Department of Thoracic Surgery[1], Shandong University; Department of Thoracic Surgery[2], The Fourth People's Hospital of Jinan; Department of Thoracic Surgery[2], Qilu Hospital of Shandong University; Department of General Surgery[3], The Fourth People's Hospital of Jinan, Jinan, China

MicroRNA-4286 promotes esophageal carcinoma development by targeting INPP4A to evoke the JAK2/STAT3 pathway activation

Meng Zhang[1,2], Hui Tian[3,*], Rongrong Li[4], Wenlong Yan[2], Rongxue Xu[2]

Received December 22, 2017, accepted January 19, 2018

[*]Corresponding author: Hui Tian, Department of Thoracic Surgery, Qilu Hospital of Shandong University, NO.107 Jinan Culture West Road, Jinan, Shandong 250012, China
huitian787@sina.com

Pharmazie 73: 342–348 (2018) *doi: 10.1691/ph.2018.7339*

This study aimed to investigate the key roles of miR-4286 in regulating the development of esophageal carcinoma, as well as its regulatory mechanism. The expression of miR-4286 in esophageal carcinoma tissues and cells was determined. Effects of abnormal expression of miR-4286 cell viability, apoptosis, migration and invasion were further investigated. Furthermore, the potential target of miR-4286 was explored, and the regulatory relationship between miR-4286 and Janus tyrosine kinase/signal transducer and activator of transcription 3 (JAK2/STAT3) pathway was elucidated. The results showed that miR-4286 was highly expressed in esophageal carcinoma tissues and cells. Overexpression of miR-4286 significantly promoted cell viability, migration and invasion. In addition, Inositol Polyphosphate 4-Phosphatase Type I Gene (INPP4A) was a target of miR-4286. The effects of miR-4286 inhibitor on cell proliferation, migration, invasion and apoptosis were significantly counteracted by knockdown of INPP4A concurrently. Furthermore, inhibition of miR-4286 suppressed the activation of JAK2/STAT3 pathway, which was reversed after miR-4286 inhibition and INPP4A knockdown at the same time. Our findings reveal that highly expression of miR-4286 may promote the development of esophageal carcinoma by targeting INPP4A to evoke the JAK2/STAT3 pathway activation. miR-4286 may serve as a promising target for esophageal carcinoma treatment.

1. Introduction

Esophageal carcinoma is a frequent malignant tumor occurring at the upper gastrointestinal tract (Holmes and Vaughan 2007; Siegel et al. 2014), in which esophageal squamous cell carcinoma (ESCC) is the predominant type (Enzinger and Mayer 2003; Jemal et al. 2011). Although great advances have been made in surgical techniques and therapies, the prognosis of patients remains poor with only 20% of overall five-year survival rate (Enzinger and Mayer 2003; Rustgi and El-Serag 2014; Siegel et al. 2012). Therefore, it is still urgent to further elucidate key mechanisms underlying esophageal cancer.

MicroRNAs (miRNAs) are endogenous small non-coding RNAs, whose dysregulation has been implicated in numerous types of cancers via negatively regulating their target genes (Abba et al. 2017; Adams et al. 2014; Bartel 2009; Trang et al. 2017). In esophageal carcinoma, several miRNAs have been identified as key regulators to mediate cancer development. For instance, miR-9 is found to be an activator to promote ESCC metastasis via repressing E-cadherin (Song et al. 2014). Downregulation of miR-21 can inhibit cell growth, invasion and promote cell apoptosis in esophageal carcinoma (Wang et al. 2013a). Upregulation of miR-429 is shown to suppress cell invasion and induce apoptosis in esophageal carcinoma (Wang et al. 2013b). Other miRNAs, such as miR-129-2 and miR-451 are also found to regulate the malignant behaviors of esophageal carcinoma cells (Kang et al. 2013; Zang et al. 2015). Therefore, identification of key miRNAs involved in esophageal carcinoma development has become a hot topic of research. Recently, miR-4286 inhibition has been found to have anti-proliferative and pro-apoptotic effects on melanoma cells (Komina et al. 2016). Moreover, a miRNA expression profiling analysis identifies upregulation of miR-4286 in metastatic cutaneous squamous cell carcinoma (Gillespie et al. 2016). Nevertheless, whether miR-4286 plays a key role in the progression of esophageal carcinoma has not been clarified.

To elucidate the crucial role and possible regulatory mechanism of miR-4286 in esophageal carcinoma, the expression of miR-4286 in esophageal carcinoma tissues and cells was determined. Effects of abnormal expression of miR-4286 cell viability, apoptosis, migration and invasion were further investigated. Besides, the potential target of miR-4286 was explored and the regulatory relationship between miR-4286 and Janus tyrosine kinase/signal transducer and activator of transcription 3 (JAK2/STAT3) pathway was elucidated. Our findings are expected to provide a theoretical basis for better designing a promising therapeutic approaches for this disease.

2. Investigations and results

2.1. miR-4286 expression was upregulated in esophageal cancer tissues and cells

To investigate whether miR-4286 play a key role in esophageal cancer, the expression of miR-4286 in esophageal cancer tissues and cell lines were detected. The results showed that miR-4286 expression in ESCC tissues was significantly higher than in non-tumor esophageal tissues ($P < 0.001$, Fig. 1A). Consistent with these results, miR-4286 expression was markedly up-regulated in ESCC (TE-1, HCE-4 and HCE-7) and adenocarcinoma cells (SKGT-4 and Bic-1) in comparison of that in normal esophageal epithelial HEEC cells ($P < 0.05$, Fig. 1B).

