

MicroRNA-455 inhibits cell proliferation and invasion of epithelial ovarian cancer by directly targeting Notch1

LIXIA XU^{1*}, HAIYAN LI^{2*}, LEI SU¹, QIAN LU³ and ZHENFENG LIU⁴

¹Department of Tumor Radiotherapy, The Sixth People's Hospital of Jinan, Shandong 250200;

²Department of Reproductive Medicine, Provincial Hospital Affiliated to Shandong University, Jinan, Shandong 250021;

Departments of ³Gynecology and ⁴Traditional Chinese Medicine, Jinan Maternity and Child Care Hospital Affiliated of Shandong University, Jinan, Shandong 250001, P.R. China

Received March 15, 2017; Accepted September 5, 2017

DOI: 10.3892/mmr.2017.7790

Abstract. Ovarian cancer (OC) is the fifth leading cause of cancer-associated mortality among women in developed countries. Numerous microRNAs are aberrantly expressed in epithelial (E) OC, and are involved in EOC formation and progression. As such, microRNAs may be investigated as diagnostic and prognostic molecular biomarkers, and therapeutic targets for patients with EOC. For instance, microRNA-455 (miR-455) is abnormally expressed in various types of human cancer. However, details on the expression level, biological roles and underlying mechanism of miR-455 in EOC remain unclear. In the present study, the expression of miR-455 in EOC was detected, and its association with clinical characteristics was analysed. The functional roles and underlying mechanisms of miR-455 in EOC were also investigated. The results revealed that miR-455 expression in EOC tissues and cell lines was significantly downregulated. Furthermore, miR-455 downregulation was correlated with tumor size, International Federation of Gynecology and Obstetrics stage and lymph node metastasis of patients with EOC. Restoration of miR-455 expression efficiently inhibited cell proliferation and invasion *in vitro*. Notch1 was identified as a direct target of miR-455 in EOC. The mRNA and protein expression levels of Notch1 were higher in EOC tissues compared with in normal ovarian epithelial tissues. Spearman's correlation analysis indicated that miR-455 expression was negatively correlated with the mRNA level of Notch1 in EOC tissues. Notch1 overexpression was able to restore the EOC cell

proliferation and invasion abilities suppressed by miR-455. The present study provided evidence that the dysregulation of the miR-455/Notch1 signalling pathway may be essential for EOC occurrence and development. In addition, the results confirmed the tumor-suppressive roles of miR-455 in modulating EOC proliferation and invasion through regulation of Notch1 expression. Thus, miR-455 may be a novel miRNA-based therapeutic target to treat patients with EOC.

Introduction

Ovarian cancer (OC) is the fifth leading cause of cancer-related deaths among women in developed countries and the primary cause of gynecological cancer death (1). Epithelial OC (EOC), the most common type of OC, accounts for approximately 90% of all OCs (2). According to histological origins, EOC can be divided into many different subgroups, including serous, mucinous, endometrioid, undifferentiated, clear cell and Brenner carcinomas (3,4). EOC is generally called a 'silent killer' because it does not create symptoms in patients with EOC; consequently, numerous patients are diagnosed in advanced stage with metastasis (5). Although advancements in the diagnosis of EOC patients in early stages have been achieved and efficient treatments have been developed, the prognosis of patients with EOC remains poor, and their 5-year survival rate is less than 50% (6). The exact mechanisms underlying EOC formation and progression are also unknown (7). Thus, further investigations on the molecular mechanism of EOC occurrence and development may be advantageous to identify novel biomarkers and therapeutic strategies to improve the survival of patients with this disease.

MicroRNAs (miRNAs) are endogenous, single-stranded, noncoding and short RNA molecules of approximately 22 nucleotides long (8). miRNAs serve as critical gene regulators by binding to the 3'-untranslated regions (3'-UTRs) of their target gene mRNAs in a base pairing sequence-specific manner; as a result, translational repression or mRNA degradation occurs (9). More than 60% of human protein-coding genes have been regulated by miRNAs (10). Half of the human miRNAs are located in cancer-related genomic regions, such as regions of homozygous deletion, loss of heterozygosity, oncogene and around tumor suppressor gene, breakpoint area

Correspondence to: Professor Zhenfeng Liu, Department of Traditional Chinese Medicine, Jinan Maternity and Child Care Hospital Affiliated of Shandong University, 2 Jianguoxiaojingsan Road, Jinan, Shandong 250001, P.R. China
E-mail: zfliu_jinan@163.com

*Contributed equally

Key words: ovarian cancer, microRNA-455, notch1, proliferation, invasion, treatment

and amplified region, and are implicated in tumorigenesis and tumor development (11). miRNAs are also involved in modulating different cancer-related biological processes, such as cell proliferation, cycle, apoptosis, angiogenesis, invasion, migration and metastasis (12-14). Moreover, numerous miRNAs are aberrantly expressed in various kinds of human cancers, such as EOC (15), cervical cancer (16), gastric cancer (17), lung cancer (18) and bladder cancer (19). miRNAs may act as either oncogenes or tumor suppressors in tumor initiation and progression on the basis of the biological roles of their target genes (20). Therefore, miRNAs can be investigated as diagnostic markers or potential therapeutic targets for various cancers.

miR-455 is abnormally expressed in several types of human cancer (21-23). This finding suggests that miR-455 may play important roles in these types of cancer. However, the detailed expression level, biological roles and underlying mechanism of miR-455 in EOC remain unknown. In this study, the miR-455 expression in EOC was detected, and its association with clinical characteristics was analysed. The functional roles and underlying mechanisms of miR-455 in EOC were also investigated.

Materials and methods

Clinical tissue samples. This study was approved by the Ethical Committee of the Sixth People's Hospital of Jinan, and written informed consent was obtained from all of the patients prior to the study. A total of 45 paired EOC tissues and their adjacent normal ovarian epithelial tissues were obtained from patients who received surgical resection at the Sixth People's Hospital of Jinan between January 2014 and February 2016. None of these EOC patients were treated with radiotherapy, chemotherapy or immunotherapy prior to operation. After surgery was completed, all of the tissue samples were immediately snap-frozen in liquid nitrogen and then stored at -80°C until use.

Cell lines, culture conditions and transfection. Four EOC cell lines, namely, OVCAR3, SKOV3, ES-2 and CAOV-3, were purchased from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China) and cultured in Dulbecco's modified Eagle's medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 mg/ml streptomycin (all from Gibco; Thermo Fisher Scientific, Inc.). Human normal ovarian epithelial NOEC cell line was obtained from the American Type Culture Collection (Manassas, VA, USA) and grown in Ham's F-12 with 20% FBS, 120 mg/ml streptomycin and 120 U/ml penicillin (Gibco; Thermo Fisher Scientific, Inc.). All of the cells were grown at 37°C in a humidified environment with 5% CO₂.

miR-455 mimics and negative control miRNA mimics (miR-NC) were synthesised by Shanghai GenePharma Co., Ltd. (Shanghai, China). Notch1-expressing vector (pcDNA3.1-Notch1) and empty vector (pcDNA3.1) were obtained from RiboBio (Guangzhou, China). The cells were seeded in a 6-well plate (Corning, Inc., Corning, NY, USA) at a density of 60-70% confluence, incubated overnight at 37°C

and transfected with Lipofectamine 2000 (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions.

