cells were transfected with NC; iii) miR-142-5p mimic group, cells were transfected with miR-142-5p mimic. The sequences of NC: 5′-GUGUAACACGUCUAUACGCCCA-3′ and the sequences of miR-142-5p mimic: 5′-CAUAAAGUAGAAAGCAC UACU-3′.

The RAP1A overexpression vector pcDNA3.1-RAP1A and pcDNA3.1 blank vector were produced by Chinese Academy of Sciences (Changchun, China). As to confirm the transfection efficiency of

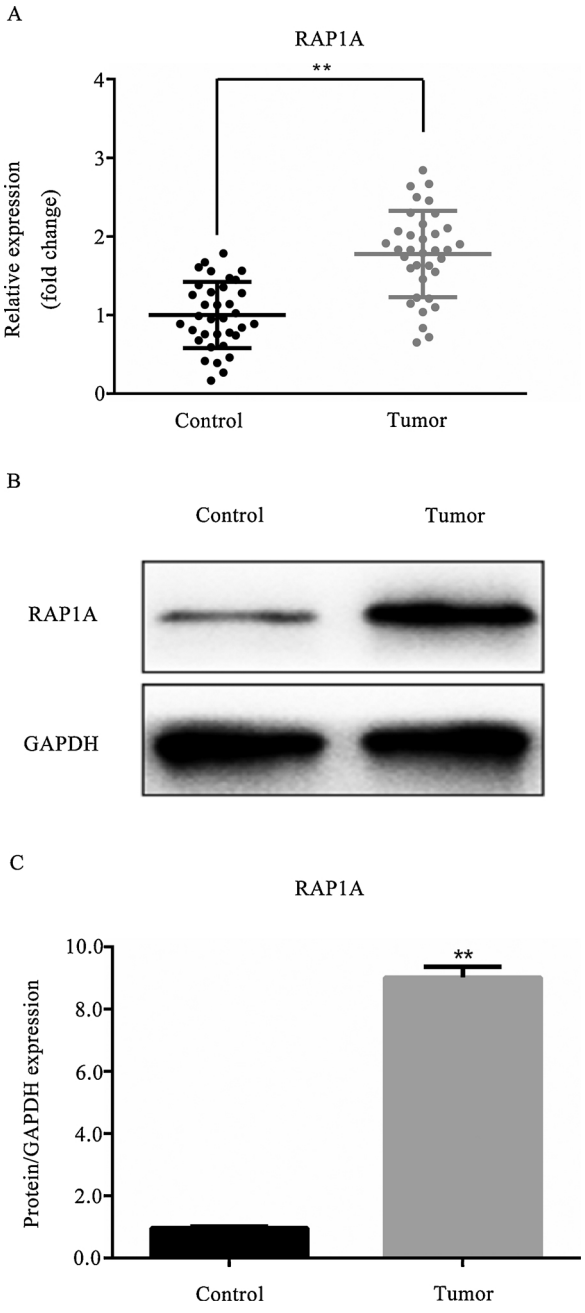

Fig. 2. RAP1 was upregulated in pancreatic cancer tumor tissues. (A) The mRNA expression level of RAP1A in pancreatic cancer tumor and para-carcinoma non-tumor tissues. (B) One of the protein expression level of RAP1A in pancreatic cancer tumor and para-carcinoma non-tumor tissues was presented. (C) The quantified protein expression levels of RAP1A in pancreatic cancer tumor and para-carcinoma non-tumor tissues (N = 3).

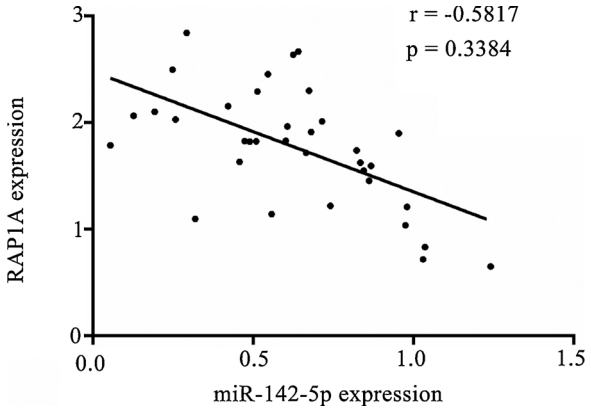

Fig. 3. miR-142-5p was negatively correlated with RAP1A.
Results of correlation analysis between the expression of miR-142-5p and the expression of RAP1A in patients with pancreatic cancer. RAP1A, Ras-related protein Rap-1A.

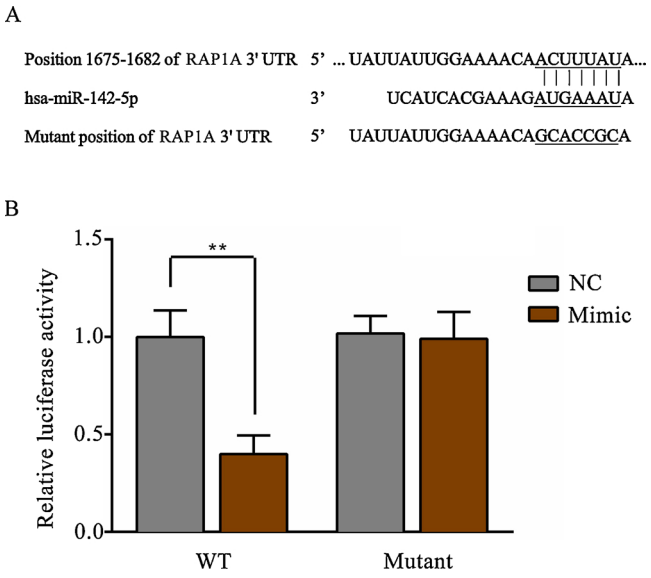

Fig. 4. miR-142-5p targets RAP1A.
(A) The predicted miR-142-5p binding site within the RAP1A 3′UTR and a mutated type of RAP1A were presented. (B) Luciferase reporter assay demonstrated that miR-142-5p bound to the wild-type but not mutant sequences within the 3′UTR of RAP1A.

