ARTICLE
Journal of Cellular Biochemistry 116:1050–1059 (2015)

Journal of Cellular Biochemistry

MiR-17–5p Up-Regulates YES1 to Modulate the Cell Cycle Progression and Apoptosis in Ovarian Cancer Cell Lines

Lan Li,[1,2] Li He,[1] Jian-Li Zhao,[1] Jing Xiao,[1] Min Liu,[1] Xin Li,[1] and Hua Tang[1]*

[1]*Tianjin Life Science Research Center and Basic Medical School, Tianjin Medical University, Tianjin 300070, China*

[2]*Maternity and Child Healthcare Hospital, Anyang City, Henan Province 455000, China*

ABSTRACT

MicroRNAs (miRNAs) are small, non-coding RNAs that participate in the regulation of gene expression. Although many studies have demonstrated the involvement of miR-17–5p in different cancers, little is known to its function in ovarian cancer. In this study, we demonstrated that overexpression of miR-17–5p was able to enhance cell proliferation by promoting G1/S transition of the cell cycle and suppressing apoptosis in ES-2 and OVCAR3 cell lines, whereas inhibition of miR-17–5p yielded the reverse phenotype. YES1 was identified as a novel target gene of miR-17–5p. Moreover, miR-17–5p was found to directly bind to the 3′UTR of YES1 mRNA and up-regulated its expression. Furthermore, knockdown of YES1 led to the suppression of proliferation and induced cell cycle arrest in ES-2 and OVCAR3 cells. Ectopic expression of YES1 was able to reverse the effects of miR-17–5p inhibition. Collectively, our results indicated that miR-17–5p might play a role in human ovarian cancer by up-regulating YES1 expression. J. Cell. Biochem. 116: 1050–1059, 2015.

KEY WORDS: MICRORNA; MiR-17-5P; OVARIAN CANCER; TARGET GENE; YES1; CELL CYCLE

Although ovarian cancer only accounts for about 3% of cancers among women, it causes more deaths than any other cancer of female reproductive system and only 45% ovarian cancer patients can survive more than 5 years after the initial diagnosis. Because of the poor survival of ovarian cancer patients, it is urgent to identify the molecular basis underling ovarian cancer progression. Recently, miRNA offers a novel molecular approach, and it has been reported to be involved in ovarian cancer pathogenesis [Donadeu et al., 2012; Sun et al., 2014].

MicroRNAs (miRNAs) are small non-coding single-stranded RNAs of approximately 22 nucleotides in length that modulate protein-coding gene expression via interacting with complementary sites within the 3′UTRs of target mRNA. miRNAs are typically transcribed from the genome as primary miRNAs, which are processed into mature miRNAs by the ribonuclease (RNase) III family members Drosha and Dicer [Bartel, 2004]. Mature miRNAs are associated with RNA-induced silencing complex (RISC) activity, which leads to mRNA translational repression or cleavage [Djuranovic et al., 2011]. Thus, miRNAs negatively regulate most genes. However, the complexity of miRNA-mediated gene regulation has been further demonstrated by observations that miRNAs can positively affect gene expression. For example, miR-10a has been shown to enhance translation by interacting with the 5′UTR of ribosomal protein mRNA [Orom et al., 2008], and miR-490–3p up-regulates ERGIC3 in human hepatocellular carcinoma cells [Zhang et al., 2013]. Evidence also indicates that miRNAs participate in a number of cellular processes, such as cell proliferation, apoptosis, and carcinogenesis [Jovanovic and Hengartner, 2006; Rao et al., 2012]. Therefore, they act as either tumor suppressors or oncogenes by regulating genes or modulating molecular pathways involved in human cancer [Volinia et al., 2006; Ma et al., 2007; Tavazoie et al., 2008].

miR-17–5p is a member of the miR-17–92 cluster, which contains seven members in total (miR-17–5p, miR-17–3p, miR-20a, miR-19a, miR-19b, miR-92a, and miR-18a) [He et al., 2005]. The oncogenic role of miR-17–5p has been reported in gastric cancer [Wu et al., 2014], colorectal cancer [Luo et al., 2012; Ma et al., 2012], hepatocellular carcinoma [Shan et al., 2013], glioma [Lu et al., 2012], and pancreatic cancer [Yu et al., 2010], whereas miR-17–5p acts as a tumor suppressor in breast cancer and cervical cancer [Hossain et al., 2006; Wei et al., 2012]. Although miR-17–5p was identified to have oncogenic or suppressive ability in several cancers, the role of miR-17–5p in human ovarian cancer has not been described to date.

Lan Li, Li He, and Jian-Li Zhao contributed equally to this work.

Grant sponsor: National Natural Science Foundation of China; Grant numbers: 31270818, 91029714, 31071191; Grant sponsor: Natural Science Foundation of Tianjin; Grant numbers: 12JCZDJC25100, 09JCZDJC17500.

*Correspondence to: Prof. Hua Tang, Tianjin Life Science Research Center and Basic Medical School, Tianjin Medical University, Tianjin 300070, China. E-mail: htang2002@yahoo.com

Manuscript Received: 14 October 2014; Manuscript Accepted: 18 December 2014

Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 5 January 2015

DOI 10.1002/jcb.25060 • © 2015 Wiley Periodicals, Inc.

In this study, we first explored the role of miR-17–5p in human ovarian cancer cell proliferation and apoptosis and to identify novel targets to aid in the elucidation of its function. We observed that miR-17–5p promoted cell proliferation and inhibited cell apoptosis though the up-regulation of v-yes-1 Yamaguchi sarcoma viral oncogene homolog 1 (YES1). Our findings provided evidence for a function of miR-17–5p in human ovarian cancer and, importantly, contributed to the understanding of the mechanisms underlying the development of ovarian cancer due to the altered modulation of miRNA pathways.

MATERIALS AND METHODS

CELL LINES, TRANSFECTION, AND RNA EXTRACTION

The ovarian cancer cell lines ES-2 and OVCAR3 were cultured at 37°C and 5% CO_2 in RPMI 1640 media (Gibco, Gaithersburg) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin. Transient transfection was performed using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. All transfections were carried out in three independent experiments. Enriched miRNAs were obtained from the ES-2 cells using a mirVana miRNA Isolation Kit (Ambion, Austin, TX) according to the manufacturer's recommendations. Total RNA was extracted using TRIzol reagent (Invitrogen).

