Effects of let-7c on the proliferation of ovarian carcinoma cells by targeted regulation of CDC25a gene expression

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Received October 9, 2016; Accepted January 3, 2018

DOI: 10.3892/ol.2018.9327

Abstract. MicroRNAs serve a role in the development of ovarian cancer (OC). The present study investigated whether let-7c is able to regulate the proliferation of OC cells by targeting cell division cycle 25A (CDC25a). The reverse transcription-quantitative polymerase chain reaction was performed to detect the expression of let-7c in OC specimens. Let-7c agomir was transfected into OC cells, and the proliferation and apoptosis of OC cells were detected. A dual-luciferase assay and western blotting were performed to analyze whether CDC25a was the target gene of let-7c as well as its interaction site. The results revealed that, in OC tissue, let-7c was downregulated when compared with normal ovarian tissue. A Cell Counting Kit-8 (CCK8) assay, colony formation assay and flow cytometry demonstrated that increased expression of let-7c was able to inhibit the proliferation and increase the apoptosis of OC cells. Western blotting revealed that upregulated let-7c is able to decrease the expression of CDC25a, and a dual-luciferase assay and a recovery assay demonstrated that let-7c was able to regulate the expression of the 3' untranslated region of CDC25a. Therefore, the roles of let-7c in inhibiting the proliferation and promoting the apoptosis of OC cells may be realized through the regulation of the expression of CDC25a. The results of the present study revealed that let-7c may be a novel target in the diagnosis and treatment of OC.

Introduction

Ovarian cancer (OC) is a common gynecological malignancy (1), ranking third among the worldwide incidence of gynecological cancers, with its mortality rate ranking the first (2). Owing to its subtle onset and atypical early-stage clinical symptoms, almost 70% of OC patients present with stage III or IV OC when diagnosed (3). With continued investigation into OC pathogenesis, the search for early diagnosis and chemotherapy-sensitive markers has gained increased attention.

MicroRNAs (miRNAs/miRs) are a class of endogenous non-coding small RNA molecules which serve roles in cell growth, differentiation, metabolism and the cell cycle (4,5). The mutation, deletion and aberrant expression of miRNAs is associated with the occurrence and development of human tumors. Upregulation of miRNAs which have tumor suppressing roles is anticipated to be a novel approach in cancer gene therapy (6,7).

Let-7 was one of the first miRNAs to be identified in humans, and the 5' end of human let-7 miRNA family (let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f-1, let-7f-2, let-7g, let-7i, miR-202 and miR-98) contains one highly conserved nucleotide seed sequence which is necessary for miRNA target binding (8,9). Through the regulation of target genes, let-7 is involved in multiple biological processes including cell proliferation, differentiation, apoptosis, hormone secretion, metabolism, immune regulation and tumorigenesis. Downregulated expression of let-7 has been observed in several tumor tissues and cells including breast, lung, prostate and hepatocellular cancer (10-13). Let-7 is abundant in ovaries, and serves an important role in reproductive control during distinct developmental stages, during which the expression levels of let-7 in ovary cells are different (14). A previous study demonstrated that let-7c is involved with various ovarian physiological and pathological processes via regulation of its target genes (15). However, the underlying molecular mechanisms of let-7c in OC are not well understood. Prior investigation has revealed that cell division cycle 25A (CDC25a) is the target gene of let-7c (16). As a cell cycle regulatory protein, the abnormal expression of CDC25a is associated with the occurrence and development of multiple tumors (17-21). In the present study, the biological functions of let-7c in the development and progression of OC were investigated. The extent by which let-7c exhibits its cancer suppressive roles through the regulation of CDC25a expression levels was also investigated.

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Key words: ovarian cancer, let-7c, cell division cycle 25a, proliferation

Materials and methods

Clinical samples. A total of 52 OC tissue samples resected in Henan Provincial Maternal and Child Health Institute (Zhengzhou, China) from March 2014 to March 2015 were selected, including 21 poorly differentiated cases, 18 mid-differentiated cases and 13 well-differentiated cases. The patients were aged between 27 and 68 years, with a mean age of 55.7 years. According to the clinical staging criteria of OC by the International Federation of Gynecology and Obstetrics (FIGO) (22), 8 cases were in stage I, 10 cases were in stage II, 19 cases were in stage III and 15 cases were in stage IV. The tissue samples included 33 cases of serous adenocarcinoma and 19 cases of mucinous adenocarcinoma. No chemotherapy, radiotherapy or immunotherapy was being performed when sampling the specimens, and all samples were confirmed using histology and imaging. In the present study, all patients provided written informed consent, and the Ethics Committee of Third Affiliated Hospital of Zhengzhou University approved the present study.