RAP1A, cells were divided into three different groups: i) control group, untransfected cells; ii) pcDNA3.1 group, cells were transfected with pcDNA3.1; iii) pcDNA3.1-RAP1A, cells were transfected with pcDNA3.1-RAP1A.

2.3. Real-time RT-qPCR analysis

Total RNA was extracted from human tissues and cell lines using TRIzol® RNA Isolation Reagents (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Then, mRNA was reversed transcribed to cDNA using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's instructions. Afterward, RT-qPCR analysis was carried out in triplicate with the SYBR Green PCR Master Mix (Takara, Japan) according to the manufacturer's protocol. For miRNA detection, U6 was used to normalize expression. To detect the target genes, GAPDH was used to normalize expression. Primers sequences were listed below: miR-142-5p forward, 5′-CAUAAAGUAGAAAGCACUACU-3′ and reverse 5′-UAGUGCUUUCUACUUUAUGUU-3′; U6 forward, 5′-CTCGCTTCGGC AGAC-3′ and reverse 5′-AACGCTTACGAATTT-3′; RAP1A forward, 5′-CAAGCTAGTAGTCCTTGGTTCAG-3′ and reverse 5′-GGAATCTTCTA TCGTTGGGTCAT-3′; GAPDH forward, 5′-AACAGCAACTCCCACTC TTC-3′ and reverse 5′−CCTGTTGCTGTAGCCGTATT-3′. The cycling conditions were as follows: initial denaturation for 10 min at 95℃, followed by 40 thermal cycles of 95℃ for 10 s, 55℃ for 10 s and 72℃ for 30 s. Finally extension at 72℃ for 10 min. Data analysis was performed using $2^{-\triangle\triangle Ct}$ method.

2.4. Cell viability

After transfection, cells were resuspended and reseeded into 96-well plates at the density of 3×10^3 cells/well. At 0, 24, 48 h, 10 μl cell-counting kit 8 (CCK-8; Dojindo, Kumamoto, Japan) solution was added into each well and incubated at 37℃ for 2 h in a humidified atmosphere. Finally, the absorbance was measured at 450 nm using a microplate reader (Bio-Rad Laboratories Inc., Hercules, CA, USA).

2.5. Cell apoptosis

At 48 h post-transfection, 2×10^5 cells were collected and stained in the dark with 5 μl of Annexin V FITC and 5 μl of propidium iodide diluted 100 μl binding buffer (Annexin V fluorescein isothiocyanate apoptosis detection kit; Beyotime Institue of Biotechnology). After incubated for another 20 min, the percentage of apoptosis cells were detected by flow cytometry (FACScan; BD Biosciences, San Jose, CA, USA).

2.6. Western blotting analysis

The total protein of the tissues and cells were extracted using a RIPA buffer (Beyotime Institute of Biotechnology) containing protease inhibitor cocktail (Pierce Biotechnology, Inc., Beijing, China). Then, the concentration of protein was measured using bicinchoninic acid protein assay (BCA; Vazyme, Nanjing, China). Afterward, the protein was loaded onto 10% SDS-PAGE gels and transferred to polyvinylidene difluoride membranes (PVDF; Sigma-Aldrich CO., St Louis MO, USA). The membranes were then blocked with 5% nonfat milk diluted in Tris-buffered saline containing 0.1%Tween-20 (TBST; T1081-500; Solarbio, Beijing, China) for 2 h at room temperature followed by incubated with primary antibodies at 4℃ overnight: rabbit anti RAP1A (1:1000; ab115776; Abcam, Cambridge, MA, USA); rabbit anti p-ERK1/2 (1:1000; ab214362; Abcam, Cambridge, MA, USA); rabbit anti ERK1/2 (1:1000; ab17942; Abcam, Cambridge, MA, USA); rabbit anti p38 (1:1000; ab170099; Abcam, Cambridge, MA, USA); rabbit anti p-p38 (1:1000; ab4822; Abcam, Cambridge, MA, USA) and rabbit anti GAPDH (1:2500; ab9485; Abcam, Cambridge, MA, USA). After washing with TBST for three times, goat anti-rabbit secondary antibody IgG H&L (1:1000; ab6940; Abcam, Cambridge, MA, USA) was incubated with the membranes at room temperature for 2 h. Finally, an enhanced chemiluminescence reagent (ECL; Pierce, Rockford, IL, USA) was applied to visualize the protein bands while ImageJ software (version 1.49; National Institues of Health, Bethesda, MD, USA) was used to semi-quantify the ratio of the grey value of target proteins. GAPDH functioned as an internal control to ensure the equal protein loading.