VECTOR CONSTRUCTIONS

Primary miR-17–5p was amplified from genomic DNA and cloned into pcDNA3 vector sites with primer sequences sense 5′-CCAGGATCCTTTATAGTTGTTAGAGTTTG-3′, sense 5′- CGGAATTC-TAATCTACTTCACTATCTGCAC-3′. The gene encoding YES1 was amplified from cDNA isolated from ES-2 cells with the primer sequences sense 5′- GCGGAATTCATAATGGGCTGCATTAAAAG-3′, antisense 5′-GGCTATCTAGATTATAAATTTTCTCCTGGCTG-3′. The product was ~1800 bp, and it was cloned into pcDNA3 vector sites (*EcoR I* and *Xba II*). YES1 siRNA was annealed and cloned into a pSilencer 2.1 neo vector. The 3′UTR of YES1 (containing the binding sites for miR-17–5p) was amplified from an ES-2 cDNA library with the primer sequences sense 5′-CGCGGATCCAGAACTTTTTCACTT-CAGTC-3′, antisense 5′- CGGAATTCTACATTTTCCTCTCTGTTCATC-3′. The product was ~300 bp and was cloned into a pcDNA3-EGFP control vector (downstream of EGFP). The mutant 3′UTR of YES1 (four nucleotides were mutated in the binding sites) was amplified from the construct (pcDNA3/EGFP-YES1–3′UTR) with primer sequences sense 5′-GGACTTGAATCCTCATATGCTCATGGTG-3′, antisense 5′- CACCATGAGCATATGAGGATTCAAGTCC-3′. The resulting vectors included pcDNA3/EGFP-YES1–3′UTR and pcDNA3/EGFP-YES1–3′UTR-mut.

REAL-TIME REVERSE TRANSCRIPTION PCR

mRNAs or miRNAs were reverse transcribed to generate cDNA using oligo-dT primers or stem-loop reverse transcription (RT) primers, respectively. Then, the housekeeping gene β-actin (for mRNA) or U6 snRNA (for miRNA) was used as an endogenous control. Target genes and controls were treated under the same conditions and analyzed by real-time RT–PCR using SYBR Premix Ex Taq™ (*TaKaRa*, Dalian, China) according to the manufacturer's protocol. The primer sequences for YES1 were sense 5′-TCCAGAACTTTTTCACTT-CAGTC-3′, and antisense 5′-TCTACATTTTCCTCTCTGTTCATC-3′; β-actin sense 5′- CGTGACATTAAGGAGAAGCTG -3′, and antisense 5′- CTAGAAGCATTTGCGGTGGAC -3′.

EGFP REPORTER ASSAY

To confirm the direct interaction between miR-17–5p and YES1 mRNA, ES-2 and OVCAR3 cells were transiently co-transfected with a fluorescent reporter plasmid and ASO-miR-17–5p, pri-miR-17–5p or their control oligonucleotides or vectors in 48-well plates. An RFP expression vector plasmid, pDsRed2-N1, was transfected together with the above vectors and used as the loading control. After transfection for 48 h, the cells were lysed using radio-immunoprecipitation assay (RIPA) lysis buffer (150 mM NaCl, 50 mM Tris–HCl, pH 7.2, 1% Triton X-100, and 0.1% SDS), and the EGFP and RFP intensities were measured with an F4500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan).

WESTERN BLOT ANALYSIS

Total protein isolated from ES-2 cells transiently transfected with ASO-miR-17–5p, pri-miR-17–5p, or control oligonucleotides was extracted using RIPA lysis buffer. Protein expression was analyzed by western blotting. Briefly, protein electrophoresis was performed in 8 and 15% SDS denaturing PAGE gels and transferred to a nitrocellulose membrane. The expression level of YES1 was evaluated by using a rabbit polyclonal anti-YES1 antibody (1:300; Proteintech), cleaved caspase-3 was detected using a rabbit polyclonal anti-caspase-3 antibody (1:600; Proteintech), and cleaved PARP was analyzed using a rabbit polyclonal anti-PARP antibody (1:50; Saier Co., China). The secondary antibody used was a goat anti-rabbit antibody (1:1000; Saier Co., China). As a loading control, GAPDH was used to normalize the expression levels of YES1, caspase-3, and PARP. The membrane was incubated with the primary antibodies overnight at 4°C and then washed and incubated with the horseradish peroxidase (HRP)-conjugated secondary antibody on the next day. Protein expression was assessed by enhanced chemiluminescence and chemiluminescent film (Fujifilm, Tokyo, Japan). LabWorks image acquisition and analysis software (UVP) was used to quantify the band intensities.

MTT ASSAY

ES-2 and OVCAR3 cells were seeded in 96-well plates at 7000 cells/well. After transient transfection for 72 h, the cells were incubated with 15 μl MTT (at a final concentration of 0.5 mg/ml) at 37°C for an additional 4 h. Then, the medium was removed, and the precipitated formazan (Sigma–Aldrich) was dissolved in 100 μl DMSO. After shaking for 20 min, the absorbance at 570 nm (A570) was detected using a μQuant Universal Microplate Spectrophotometer (Bio-Tek Instruments, Winooski).

CELL GROWTH CURVE ASSAY

For the transient transfection of cell lines, ES-2 and OVCAR3 cells were seeded in 24-well plates at 3000 cells/well. Following a 48 h transfection, cell numbers were counted every day for 7 days.

TUNEL ASSAY

The free 3′-OH strand breaks resulting from DNA degradation were detected using the TUNEL technique. After washing with PBS, cells were fixed in 4% paraformaldehyde for 30 min at room temperature, followed by permeabilization with 0.1% Triton X-100 for 5 min at room temperature. The TUNEL assay was performed according to the manufacturer's instructions (In Situ Cell Death Detection Kit, AP; Roche Molecular Biochemicals, Mannheim, Germany). The preparations were then rinsed with Tris-buffered saline and incubated for 10 min at room temperature using a 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium (BCIP/NBT) liquid substrate system (Sigma–Aldrich). Thereafter, the cells were incubated with a guinea pig anti-insulin antibody as described above, followed by detection with streptavidin-biotin-horseradish peroxidase complex (Zymed Laboratories Inc., South San Francisco, CA). Similar to the TUNEL reaction, we used the DNA-binding dye, propidium iodide (Sigma–Aldrich), to assess the cellular nuclei. The cells were washed with PBS (without paraformaldehyde fixation), incubated for 10 min on ice with 10 μg/ml propidium iodide in PBS, washed again with PBS, and embedded in fluorescent mounting medium (DAKO Corp.,). The samples were immediately evaluated by fluorescence microscopy for positively stained cells.

FLOW CYTOMETRY ANALYSIS

All cells were harvested and fixed in 95% ethanol at −20°C overnight and then resuspended in buffer (5 mM EDTA and PBS, pH 7.4) for approximately 1 h prior to analysis. DNA was stained with 40 μg/ml propidium iodide (Sigma–Aldrich), and RNA was removed using 400 μg/ml RNase A (Sigma–Aldrich). The cells were subsequently filtered through a 35 μm cell strainer mesh (Becton Dickinson, North Ryde, NSW, Australia) and analyzed using a FACSCalibur System (BD Biosciences, San Jose, CA); cell cycle phase was determined by analysis with CellQuest.

STATISTICAL ANALYSIS

All experiments were performed three times, and the results are expressed as the mean ± standard deviation (SD). The data were statistically analyzed using Student's *t*-test for the comparison of two groups, and $P < 0.05$ was considered to be significant in two-sided tests.