RNA isolation and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The total RNA of tissue samples and cell lines was extracted according to the instruction manual of TRIzol reagent (Invitrogen Life Technologies). To quantify miR-455, total RNA was reverse-transcribed using a TaqMan MicroRNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA) to synthesize cDNA, and qPCR was performed with TaqMan MicroRNA PCR kit (Applied Biosystems). For Notch1 mRNA expression, cDNA was synthesized using a PrimeScript RT Reagent kit (Takara Biotechnology Co., Ltd., Dalian, China), and quantification of Notch1 mRNA was performed using a SYBR Premix Ex Taq™ kit (Takara Biotechnology Co., Ltd.). U6 and GAPDH were used as the internal control for miR-455 and Notch1 mRNA, respectively. The primers used for miR-455 were 5'-GAACTG CAGTCCATGGGGATA-3' (forward) and 5'-GCAGGGTCC GAGGTATTC-3' (reverse). The primers for U6 5'-CTCGCT TCGGCAGCACCA-3' (forward) and 5'-AACGCTTCACGA ATTTGCGT-3' (reverse). The primers for Notch1 were 5'-GTG GATGACCTAGGCAAGTCG-3' (forward) and 5'-GTCTCC TCCTTGTTGTTCTGC-3' (reverse). The primers for GAPDH were 5'-GCACCGTCAAGGCTGAGAAC-3' (forward) and 5'-TGGTGAAGACGCCAGTGGA-3' (reverse). 2^{-ΔΔCT} method was used to calculate the relative expression (24).

Cell Counting Kit-8 (CCK-8) assay. CCK-8 assay (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) was used for analyzing the cell proliferation following the manufacturer's instructions. Briefly, transfected cells were collected at 24 h post-transfection, and reseeded into 96-well plates at a density of 3x10³ cells/well. Cells were incubated at 37°C and cell proliferation was examined every 24 h according to the manufacturer's instructions (0, 24, 48, 72 h). 10 μl of CCK-8 solution was added into each group of cells and incubated at 37°C for 4 h. The absorbance of each sample was determined at a wavelength of 450 nm using an automatic multi-well spectrophotometer (Bio-Rad Laboratories, Inc., Hercules, CA, USA). All samples were analyzed in triplicate and repeated three times.

Cell invasion assay. Cell invasion assays were conducted using Transwell chamber inserts (Costar; Corning Life Sciences, Cambridge, MA, USA) with Matrigel (BD Biosciences, San Jose, CA, USA) according to the manufacturer's protocol. In briefly, transfected cells were collected at 48 h post-transfection, and seeded into the upper chamber of the insert at a density 5x10⁴ in 200 μl FBS-free medium. The bottom of the insert was filled with DMEM containing 20% FBS to serve as chemoattractant. After 20 h of incubation, the cells remaining on the upper membrane were carefully removed using cotton swabs. The invasive cells were fixed in 100% methanol, stained with 0.5% crystal violet, washed with PBS, and dried in air. Photographs of five manually selected fields of the invasive cells were taken at x200 magnification and counted under an inverted light microscope (Olympus Corporation, Tokyo, Japan).

Table I. Correlation between the expression of microRNA-455 and clinicopathological variables of epithelial ovarian cancer patients.

Clinical variables	Case no.	microRNA-455 expression		P-value
		Low	High	
Age (years)				0.300
<50	21	9	12	
≥50	24	14	10	
Tumor size (cm)				0.023
<5	20	6	14	
≥5	25	17	8	
FIGO stage				0.004
I-II	25	9	16	
III-IV	20	14	6	
Differentiation				0.449
I-II	21	12	9	
III	24	11	13	
Histological subtype				0.242
Serous	38	18	20	
Non-serous	7	5	2	
Lymph node metastasis				0.020
No	22	8	14	
Yes	23	15	8	

FIGO, International Federation of Gynecology and Obstetrics.

miR-455 target prediction. miRNA target prediction algorithms: TargetScan (release 7.0, <http://www.targetscan.org/>) and PicTar (<http://pictar.mdc-berlin.de/>) were used to forecast the putative targets of miR-455.

Luciferase reporter assay. Luciferase reporter vector, pGL3-Notch1-3'UTR wild-type (Wt) and pGL3-Notch1-3'UTR mutant (Mut), were synthesized by Shanghai GenePharma Co., Ltd. For luciferase assays, cells were plated in 24-well plates at a density of 1.5×10^5 cells/well, and then were co-transfected with pGL3-Notch1-3'UTR Wt or pGL3-Notch1-3'UTR Mut, and miR-455 mimic or miR-NC, using Lipofectamine 2000 following to the manufacturer's instructions. After 48 h of incubation, luciferase activity was detected using the Dual-Luciferase reporter assay system (Promega, Mannheim, Germany) according to the manufacturer's protocol. *Renilla* luciferase activity was used to normalize to firefly luciferase activity.

Western blot analysis. Protein was extracted from tissues or cells with radioimmunoprecipitation assay lysis buffer (Beyotime Institute of Biotechnology, Haimen, China) containing proteinase inhibitor (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) on ice for 30 min. After centrifugation at 4°C for 15 min at 13,000 rpm, the protein

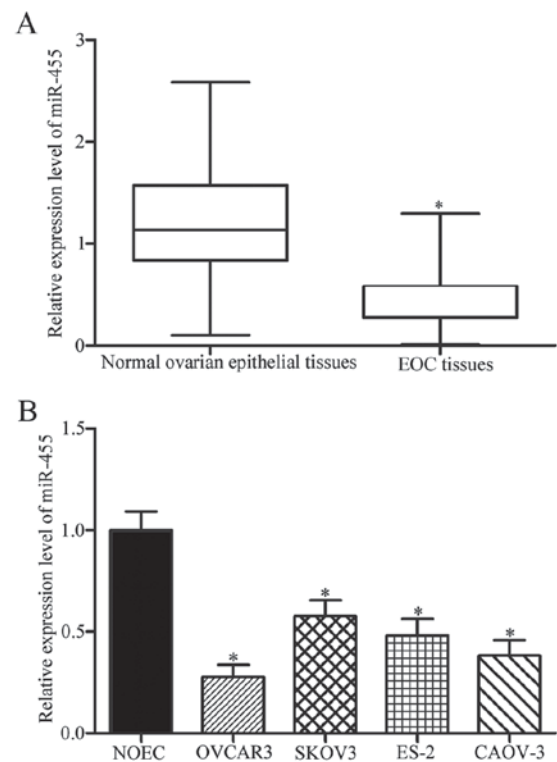


Figure 1. Expression of miR-455 was downregulated in EOC tissues and cell lines. (A) miR-455 expression levels in EOC tissues and their adjacent normal ovarian epithelial tissues were detected through RT-qPCR. (B) RT-qPCR analysis of the miR-455 expression in four ovarian cancer cell lines (OVCAR3, SKOV3, ES-2 and CAO-V-3) and normal ovarian epithelial NOEC cell line. * $P < 0.05$ vs. normal tissue or cell line. EOC, epithelial ovarian cancer; miR, microRNA; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