2.7. miRNA target prediction

TargetScan (http://www.targetscan.org) was used to predict the

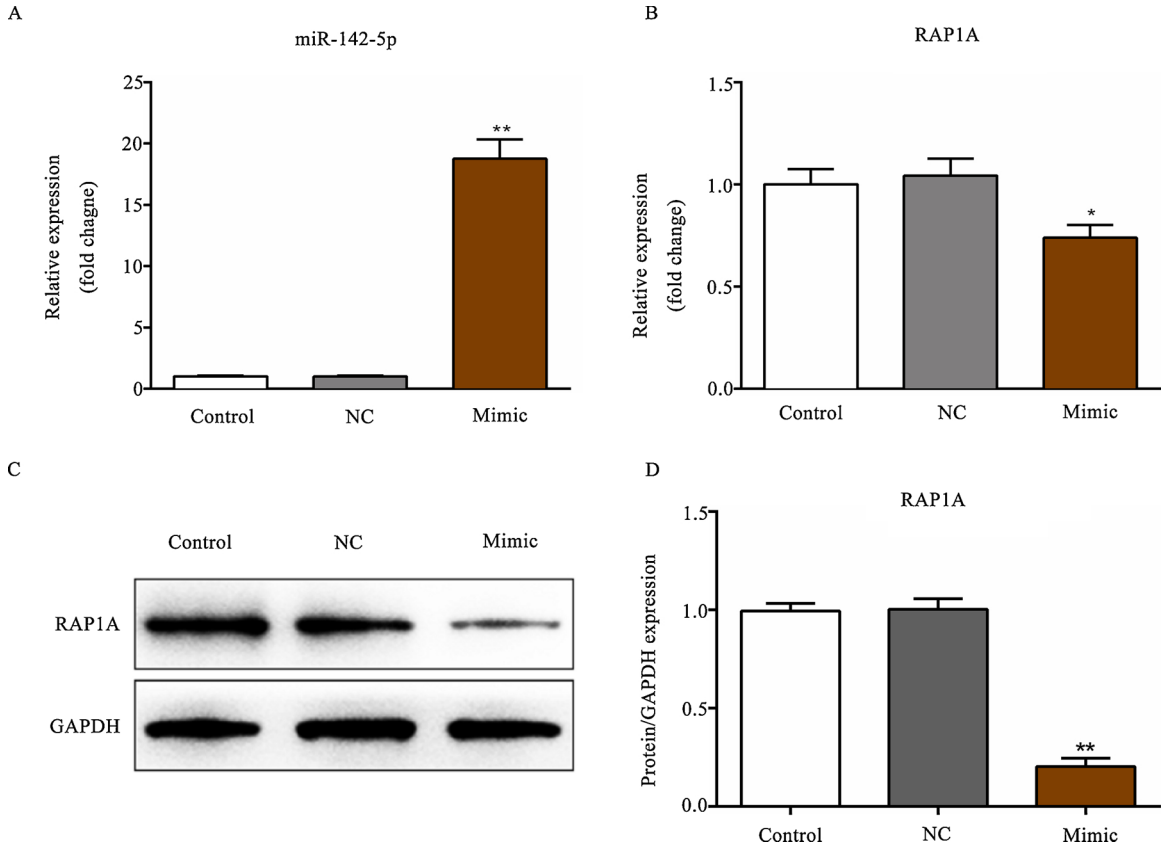

Fig. 5. Transfection efficiency of miR-142-5p.
(A) The expression level of miR-142-5p in different groups. (B) The mRNA expression level of RAP1A after transfected with miR-142-5p mimic. (C) One of the protein expression level of RAP1A after transfected with miR-142-5p mimic. (D) The protein expression level of RAP1A after transfected with miR-142-5p mimic (N = 3).

putative target and binding sequence of miR-142-5p.

2.8. Luciferase reporter assay

The luciferase reporter assay was carried out to determine whether there is an interaction between miR-142-5p and RAP1A. Briefly, the wild type or mutant of the RAP1A 3′UTR fragment containing the putative binding sequence was amplified from PCR. Then, the PCR product was cloned into the luciferase reporter plasmid vector pGL3-Basic (Biovector, Beijing, China). Afterward, HPDE6-C7 cells were co-transfected with the plasmid containing the mutant type or wild type of RAP1A 3′-UTR and miR-142-5p mimic or NC. The transfected cells were incubated for 48 h at 37°C in a humidified atmosphere and were analyzed for luciferase activity using the dual-luciferase reporter assay kit (Thermo Fisher Scientific, Waltham, MA, USA).

2.9. Statistical analysis

All the data were presented as the mean ± standard deviation in the present study. Statistical analysis was carried out using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Student's *t*-test was applied to distinguish differences between two groups, while one-way analysis of variance (ANOVA) followed with Newman-Keuls test was applied to analyze data among three or more groups. Pearson's analysis was used to evaluate the correlation between miR-142-5p and RAP1A. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. miR-142-5p is downregulated in pancreatic cancer tumor tissues

As demonstrated in Fig. 1, the expression level of miR-142-5p was significantly downregulated in pancreatic cancer tumor tissues, in contrast with para-carcinoma non-tumor tissues ($^{**}P < 0.01$, tumor vs control group).

3.2. RAP1A is upregulated in pancreatic cancer tumor tissues

As demonstrated in Fig. 2A, the mRNA expression level of RAP1A was significantly upregulated in pancreatic cancer tumor tissues, compared with para-carcinoma non-tumor tissues ($^{**}P < 0.01$, tumor vs control group). Meanwhile, the protein results in Fig. 2B and C presented the similar trend of variation as well ($^{**}P < 0.01$, tumor vs control group).