Cell culture and transfection. OC cell lines SKOV3 and ES2 were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China), cultured in RPMI-1640 cell culture medium containing 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 37˚C with 5% CO_2.

During transfection, the OC cells were seeded in 6-well plates at a concentration of $5x10^4$ cells/well, and when 80% of the cells had fused, 50 nM let-7c agomir was transfected into ES2 and SKOV3 cells using a Lipofectamine™ 2000 kit (Invitrogen, Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Let-7c agomir (5'-UGAGGUAGU AGGGUUGUAUGGUU-3') and the negative control (5'-CAG UACUUUUGUGUAGUACAA-3') were designed and synthesized by Guangzhou Ribobio Co., Ltd. (Guangzhou, China). The culture medium was changed 6 h after transfection for a further 24 h in culture, and the cells were then collected for various assays.

RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The total RNA in the OC and normal ovarian tissues was extracted using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). An RNA extraction kit (Invitrogen; Thermo Fisher Scientific, Inc.) was used according to the manufacturer's protocol to extract the total RNA from the OC cells. A NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Inc.) was used to determine the concentration and purity of the extracted RNA, with the optical density 260/280 nm value close to 1.8, indicating that the purity complied with the test requirements.

RT-qPCR was performed to detect the expression of let-7c in these specimens. An RNA Reverse Transcription kit (Thermo Fisher Scientific, Inc.) was used according to the manufacturer's protocol to reverse-transcribe 1.0 μg total RNA into cDNA; ABI Power SYBR-Green PCR Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.) qPCR amplification was performed according to the manufacturer's protocol to detect the target fragments. An ABI 7500 Fast Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used according to the manufacturer's protocol to perform PCR, with a *Homo sapiens* let-7c specific primer sequence (Applied Biosystems; Thermo Fisher Scientific, Inc.). The copy number of RNU6B was used as the correction base, to detect the concentration of let-7c, which was expressed as a relative expression level using the $2^{-\Delta\Delta Cq}$ method (23). The primer sequences used were as follows: miRNA-let-7c: 5'-GCGCGTGAGGTAGTAGGTT-3' (sense) and 5'-GTGCAGGGTCCGAGGT' (anti-sense); U6 5'-GCG CGTCGAAGCGTTC-3' (sense) and 5'-GTGAGGGTCCGA GGT-3' (anti-sense). Hot start PCR conditions were 10 sec at 95˚C, 20 sec at 60˚C and 10 sec at 72˚C for 40 cycles.

Western blot analysis. The protein samples of each group were collected. The cells were digested with 0.25% trypsin and the cell stocks were centrifuged at 156 x g for 5 min at 4˚C. The cells were resuspended with 1 ml phosphate suffered saline (PBS) and centrifuged at 156 x g for 5 min at 4˚C. These cells were then lysed with radioimunoprecipitation assay buffer (Beyotime Institute of Biotechnology, Haimen, China) to lyse the cells, and coomassie brilliant blue was used to determine the protein concentration. Following separation using SDS-PAGE (10% gel), the required proteins (50 μg) were transferred onto a polyvinylidene difluoride film, followed by 1 h of blocking using 5% skimmed milk in Tris-buffered saline with Tween-20, and overnight incubation at 4˚C with agitation with diluted primary antibody (1:500; rabbit anti-human CDC25a antibody; cat. no. SC-97; Santa Cruz Biotechnology, Inc., Dallas, TX, USA). Following washing using Tris-buffered saline containing Tween-20 (TBST) for 4x15 min, diluted secondary antibody (1:1,000; horseradish peroxidase-labeled IgG; cat. no. SC-2357; Santa Cruz Biotechnology, Inc.) was added for 1 h to incubate, followed by TBST washing for 4x15 min. An ECL Western Blotting Detection kit was then used to detect the signals (GE Healthcare, Chicago, IL, USA) with GAPDH (1:500; cat. no. sc-47724; Santa Cruz Biotechnology, Inc.) as the reference to calculate the relative expression of the proteins.

Detection of cellular proliferation using Cell Counting Kit-8 (CCK-8). CCK-8 contains WST-8 [chemical name, 2-(2-meth oxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfonic acid benzene)-2H-tetrazolium monosodium salt], which is able to be reduced to a highly water-soluble formazan dye by the dehydrogenase in the electron carrier 1-methoxy-5-methylphenazinium dimethyl sulfate in cells. The amount of formazan produced is proportional to the number of viable cells. Therefore, this trait may be utilized directly as a cell proliferation assay. A CCK-8 kit (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) was used to detect the effect of let-7c on cellular proliferation. Cells in the exponential growth phase were seeded in 96-well plates ($2x10^3$ cells/well), with 10 μl WST-8 solution added into each well at 24, 48, 72 and 96 h respectively, according to the manufacturer's protocol to detect the cell proliferation. Results were expressed as the optical density value of each well at 450 nm.