RESULTS

MIR-17–5P PROMOTES THE PROLIFERATION OF ES-2 AND OVCAR3 CELLS

To investigate whether miR-17–5p affects cell growth in human ovarian cancer, MTT assay and growth curve assay were performed on ES-2 and OVCAR3 cell lines. First, we validated the effect of the miR-17–5p overexpression vector (pri-miR-17–5p) and miR-17–5p antisense oligonucleotides (ASO-miR-17–5p) in both ES-2 and OVCAR3 cells by real-time PCR (Fig. 1A). Second, as shown in Fig. 1B, the cell number of ES-2 and OVCAR3 cells transfected with ASO-miR-17–5p were reduced compared with the ASO control, whereas pri-miR-17–5p increased cell number compared with the control (Fig. 1B). Finally, we used a cell growth curve assay to monitor the proliferative capacities of ES-2 and OVCAR3 cells after transfection. The results showed significant inhibition of the growth of ES-2 and OVCAR3 cells transfected with ASO-miR-17–5p compared with the ASO control, and an opposite result was observed in cells transfected with pri-miR-17–5p (Fig. 1C). Collectively, these data indicate that miR-17–5p may contribute to the growth of ES-2 and OVCAR3 cells.

MIR-17–5P INHIBITS APOPTOSIS IN HUMAN OVARIAN CANCER CELLS

To further evaluate the reduced cell number of the ES-2 and OVCAR3 cells due to the inhibition of miR-17–5p, we used an In Situ Cell Death Detection Kit (Roche) to detect apoptosis rates. We found that there was a significant increase in the apoptosis rate in ES-2 cells transfected with ASO-miR-17–5p compared with the ASO control cells (Fig. 2A). In addition, a reduction in the apoptosis rate in ES-2 cells transfected with pri-miR-17–5p compared with the control was also observed. Consistent with the results obtained for ES-2, miR-17–5p played a similar role in OVCAR3 cells (Fig. 2A).

To explore the molecular mechanisms underlying the phenomenon of apoptosis, we used western blotting to detect the protein levels of cleaved caspase-3 and PARP. These proteins were up-regulated in the two cell lines transfected with ASO-miR-17–5p compared with the ASO controls, but they were down-regulated in cells transfected with pri-miR-17–5p compared with the controls (Fig. 2B). These data indicate that miR-17–5p may affect apoptosis though the caspase pathway.

MIR-17–5P FACILITATES G1/S PHASE TRANSITION IN HUMAN OVARIAN CANCER CELL LINES

To investigate whether an alteration in cell cycle progression is responsible for the promotion of cell growth by miR-17–5p in ovarian cancer cells, we performed flow cytometry analysis to assess cell cycle progression. The inhibition of miR-17–5p in ES-2 cells resulted in an increase in cells at G1 phase (from 48.1% to 62.48%) and a decrease in cells at S phase (from 21.06% to 14.88%) (Fig. 2C, left). By contrast, the overexpression of miR-17–5p in ES-2 cells led to a decrease in cells at G1 phase (from 53.19% to 42.75%) and an increase in cells at S phase (from 21.11% to 29.91%). This same phenomenon was observed in OVCAR3 cells transfected with pri-miR-17–5p or ASO-miR-17–5p (Fig. 2C, right). Therefore, these results suggest that miR-17–5p might play a role in G1/S phase transition in human ovarian cell lines.

YES1 IS A DIRECT TARGET GENE OF MIR-17–5P

To elucidate the activity of miR-17–5p in ES-2 and OVCAR3 cells, we used algorithm programs (PicTar, TargetScan, and miRBase Targets) to select the potential target genes of miR-17–5p. We chose YES1 due to its involvement in the promotion of tumor proliferation and the inhibition of apoptosis, which were consistent with the effects of miR-17–5p on the phenotypes of ES-2 and OVCAR3 cells. To verify the regulatory role of miR-17–5p in endogenous YES1 expression, real-time PCR and western blotting were performed to detect the effects of miR-17–5p on YES1 mRNA and protein levels. In ES-2 cells, we found that the inhibition of miR-17–5p reduced YES1