3.3. miR-142-5p negatively correlates with RAP1A

As demonstrated in Fig. 3, the expression of miR-142-5p was negatively correlated with the expression of RAP1A ($r = -0.5817$, $p = 0.3384$).

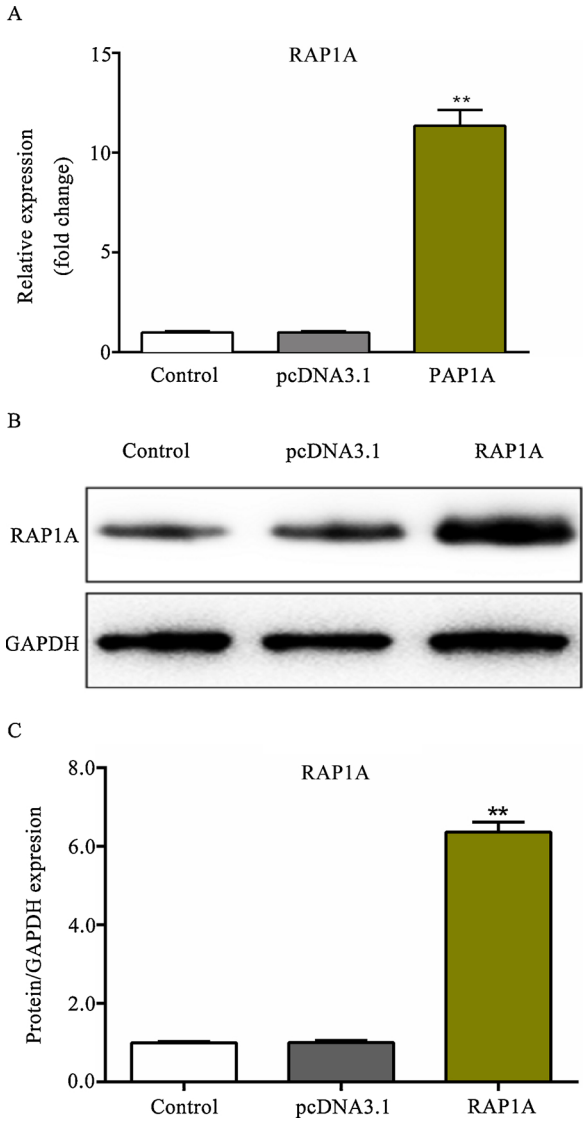

Fig. 6. Transfection efficiency of RAP1A.
(A) The mRNA expression level of RAP1A in different groups. (B) One of the protein expression level of RAP1A in different groups. (C) The protein expression level of RAP1A in different groups (N = 3).

3.4. RAP1A is a downstream target of miR-142-5p

As demonstrated in Fig. 4A, the 3′-UTR of RAP1A contained the binding sequences of miR-142-5p. Furthermore, the miR-142-5p mimic prominently decreased the luciferase activity in the WT group, in contrast with that in the NC group (Fig. 4B); whereas, there was no significant difference between in the mutant groups ($^{**}P < 0.01$, mimic vs NC group).

3.5. Transfection efficiency of miR-142-5p

As demonstrated in Fig. 5A, the expression level of miR-142-5p was markedly increased in miR-142-5p mimic group, as compared with NC group ($^{**}P < 0.01$, mimic vs NC group); while there was no variation between NC and control group.

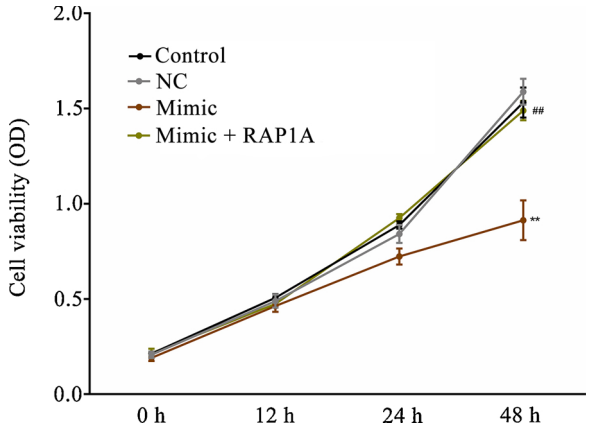

Fig. 7. miR-142-5p significantly decreases pancreatic cancer cell proliferation. Pancreatic cell viability in different groups was determined by CCK-8 assay.

Moreover, after transfection with miR-142-5p mimic and NC, the expression level of RAP1A was detected accordingly. As demonstrated in Fig. 5B, the mRNA expression level of RAP1A was markedly decreased in miR-142-5p mimic group, in contrast with NC group ($^{**}P < 0.01$, mimic vs NC group); while there was no significant difference between NC and control group. Meanwhile, the protein results in Fig. 5C and D presented the same trend of variation as well ($^{**}P < 0.01$, mimic vs NC group).

3.6. Transfection efficiency of RAP1A

After transfection with pc DNA3.1 or pcDNA3.1-RAP1A, the mRNA expression levels of RAP1A was detected accordingly. As demonstrated in Fig. 6A, the expression level of RAP1A was prominently increased in the pcDNA3.1-RAP1A group, compared with pcDNA3.1 and control group ($^{**}P < 0.01$, RAP1A vs pcDNA3.1 group). Meanwhile, the protein results of RAP1A in Fig. 6B and C presented the similar trend of variation as well ($^{**}P < 0.01$, RAP1A vs pcDNA3.1 group).