2.2. Abnormal expression of miR-4286 altered esophageal cancer cell viability and apoptosis

To further detect the role of miR-4286 in esophageal cancer, TE-1, HCE-7 and Bic-1 were then transfected with miR-4286 mimics, mimic NC, miR-4286 inhibitor, or inhibitor NC. Effects of abnormal expression of miR-4286 on cell viability and apop-

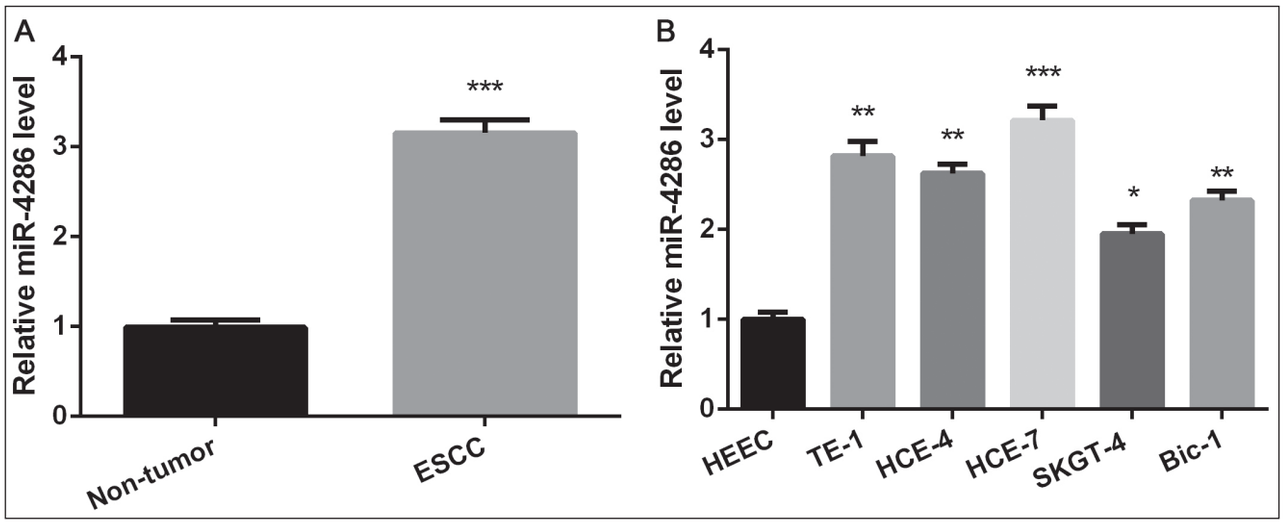

Fig. 1: Expression of miR-4286 in esophageal carcinoma tissues and cells. A: The expression of miR-4286 in esophageal squamous cell carcinoma (ESCC) tissues and non-tumor tissues. B: The expression of miR-4286 in ESCC cell lines (TE-1, HCE-4 and HCE-7), adenocarcinoma cell lines (SKGT-4 and Bic-1) and normal esophageal epithelial cell line HEEC. Error bars indicate means±SD. *, $p < 0.05$, **, $p < 0.01$ and ***, $p < 0.001$.

tosis were then detected. As shown in Fig. 2A, qRT-PCR analysis confirmed that miR-4286 was successfully overexpressed or knocked down in TE-1, HCE-7 and Bic-1 cells after transfection of miR-4286 mimics or miR-4286 inhibitor, respectively ($P < 0.05$). In addition, miR-4286 mimic significantly promoted the viabilities of TE-1, HCE-7 and Bic-1 cells ($P < 0.05$, Fig. 2B), but had no marked effects on apoptosis of these cells ($P > 0.05$, Fig. 2C). Reversely, miR-4286 inhibitor obviously inhibited the viabilities of TE-1, HCE-7 and Bic-1 cells ($P < 0.05$, Figure 2B) and induced the apoptosis of these cells ($P > 0.05$, Fig. 2C). Furthermore, the expression levels of apoptosis-related proteins in different transfected HCE-7 cells were determined. The results showed that miR-4286 inhibitor could inhibit Bcl-2 expression and increased the expression levels of Bax, cleaved-caspase-3 and cleaved-Caspase-9 (Fig. 2D).

2.3. Abnormal expression of miR-4286 altered cell migration and invasion

Effects of abnormal expression of miR-4286 on cell viability and apoptosis were then detected after different transfections. The results showed that miR-4286 mimic significantly promoted the migration and invasion of TE-1, HCE-7 and Bic-1 cells ($P < 0.05$, Fig. 3A and 3B), whereas miR-4286 inhibitor resulted in opposite effects but had no markedly effects on apoptosis of these cells ($P > 0.05$, Fig. 3A and 3B). Moreover, the expression levels of EMT markers in different transfected HCE-7 cells were detected to further explore the possible mechanism of cell migration and invasion. The results showed that miR-4286 mimic transfection resulted in the marked decrease of E-cadhein expression and obvious increase in the expressions of N-cadherin, vimentin, ZEB1, and snail were increased (Fig. 3C). The opposite expression changes of these EMT markers were obtained after miR-4286 inhibitor transfection (Fig. 3C).

2.4. INPP4A is a target of miR-4286

By means of the TargetScan (http://www.targetscan.org/cgi-bin/targetscan/vert_71/view_gene.cgi?rs=ENST00000409016.4&taxid=9606&members=miR-4286&showcnc=0&shownc=0&showncf1=1&showncf2=1&subset=1), we predicted the potential target of miR-4286 in this study to explore the regulatory mechanism of miR-4286 in esophageal cancer. As shown in Fig. 4A, INPP4A was predicted as a potential target of miR-4286. Moreover, dual luciferase activity assay confirmed that miR-4286 directly interacted with INPP4A 3'UTR in HCE-7 cells (Fig. 4B). In addition, miR-4286 mimic transfection increased both mRNA and protein expression levels of INPP4A in HCE-7 cells, while miR-4286 inhibitor transfection markedly decreased INPP4A expression ($P<0.05$, Fig. 4C and 4D). These data indicated that INPP4A expression was negatively regulated by miR-4286.