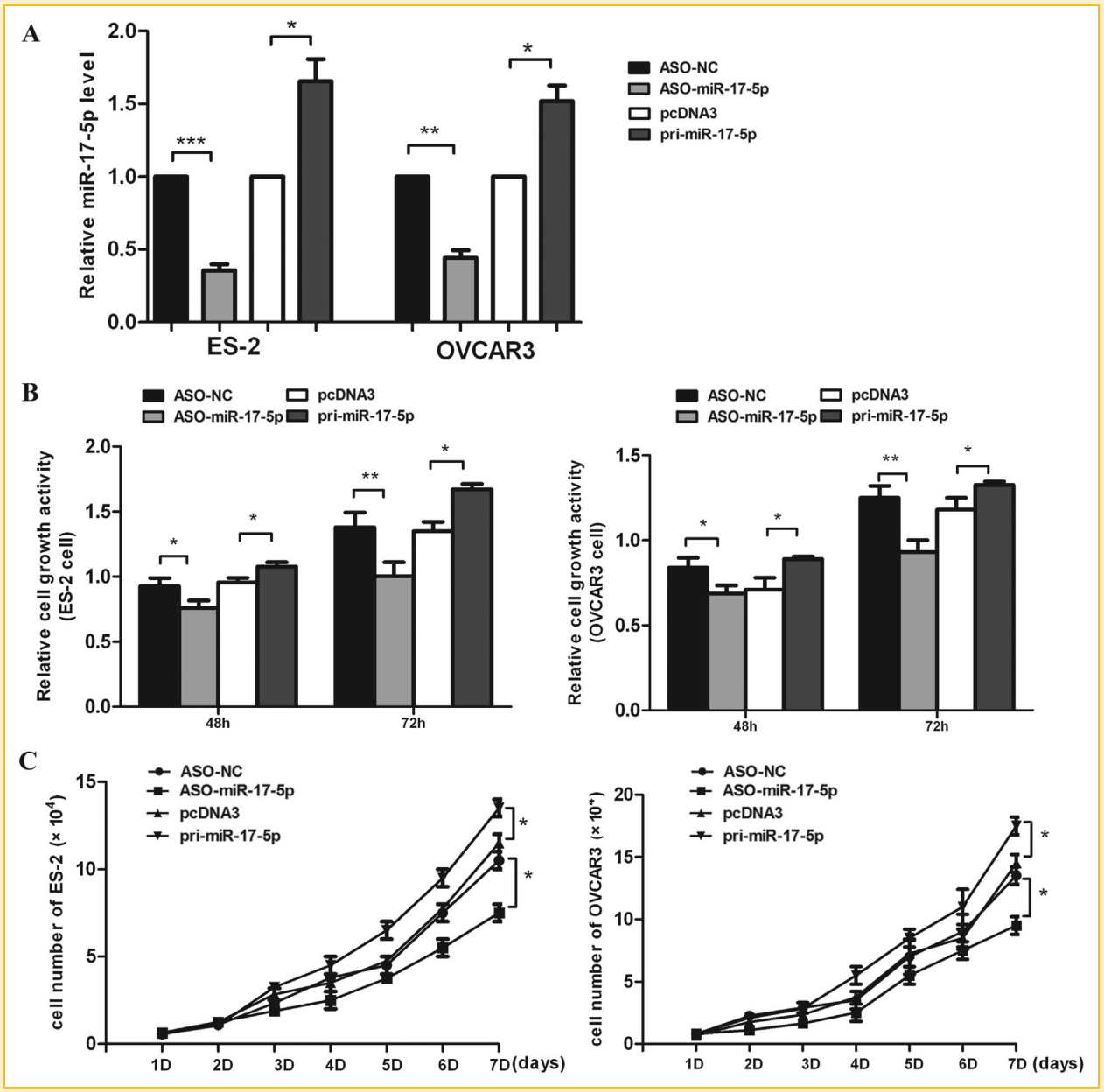

Fig. 1. miR-17-5p promotes proliferation of ES-2 and OVCAR3 cells. A. Real-time PCR was performed to detect the miR-17-5p levels in ES-2 and OVCAR3 cells treated with ASO-miR-17-5p or pri-miR-17-5p. The control was normalized to 1. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). B. An MTT assay was used to measure ES-2 (left) and OVCAR3 (right) cell viability at 48 and 72 h after transfection. C. The curved diagram shows the effects of miR-17-5p on the long-term proliferative activities of the ES-2 and OVCAR3 cells. The bars represent the mean ± SD of three independent experiments (*$P < 0.05$, **$P < 0.01$).

mRNA levels by 60% compared with cells transfected with the ASO control, and overexpression of miR-17-5p led to an increase in YES1 mRNA of approximately 60% (Fig. 3A). In accord with these results, the overexpression of miR-17-5p increased the YES1 protein level, and it was reduced following the inhibition of miR-17-5p (Fig. 3B). The results obtained from OVCAR3 cells were in consistent with those in ES-2 cells. Next, to determine whether the interaction between miR-17-5p and YES1 mRNA was direct, we performed EGFP fluorescent reporter assays. First, we constructed EGFP reporter vectors carrying the YES1 3′UTR or a mutant YES1 3′UTR downstream of an EGFP reporter gene. The binding sites for miR-17-5p in the 3′UTR of YES1 are illustrated in Fig. 3C. Next, pcDNA3/EGFP-TES1-3′UTR or pcDNA3/EGFP-YES1-3′UTR-mut was co-transfected with pri-miR-17-5p or ASO-miR-17-5p into ES-2 and OVCAR3 cells. Compared with the empty vector, the fluorescence intensity was increased by 20% in cells co-transfected with pri-miR-17-5p and pcDNA3/EGFP-YES1-3′UTR. In contrast, the fluorescence intensity was reduced by 55% in cells co-transfected with

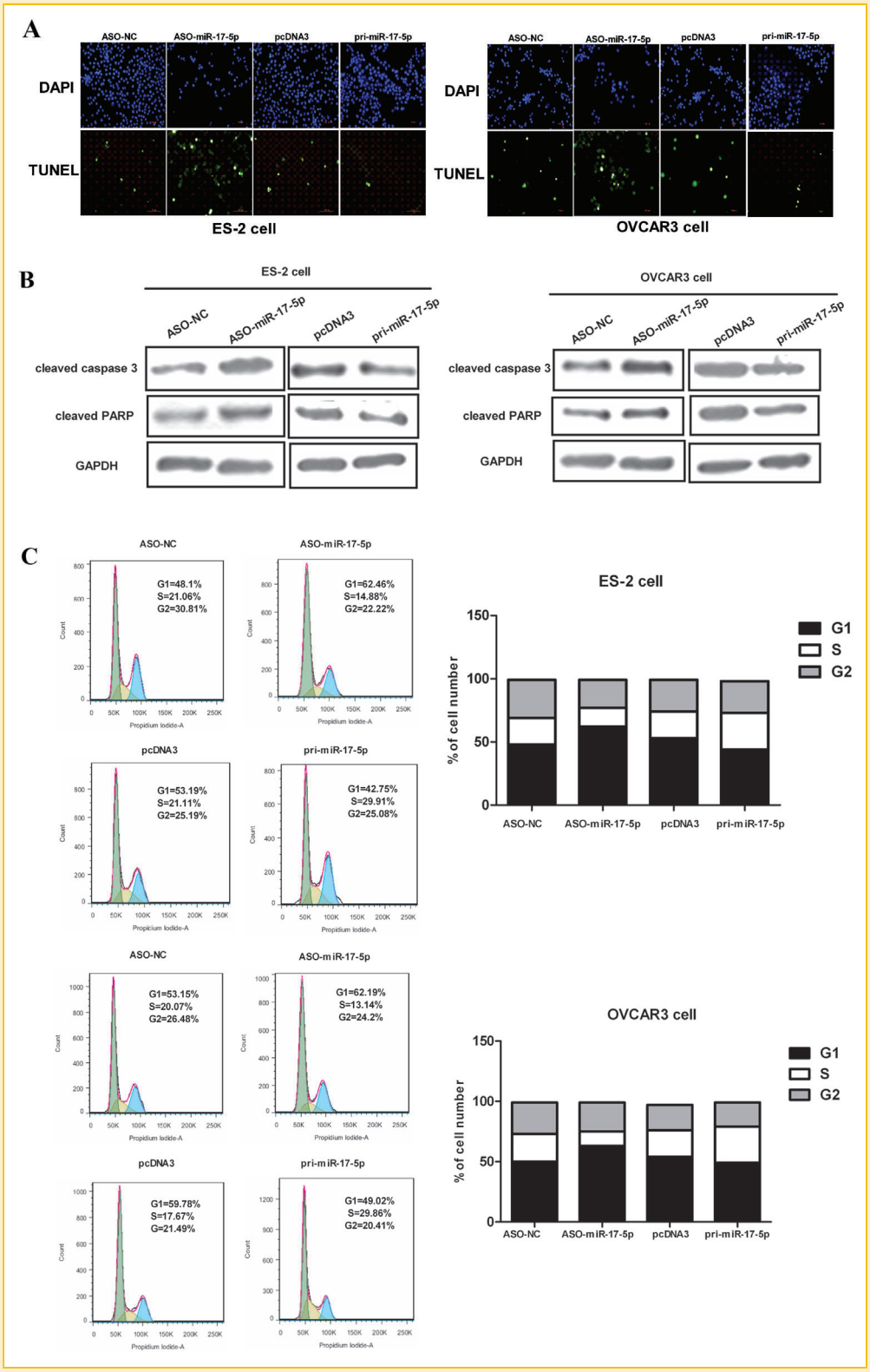

Fig. 2. miR-17-5p inhibits apoptosis and facilitates the G1/S phase transition in human ovarian cancer cell lines. A. Apoptosis of ES-2 cells and OVCAR3 cells treated with ASO-miR-17-5p or pri-miR-17-5p was detected using the TUNEL assay. B. Western blotting was used to detect the protein expression levels of cleaved caspase-3 and PARP in ES-2 and OVCAR3 cells treated with ASO-miR-17-5p or pri-miR-17-5p. C. The cell cycle profiles of ES-2 and OVCAR3 cells treated with ASO-miR-17-5p or pri-miR-17-5p were detected by flow cytometry.