3.7. miR-142-5p significantly decreases pancreatic cancer cell viability

As demonstrated in Fig. 7, cell viability was reduced in miR-142-5p mimic group compared with NC and control group, whereas there was no significant difference between NC and control group ($^{**}P < 0.01$, mimic vs NC group). Meanwhile, the variation of cell viability induced by transfected with miR-142-5p mimic could be revered by co-transfected with pcDNA3.1-RAP1A ($^{\#\#}P < 0.01$, mimic + RAP1A vs mimic group).

3.8. miR-142-5p significantly promotes pancreatic cancer cell apoptosis

As demonstrated in Fig. 8A–E, cell apoptosis was increased in miR-142-5p mimic group compared with NC and control group, whereas there was no significant difference between NC and control group ($^{**}P < 0.01$, mimic vs NC group). Meanwhile, the variation of cell apoptosis induced by transfected with miR-142-5p mimic could be reversed by co-transfected with pcDNA3.1-RAP1A ($^{\#\#}P < 0.01$, mimic + RAP1A vs mimic group).

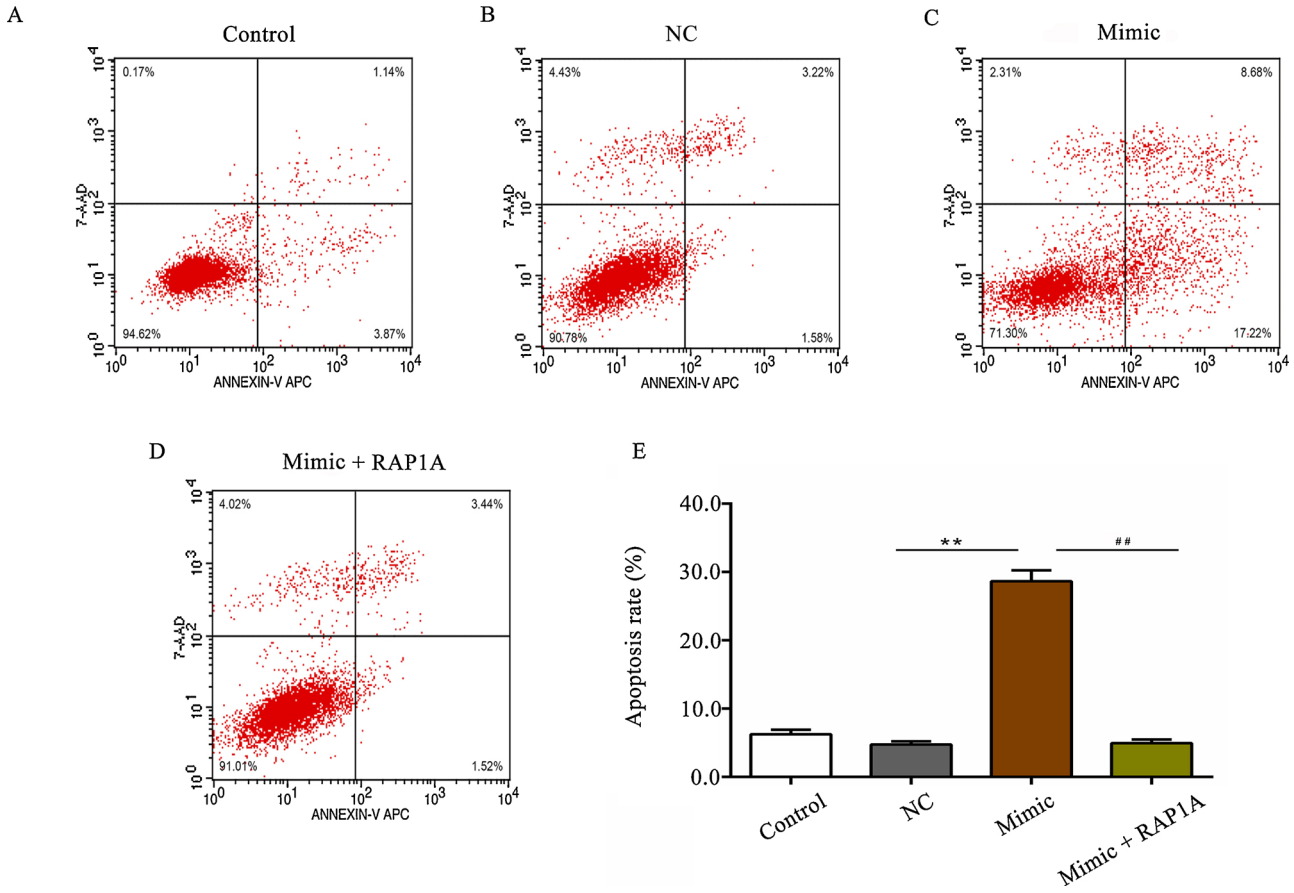

Fig. 8. miR-142-5p significantly increases pancreatic cancer cell apoptosis.
(A) Control group; (B) NC group; (C) Mimic group; (D) Mimic + RAP1A group; (E) The corresponding results of A–D were presented.