2.5. Knockdown of miR-4286 inhibited proliferation, migration and invasion and promoted apoptosis by upregulating INPP4A

To determine whether the effects of miR-4286 on cell behaviors by targeting INPP4A, HCE-7 cells were cotransfected with miR-4286 inhibitor and si-INPP4A. As shown in Fig. 5A, INPP4A expression was significantly down-regulated in HCE-7 cells after si-INPP4A transfection, which was markedly reversed by miR-4286 inhibitor after cotransfection with miR-4286 inhibitor and si-INPP4A ($P < 0.01$, Fig. 5A). Knockdown of INPP4A markedly increased cell proliferation, inhibited apoptosis and promoted migration and invasion ($P<0.05$, Figure 5B-E). Moreover, the effects of miR-4286 inhibitor on cell proliferation, migration, invasion and apoptosis were significantly counteracted by knockdown of INPP4A after cells were cotransfected with miR-4286 inhibitor and si-INPP4A ($P<0.05$, Fig. 5B-E). These data indicated that knockdown of miR-4286 inhibited proliferation, migration and invasion and promoted apoptosis possible by up-regulating INPP4A

2.6. miR-4286 activated JAK2/STAT3 pathway by regulation of INPP4A

It is reported that activation of the JAK2/STAT3 pathway is a key mechanism to mediate the progression of human esophageal carcinoma (You et al. 2012), thus, we investigated the relationship between miR-4286 and JAK2/STAT3 pathway to further explore the regulatory mechanism of miR-4286. As shown in Fig. 6A, the expression levels of JAK2 and STAT3 were significantly increased in HCE-7 cells after overexpression of miR-4286, while obviously decreased after miR-4286 inhibition. Furthermore, we found the expression levels of JAK2 and STAT3 were also significantly increased in HCE-7 cells after knockdown of INPP4A, which were reversed after cotransfection with miR-4286 inhibitor and si-INPP4A (Figure 6B). These data indicated that miR-4286 might activate JAK2/STAT3 pathway in esophageal carcinoma via regulation of INPP4A.

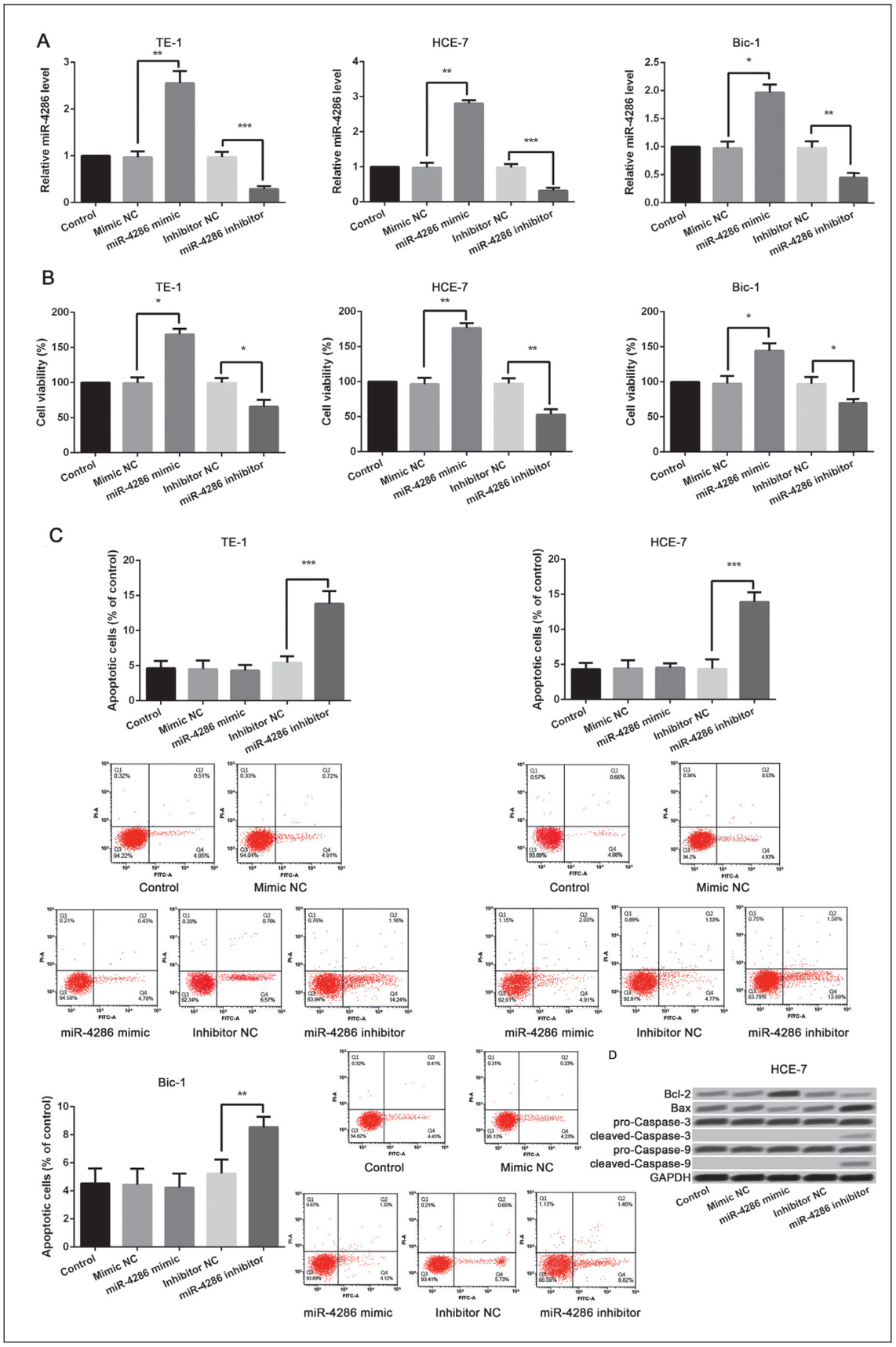

Fig. 2: Abnormal expression of miR-4286 altered esophageal cancer cell viability and apoptosis. TE-1, HCE-7 and Bic-1 were then transfected with miR-4286 mimics, mimic NC, miR-4286 inhibitor, or inhibitor NC. A: qRT-PCR analysis showed the expression of miR-4286 in TE-1, HCE-7 and Bic-1 cells after transfection. B: MTT assay showed the viabilities of TE-1, HCE-7 and Bic-1 cells after transfection. C: Flow cytometry showed the apoptosis of TE-1, HCE-7 and Bic-1 cells after transfection. D: Western blot showed the expression of apoptosis-related protein in HCE-7 cells after transfection. Error bars indicate means ± SD. *, $p < 0.05$, **, $p < 0.01$ and ***, $p < 0.001$.