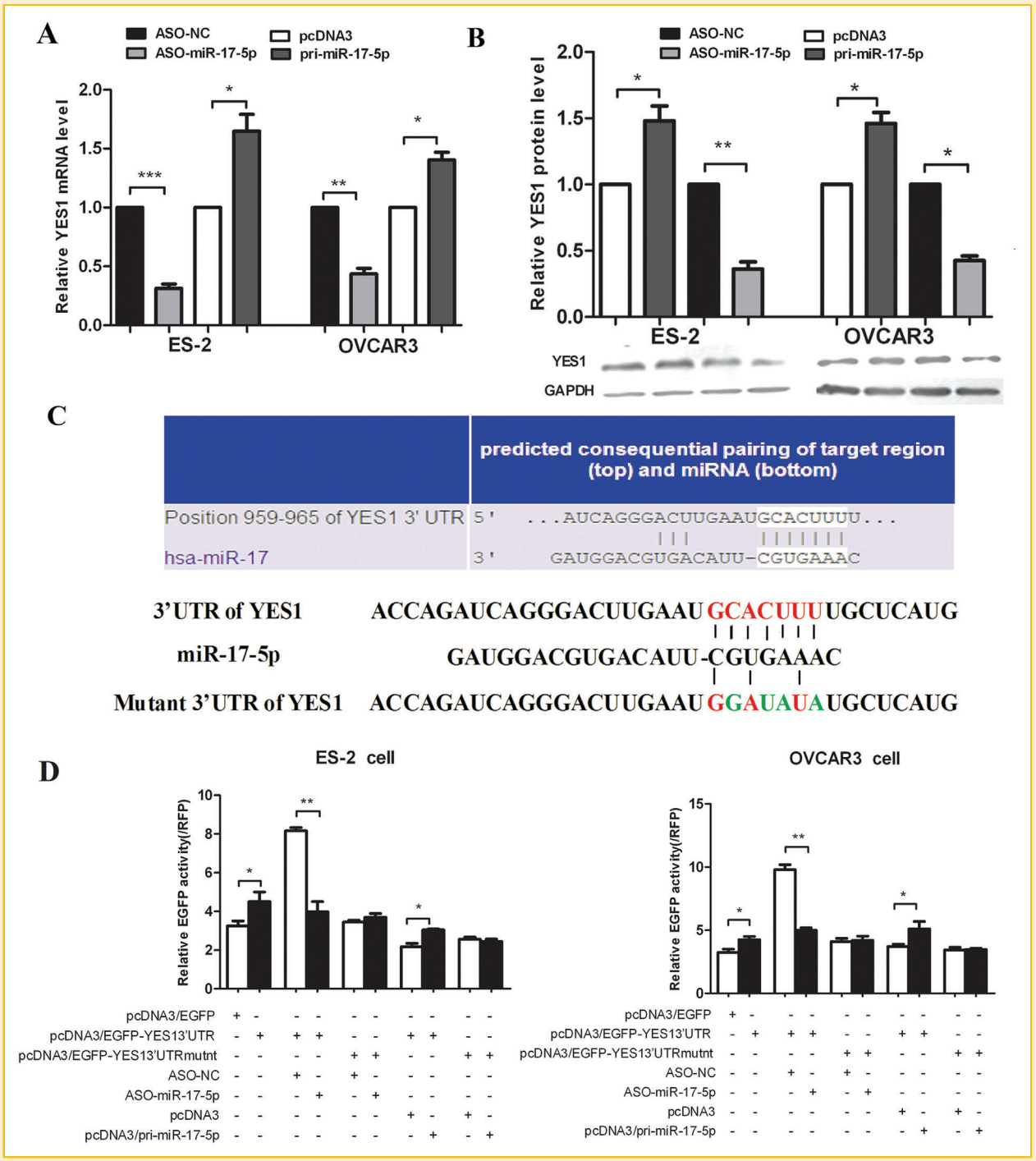

Fig. 3. YES1 is a direct target of miR-17-5p. A. Real-time PCR was performed to detect the YES1 mRNA level in ES-2 and OVCAR3 cells treated with ASO-miR-17-5p or pri-miR-17-5p. The control was normalized to 1. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). B. Western blotting was used to detect the protein expression level of YES1 in ES-2 and OVCAR3 cells transfected with ASO-miR-17-5p or pri-miR-17-5p. C. Complementary sequences of miR-17-5p to the YES1 mRNA 3′UTR were obtained using publicly available algorithms. The mutant YES1 3′UTR bearing four mutated bases is also shown. D. An EGFP reporter assay was performed to confirm direct regulation by miR-17-5p at the 3′UTR of YES1. Bars indicate the normalized mean fluorescence intensity ± SD for three independent experiments (*$P < 0.05$, **$P < 0.01$).