3.9. miR-142-5p regulates pancreatic cancer cell proliferation and apoptosis through MAPK signaling pathway

As demonstrated in Fig. 9A and B, transfection with miR-142-5p mimic could inhibit p-ERK1/2, phosphate p38 mitogen-activated protein kinases (p-p38); however, the variation induced by miR-142-5p mimic could be reversed by co-transfected with pcDNA3.1-RAP1A ($^{**}P < 0.01$, mimic vs NC group; $^{\#\#}P < 0.01$, mimic + RAP1A vs mimic group). Collectively, miR-142-5p could regulate cell proliferation and apoptosis by inhibition of MAPK signaling pathway.

4. Discussion

microRNAs are small, non-coding RNA molecules involved in regulating cell proliferation, apoptosis, differentiation, invasion and migration in cancer progression [18–20]. Accumulating evidence has suggested that microRNAs act either as oncogenes or tumor suppressor genes in pancreatic cancer [21,22]. A previous study revealed that miR-142-5p overexpression may inhibit pancreatic cancer growth [23]. Consistent with the previous study, in this study, our results revealed that miR-142-5p was downregulated in pancreatic cancer tumor tissues. Moreover, miR-142-5p overexpression was demonstrated to inhibit pancreatic cancer cell proliferation but promote apoptosis, indicating that miR-142-5p functioned as a tumor suppressor gene in pancreatic cancer. As known, microRNAs regulate gene expressions by degradation of target mRNAs or suppressing of their translation. A previous study demonstrated that RAP1A was a target gene of miR-142-5p involved in neurotrophin-mediated cell survival [24]. Our study demonstrated that miR-142-5p negatively regulated RAP1A expression by binding to the 3′UTR of RAP1A. After confirming that RAP1A was upregulated in pancreatic cancer tissues, the results also demonstrated that RAP1A could reverse the inhibition of pancreatic cell proliferation and increased apoptosis induced by miR-142-5p mimic. Thus, miR-142-5p may inhibit pancreatic cancer cell proliferation but promote apoptosis by targeting RAP1A, which may have potential therapeutic implications concerning clinical treatment.

ERK1/2 and p38 belonging to of MAPK signaling pathway, are important cellular protein kinases. Previous studies have reported that MAPK signaling pathway is involved in regulating cell proliferation and apoptosis [25–27]. The activation of ERK1/2 and p38 through phosphorylated forms in the regulation of cell growth and apoptosis [28,29]. In pancreatic cancer, the suppression of MAPK signaling pathway may inhibit cell growth [30]. Meanwhile, a previous study has demonstrated that overexpression of RAP1A is involved in regulating MAPK signaling pathway by activation of p-ERK1/2 and p-p38 [31]. In the present study, our data demonstrated that miR-142-5p inhibited the expression levels of RAP1A, p-ERK1/2 and p-p38 in pancreatic cancer cells.

In conclusion, our study demonstrates that miR-142-5p inhibits pancreatic cancer cell proliferation but promotes apoptosis by targeting oncogene RAP1A through the MAPK signaling pathway, indicating that targeting miR-142-5p may provide a novel strategy for the treatment of pancreatic cancer.

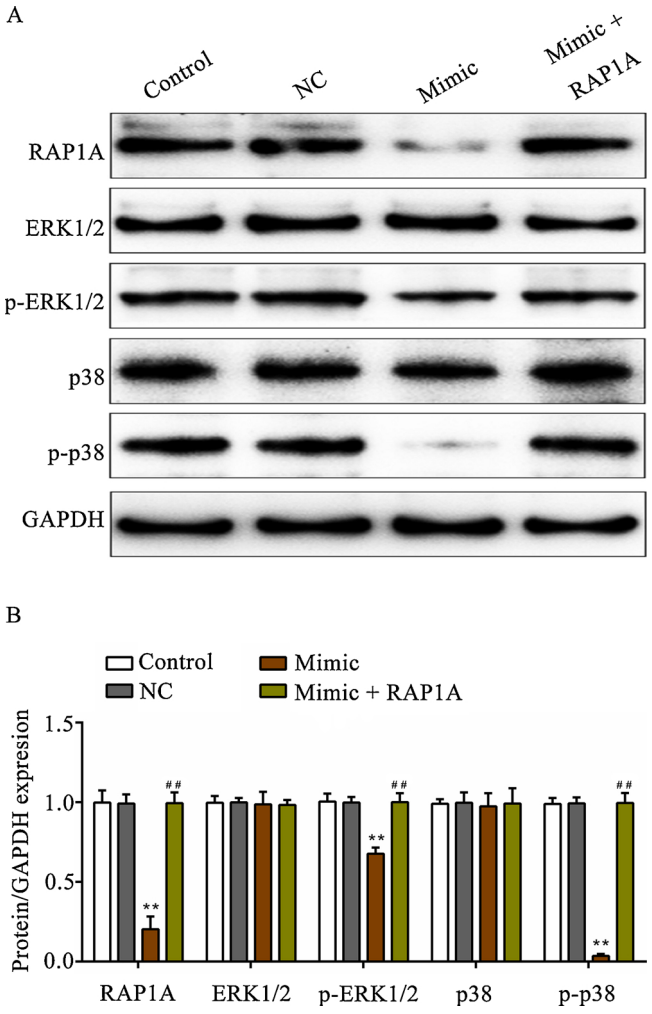

Fig. 9. miR-142-5p regulates pancreatic cancer cell proliferation and apoptosis through MAPK signaling pathway.
(A) The protein expression levels of related-factors in different groups. (B) The quantified result of A was presented.

Conflict of interest

None.

Disclosure

None.

Availability of materials and data

They are available on special request.