concentration in the supernatant was examined using the BCA protein assay (Pierce Biotechnology, Inc., Rockford, IL, USA). Equal amounts of protein were resolved on a 10% SDS denaturing polyacrylamide gel and transferred onto polyvinylidene difluoride membranes (EMD Millipore, Billerica, MA, USA). Subsequently, the membranes were blocked with 5% nonfat milk in Tris-buffered saline with 0.05% Tween-20 (TBST) for 1 h at room temperature, and next incubated with primary antibodies overnight at 4°C: Mouse anti-human monoclonal Notch1 antibody (dilution, 1:1,000; cat no. sc-373891) and mouse anti-human monoclonal GAPDH antibody (dilution, 1:1,000; cat no. sc-47724) (both from Santa Cruz Biotechnology, Inc., Dallas, TX, USA). The membranes were then washed with TBST and incubated with horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG second antibodies (dilution, 1:5,000; cat no. sc-2005; Santa Cruz Biotechnology, Inc.) at room temperature for 2 h. Finally, bands were visualized using an enhanced luminol-based chemiluminescence detection kit (Pierce Biotechnology, Inc.). GAPDH was used as a loading control.

Statistical analysis. Data are presented as the mean \pm standard deviation and statistical analysis was performed with Student's t-tests or one-way analysis of variance plus multiple comparisons using SPSS 14.0 (SPSS Inc., Chicago, IL, USA). Spearman's correlation analysis was used to analyze the inverse relationship of miR-455 and

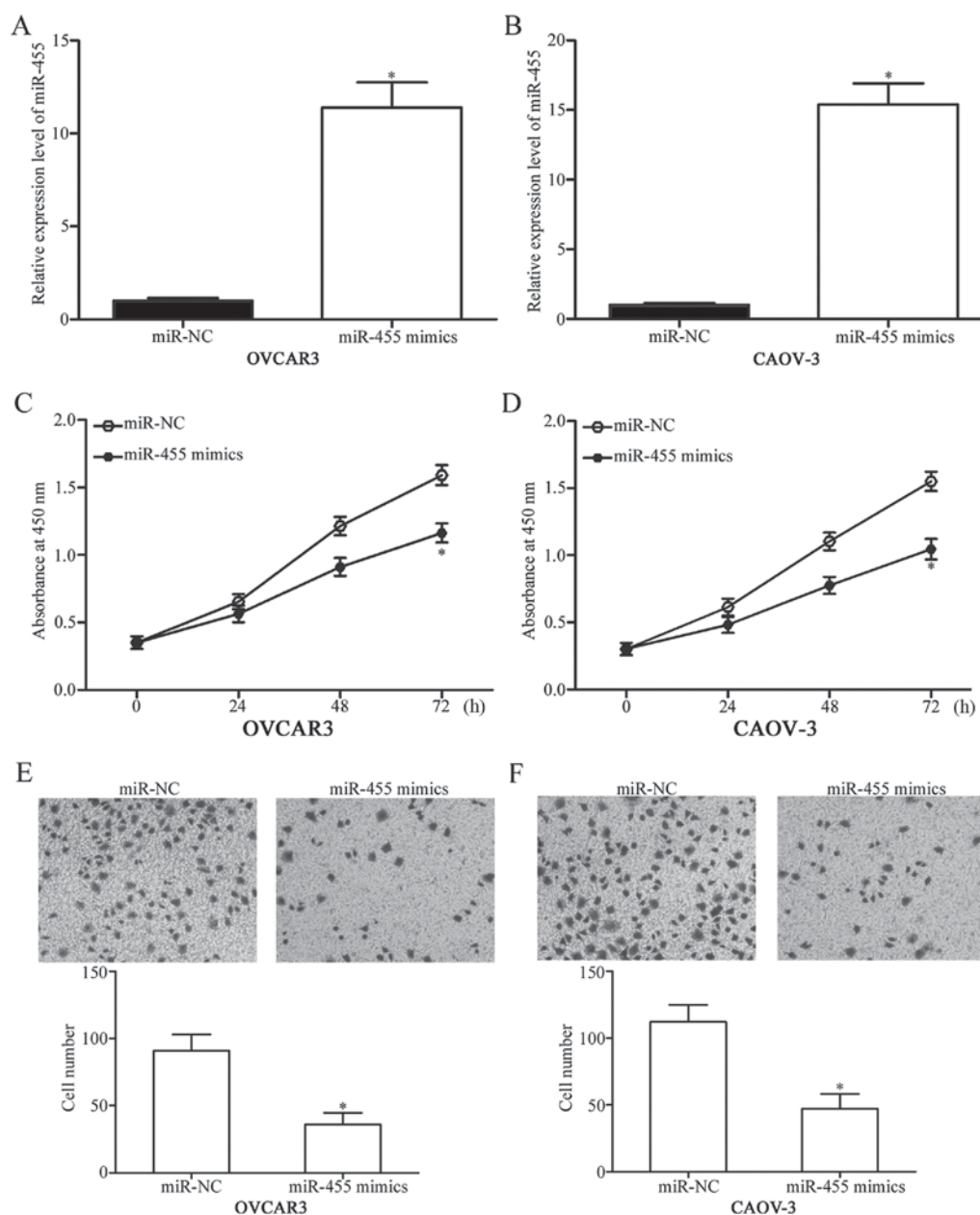


Figure 2. miR-455 overexpression inhibited the proliferation and invasion of EOC cells. (A and B) Transfection efficiency of miR-455 mimic in OVCAR3 and CAOV-3 cells, as revealed by reverse transcription-quantitative polymerase chain reaction 48 h after transfection with miR-NC as a negative control. (C and D) CCK-8 assay was performed to examine the effects of miR-455 on the proliferation of OVCAR3 and CAOV-3 cells. Compared with the proliferation of the cells transfected with miR-NC, the proliferation of OVCAR3 and CAOV-3 cells transfected with miR-455 mimic was reduced. (E and F) Cell invasion assay of OVCAR3 and CAOV-3 cells transfected with miR-455 mimic or miR-NC. Representative images are shown with the quantification of five randomly selected fields. * $P < 0.05$ vs. NC. EOC, epithelial ovarian cancer; CCK-8, Cell Counting Kit-8; miR, microRNA; NC, negative control.