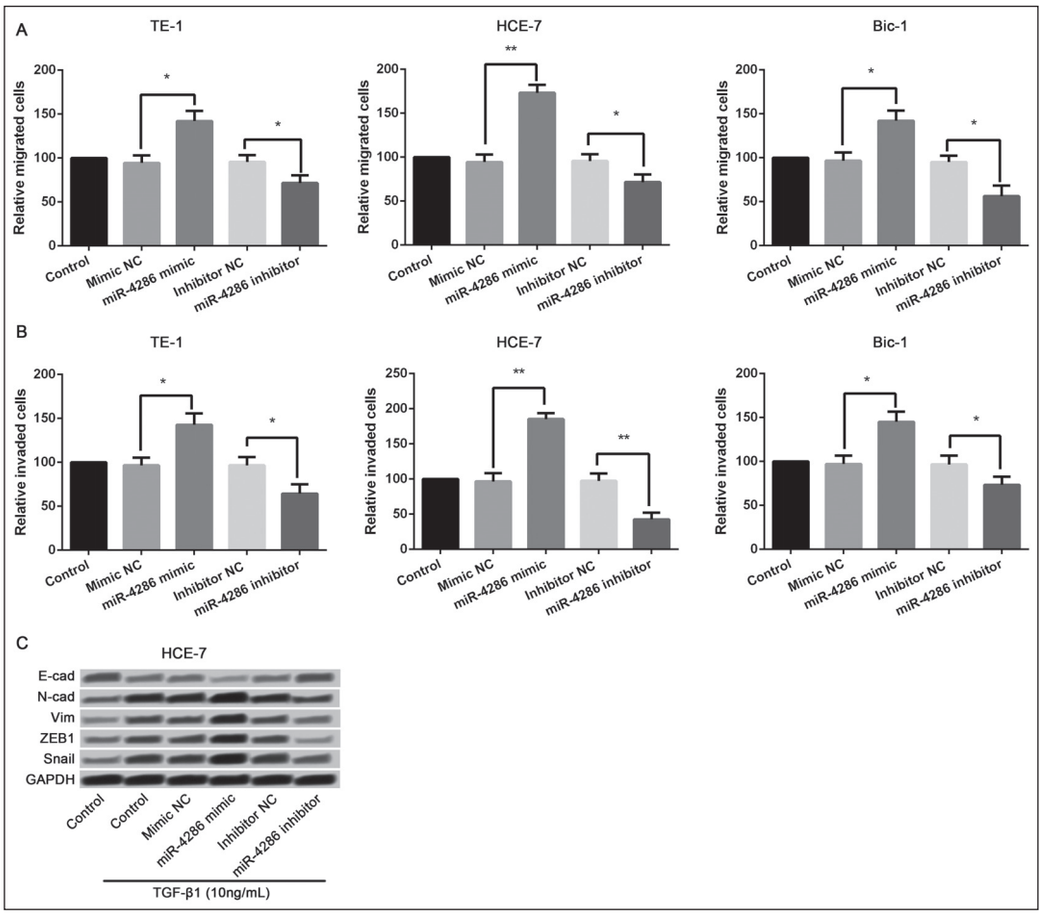

Fig. 3: Abnormal expression of miR-4286 altered esophageal cancer cell migration and invasion. TE-1, HCE-7 and Bic-1 were then transfected with miR-4286 mimics, mimic NC, miR-4286 inhibitor, or inhibitor NC. A: Transwell assay showed the migration of TE-1, HCE-7 and Bic-1 cells after transfection. B: Transwell assay showed the invasion of TE-1, HCE-7 and Bic-1 cells after transfection. C: Western blot showed the expression of epithelial-mesenchymal transition markers (including E-cadherin, N-cadherin, Vimentin, ZEB1, and snail) in HCE-7 cells after transfection. Error bars indicate means ± SD. *, $p < 0.05$, and **, $p < 0.01$.