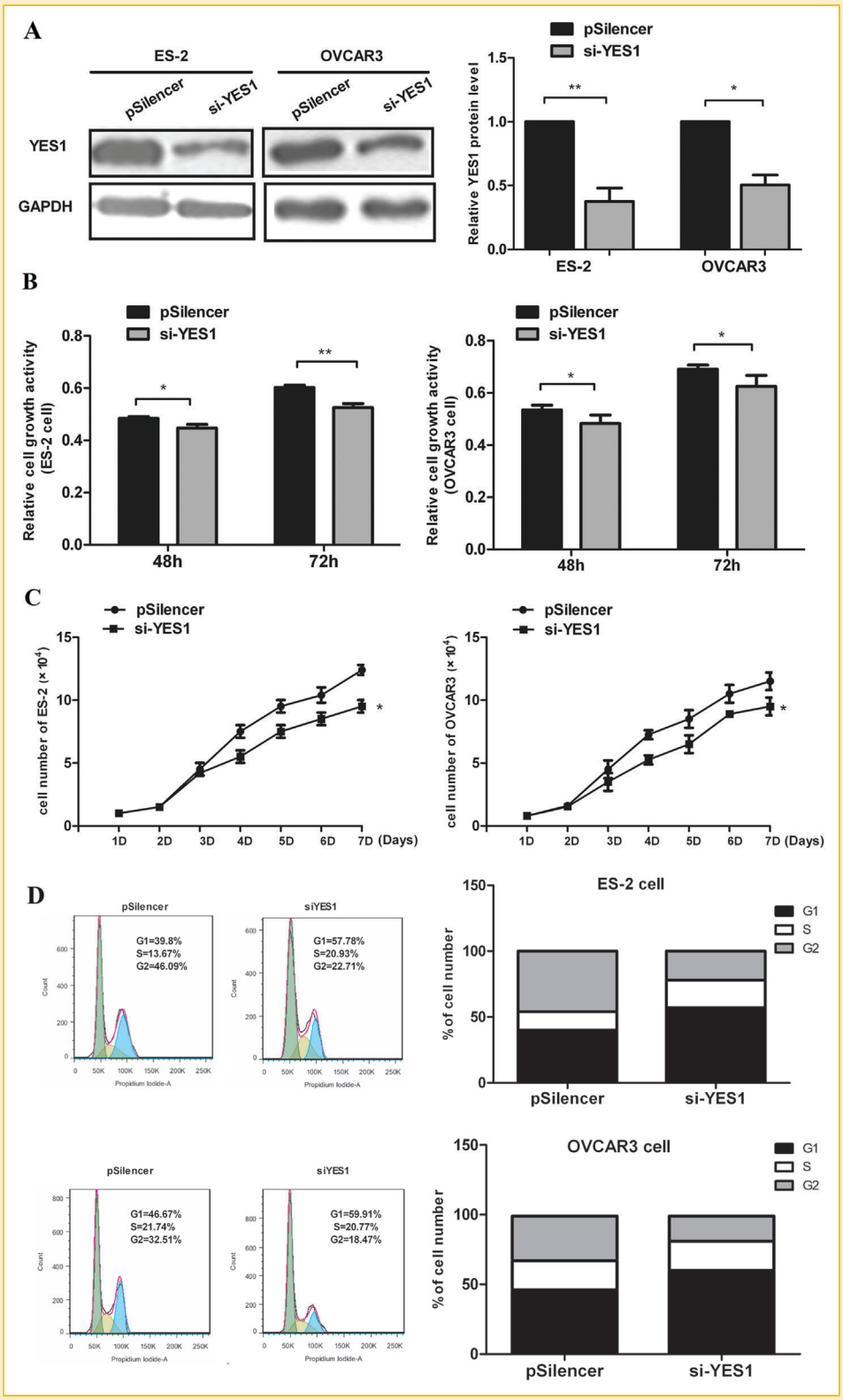

Fig. 4. Knockdown of YES1 suppresses cell proliferation and inhibits cell cycle progression. A. Western blotting was used to detect the efficiency of pSilencer/sh-YES1 (si-YES1) in suppressing protein expression. GAPDH was used as an internal control for quantitation normalization. B. An MTT assay was used to determine the effects of YES1 on the proliferation of ES-2 and OVCAR3 cells. C. A growth curve assay was performed to evaluate the effects of si-YES1 on the long-term proliferation of ES-2 and OVCAR3 cells. D. The cell cycle profiles of ES-2 and OVCAR3 cells treated with si-YES1 was detected by flow cytometry. Bars indicate the normalized mean fluorescence intensity ± SD for three independent experiments (*$P < 0.05$, **$P < 0.01$).

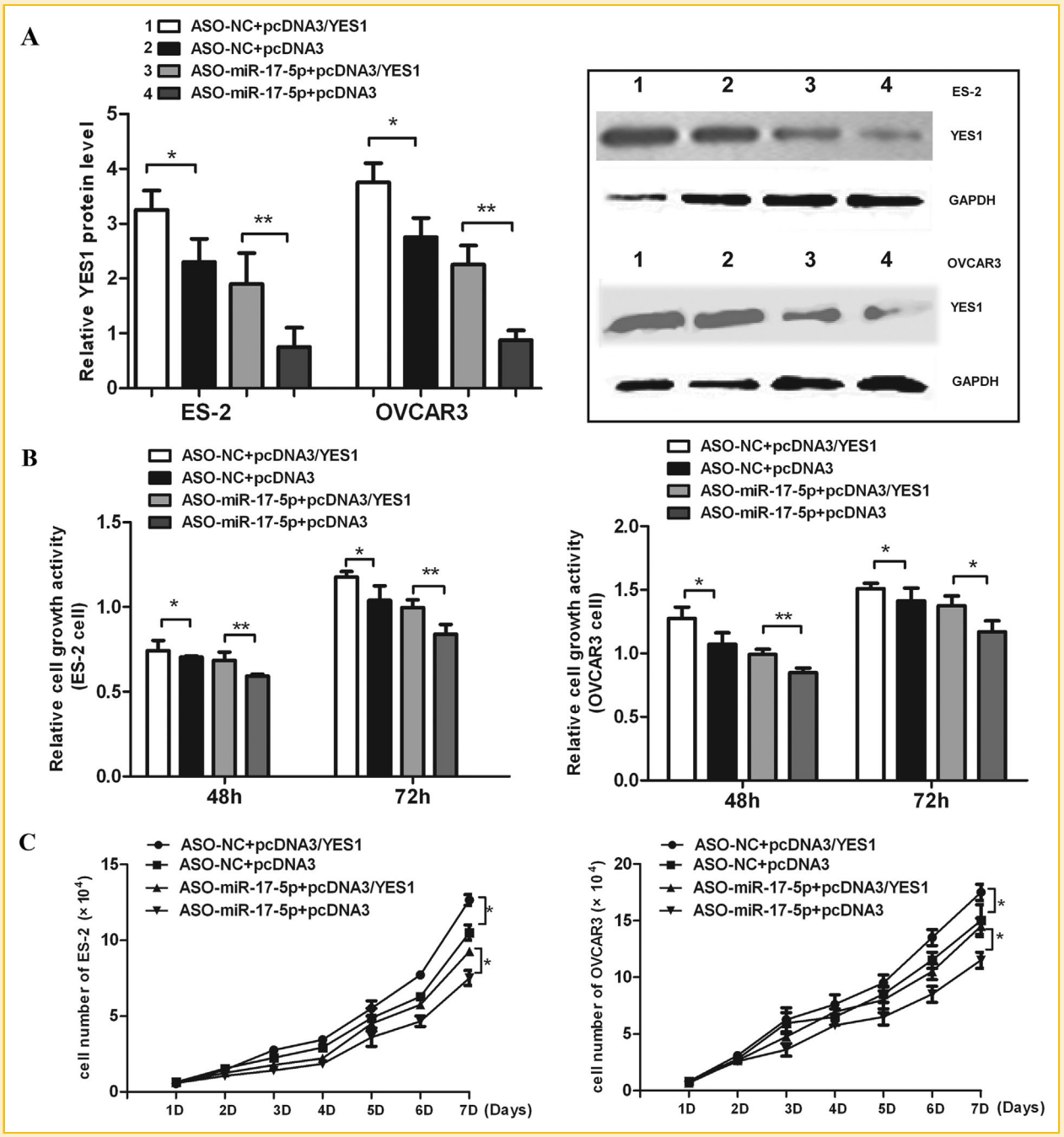

Fig. 5. Ectopic expression of YES1 restores the influence of ASO-miR-17-5p on cell growth. A. Cells were transfected with pcDNA3/YES1 or pcDNA3 with or without ASO-miR-17-5p. After 48 h, the YES1 protein level was detected by western blotting. B. MTT assay and C. growth curve assay were performed to assess cell growth in the rescue experiment. Bars indicate the normalized mean fluorescence intensity ± SD for three independent experiments (*$P < 0.05$, **$P < 0.01$).