Notch1 mRNA expression level. $P < 0.05$ was regarded as statistically significant.

Results

miR-455 is downregulated in EOC tissue samples and cell lines. miR-455 expression was detected in 45 paired EOC tissues and their adjacent normal ovarian epithelial tissues through quantitative reverse transcription polymerase chain reaction (RT-qPCR). Compared with the levels in adjacent normal ovarian epithelial tissues, miR-455 was significantly downregulated in EOC tissues (Fig. 1A, $P < 0.05$). Consistent with this observation, our results confirmed that miR-455 was

also downregulated in OVCAR3, SKOV3, ES-2 and CAOV-3 in comparison to the normal ovarian epithelial NOEC cell line (Fig. 1B, $P < 0.05$). These results suggested that miR-455 expression was impaired in EOC and might participate in EOC progression.

Association between miR-455 and clinicopathological features in EOC patients. To evaluate the associations between miR-455 downregulation and various clinicopathological features of EOC patients, we chose the median values of miR-455 in EOC tissues as cutoff values and divided the EOC patients into low miR-455 group ($n = 23$) and high miR-455 group ($n = 22$). In Table I, the low expression levels of miR-455

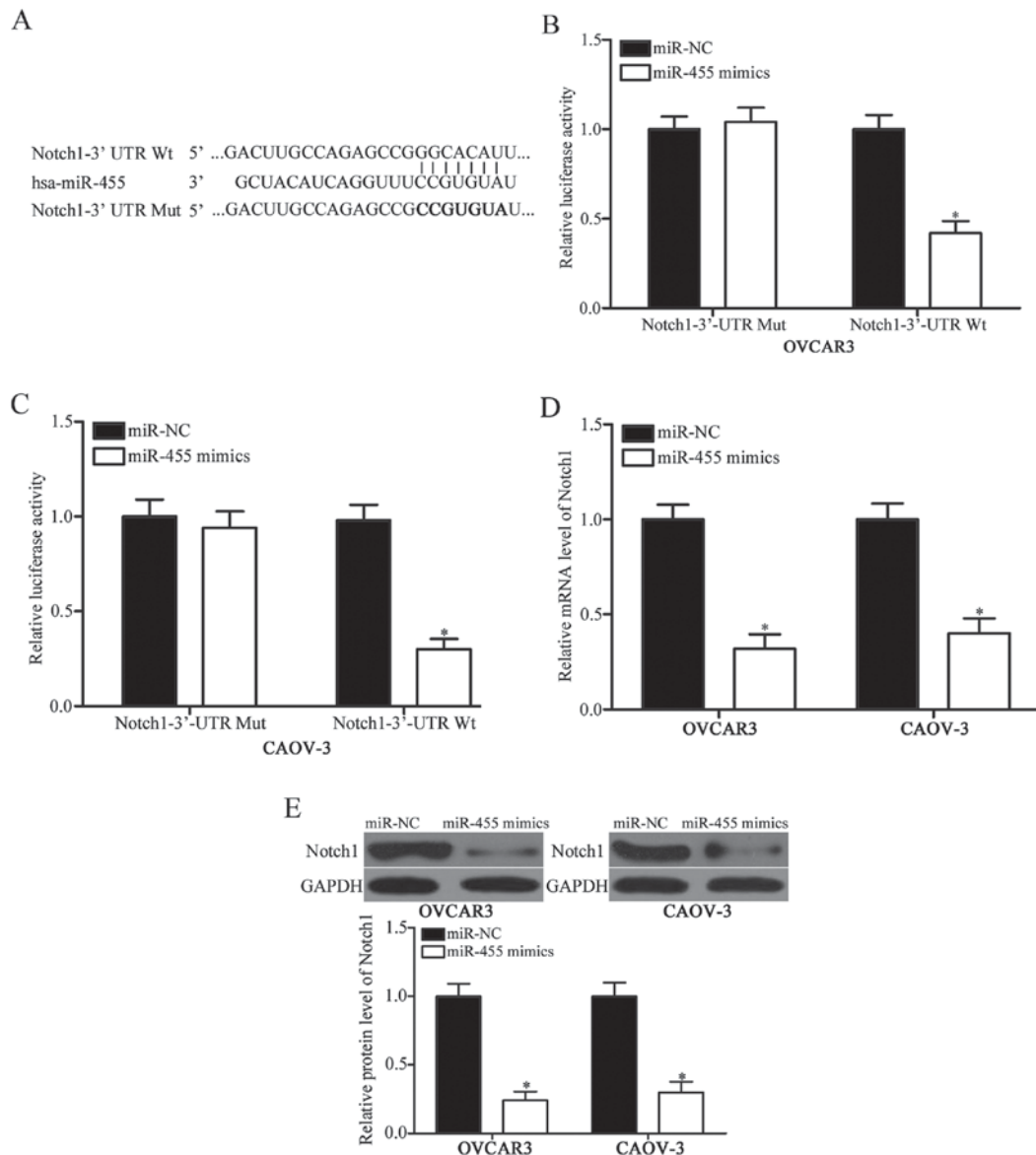


Figure 3. Notch1 is a direct target gene of miR-455 in EOC. (A) Putative binding sites of miR-455 on the 3'-UTR of Notch1. (B and C) Relative luciferase activity was detected in OVCAR3 and CAOV-3 cells co-transfected with Wt or Mut vector and miR-455 mimic or miR-NC after 48 h. (D and E) MRNA and protein expression levels of Notch1 in OVCAR3 and CAOV-3 cells transfected with miR-455 mimics or miR-NC were determined through reverse transcription-quantitative polymerase chain reaction and western blot analysis, respectively. * $P < 0.05$ vs. NC. EOC, epithelial ovarian cancer; 3'-UTR, 3'-untranslated region; miR, microRNA; NC, negative control; Mut, mutant; wt, wild-type.

were significantly correlated with the tumor size ($P = 0.023$), FIGO stage ($P = 0.004$) and lymph node metastasis ($P = 0.020$) of patients with EOC. Conversely, no significant associations were identified between miR-455 expression levels and other clinicopathological variables, including age ($P = 0.300$), differentiation ($P = 0.449$) and histological subtype ($P = 0.242$).

miR-455 overexpression inhibits cell proliferation and invasion of EOC. The cells were transfected with miR-455 mimics to increase the endogenous miR-455 expression and to investigate the potential biological roles of miR-455 in EOC, OVCAR3 and CAOV-3. Using RT-qPCR, we found that miR-455 was markedly upregulated in OVCAR3 and CAOV-3 cells transfected with the miR-455 mimics compared with the cells transfected with miR-NC (Fig. 2A and B, $P < 0.05$). The effects of miR-455 overexpression on the

proliferation of EOC cells were examined through CCK-8 assay. In Fig. 2C and D, the miR-455 upregulation inhibited the proliferation of OVCAR3 and CAOV-3 cells. A cell invasion assay was conducted to evaluate the effects of miR-455 on the invasion capacity of EOC cells. In Fig. 2E and F, the restored expression of miR-455 decreased the invasion capacities of OVCAR3 and CAOV-3 cells ($P < 0.05$). These observations revealed that miR-455 functioned as a tumor suppressor in EOC.