Plate clone assay. Low melting point agarose solution (0.6%) was mixed with RPMI-1640 medium containing 10% FBS (1:1), added into 6-well plates, and allowed to cool and solidify at room temperature. The cells of each group in the exponential growth phase following transfection were suspended

in the aforementioned culture medium, and then added into 6-well plates in which the lower gel layer had already solidified. Following a 12-day incubation at 37˚C, the cells were stained using 1 ml 1% crystal violet to count the cell clones with the naked eye and light microscopy (x100 magnification). The cell clones with more than 50 cells were counted as one monoclone, and the mean number of clones on the plate was used to calculate the cell clone ability.

Detection of apoptosis by flow cytometry. The cells were digested by 0.2% trypsin at 37˚C for 2 min and centrifuged at 156 x g for 5 min at 4˚C, and the cells were counted following suspension in PBS ($1x10^6$ cells/ml) 72 h post-transfection. An annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) Apoptosis Detection kit (BestBio, Shanghai, China) was used to detect the proportion of cells undergoing apoptosis and flow cytometry (BD Biosciences, Franklin Lakes, NJ, USA) was used to determine the cell apoptosis within 30 min.

Dual-luciferase reporter gene assay. Three target gene prediction databases, TargetScan (http://www.targetscan.org), PicTar (http://pictar.mdc-berlin.de) and miRanda (http://microrna.sanger.ac.uk), were used to predict the target gene of let-7c, with CDC25a identified as the potential target. The DNA of healthy human subjects was extracted using a Human blood genomic DNA extraction kit (Promega Corporation, Madison, WI, USA) following the provision of written informed consent, and the CDC25a 3' untranslated region (UTR) that included the let-7c-binding sites was amplified using PCR. The reaction protocols were as follows: 95˚C pre-degeneration, cycle 95˚C for 10 sec degeneration; 1˚C annealing from 65˚C each cycle, 72˚C extension for 2 min, for 10 cycles; 55˚C annealing, 15 cycles of; 72˚C for 7 min and 4˚C preservation. TaqMan Universal PCR Master Mix (Applied Biosystems). The primer sequences of CDC25A used were as follows: 5'-CCGCTCGAG GCGGCAGGACCAGCCAG-3' (sense) and 5'-GAATGCGGC CGCTCAGAGCTTCCA ACAGTTGGTTAG-3' (anti-sense). The amplified CDC25a was then recovered by AxyPrep DNA gel recovery kit (Axygen; Corning Incorporated, Corning, NY, USA) following 1% agarose electrophoresis, and connected with pmirGLO carrier (pmirGLO-CDC25a-wt, wild-type CDC25a) using T4 DNA ligase (Takara Bio, Inc., Otsu, Japan); mutagenic primers targeting the CDC25a 3'UTR seed region were then designed, and amplified using the overlap method, and inserted into the multi-cloning site of pmirGLO vector (pmirGLO-CDC25a-mut, mutant CDC25a). Lipofectamine 2000 was then used to co-transfect the recombinant vectors and let-7c agomir or negative control into OC cells; 48 h later, a Dual-Luciferase Reporter assay system (Promega Corporation, Madison, WI, USA) was used to measure the dual-luciferase signals in each group, with the *Renilla* luciferase signal as the reference; each group was tested in triplicate.

Recovery assay. The CDC25a fragment free of 3'UTR was amplified and inserted into the eukaryotic expression vector pcDNA3.1 (pcDNA3.1-CDC25a). pcDNA3.1-CDC25a was then transfected into SKOV3 cells; meanwhile, let-7c agomir or negative control was also transfected using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The cells of each group were collected 48 h later, expression levels of CDC25a protein were investigated using western blotting; and apoptosis was investigated using flow cytometry.