References

[1] X. Chen, S. Ma, Z. Zhang, Analysis of the clinical characteristics of pancreatic carcinoma in northern China, Pancreas 39 (2010) 1116–1118, https://doi.org/10.1097/mpa.0b013e3181dbc5a6.
[2] R. Siegel, D. Naishadham, A. Jemal, Cancer statistics, 2013, CA Cancer J. Clin. 63 (2013) 11–30, https://doi.org/10.3322/caac.21166.
[3] A. Vincent, J. Herman, R. Schulick, R.H. Hruban, M. Goggins, Pancreatic cancer, Lancet 378 (2011) 607–620, https://doi.org/10.1016/S0140-6736(10)62307-0.
[4] H. Li, T. Yang, D. Shang, Z. Sun, miR-1254 promotes lung cancer cell proliferation by targeting SFRP1, Biomed. Pharmacother. 92 (2017) 913–918, https://doi.org/10.1016/j.biopha.2017.05.116.
[5] Z.D. Lv, H.N. Xin, Z.C. Yang, W.J. Wang, J.J. Dong, L.Y. Jin, F.N. Li, miR-135b promotes proliferation and metastasis by targeting APC in triple-negative breast cancer, J. Cell. Physiol. 9 (2019), https://doi.org/10.1002/jcp.27906.
[6] Z.Y. Feng, X.H. Xu, D.Z. Cen, C.Y. Luo, S.B. Wu, miR-590-3p promotes colon cancer cell proliferation via Wnt/β-catenin signaling pathway by inhibiting WIF1 and DKK1, Eur. Rev. Med. Pharmacol. Sci. 21 (2017) 4844–4852.
[7] H. Yan, Q. Li, J. Wu, W. Hu, J. Jiang, L. Shi, X. Yang, D. Zhu, M. Ji, C. Wu, miR-629 promotes human pancreatic cancer progression by targeting FOXO3, Cell Death Dis. 8 (2017) e3154, https://doi.org/10.1038/cddis.2017.525.
[8] A. Shrestha, R.T. Mukhametshina, S. Taqhizadeh, E. Vasquez-Pacheco, H. Cabrera-Fuentes, A. Rizvanov, B. Mari, G. Carraro, S. Bellusci, microRNA-142 is a multi-faceted regulator in organogenesis, homeostasis, and disease, Dev. Dyn. 246 (2017) 285–290, https://doi.org/10.1002/dvdy.24477.
[9] F.H. Tsang, S.L. Au, L. Wei, D.H. Fan, J.M. Lee, C.C. Wong, I.O. Ng, C.M. Wong, MicroRNA-142-3p and microRNA-142-5p are downregulated in hepatocellular carcinoma and exhibit synergistic effects on cell motility, Front. Med. 9 (2015) 331–343, https://doi.org/10.1007/s11684-015-0409-8.
[10] Y.H. Su, Z. Zhou, K.P. Yang, X.G. Wang, Y. Zhu, X.E. Fa, miR-142-5p and miR-9 may be involved in squamous lung cancer by regulating cell cycle related genes, Eur. Rev. Med. Pharmacol. Sci. 17 (2013) 3213–3220.
[11] (a) F. Talebi, S. Ghorbani, W.F. Chan, R. Boqhozian, F. Masoumi, S. Ghasemi, M. Vojgani, C. Power, F. Noorbakhsh, microRNA-142 regulates inflammation and T cell differentiation in an animal model of multiple sclerosis, J. Neuroinflammation 14 (2017) 55, https://doi.org/10.1186/s12974-017-0832-7;
(b) Z. Wang, Z. Liu, X. Fang, H. Yang, miR-142-5p suppresses tumorigenesis by targeting PIK3CA in non-small cell lung cancer, Cell. Physiol. Biochem. 43 (2017) 2505–2515, https://doi.org/10.1159/000484459.
[12] W. Xu, W. Wang, microRNA-142-5p modulates breast cancer cell proliferation and apoptosis by targeting phosphatase and tensin homolog, Mol Mel Rep 17 (2018) 7529–7536, https://doi.org/10.3892/mmr.2018.8812.
[13] Y. Yan, B. Yang, S. Lin, R. Xing, Y. Lu, Downregulation of miR-142-5p promotes tumor metastasis through directly regulating CYR61 expression in gastric cancer, Gastric Cancer 3 (2018), https://doi.org/10.1007/s10120-018-0872-4.
[14] K. Ohuchida, K. Mizumoto, T. Kawashima, H. Fujita, T. Moriyama, T. Ohtsuka, J. Ueda, E. Nagai, M. Hashizume, M. Tanaka, microRNA expression as a predictive marker for gemcitabine response after surgical resection of pancreatic cancer, Ann. Surg. Oncol. 18 (2011) 2381–2387, https://doi.org/10.1245/s10434-011-1602-x.
[15] L. Du, M.C. Subauste, C. DeSevo, Z. Zhao, M. Baker, R. Borkowski, J.J. Schageman, R. Greer, C.R. Yang, M. Suraokar, et al., miR-337-3p and its targets STAT3 and RAP1A modulate taxane sensitivity in non-small cell lung cancers, PLoS One 7 (2012) e39167, , https://doi.org/10.1371/journal.pone.0039167.
[16] J. Xiang, C. Bian, H. Wang, S. Huang, D. Wu, miR-203 down-regulates RAP1A and suppresses cell proliferation, adhesion and invasion in prostate cancer, J. Exp. Clin. Cancer Res. 34 (2015) 8, https://doi.org/10.1186/s13046-015-0125-x.
[17] M. Huang, S. Anand, E.A. Murphy, J.S. Desgrosellier, D.G. Stupack, S.J. Shattil, D.D. Schlaepfer, D.A. Cheresh, EGFR-dependent pancreatic carcinoma cell metastasis through RAP1 activation, Oncogene 31 (2012) 2783–2793, https://doi.org/10.1038/onc.2011.450.
[18] B. Zhang, J. Yi, C.L. Zhang, Q.H. Zhang, J.F. Xu, H.Q. Shen, D.W. Ge, miR-146a inhibits proliferation and induces apoptosis in murine osteoblastic MC3T3-E1 by regulating Bcl2, Eur. Rev. Med. Pharmacol. Sci. 21 (2017) 3754–3762.
[19] B. Li, X. Wang, I.Y. Choi, Y.C. Wang, S. Liu, A.T. Pham, H. Moon, D.J. Smith, D.S. Rao, M.P. Boldin, L. Yang, miR-146a modulates autoreactive Th17 cell differentiation and regulates organ-specific autoimmunity, J. Clin. Invest. 127 (2017) 3702–3716, https://doi.org/10.1172/JCI94012.
[20] D. Yang, G. Du, A. Xu, X. Xi, D. Li, Expression of miR-149-3p inhibits proliferation, migration and invasion of bladder cancer by targeting S100A4, Am. J. Cancer Res. 7 (2017) 2209–2219, https://doi.org/10.1136/jim-2017-000598.
[21] H. Diao, Z. Ye, R. Qin, miR-23a acts as an oncogene in pancreatic carcinoma by targeting FOXP2, J. Investig. Med. 66 (2018) 676–683, https://doi.org/10.1136/jim-2017-000598.
[22] X. Wu, W. Chen, H. Cai, J. Hu, B. Wu, Y. Jiang, X. Chen, D. Sun, Y. An, miR-216b inhibits pancreatic cancer cell progression and promotes apoptosis by down-regulating KRAS, Arch. Med. Sci. 14 (2018) 1321–1332, https://doi.org/10.5114/aoms.2018.72564.
[23] L. Jia, Q. Xi, H. Wang, Z. Zhang, H. Liu, Y. Cheng, X. Guo, J. Zhang, Q. Zhang, L. Zhang, et al., miR-142-5p regulates tumor cell PD-L1 expression and enhances anti-tumor immunity, Biochem. Biophys. Res. Commun. 488 (2017) 425–431, https://doi.org/10.1016/j.bbrc.2017.05.074.
[24] M. Colitti, S. Sgorlon, D. Licastro, B. Stefanon, Differential expression of miRNAs in milk exosomes of cows subjected to group relocation, Res. Vet. Sci. 122 (2019) 148–155, https://doi.org/10.1016/j.rvsc.2018.11.024.
[25] Y. Sun, W.Z. Liu, T. Liu, X. Feng, N. Yang, H.F. Zhou, Signaling pathway of MAPK/ERK in cell proliferation, differentiation, migration, senescence and apoptosis, J. Recept. Signal Transduct. Res. 35 (2015) 600–604, https://doi.org/10.3109/10799893.2015.1030412.
[26] Y. Wang, X. Wang, X. Su, T. Liu, HIF-2α affects proliferation and apoptosis of MG-53 osteosarcoma cells through MAPK signaling, Mol. Med. Rep. 15 (2017) 2174–2178, https://doi.org/10.3892/mmr.2017.6243.
[27] J. Yang, J.N. Zhang, W.L. Chen, G.S. Wang, Q. Mao, S.Q. Li, W.H. Xiong, Y.Y. LiN, J.W. Ge, et al., Effects of AQP5 gene silencing on proliferation, migration and