Notch1 is a direct target of miR-455 in EOC. Bioinformatics analysis was conducted to predict the putative targets of miR-455 and to investigate the molecular mechanism by which miR-455 repressed EOC cell proliferation and invasion. Numerous genes were identified as potential targets of miR-455, and Notch1 (Fig. 3A) was selected for further confirmation

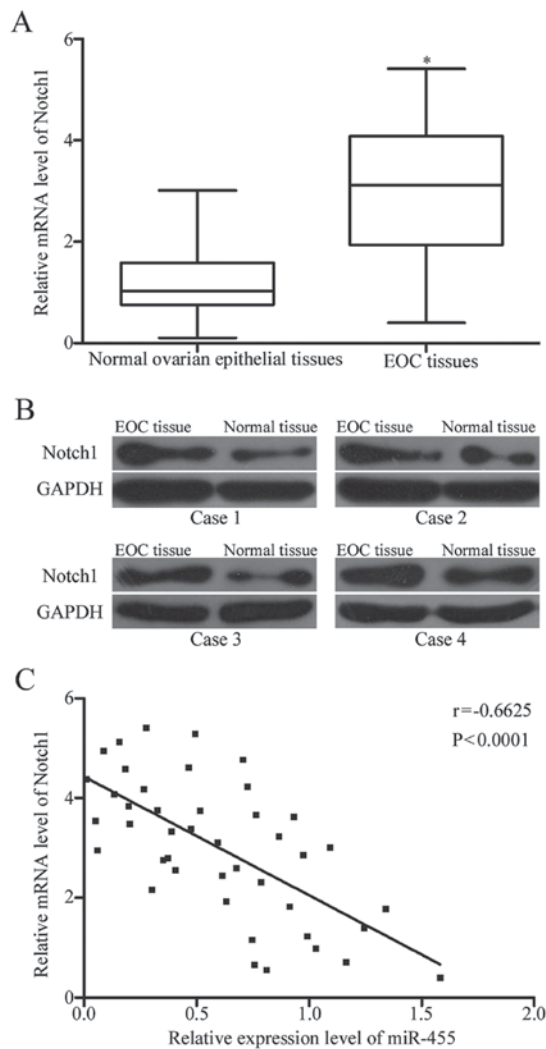


Figure 4. Notch1 was upregulated in EOC tissues and negatively correlated with miR-455 expression. (A and B) mRNA and protein expression levels of Notch1 in EOC tissues and their adjacent normal ovarian epithelial tissues were examined through reverse transcription-quantitative polymerase chain reaction and western blot analysis, respectively. (C) Correlation between the expression of miR-455 and Notch1 mRNA was evaluated through Spearman's correlation analysis. * $P < 0.05$ vs. normal tissue. EOC, epithelial ovarian cancer; miR, microRNA.

because Notch1 was involved in EOC initiation and progression. A luciferase reporter assay was carried out to investigate whether Notch1 was a target for miR-455. OVCAR3 and CAOV-3 cells were co-transfected with pGL3-Notch1-3'-UTR Wt or pGL3-Notch1-3'-UTR Mut and miR-455 mimics or miR-NC. We found that the upregulation of miR-455 significantly reduced the luciferase activity of pGL3-Notch1-3'-UTR Wt (Fig. 3B and C, $P < 0.05$). By contrast, the cells transfected with pGL3-Notch1-3'-UTR Mut were unaffected. This finding suggested that miR-455 could directly target the 3'-UTR of Notch1. To confirm the endogenous regulatory role of miR-455 in relation to Notch1 in EOC cells, we determined the Notch1 expression in OVCAR3 and CAOV-3 cells transfected with miR-455 mimic or miR-NC. In Fig. 3D and 3E, the mRNA and protein levels of Notch1 were downregulated in OVCAR3 and CAOV-3 cells transfected with miR-455 mimic ($P < 0.05$). These results suggested that Notch1 might be a direct target of miR-455 in EOC.

Inverse correlation between Notch1 and miR-455 expression in EOC tissues. To explore the association between Notch1 and miR-455, we examined the Notch1 expression in 45 paired EOC tissues and their adjacent normal ovarian epithelial tissues through RT-qPCR and Western blot analysis. The results showed that the expression of Notch1 was higher in EOC tissues at mRNA and protein levels than in normal ovarian epithelial tissues (Fig. 4A and B, $P < 0.05$). Spearman's correlation analysis was conducted to validate the relationship between Notch1 mRNA and miR-455 expression in these clinical tissues. We found that miR-455 expression was negatively correlated with the mRNA level of Notch1 (Fig. 4C; $r = -0.6625$, $P < 0.0001$). This observation supported that Notch1 was a target gene of miR-455 in EOC.

Restored expression of Notch1 can rescue the inhibitory effects on EOC cells induced by miR-455. pcDNA3.1-Notch1 was transfected into OVCAR3 and CAOV-3 cells and rescue experiments were performed to validate whether Notch1 can mediate the tumor-suppressive effects of miR-455 on EOC cells. After transfection was completed, western blot analysis confirmed that Notch1 was significantly upregulated in OVCAR3 and CAOV-3 cells transfected with pcDNA3.1-Notch1 (Fig. 5A, $P < 0.05$). We also found that the upregulation of Notch1 could reverse the inhibitory effects of miR-455 overexpression on cell proliferation (Fig. 5B and C, $P < 0.05$) and invasion (Fig. 5D and E, $P < 0.05$) in OVCAR3 and CAOV-3 cells. Overall, these results implied that miR-455 inhibited the cell proliferation and invasion of EOC partially by downregulating Notch1.

Discussion

miRNAs play important roles in cancer by acting as tumor suppressors or oncogenes (25-27). Many aberrantly expressed miRNAs have been reported in EOC and involved in EOC cell proliferation, invasion, migration, metastasis and apoptosis (13,28,29). Therefore, miRNAs may be investigated as diagnostic and prognostic molecular biomarkers and therapeutic targets for patients with EOC. In this study, the miR-455 expression was downregulated in EOC tissues and cell lines. Low miR-455 expression was correlated with the tumor size, FIGO stage and lymph node metastasis of EOC patients. In addition, the ectopic expression of miR-455 inhibited the cell proliferation and invasion of EOC. Notch1 was also identified as a direct target of miR-455 in EOC. These data suggested that miR-455 might be implicated in the carcinogenesis and progression of EOC.