ASO-miR-17-5p and pcDNA3/EGFP-YES1-3′UTR (Fig. 3D). However, neither the overexpression nor the inhibition of miR-17-5p affected the fluorescence intensity of cells transfected with pcDNA3/EGFP-YES1-3′UTR-mut. On the basis of these data, we conclude that YES1 is a direct target gene of miR-17-5p and that miR-17-5p can up-regulate YES1 expression.

KNOCKDOWN OF YES1 SUPPRESSES CELL PROLIFERATION AND INHIBITS CELL CYCLE PROGRESSION

To determine the function of YES1 in proliferation and the cell cycle, we constructed a YES1 siRNA expression vector (si-YES1). Western blotting showed that the YES1 endogenous protein level was effectively suppressed by si-YES1 (Fig. 4A). Knockdown of YES1

suppressed cell growth in ES-2 and OVCAR3 cells, similar to the effects observed for ASO-miR-17–5p (Fig. 4B and C). We also observed that YES1 knockdown caused an increase in cells at G1 phase and a decrease in cells at S phase, which was consistent with the results of miR-17–5p inhibition (Fig. 4D). In conclusion, these data indicate that the knockdown of YES1 suppress ovarian cancer cell proliferation and induced cell cycle arrest.

ECTOPIC EXPRESSION OF YES1 RESTORES THE INFLUENCE OF ASO-MIR-17–5P ON CELL GROWTH

To confirm that the phenotype of miR-17–5p was mediated by YES1 rather than by other genes, we carried out rescue experiments. First, we constructed an overexpression vector (pcDNA3/YES1) lacking the 3′UTR, and confirmed the expression of YES1 in ES-2 and OVCAR3 cells by western blotting (Fig. 5A). Then, ES-2 and OVCAR3 cells were transfected with ASO-miR-17–5p plus pcDNA3/YES1 or ASO-miR-17–5p plus pcDNA3. We found that the ectopic expression of YES1 counteracted the effects of ASO-miR-17–5p on cell growth (Fig. 5B and C). Therefore, these data indicate that YES1 is involved in the regulation of cell growth by miR-17–5p in human ovarian cells.

DISCUSSION

It is known that miRNAs are regulators of many genes at the post-transcriptional level and that their aberrant expression is related to cancer initiation, development, and prognosis. The understanding of the molecules and pathways involved in the progression of human ovarian cancer will aid in the development of new biomarkers for diagnosis and new therapeutic targets. In this study, we provided evidence that miR-17–5p might play a role in the growth and proliferation of human ovarian cancer cells.

Previous studies have shown that miR-17–5p is involved in the processes of cancer cell growth, proliferation, migration, and invasion. For example, miR-17–5p increases the proliferation and growth of gastric cancer cells and functions as a pro-proliferative factor by repressing SOCS6 [Wu et al., 2014]. Overexpression of miR-17–5p promotes cell proliferation, tumor progression, and cell cycle progression by targeting RND3 in colorectal carcinoma [Luo et al., 2012]. miR-17–5p is up-regulated in HCC and contributes to the metastatic activity of HuH-7 through the p38 mitogen-activated kinase/heat shock protein 27 pathway [Cloonan et al., 2008]. In pancreatic cancer, overexpression of miR-17–5p in pancreatic cancer cells promotes cell growth and invasion [Yu et al., 2010]. However, miR-17–5p acts as a tumor suppressor in breast cancer cells, in which overexpressed miR-17–5p can suppress cell proliferation by repressing the expression of AIB1 and CCND1 [Hossain et al., 2006]. Overexpression of miR-17–5p can also suppress cervical cancer cell proliferation by targeting TP53INP1 [Wei et al., 2012]. Taken together, these findings suggest that miR-17–5p is able to act as both an oncogene and a tumor suppressor in different cellular contexts. In our study, miR-17–5p was able to promote cell growth by inhibiting apoptosis induced by the caspase-3 pathway and by facilitating G1/S phase transition in human ovarian cancer cells. Thus, miR-17–5p may function as an oncogene in human ovarian cancer.

miRNAs are known to participate in a wide variety of cellular functions by regulating gene expression via binding complementary sites in the 3′UTRs of mRNA sequences. In our study, we used bioinformatics to predict target genes and selected YES1 as the candidate target gene. An EGFP fluorescence reporter assay verified that YES1 is a direct target of miR-17–5p, but surprisingly, miR-17–5p positively regulated this protein. Real-time PCR and western blotting results further supported the positive regulatory role of miR-17–5p in endogenous YES1 expression at both the mRNA and protein levels. These results are in contrast with previous reports suggesting that most miRNAs negatively regulate gene expression. Several important ways that miRNAs are able to up-regulate gene expression have been revealed, but the precise underlying mechanism remains elusive. For example, miR-744 and miR-1186 up-regulate cyclin B1 by binding to its promoter [Huang et al., 2012]. miR-122 can enhance hepatitis C viral (HCV) gene replication by binding to the 5′UTR of the viral genome [Shimakami et al., 2012]. miR-369–3 activates mRNA translation by targeting AU-rich elements in 3′UTRs under serum starvation [Vasudevan and Steitz, 2007; Vasudevan et al., 2007]. Despite our findings, the mechanism underlying the miR-17–5p-mediated up-regulation of YES1 needs to be further explored.

YES1, which is known as v-yes-1 Yamaguchi sarcoma viral oncogene homolog 1, is the cellular homolog of the Yamaguchi sarcoma virus oncogene. It is located on chromosome 18. YES1 belongs to the Src kinase family, and its tyrosine kinase activity has been shown to be elevated in colonic adenomas compared with its activity in adjacent normal mucosa [Pena et al., 1995; Thomas and Brugge, 1997]. In addition, a number of studies have shown that up-regulation of YES1 is important for the growth and transformation of intestinal cells [Pena et al., 1995; Kleber et al., 2008]. Resently, Gregersen et al. have reported YES is over-expression in colon cancer and is targeted by miR-145 [Gregersen et al., 2010; Sun et al., 2014]. In accord with these findings, our study demonstrated that the knockdown of YES1 inhibited human ovarian cell growth by inducing G1/S arrest. Furthermore, YES1 knockdown was able to reverse the cellular activities induced by miR-17–5p overexpression, indicating that miR-17–5p stimulated these cell functions, at least partially, by regulating YES1.

Taken together, our results demonstrate that miR-17–5p promotes cell growth by inhibiting apoptosis and enhancing G1/S phase transition due to its up-regulation of YES1 expression in human ovarian cancer cell lines. Further exploration of these molecules and an improved understanding of miRNAs will facilitate the development of more effective therapies against human ovarian cancer.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (Nos. 31270818, 91029714, and 31071191) and the Natural Science Foundation of Tianjin (12JCZDJC25100 and 09JCZDJC17500).