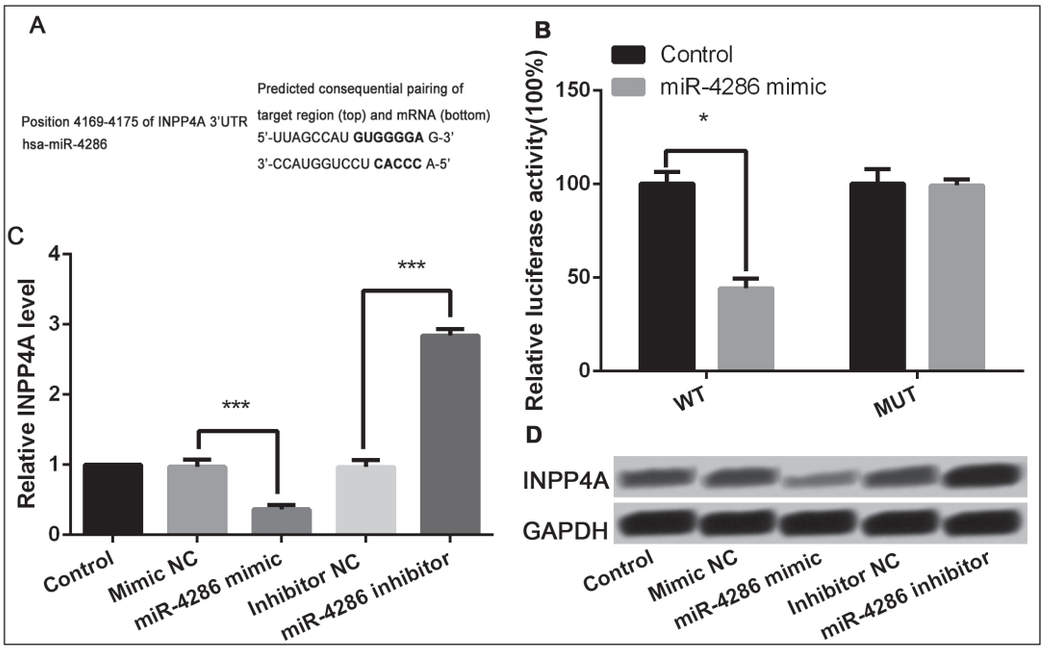

Fig. 4: INPP4A was the direct target of miR-4286. A: The predicted binding sequence of INPP4A and miR-4286 using TargetScan. B: Luciferase report assay showed that miR-4286 directly targeted the INPP4A 3'UTR. C: The mRNA expression of INPP4A in different transfection groups determined by qRT-PCR. D: The protein expression of IL6 in different transfection groups determined by western blot. Error bars indicate means ± SD. *, $p < 0.05$, and ***, $p < 0.001$.

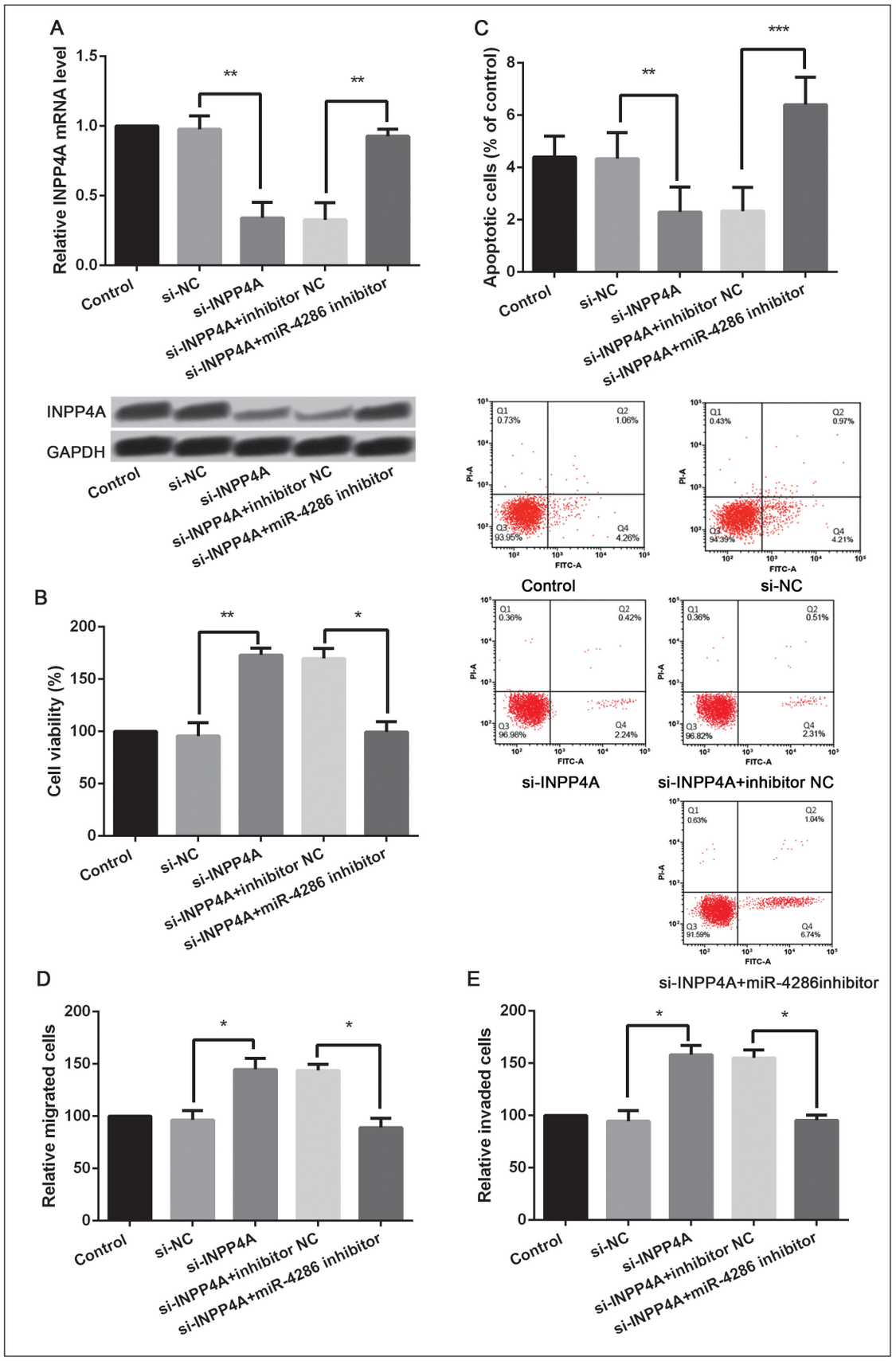

Fig. 5: Knockdown of miR-4286 inhibited proliferation, migration and invasion and promoted apoptosis by up-regulating INPP4A. HCE-7 cells were transfected with si-INPP4A, si-NC, inhibitor NC+si-INPP4A, and miR-4286 inhibitor+si-INPP4A. A: The mRNA expression of INPP4A in different transfection groups. B: MTT assay showed the cell viability in different transfected groups after transfection. C: Flow cytometry showed the apoptosis in different transfected groups after transfection. D: Transwell assay showed cell migration of different transfected groups after transfection. E: Transwell assay showed cell invasion of different transfected groups after transfection. Error bars indicate means±SD. *, $p < 0.05$, **, $p < 0.01$ and ***, $p < 0.001$.