Aberrant miR-455 expression has been observed in various human cancers. For instance, miR-455 is weakly expressed in hepatocellular carcinoma. miR-455 expression is correlated with multiple tumor nodes, high Edmondson-Steiner grading, advanced tumor node metastasis stage and venous infiltration of hepatocellular carcinoma patients. miR-455 is also validated as a novel prognostic indicator to predict the 5-year overall and disease-free survival of hepatocellular carcinoma patients (21). In gastric cancer, the miR-455 expression level is downregulated in tumor tissues and related to advanced clinical stage for patients with gastric cancer (22). miR-455

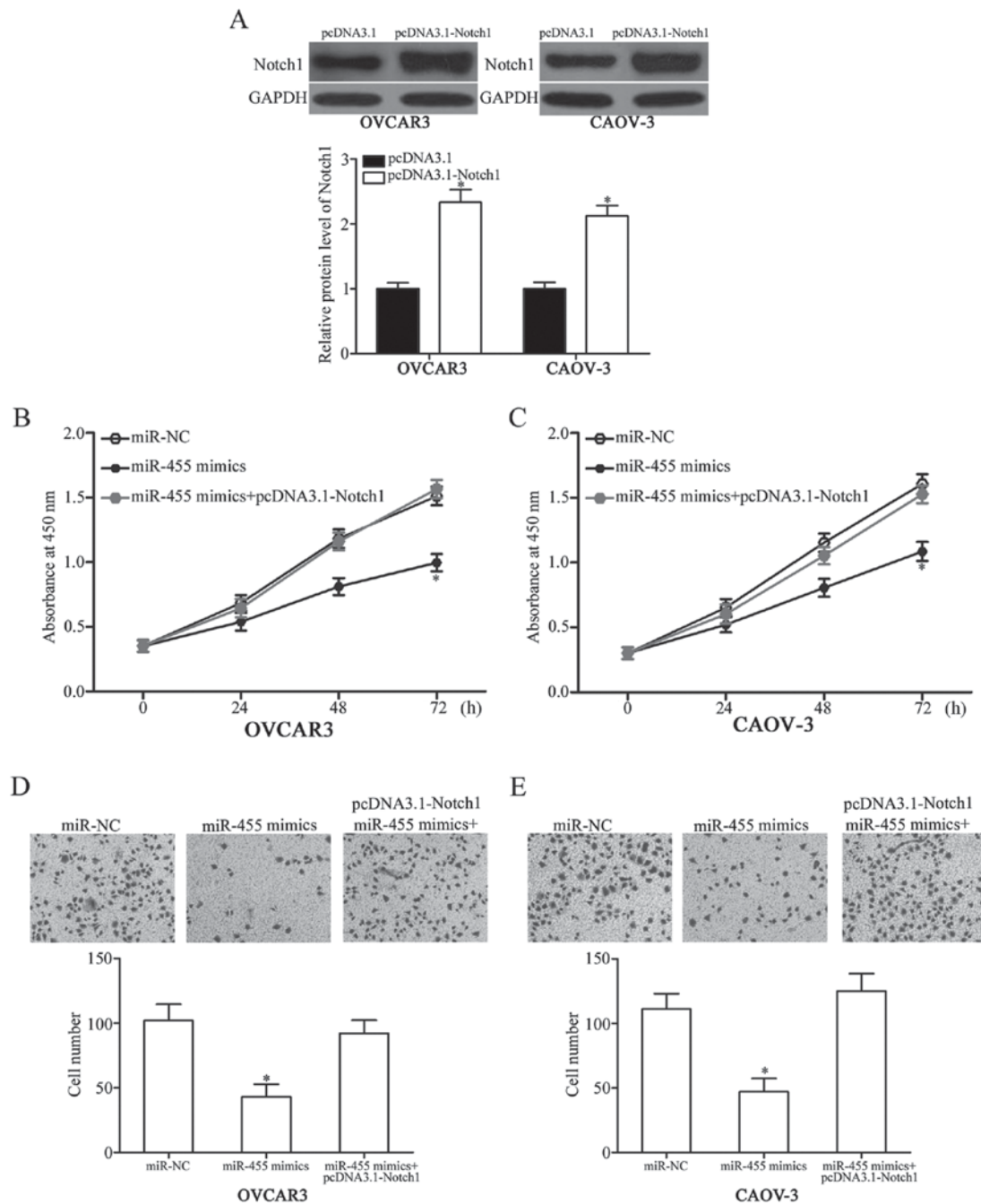


Figure 5. Restoration of Notch1 rescued the effects of miR-455 in EOC cells. (A) Notch1 protein was significantly upregulated in OVCAR3 and CAOV-3 cells transfected with pcDNA3.1-Notch1. Cell proliferation (B and C) and invasion (D and E) were determined in OVCAR3 and CAOV-3 cells transfected with miR-NC, miR-455 mimics or miR-455 mimics with pcDNA3.1-Notch1. * $P < 0.05$ vs. NC. EOC, epithelial ovarian cancer; NC, negative control; miR, microRNA.

downregulation is also observed in nonsmall cell lung cancer (23) and colorectal cancer (30). However, miR-455 expression is increased in oral squamous cancer tissues and cell lines (31). These findings suggested that miR-455 expression exhibits tissue specificity and may be a diagnostic and prognostic marker for cancers.

miR-455 is implicated in the development of many tumors. Qin *et al* (21) found that miR-455 upregulation suppresses cell migration and invasion in hepatocellular carcinoma. Liu *et al* (22) demonstrated that miR-455 overexpression inhibits gastric cancer cell proliferation and invasion *in vitro*. Li *et al* (23) revealed that enforced miR-455

expression attenuates the cell growth and metastasis of nonsmall cell lung cancer. Chai *et al* (30) reported that the restored miR-455 expression represses the cell proliferation and invasion of colorectal cancer. On the contrary, miR-455 performs oncogenic roles in oral squamous cancer by promoting tumor cell proliferation and invasion (31). This contradiction may be explained by the 'imperfect complementarity' of the interactions between miRNAs and their target genes (32). These findings also suggested that miR-455 is involved in the tumorigenesis and progression of these cancer types and is a promising therapeutic target for the treatment of cancer.

miRNAs perform its biological roles by negatively regulating their target genes (9). Therefore, the identification and characterisation of the targets of altered miRNAs may help elucidate the molecular mechanisms involved in carcinogenesis and progression. Previous studies identified several miR-455 targets, including Runx2 (21) in hepatocellular carcinoma, RAB18 (22) in gastric cancer, ZEB1 (23) in lung cancer, RAF1 (30) in colorectal cancer and UBE2B (31) in oral squamous cancer. In this study, Notch1 was validated as a novel direct target of miR-455 in EOC. TargetScan and Pictar predicted that Notch1 was a potential miR-455 target. Secondly, luciferase reporter assay revealed that miR-455 could directly target the 3'-UTR of Notch1. Thirdly, RT-qPCR and Western blot analysis revealed that miR-455 reduced Notch1 expression at the mRNA and protein levels in EOC cells. Fourthly, Notch1 was upregulated in EOC tissues and inversely correlated with miR-455 expression level. Finally, rescue experiments demonstrated that the Notch1 upregulation could reverse the inhibitory effects of miR-455 overexpression in EOC cells. These findings indicated that miR-455 was involved in EOC carcinogenesis and progression by directly targeting Notch1.