Statistical analysis. SPSS (version 18.0; SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Data are expressed as the mean ± standard deviation. The differences between groups was analyzed using Student's t-test or analysis of variance with Student-Newman-Keuls method as a post-hoc test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Expression of let-7c in OC tissue. The expression levels of let-7c in 52 OC cases determined using RT-qPCR demonstrated that, compared with normal tissue, let-7c was significantly down-regulated in the OC tissue ($P<0.05$; Fig. 1A); further analysis of the association of let-7c expression with the pathological factors of patients revealed that let-7c was associated with lymph node metastasis (Fig. 1B) and clinical stage ($P<0.05$; Fig. 1C and Table I), the expression levels of let-7c in samples from patients with lymph node metastasis were decreased compared with those with no lymph node metastasis, and were decreased in the higher clinical grading group; however, there was no observable association with age, pathological type or differentiation degree ($P>0.05$; Table I).

Effects of let-7c on the proliferation of OC cells. CCK-8 and plate clone assays were performed to analyze the effects of let-7c on the proliferation of OC cells. A CCK-8 assay revealed that, after 48 h of transfection of the let-7c agomir into the OC cell lines, the relative proliferation rates of SKOV3 and ES2 were significantly decreased compared with the blank group and the control group ($P<0.05$), and no statistically significant difference was identified between the blank group and the control group at any time point ($P>0.05$; Fig. 2). The plate clone assay demonstrated that the let-7c agomir significantly decreased the number of OC cell colonies ($P<0.05$; Fig. 3), suggesting that the overexpression of let-7c inhibited the proliferative abilities of the OC cell lines SKOV3 and ES2.

Effects of let-7c on apoptosis of OC cells. Flow cytometry revealed that, following transfection with let-7c agomir, the apoptotic rates of SKOV3 and ES2 cells were significantly increased compared with the control group ($P<0.05$; Fig. 4), suggesting that the overexpression of let-7c leads to the apoptosis of SKOV3 and ES2 cells.

Expression inhibition of CDC25a mRNA 3'UTR by let-7c. The miRNA bioinformatics database analysis identified that CDC25a was the target gene of let-7c (Fig. 5A). The present study inserted human CDC25a wild-type and mut-type 3'UTRs into the dual-luciferase reporter vector pmirGLO, and co-transfected this recombinant vector and let-7c agomir or negative control into OC cells to detect the fluorescent signal changes in each group. The results demonstrate that following co-transfection with let-7c agomir and pmirGLO-CDC25a-wt, luciferase activity was significantly inhibited ($P<0.05$; Fig. 5B); however, the co-transfection of let-7c agomir and

Table I. Association between let-7c expression and clinical pathological factors in OC.

Clinicopathological characteristic	n	let-7c expression	P-value
Age, years			0.087
<50	23	0.4741±0.0770	
≥50	29	0.5132±0.0828	
Tissue type			0.844
Serous adenocarcinoma	33	0.4976±0.0853	
Mucinous adenocarcinoma	19	0.4929±0.7768	
Differentiation			0.263
High	21	0.4819±0.0744	
Medium	18	0.4892±0.0924	
Poor	13	0.5278±0.0752	
Clinical stage			0.015[a]
I and II	18	0.5333±0.0831	
III and IV	34	0.4761±0.0750	
Lymph node metastasis			0.033[a]
No (-)	24	0.5218±0.0833	
Yes (+)	28	0.4737±0.0751	

Values are the median ± standard deviation. [a]P<0.05.

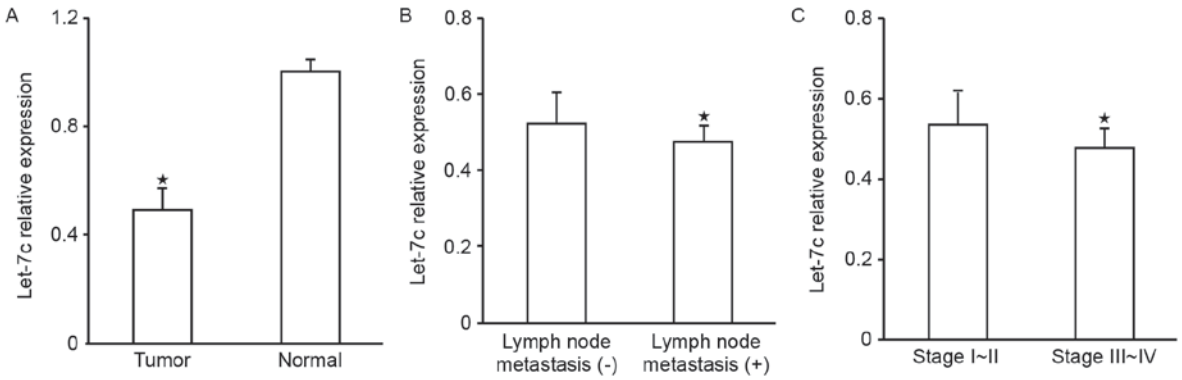

Figure 1. Detection of let-7c expression level in OC tissue using the reverse transcription-quantitative polymerase chain reaction. (A) Let-7c was significantly increased in OC tissue compared with normal tissue (*P<0.05). (B) Let-7c expression levels are significantly decreased in metastatic lymph nodes compared with non-metastatic lymph node tissue (*P<0.05). (C) In OC tissues, the higher the clinical stage, the lower the let-7c expression level (*P<0.05). OC, ovarian cancer.