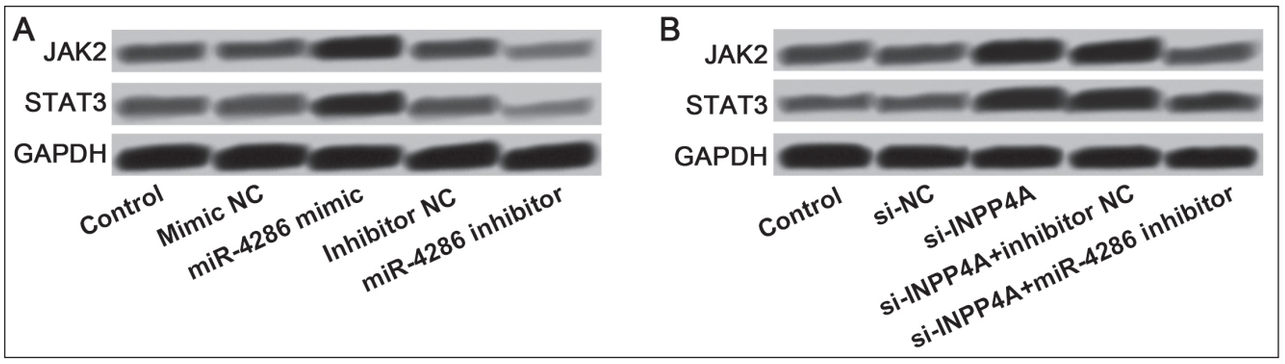

Fig. 6: Protein expression of JAK2 and STAT3 in different transfected groups. Error bars indicate means±SD. *, $p < 0.05$, **, $p < 0.01$ and ***, $p < 0.001$.

3. Discussion

The present study investigated the role of miR-4286 in esophageal carcinoma. The results showed that miR-4286 was highly expressed in esophageal carcinoma tissues and cells. Overexpression of miR-4286 significantly promoted cell viability, migration and invasion. In addition, INPP4A was a target of miR-4286. The effects of miR-4286 inhibitor on cell proliferation, migration, invasion and apoptosis were significantly counteracted by knockdown of INPP4A concurrently. Furthermore, inhibition of miR-4286 suppressed the activation of JAK2/STAT3 pathway, which was reversed after miR-4286 inhibition and INPP4A knockdown at the same time. These data suggest the possible regulatory mechanism of miR-4286 in esophageal cancer development and merit further discussion.

In esophageal cancer, several miRNAs have been found to play a key role in cancer development via regulating the expression of their target mRNAs (Li et al. 2017; Yang et al. 2014; Yuan et al. 2011). Intriguingly, INPP4A was identified as a direct target of miR-4286 in this study. INPP4A, a PtdIns(3,4)P2 phosphatase, can inhibit glutamate excitotoxicity in the central nervous system and subsequently protect neurons from excitotoxic cell death (Sasaki et al. 2010). Upregulation of miR-935 is found to regulate pancreatic carcinoma development through repressing INPP4A (Wang et al. 2016). Moreover, miR-940 contributes to the malignant development of bladder cancer possible via targeting INPP4A (Wang et al. 2018). Although the role of miR-4286 and INPP4A in esophageal cancer has not been fully investigated, we hypothesize that high expression of miR-4286 may promote esophageal cancer development via targeting INPP4A.

Furthermore, another important aspect of this study showed that inhibition of miR-4286 suppressed the activation of JAK2/STAT3 pathway in HCE-7 cells, which was reversed after miR-4286 inhibition and INPP4A knockdown at the same time. It has been reported that blockade of the JAK2/STAT3 pathway activation resulted in a reduced gastric cancer growth (Judd et al. 2014). Inhibiting the activation of JAK2/STAT3 signaling can induce cell apoptosis in colorectal cancer via the mitochondrial pathway (Du et al. 2012). Moreover, B7-H4, one of the costimulatory molecules of the B7 family, can promote ESCC cell proliferation by activation of interleukin-6/ STAT3pathway (Chen et al. 2016). Liu et al. (2016) demonstrated that miR-143 could suppress ESCC cell proliferation and invasion through targeting STAT3 . Importantly, a JAK2 inhibitor is shown to inhibit the inflammation and growth of ESCC via the JAK/STAT3 pathway (Fang et al. 2015). These findings imply the crucial effects of JAK/STAT pathway on mediating esophageal cancer development. Based on our results, we hypothesize that miR-4286 may promote esophageal carcinoma development via regulation of INPP4A to activate the JAK2/STAT3 pathway.

Thus, miR-4286 may serve as a promising target for esophageal carcinoma treatment.

4. Experimental

4.1. Patients

From March 2014 to Oct 2016, total 60 ESCC patients who underwent esophagectomy in our hospital were recruited. Primary ESCC tissues and adjacentnormal esophageal tissues were collected and their histological confirmation was performed by the pathologist. None of these patients had a history of chemotherapyor radiotherapy before the surgery. The clinicopathological features of all patients are shown in the Table. All patients were informed consent and this study was approved by the ethical committees of our hospital.

Table: Characteristics of ESCC patients enrolled in the study

Clinicopathologic characteristics		N of cases
Age (years)	<40	9
	40-60	41
	>60	16
Gender	Male	48
	Female	18
Grade of differentiation	Well	22
	Moderate	32
	Poorly	12
Degree of tumor invasion	Submucosa	3
	Muscularispropria	60
	Adventitia	3
Lymph node metastasis	Negative	56
	Positive	10

4.2. Cell lines and culture

The ESCC cell lines (TE-1, HCE-4 and HCE-7), adenocarcinoma cell lines (SKGT-4 and Bic-1) and normal esophageal epithelial cell line HEEC were purchased from American Tissue Culture Collection (ATCC, Rockville, MD, USA). These cells were grown in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum in a 37°C humidified incubator with 95% air and 5% CO_2.

4.3. Cell transfection

The miR-4286 mimics, inhibitor or scrambled oligonucleotides (negative mimic NC or inhibitor NC), small interference RNAs (siRNAs) targeting Inositol Polyphosphate 4-Phosphatase Type I Gene (INPP4A) (si-INPP4A) and its negative control siRNAs (si-NC) were designed and synthesized by Ribobio (Guangzhou, China). TE-1, HCE-7 and Bic-1 were then transfected with miR-4286 mimics, inhibitor, mimic NC or inhibitor NC using Lipofectamine 2000 reagent (Invitrogen) following the protocols provided by manufacturer. Moreover, TGF-β1 (10 ng/mL) was used to induce epithelial-mesenchymal transition (EMT).