Notch signalling pathway consists of three components: Notch ligands, Notch receptors 1-4 and downstream target genes (33). Notch1, a member of Notch receptors, is a highly conserved I type transmembrane glycoprotein (34). Notch1 is aberrantly and highly expressed in multiple human cancers, such as breast cancer (35), colorectal cancer (36), bladder cancer (37), gastric cancer (38), renal cell carcinoma (39) and oesophageal squamous cell cancer (40). Moreover, Notch1 upregulation contributes to cancer cell proliferation, apoptosis, angiogenesis, migration, invasion and metastasis (41-43). In EOC, Notch1 expression is higher in tumor tissues and cell lines than in matched normal tissues and normal tissues, respectively (44,45). Notch1 expression is associated with EOC tumor stage, differentiation status and FIGO stage (44,45). High EOC expression is correlated strongly with poor prognosis (45). Functional assays revealed that Notch1 participates in regulating EOC cell proliferation, apoptosis, invasion and angiogenesis (46-49). Indeed, our work confirmed that miR-455 upregulation inhibited EOC cell proliferation and invasion through the negative regulation of Notch1. These findings suggested that the miR-455/Notch1 signalling axis may provide efficient therapeutic targets for the treatment of EOC patients.

In conclusion, miR-455 was downregulated in EOC tissues and cell lines. Low Notch1 expression was correlated with tumor size, FIGO stage and lymph node metastasis. miR-455 inhibited EOC cell proliferation and invasion by directly targeting Notch1. However, the weakness of this study is that we do not explore the effects of miR-455 on EOC cell growth and metastasis *in vivo*. Besides, future studies needed to investigate whether the miR-455/Notch1 pathway might be exploited in a therapeutic approach for the treatment of patients with EOC.

References

1. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2016. *CA Cancer J Clin* 66: 7-30, 2016.
2. Mutch DG and Prat J: 2014 FIGO staging for ovarian, fallopian tube and peritoneal cancer. *Gynecol Oncol* 133: 401-404, 2014.
3. Nodin B, Zendeirokh N, Sundström M and Jirström K: Clinicopathological correlates and prognostic significance of KRAS mutation status in a pooled prospective cohort of epithelial ovarian cancer. *Diagn Pathol* 8: 106, 2013.
4. Dong R, Liu X, Zhang Q, Jiang Z, Li Y, Wei Y, Li Y, Yang Q, Liu J, Wei JJ, *et al*: miR-145 inhibits tumor growth and metastasis by targeting metadherin in high-grade serous ovarian carcinoma. *Oncotarget* 5: 10816-10829, 2014.
5. Pereira A, Magrina JF, Magtibay PM, Pérez-Medina T, Fernández A and Peregrin I: The impact of peritoneal metastases in epithelial ovarian cancer with positive nodes. *Int J Gynecol Cancer* 21: 1375-1379, 2011.
6. Candido-dos-Reis FJ, Song H, Goode EL, Cunningham JM, Fridley BL, Larson MC, Alsop K, Dicks E, Harrington P, Ramus SJ, *et al*: Germline mutation in BRCA1 or BRCA2 and ten-year survival for women diagnosed with epithelial ovarian cancer. *Clin Cancer Res* 21: 652-657, 2015.
7. Bi L, Yang Q, Yuan J, Miao Q, Duan L, Li F and Wang S: MicroRNA-127-3p acts as a tumor suppressor in epithelial ovarian cancer by regulating the BAG5 gene. *Oncol Rep* 36: 2563-2570, 2016.
8. Moss EG: MicroRNAs: Hidden in the genome. *Curr Biol* 12: R138-R140, 2002.
9. Alvarez-Garcia I and Miska EA: MicroRNA functions in animal development and human disease. *Development* 132: 4653-4662, 2005.
10. Friedman RC, Farh KK, Burge CB and Bartel DP: Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res* 19: 92-105, 2009.
11. Zaman MS, Maher DM, Khan S, Jaggi M and Chauhan SC: Current status and implications of microRNAs in ovarian cancer diagnosis and therapy. *J Ovarian Res* 5: 44, 2012.
12. Chen L, Zhang F, Sheng XG, Zhang SQ, Chen YT and Liu BW: MicroRNA-106a regulates phosphatase and tensin homologue expression and promotes the proliferation and invasion of ovarian cancer cells. *Oncol Rep* 36: 2135-2141, 2016.
13. Liang T, Li L, Cheng Y, Ren C and Zhang G: MicroRNA-194 promotes the growth, migration, and invasion of ovarian carcinoma cells by targeting protein tyrosine phosphatase nonreceptor type 12. *Oncotargets Ther* 9: 4307-4315, 2016.
14. Lin ZY, Chen G, Zhang YQ, He HC, Liang YX, Ye JH, Liang YK, Mo RJ, Lu JM, Zhuo YJ, *et al*: MicroRNA-30d promotes angiogenesis and tumor growth via MYPT1/c-JUN/VEGFA pathway and predicts aggressive outcome in prostate cancer. *Mol Cancer* 16: 48, 2017.
15. Cong J, Liu R, Wang X, Wang J, Wang H and Hou J: Low miR-498 expression levels are associated with poor prognosis in ovarian cancer. *Eur Rev Med Pharmacol Sci* 19: 4762-4765, 2015.
16. Zhou X, Yue Y, Wang R, Gong B and Duan Z: MicroRNA-145 inhibits tumorigenesis and invasion of cervical cancer stem cells. *Int J Oncol* 50: 853-862, 2017.
17. Li C, Lu S and Shi Y: MicroRNA-187 promotes growth and metastasis of gastric cancer by inhibiting FOXA2. *Oncol Rep* 37: 1747-1755, 2017.
18. Zeng Y, Zhu J, Shen D, Qin H, Lei Z, Li W, Liu Z and Huang JA: MicroRNA-205 targets SMAD4 in non-small cell lung cancer and promotes lung cancer cell growth in vitro and in vivo. *Oncotarget* 8: 30817-30829, 2017.
19. Guo J, Cao R, Yu X, Xiao Z and Chen Z: MicroRNA-223-3p inhibits human bladder cancer cell migration and invasion. *Tumour Biol* 39: 1010428317691678, 2017.
20. Bartel DP: MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell* 116: 281-297, 2004.
21. Qin L, Zhang Y, Lin J, Shentu Y and Xie X: MicroRNA-455 regulates migration and invasion of human hepatocellular carcinoma by targeting Runx2. *Oncol Rep* 36: 3325-3332, 2016.
22. Liu J, Zhang J, Li Y, Wang L, Sui B and Dai D: miR-455-5p acts as a novel tumor suppressor in gastric cancer by down-regulating RAB18. *Gene* 592: 308-315, 2016.
23. Li YJ, Ping C, Tang J and Zhang W: MicroRNA-455 suppresses non-small cell lung cancer through targeting ZEB1. *Cell Biol Int* 40: 621-628, 2016.
24. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.