REFERENCES

Bartel DP. 2004. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116:281–297.

Cloonan N, Brown MK, Steptoe AL, Wani S, Chan WL, Forrest AR, Kolle G, Gabrielli B, Grimmond SM. 2008. The miR-17–5p microRNA is a key regulator of the G1/S phase cell cycle transition. Genome Biol 9:R127.

Djuranovic S, Nahvi A, Green R. 2011. A parsimonious model for gene regulation by miRNAs. Science 331:550–553.

Donadeu FX, Schauer SN, Sontakke SD. 2012. Involvement of miRNAs in ovarian follicular and luteal development. J Endocrinol 215:323–334.

Gregersen LH, Jacobsen AB, Frankel LB, Wen J, Krogh A, Lund AH. 2010. MicroRNA-145 targets YES and STAT1 in colon cancer cells. PloS ONE 5: e8836.

He L, Thomson JM, Hemann MT, Hernando-Monge E, Mu D, Goodson S, Powers S, Cordon-Cardo C, Lowe SW, Hannon GJ, Hammond SM. 2005. A microRNA polycistron as a potential human oncogene. Nature 435:828–833.

Hossain A, Kuo MT, Saunders GF. 2006. Mir-17–5p regulates breast cancer cell proliferation by inhibiting translation of AIB1 mRNA. Mol Cell Biol 26:8191–8201.

Huang V, Place RF, Portnoy V, Wang J, Qi Z, Jia Z, Yu A, Shuman M, Yu J, Li LC. 2012. Upregulation of Cyclin B1 by miRNA and its implications in cancer. Nucleic Acids Res 40:1695–1707.

Jovanovic M, Hengartner MO. 2006. MiRNAs and apoptosis: RNAs to die for. Oncogene 25:6176–6187.

Kleber S, Sancho-Martinez I, Wiestler B, Beisel A, Gieffers C, Hill O, Thiemann M, Mueller W, Sykora J, Kuhn A, Schreglmann N, Letellier E, Zuliani C, Klussmann S, Teodorczyk M, Grone HJ, Ganten TM, Sultmann H, Tuttenberg J, von Deimling A, Regnier-Vigouroux A, Herold-Mende C, Martin-Villalba A. 2008. Yes and PI3K bind CD95 to signal invasion of glioblastoma. Cancer Cell 13:235–248.

Lu S, Wang S, Geng S, Ma S, Liang Z, Jiao B. 2012. Increased expression of microRNA-17 predicts poor prognosis in human glioma. J Biomed Biotechnol 2012:970761.

Luo H, Zou J, Dong Z, Zeng Q, Wu D, Liu L. 2012. Up-regulated miR-17 promotes cell proliferation, tumour growth and cell cycle progression by targeting the RND3 tumour suppressor gene in colorectal carcinoma. Biochem J 442:311–321.

Ma L, Teruya-Feldstein J, Weinberg RA. 2007. Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. Nature 449:682–688.

Ma Y, Zhang P, Wang F, Zhang H, Yang Y, Shi C, Xia Y, Peng J, Liu W, Yang Z, Qin H. 2012. Elevated oncofoetal miR-17–5p expression regulates colorectal cancer progression by repressing its target gene P130. Nat Commun 3:1291.

Orom UA, Nielsen FC, Lund AH. 2008. MicroRNA-10a binds the 5′UTR of ribosomal protein mRNAs and enhances their translation. Mol Cell 30:460–471.

Pena SV, Melhem MF, Meisler AI, Cartwright CA. 1995. Elevated c-yes tyrosine kinase activity in premalignant lesions of the colon. Gastroenterology 108:117–124.

Rao E, Jiang C, Ji M, Huang X, Iqbal J, Lenz G, Wright G, Staudt LM, Zhao Y, McKeithan TW, Chan WC, Fu K. 2012. The miRNA-17 approximately 92 cluster mediates chemoresistance and enhances tumor growth in mantle cell lymphoma via PI3K/AKT pathway activation. Leukemia 26:1064–1072.

Shan SW, Fang L, Shatseva T, Rutnam ZJ, Yang X, Du W, Lu WY, Xuan JW, Deng Z, Yang BB. 2013. Mature miR-17–5p and passenger miR-17–3p induce hepatocellular carcinoma by targeting PTEN, GalNT7 and vimentin in different signal pathways. J Cell Sci 126:1517–1530.

Shimakami T, Yamane D, Jangra RK, Kempf BJ, Spaniel C, Barton DJ, Lemon SM. 2012. Stabilization of hepatitis C virus RNA by an Ago2-miR-122 complex. Proc Natl Acad Sci USA 109:941–946.

Sun N, Zhang Q, Xu C, Zhao Q, Ma Y, Lu X, Wang L, Li W. 2014. Molecular regulation of ovarian cancer cell invasion. Tumour Biol 35:11359–11366.

Tavazoie SF, Alarcon C, Oskarsson T, Padua D, Wang Q, Bos PD, Gerald WL, Massague J. 2008. Endogenous human microRNAs that suppress breast cancer metastasis. Nature 451:147–152.

Thomas SM, Brugge JS. 1997. Cellular functions regulated by Src family kinases. Annu Rev Cell Dev Biol 13:513–609.

Vasudevan S, Steitz JA. 2007. AU-rich-element-mediated upregulation of translation by FXR1 and Argonaute 2. Cell 128:1105–1118.

Vasudevan S, Tong Y, Steitz JA. 2007. Switching from repression to activation: microRNAs can up-regulate translation. Science 318:1931–1934.

Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, Yanaihara N, Lanza G, Scarpa A, Vecchione A, Negrini M, Harris CC, Croce CM. 2006. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci USA 103:2257–2261.

Wei Q, Li YX, Liu M, Li X, Tang H. 2012. MiR-17–5p targets TP53INP1 and regulates cell proliferation and apoptosis of cervical cancer cells. IUBMB Life 64:697–704.

Wu Q, Luo G, Yang Z, Zhu F, An Y, Shi Y, Fan D. 2014. MiR-17–5p promotes proliferation by targeting SOCS6 in gastric cancer cells. FEBS Lett 588:2055–2062.

Yu J, Ohuchida K, Mizumoto K, Fujita H, Nakata K, Tanaka M. 2010. MicroRNA miR-17–5p is overexpressed in pancreatic cancer, associated with a poor prognosis, and involved in cancer cell proliferation and invasion. Cancer Biol Ther 10:748–757.

Zhang LY, Liu M, Li X, Tang H. 2013. MiR-490–3p modulates cell growth and epithelial to mesenchymal transition of hepatocellular carcinoma cells by targeting endoplasmic reticulum-Golgi intermediate compartment protein 3 (ERGIC3). J Biol Chem 288:4035–4047.