pmirGLO-CDC25a-mut demonstrated no significant change in luciferase activity (P>0.05; Fig. 5B), suggesting that let-7c may combine with the CDC25a 3'UTR seed region, thus resulting in the downregulation of the CDC25a gene. Western blotting results also revealed that let-7c agomir was able to decrease the expression of CDC25a protein in OC cells (Fig. 5C).

Overexpression of CDC25a is able to recover the pro-apoptotic effect of let-7c towards OC cells. In order to further explore the targets of let-7c, pcDNA3.1-CDC25a was constructed and co-transfected alongside let-7c agomir or a negative control into SKOV3 cells; furthermore, let-7c agomir or a negative control were separately transfected into SKOV3 cells. An apoptosis assay demonstrated that when only let-7c agomir was transfected, was the apoptotic rate significantly increased; however, the apoptotic rate was significantly decreased when pcDNA3.1-CDC25a was transfected or pcDNA3.1-CDC25a and let-7c agomir were co-transfected into the cells (Fig. 6A). Western blot analysis revealed that, following the co-transfection of pcDNA3.1-CDC25a and let-7c agomir, CDC25a protein was increased, indicating that pcDNA3.1-CDC25a is able to recover the inhibition of let-7c towards the expression of CDC25a protein (P<0.05; Fig. 6B). This further illustrates that let-7c may act on the CDC25a 3'UTR seed region, thus regulating the CDC25a expression exhibiting pro-apoptotic effects towards OC.

Discussion

miRNAs are able to regulate transcription by binding to the 3'UTR of target genes, thus serving a role in a variety

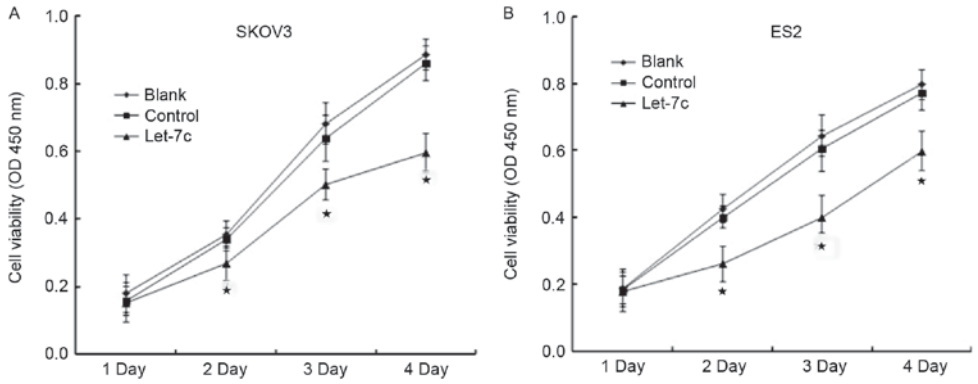

Figure 2. Let-7c agomir significantly inhibits the proliferation of OC cells. A Cell Counting Kit-8 assay revealed that compared with the blank group and the control group, the proliferation abilities of (A) SKOV3 and (B) ES2 were significantly decreased at 2, 3 and 4 days after let-7c agomir transfection compared with the control (*P<0.05). OD, optical density. OC, ovarian cancer.