4.4. Vector construction and dual-luciferase reporter assay

The 3'-UTRs of INPP4A containing miR-4286 binding sites were synthesized by Sangon Biotech (Shanghai, China) and then cloned into the downstream of the luciferase reporter in the pmirGLO Dual-Luciferase miRNA Target Expression Vector

(Promega, Madison, WI, USA). HCE-7 cells were seeded in a 24-well plate and then transfected with 100 ng of the constructed luciferase vector (pmirGLO) and 20 nM miR-4286 mimic or mimic control. At 48 h post-transfection, cells were harvested and their luciferase activity was measured with the Dual-Glo luciferase assay kit (Promega).

4.5. Real-time PCR

Trizol reagent (Invitrogen, USA) was used to extract total mRNA. After measuring concentration of isolated RNA by SMA 400 UV-VIS (Merinton, Shanghai, China), total RNA was used as the template for reverse-transcription into cDNA using a miScript II reverse transcription kit (Qiagen, USA). Real-time PCR was then used to detect the relative expression of target genes using a SYBR Green PCR kit (Applied Biosystem, USA). Fluorescent signals were quantified to determine CT values of all samples. Using GAPDH or U6 as the internal references, the relative expressions of mRNA and miRNA were respectively calculated with the $2^{-\Delta\Delta CT}$ method as previously described (Livak KJ 2001).

4.6. MTT assay

Log-phased cells (5×10^3) in different transfected groups were seeded into 96-well plates that were filled with DMEM medium containing 10% FBS. After being cultured for 24 h, 20 μL of sterile MTT reagents were added into each well to cultivate cells. Four hours later, supernatants were removed and 150 μL dimethylsulfoxide (DMSO) was added to culture cells until complete resolving of the crystal violet. Consequently, the absorbance value at 570 nm was measured by a microplate reader (Bio-Rad, USA) to assess cell viability of each well.

4.7. Apoptosis analysis

Cells in different transfected groups were trypsinized, washed with ice-cold PBS and suspended in annexin V-binding buffer. By Annexin V and propidium iodide (PI) dual labeling using the Annexin V-FITC kit (Biosea Biotechnology Co., Beijing, China), the apoptotic cells were detected and then analyzed with a FACS Calibur flow cytometer (Becton–Dickinson, San Jose, CA).

4.8. Transwell assay

Cell migration and invasion were evaluated using Transwell chambers (Hyclone, USA). For invasion detection, the transwell chamber was precoated with 50 mg/L Matrigel dilution (Sigma-Aldrich, Shanghai, China). After 48 h of post-transfection, cells were cultured in serum-free medium for 24 h. The transwell chamber was then put into a 24-well plate that had filled with 0.5 mL DMEM medium containing 10% FBS). The 100-μL cell suspension was added inside the chamber with serum-free medium. After 48h of incubation, the chamber was fixed in cold ethanol, followed by 30-min staining of crystal violet solution. The number of migrated or invaded cells that had translocated to the lower side of the membrane was counted under an inverted microscope.

4.9. Western blot

Cells that were cultured for 48 h after transfection were lysed with radioimmunoprecipitation (RIPA) buffer (Sangon Biotech). After brief sonication, protein extracts per cell lysate were then collected by being centrifuged at 10,000 g for 10 min at 4°C. The protein concentration was then determined with a BCA protein assay kit (Pierce, Rockford, IL). For western blot, protein extracts were subjected to a 10 % SDS-PAGE and then blotted onto polyvinylidene fluoride (PVDF, Millipore, Bedford, MA, USA) membranes. Followed by blockade with Tris-buffered saline-Tween 20 (TBST) containing 5% non-fat milk for 1 h, the membranes were incubated with first antibodies to Bcl-2, Bax, pro-caspase-3, cleaved caspase-3, pro-caspase-9, cleaved caspase-9, E-cadherin, N-cadherin, Vimentin, ZEB1, snail, INPP4A, JAK2, STAT3 and GAPDH with a 1:1,000 dilution at 4°C overnight. All these antibodies were purchased from Abcam (Cambridge, UK). GAPDH served as the internal control. After that, the membranes were incubated with horseradish peroxidase-labeled secondary antibody (1:1000 dilution) at 37 °C for 1 h. Using the enhanced chemiluminescence (ECL) method, the protein signals were detected after incubation with a chromogenic substrate.

4.10. Statistical analysis

All experiments mentioned above were carried out independently for three times and the data were presented as the mean±standard deviation (SD). Independent samplet test was used for assessing the significance of the paired data. Post hoc Tukey test was applied to analyze the difference among groups. Statistical analyses were conducted using Graph Prism 5.0 software (GraphPad Prism, San Diego, CA). $P < 0.05$ was considered statistically significant.

Conflicts of interest: None declared.

References

Abba ML, Patil N, Leupold JH, Moniuszko M, Utikal J, Niklinski J, Allgayer H (2017) MicroRNAs as novel targets and tools in cancer therapy. Cancer Lett 387: 84-94.

Adams BD, Kasinski AL, Slack FJ (2014) Aberrant regulation and function of microRNAs in cancer. Current Biol 24: R762-R776.

Bartel DP (2009) MicroRNAs: Target recognition and regulatory functions. Cell 136: 215-233.

Chen X, Wang L, Wang W, Zhao L, Shan B (2016) B7-H4 facilitates proliferation of esophageal squamous cell carcinoma cells through promoting interleukin-6/signal transducer and activator of transcription 3 pathway activation. Cancer Sci 107: 944-954.

Du W, Hong J, Wang YC, Zhang YJ, Wang P, Su WY, Lin YW, Lu R, Zou WP, Xiong H (2012) Inhibition of JAK2/STAT3 signalling induces colorectal cancer cell apoptosis via mitochondrial pathway. J Cell Mol Med 16: 1878-1888.