25. Zhao Y, Dong Q and Wang E: MicroRNA-320 inhibits invasion and induces apoptosis by targeting CRKL and inhibiting ERK and AKT signaling in gastric cancer cells. *Onco Targets Ther* 10: 1049-1058, 2017.
26. Shen Y, Ye YF, Ruan LW, Bao L, Wu MW and Zhou Y: Inhibition of miR-660-5p expression suppresses tumor development and metastasis in human breast cancer. *Genet Mol Res* 16: 2017.
27. Zhuo HC, Song YF, Ye J, Lai GX and Liu DL: MicroRNA-154 functions as a tumor suppressor and directly targets HMGA2 in human non-small cell lung cancer. *Genet Mol Res* 15: 2016.
28. Xiaohong Z, Lichun F, Na X, Kejian Z, Xiaolan X and Shaosheng W: MiR-203 promotes the growth and migration of ovarian cancer cells by enhancing glycolytic pathway. *Tumour Biol* 37: 14989-14997, 2016.
29. Zhao X, Zhou Y, Chen YU and Yu F: miR-494 inhibits ovarian cancer cell proliferation and promotes apoptosis by targeting FGFR2. *Oncol Lett* 11: 4245-4251, 2016.
30. Chai J, Wang S, Han D, Dong W, Xie C and Guo H: MicroRNA-455 inhibits proliferation and invasion of colorectal cancer by targeting RAF proto-oncogene serine/threonine-protein kinase. *Tumour Biol* 36: 1313-1321, 2015.
31. Cheng CM, Shiah SG, Huang CC, Hsiao JR and Chang JY: Up-regulation of miR-455-5p by the TGF- β -SMAD signaling axis promotes the proliferation of oral squamous cancer cells by targeting UBE2B. *J Pathol* 240: 38-49, 2016.
32. Yu Z, Ni L, Chen D, Zhang Q, Su Z, Wang Y, Yu W, Wu X, Ye J, Yang S, *et al*: Identification of miR-7 as an oncogene in renal cell carcinoma. *J Mol Histol* 44: 669-677, 2013.
33. Qiao L and Wong BC: Role of Notch signaling in colorectal cancer. *Carcinogenesis* 30: 1979-1986, 2009.
34. Purow BW, Haque RM, Noel MW, Su Q, Burdick MJ, Lee J, Sundaresan T, Pastorino S, Park JK, Mikolaenko I, *et al*: Expression of Notch-1 and its ligands, Delta-like-1 and Jagged-1, is critical for glioma cell survival and proliferation. *Cancer Res* 65: 2353-2363, 2005.
35. Zhong Y, Shen S, Zhou Y, Mao F, Lin Y, Guan J, Xu Y, Zhang S, Liu X and Sun Q: NOTCH1 is a poor prognostic factor for breast cancer and is associated with breast cancer stem cells. *Onco Targets Ther* 9: 6865-6871, 2016.
36. Chu D, Zhou Y, Zhang Z, Li Y, Li J, Zheng J, Zhang H, Zhao Q, Wang W, Wang R and Ji G: Notch1 expression, which is related to p65 Status, is an independent predictor of prognosis in colorectal cancer. *Clin Cancer Res* 17: 5686-5694, 2011.
37. Sima J, Zhu MY, Ai Q, Zhang C, Yao ZY, Huang QB and Zhang X: Expression analysis of NOTCH1/HES1/PTEN signaling pathway in invasive bladder transitional cell carcinoma. *Zhonghua Yi Xue Za Zhi* 92: 964-967, 2012 (In Chinese).
38. Zhang H, Wang X, Xu J and Sun Y: Notch1 activation is a poor prognostic factor in patients with gastric cancer. *Br J Cancer* 110: 2283-2290, 2014.
39. Ai Q, Ma X, Huang Q, Liu S, Shi T, Zhang C, Zhu M, Zhang Y, Wang B, Ni D, *et al*: High-level expression of Notch1 increased the risk of metastasis in T1 stage clear cell renal cell carcinoma. *PLoS One* 7: e35022, 2012.
40. Ogawa R, Ishiguro H, Kimura M, Funahashi H, Wakasugi T, Ando T, Shiozaki M and Takeyama H: NOTCH1 expression predicts patient prognosis in esophageal squamous cell cancer. *Eur Surg Res* 51: 101-107, 2013.
41. Won HY, Lee JY, Shin DH, Park JH, Nam JS, Kim HC and Kong G: Loss of Mel-18 enhances breast cancer stem cell activity and tumorigenicity through activating Notch signaling mediated by the Wnt/TCF pathway. *FASEB J* 26: 5002-5013, 2012.
42. Sharma A, Paranjape AN, Rangarajan A and Dighe RR: A monoclonal antibody against human Notch1 ligand-binding domain depletes subpopulation of putative breast cancer stem-like cells. *Mol Cancer Ther* 11: 77-86, 2012.
43. Al-Hussaini H, Subramanyam D, Reedijk M and Sridhar SS: Notch signaling pathway as a therapeutic target in breast cancer. *Mol Cancer Ther* 10: 9-15, 2011.
44. Wang M, Wang J, Wang L, Wu L and Xin X: Notch1 expression correlates with tumor differentiation status in ovarian carcinoma. *Med Oncol* 27: 1329-1335, 2010.
45. Alniaimi AN, Demorest-Hayes K, Alexander VM, Seo S, Yang D and Rose S: Increased Notch1 expression is associated with poor overall survival in patients with ovarian cancer. *Int J Gynecol Cancer* 25: 208-213, 2015.
46. Rose SL, Kunnimalaiyaan M, Drenzek J and Seiler N: Notch 1 signaling is active in ovarian cancer. *Gynecol Oncol* 117: 130-133, 2010.
47. Liang T, Guo Q, Li L, Cheng Y, Ren C and Zhang G: MicroRNA-433 inhibits migration and invasion of ovarian cancer cells via targeting Notch1. *Neoplasia* 63: 696-704, 2016.
48. Gao Y, Rankin GO, Tu Y and Chen YC: Theaflavin-3, 3'-digallate decreases human ovarian carcinoma OVCAR-3 cell-induced angiogenesis via Akt and Notch-1 pathways, not via MAPK pathways. *Int J Oncol* 48: 281-292, 2016.
49. Zou W, Ma X, Hua W, Chen B and Cai G: Caveolin-1 mediates chemoresistance in cisplatin-resistant ovarian cancer cells by targeting apoptosis through the Notch-1/Akt/NF- κ B pathway. *Oncol Rep* 34: 3256-3263, 2015.