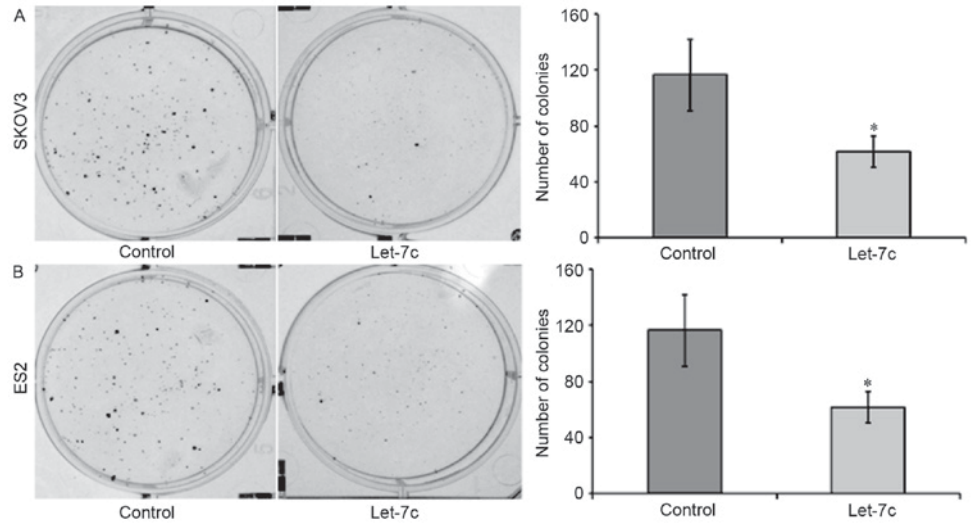

Figure 3. Overexpression of let-7c inhibits the colony formation ability of OC cells. The let-7c agomir-transfected OC cell lines (A) SKOV3 and (B) ES2 exhibited significantly decreased cell colony numbers compared with control cells (*P<0.05). OC, ovarian cancer.

of physiological processes (24). A previous study revealed that the expression profiles of miRNAs in tumor tissue and normal tissue exhibit significant differences, and the expression levels of miRNAs are associated with the development, differentiation, metastasis and prognosis of tumor cells (25). Previous studies have analyzed miRNA expression profiles in order to investigate miRNAs in a variety of malignant tumors, including OC and OC cell lines, expecting to identify miRNAs with diagnostic potential (26,27). Zhang *et al* (28) detected the expression of 173 mature miRNAs in OC cell lines and human ovarian surface epithelial cell lines, and identified 35 miRNAs with significant expression differences, among which 31 were downregulated and four were upregulated. Iorio *et al* (29) analyzed the miRNA expression profiles in OC tissue, and revealed that miRNA was not only differentially expressed between tumor and normal tissues, but also differentially expressed in different histological subtypes.

miRNAs themselves may become the target of cancer therapies which alter the expression of miRNA so as to regulate the target genes and treat cancer. Mature let-7 is able to bind to the 3'UTR of its target gene and degrade the target mRNA or inhibit its translation. Han *et al* (30) overexpressed let -7c, which inhibited the invasion and metastasis of colorectal cancer cells. Zhao *et al* (31) demonstrated that let-7c acts directly on downstream genes mitogen-activated protein kinase kinase kinase kinase 3 and integrin subunit β3, thus inhibiting the invasion and metastasis of non-small cell lung cancer. Wang *et al* (32) demonstrated that let-7c inhibits the proliferation of lung cancer cells by acting on tribbles homolog 2. Therefore, during the development of OC, the similar role of let-7c as a cancer suppressing gene may also result from its regulation of target genes. The present study detected the let-7c expression levels in OC tissue and normal ovarian tissue, and demonstrated that let-7c was downregulated in OC, with its level associated with lymph node metastasis and clinical stage, suggesting that miR-26a has certain associations with the occurrence of OC. At the same time, following transfection of let-7c agomir into SKOV3 and ES2 cells, it was revealed that the OC cell proliferation was decreased; however, the apoptotic rate was increased, suggesting that let-7ca is able to inhibit the proliferation of OC, consistent with its biological effects in other tumors.

Bioinformatics analysis revealed that CDC25a may be a potential target site of let-7c. The CDC25 gene can express