apoptosis of human glioma cells through regulating EGFR/ERK/p38 MAPK signaling pathway, Oncotarget 8 (2017) 38444–38455, https://doi.org/10.18632/oncotarget.16461.
[28] D.N. Dhanasekaran, G.L. Johnson, MAPKs: function, regulation, role in cancer and therapeutic targeting, Oncogene 26 (2007) 3097–3099, https://doi.org/10.1038/sj.onc.1210395.
[29] J. Gupta, I. del Barco Barrantes, A. Igea, S. Sakellarious, I.S. Pateras, V.G. Gorgoulis, A.R. Nebreda, Dual function of p38α MAPK in colon cancer: suppression of colitis-associated tumor initiation but requirement for cancer cell survival, Cancer Cell 25 (2014) 484–500, https://doi.org/10.1016/j.ccr.2014.02.019.
[30] J. Zhou, S.D. Song, S.B. He, X.G. Zhu, Y. Zhang, B. Yi, B. Zhang, G.Z. Qin, D.C. Li, microRNA-375 targets PDK1 in pancreatic carcinoma and suppresses cell growth through the Akt signaling pathway, Int. J. Mol. Med. 28 (2014) 950–956, https://doi.org/10.3892/ijmm.2014.1638.
[31] T. Zhang, K.F. Jiang, X.Y. Zhu, G. Zhao, H.C. Wu, G.Z. Deng, C.W. Qiu, miR-433 inhibits breast cancer cell growth via the MAPK signaling pathway by targeting RAP1A, Int. J. Biol. Sci. 14 (2018) 622–632, https://doi.org/10.7150/ijbs.24223.