Enzinger PC, Mayer RJ (2003a) Esophageal cancer. New Engl J Med 349: 2241-2252.

Fang J, Chu L, Li C, Chen Y, Hu F, Zhang X, Zhao H, Liu Z, Xu Q (2015) JAK2 inhibitor blocks the inflammation and growth of esophageal squamous cell carcinoma in vitro through the JAK/STAT3 pathway. Oncolo Rep 33: 494-502.

Gillespie J, Skeeles LE, Allain DC, Kent MN, Peters SB, Nagarajan P, Yu L, Teknos TN, Olencki T, Toland AE (2016) MicroRNA expression profiling in metastatic cutaneous squamous cell carcinoma. J Eur Acad Dermatol Venereol 30: 1043-1045.

Holmes RS, Vaughan TL (2007) Epidemiology and pathogenesis of esophageal cancer. Sem Rad Oncol 17: 2-9.

Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011) Global cancer statistics. CA Canver J Clin 61: 69-90.

Judd LM, Menheniott TR, Ling H, Jackson CB, Howlett M, Kalantzis A, Priebe W, Giraud AS (2014) Inhibition of the JAK2/STAT3 pathway reduces gastric cancer growth in vitro and in vivo. PloS one 9: e95993.

Kang M, Li Y, Liu W, Wang R, Tang A, Hao H, Liu Z, Ou H (2013) miR-129-2 suppresses proliferation and migration of esophageal carcinoma cells through downregulation of SOX4 expression. Int J Mol Med 32: 51-58.

Komina A, Palkina N, Aksenenko M, Tsyrenzhapova S, Ruksha T (2016) Antiproliferative and pro-apoptotic effects of MiR-4286 inhibition in melanoma cells. PloS one 11: e0168229.

Li B, Xu W, Han L, Chan K, Tsao S, Lee N, Law S, Xu L, Li E, Chan K (2017) MicroRNA-377 suppresses initiation and progression of esophageal cancer by inhibiting CD133 and VEGF. Oncogene 36: 3086-4000.

Liu J, Mao Y, Zhang D, Hao S, Zhang Z, Li Z, Li B (2016) MiR-143 inhibits tumor cell proliferation and invasion by targeting STAT3 in esophageal squamous cell carcinoma. Cancer Lett 373: 97-108.

Livak KJ ST (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. Methods 25: 402-408.

Rustgi AK, El-Serag HB (2014) Esophageal carcinoma. New Engl J Med 371: 2499-2509.

Sasaki J, Kofuji S, Itoh R, Momiyama T, Takayama K, Murakami H, Chida S, Tsuya Y, Takasuga S, Eguchi S (2010) The PtdIns(3,4)P(2) phosphatase INPP4A is a suppressor of excitotoxic neuronal death. Nature 465: 497-501.

Siegel R, Naishadham D, Jemal A (2012) Cancer statistics for Hispanics/Latinos, 2012. CA Cancer J Clin 62: 283-298.

Siegel RL, Ma J, Zou Z, Jemal A (2014) Cancer Statistics, 2014. CA Cancer J Clin 65.

Song Y, Li J, Zhu Y, Dai Y, Zeng T, Liu L, Li J, Wang H, Qin Y, Zeng M (2014) MicroRNA-9 promotes tumor metastasis via repressing E-cadherin in esophageal squamous cell carcinoma. Oncotarget 5: 11669.

Trang P, Weidhaas JB, Slack FJ (2017) MicroRNAs and Cancer. In: Coleman WB, Tsongalis GJ (ed.) The Molecular Basis of Human Cancer, pp 277-286.

Wang C, Feng Z, Jiang K, Zuo X (2016) Upregulation of microRNA-935 promotes the malignant behaviors of pancreatic carcinoma PANC-1 cells via targeting inositol polyphosphate 4-phosphatase type I gene (INPP4A). Oncol Res 25: 559-569.

Wang N, Zhang C-Q, He J-H, Duan X-F, Wang Y-Y, Ji X, Zang W-Q, Li M, Ma Y-Y, Wang T (2013a) MiR-21 down-regulation suppresses cell growth, invasion and induces cell apoptosis by targeting FASL, TIMP3, and RECK genes in esophageal carcinoma. Digest Dis Sci 58: 1863-1870.

Wang Y, Li M, Zang W, Ma Y, Wang N, Li P, Wang T, Zhao G (2013b) MiR-429 up-regulation induces apoptosis and suppresses invasion by targeting Bcl-2 and SP-1 in esophageal carcinoma. Cell Oncol 36: 385-394.

Wang R, Wu Y, Huang W, Chen W (2018) MicroRNA-940 Targets INPP4A or GSK3β and activates the Wnt/β-catenin pathway to regulate the malignant behavior of bladder cancer cells. Oncol Res 26: 145-155.

Yang M, Liu R, Li X, Liao J, Pu Y, Pan E, Yin L, Wang Y (2014) miRNA-183 suppresses apoptosis and promotes proliferation in esophageal cancer by targeting PDCD4. Molecules Cells 37: 873-880.

You Z, Xu D, Ji J, Guo W, Zhu W, He J (2012) JAK/STAT signal pathway activation promotes progression and survival of human oesophageal squamous cell carcinoma. Clin Transl Oncol 14: 143-149.

Yuan Y, Zeng Z-Y, Liu X-H, Gong D-J, Tao J, Cheng H-Z, Huang S-D (2011) MicroRNA-203 inhibits cell proliferation by repressing ΔNp63 expression in human esophageal squamous cell carcinoma. BMC Cancer 11: 57.

Zang W-Q, Yang X, Wang T, Wang Y-Y, Du Y-W, Chen X-N, Li M, Zhao G-Q (2015) MiR-451 inhibits proliferation of esophageal carcinoma cell line EC9706 by targeting CDKN2D and MAP3K1. World J Gastroenterol 21: 5867-5876.