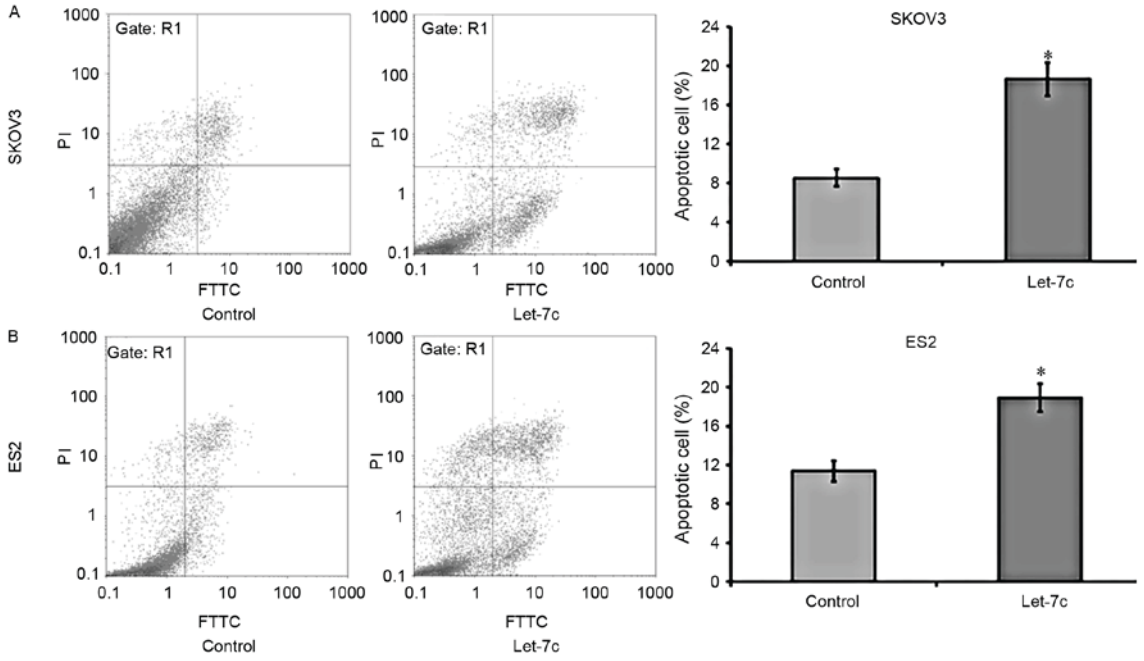

Figure 4. Overexpression of let-7c may lead to the apoptosis of SKOV3 and ES2 cells. The apoptotic rates of (A) SKOV3 and (B) ES2 cells were significantly increased when compared with the control group following transfection with let-7c agomir (*P<0.05). PI, propidium iodide.

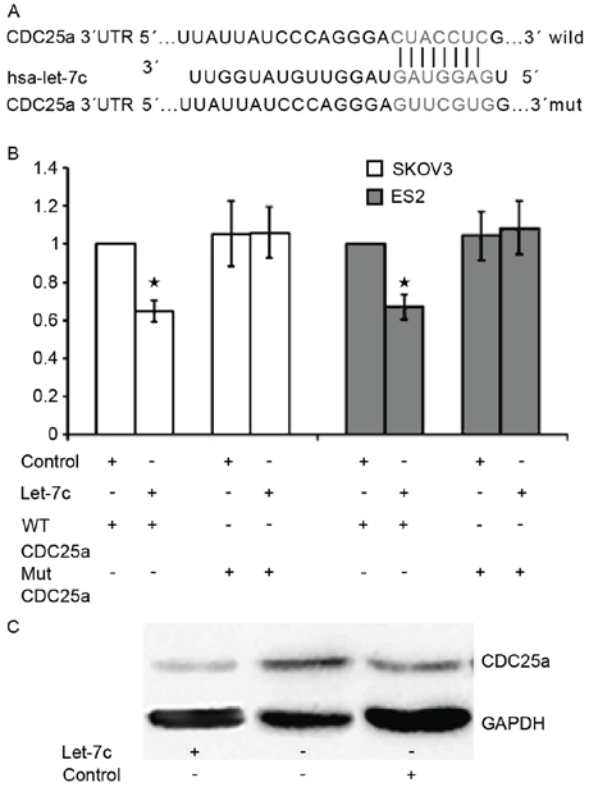

Figure 5. Let-7c acts on CDC25a 3'UTR and regulates its expression. (A) Let-7c acts on CDC25a 3'UTR seed region sequence. (B) A dual-luciferase reporter gene assay demonstrated that the co-transfection of let-7c agomir and pmirGLO-CDC25a-wt significantly decreases the luciferase activity of SKOV3 cells when compared with the control group (miR-NC and pmirGLO-CDC25a-wt co-transfection group; *P<0.05); the co-transfection of let-7c agomir and pmirGLO-CDC25a-mut does not significantly alter the luciferase activity in comparison with the control group (negative control and pmirGLO-CDC25a-mut co-transfection group) (*P>0.05). (C) Western blot analysis revealed that the overexpression of let-7c suppresses the CDC25a protein level in SKOV3 cells. CDC25a, cell division cycle 25a; Hsa, *Homo sapiens*; WT, wild-type; Mut, mutant.

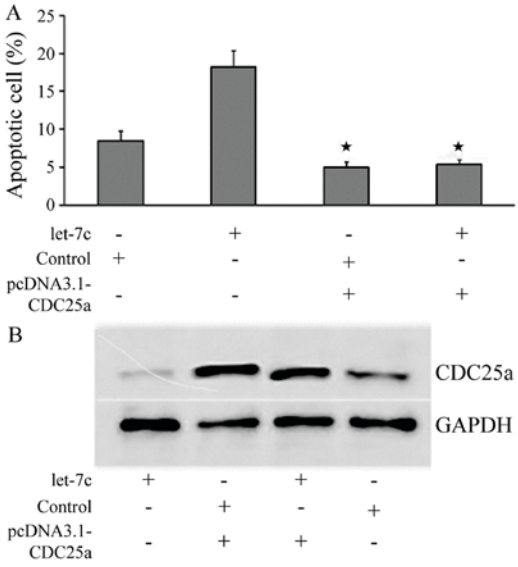

Figure 6. Overexpression of CDC25a may recover the pro-apoptotic effects of let-7c towards OC cells. (A) The co-transfection of CDC25a 3'UTR-free pcDNA3.1-CDC25a and let-7c agomir into SKOV3 cells significantly decreased the apoptotic rate of the cells (*P<0.05). (B) Western blot analysis revealed that CDC25a 3'UTR-free pcDNA3.1-CDC25a is able to recover the let-7c agomir-inhibited expression of CDC25a protein in SKOV3 cells, thus resulting in the upregulation of CDC25a protein. CDC25a, cell division cycle 25a.

CDC25 phosphatase protein, a cell cycle regulatory protein which serves a role in the normal cell cycle (33). In a series of human malignancies including hepatocellular carcinoma, ovarian cancer, colorectal cancer, esophageal cancer and non-Hodgkin's lymphoma, CDC25a exhibits increased expression levels (17-21). CDC25a exhibits pro-cancer traits in two distinct manners. First, it is able to promote cell proliferation (34). When cells enter S phase, CDC25a activates the cyclin

E-cyclin dependent kinase-2 (CDK2) and cyclin A-CDK2 complexes by dephosphorylation, thus promoting cells to enter S phase (35). If the CDC25a protein is overexpressed, it may cause cells to rapidly enter S phase from G phase, followed by an increase in DNA synthesis, malignant cell proliferation or even development of cancer (36). Secondly, CCD25a is considered to be a checkpoint gene, and acts simultaneously on two checkpoints, the G_1/S phase and the G_2/M phase, thus serving roles in DNA damage and repair (37). Therefore, the overexpression of CDC25a may lead to disorders of cell cycle regulation and decreased response to DNA damage, so abnormal cell proliferation or even tumors may be observed as a result of CDC25a overexpression.

Western blotting revealed that the overexpression of let-7c may lead to the downregulation of CDC25a protein in OC cells. CDC25a 3'UTR was then cloned into the dual-luciferase reporter vector pmirGLO, and the results revealed that let-7c is able to bind to the CDC25a 3'UTR seed region, thus negatively regulating its expression. The overexpression of CDC25a revealed by the recovery assay may restore the pro-apoptotic effects of let-7c towards OC cells. This further demonstrates that let-7c may bind to CDC25a mRNA 3'UTR, thus regulating its expression, and inhibiting the proliferation and inducing the apoptosis of OC cells. Therefore, CDC25a may possibly act as a target gene of let-7c, thus regulating the malignant proliferation of OC cells.

The development of OC is regulated by a complex network of numerous cytokines, enzymes and genes, among which let-7c serves a role as a tumor suppressor, thus affecting the development, as well as staging and treatment, of tumors. Previous research has also demonstrated that let-7c may bind to several oncoproteins and several key regulatory factors involved in the mitotic pathway and regulate their expression (38). CDC25a is only one target gene of let-7c (16,39). The activities of CDC25a are regulated by multiple mechanisms, including the ubiquitin ligase anaphase-promoting complex/cyclosome (40) and transforming growth factor β (41). Therefore, the present study demonstrated only that let-7c may target CDC25a, thus inhibiting the proliferation of OC cells; further research into other biological functions of let-7c is required.

The abnormal expression of let-7c in OC tissue is associated with the proliferation of OC; let-7c may act on CDC25a 3'UTR and inhibit its expression, thus serving a similar role to a tumor suppressor gene. Further in-depth studies into the target genes and mechanisms of let-7c may reveal its potential as a novel diagnostic and therapeutic target in OC.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

XZ designed the research. WG, WZ, QZ, ZB and JC conducted the experiments. TJ TC, DL and CL analyzed the data. The manuscript was drafted by WG and WZ. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Third Affiliated Hospital of Zhengzhou University and written informed consent was obtained from all participants.

Patient consent for publication

All participants provided written informed consent for publication.

Competing interests

The authors declare that they have no competing interests.

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