

Downregulation of DNMT3a expression increases miR-182-induced apoptosis of ovarian cancer through caspase-3 and caspase-9-mediated apoptosis and DNA damage response

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Abstract. In the present study, DNA (cytosine-5)-methyltransferase 3 α (DNMT3a) is explored as an anticancer molecule in ovarian cancer treatment, and also the mechanistic link between DNMT3a and its regulatory signaling pathway in Caov-3 cells is provided. Firstly, DNMT3a protein expression in 12 freshly resected ovarian cancer patient tissues and tissues from 8 ovariectomized patients was assessed. In the ovarian cancer tissues, DNMT3a expression was upregulated and miR-182 expression was downregulated. DNMT3a overexpression inhibited miR-182 expression and caspase-3 and -9 activity and suppressed p53 and c-Myc protein expression in Caov-3 cells. Secondly, miR-182 overexpression increased Caov-3 cell apoptosis, which however was reduced by DNMT3a (DNMT3 plasmid) overexpression. Downregulation of DNMT3a expression activated miR-182 expression and caspase-3 and -9 activity, and promoted p53 and c-Myc protein expression in Caov-3 cells. Collectively, a valuable anticancer mechanism of ovarian cancer was elucidated, by which downregulation of DNMT3a regulated miR-182 via caspase-3 and -9-mediated apoptosis and DNA damage response, which suggests that DNMT3a may be used as a potential strategy for therapeutic intervention in ovarian cancer.

Introduction

As one of the three malignant tumors of the gynecologic reproductive system, the morbidity of ovarian cancer is ranked only second to cervical and endometrial cancer. Moreover,

its mortality rate is highest among all female reproductive tract malignant tumors (1). The majority (up to 85-90%) of ovarian malignant carcinomas originate from the coelomic epithelium (2,3). Since the ovary is deeply located in the pelvic cavity and there is no specificity for incipient symptoms of ovarian cancer, epithelial ovarian carcinoma develops rapidly, and is prone to metastasis and wide dissemination (2); 70-80% of patients are diagnosed at stage III and IV while seeking medical services for ascites or pelvic mass (4).

Ovarian cancer is a multifactor disease with genetic susceptibility (3). Gene mutations in patients lead to differences in ovarian cancer susceptibility. Ovarian cancer is difficult to be definitely diagnosed at an early stage, and thus patients are at an advanced stage when diagnosed (5). Studying molecular markers with specificity is of great significance in diagnosing ovarian cancer early and improving the patient survival rate (6). DNA damage epigenetics, such as DNA abnormal demethylation and hyperphosphorylation, has become a new 'hot' research topic in recent years (7). DNA damage is closely associated with cancer-suppressor genes aroused by abnormal DNA transfer to daughter cells, therefore it is an essential mechanism for tumors to inhibit gene inactivation (7). In addition, DNA damage response is extremely important for monitoring the organism and rehabilitation mechanism (8).

DNA (cytosine-5)-methyltransferase 3 α (DNMT3a) encoding DNA methyltransferase 3A has been investigated in recent years, and the DNA methylation process may have catalytic action (9). In general, the DNA methylation level is believed to be highly correlated to tumor occurrence. Gene mutations in AML patients reach 20%. DNMT3a and b are *de novo* methylation enzymes, and also contribute to maintaining DNA methylation patterns in embryonic stem (ES) and somatic cells. The inactivation of DNMT3a and b results in the gradual loss of DNA methylation. *De novo* methylation activities are coordinated to permit the faithful inheritance of DNA methylation and would tip the balance of DNA methylation inheritance to increase DNA methylation.

As a type of non-coding small RNAs, microRNAs (miRNAs) inhibit the function of target mRNAs by inhibiting

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Table I. Characteristics of the primers used for qRT-PCR.

| Primer | Sequence (5'-3') (sense) | Sequence (5'-3') (antisense) |
|----------------|--------------------------|------------------------------|
| DNMT3a | TATGAACAGGCCGTTGGCATC | AAGAGGTGGCGGATGACTGG |
| β -actin | CACGAACTACCTTCAACTCC | CATACTCCTGCTTGCTGATC |
| miR-182 | TGCGGTTTGGCAATGGTAGAAC | TGCGGTTTGGCAATGGTAGAAC |
| U6 | GCTTGCTTCGGCAGCACATATAC | TGCATGTCATCCTTGCTCAGGG |

translation or inducing degradation (10), which regulates cell differentiation, proliferation and apoptosis. Research shows that various tumor tissues have abnormal expression or mutation of miRNAs, which have an effect on tumor growth, invasion and metastasis (10,11). miR-182 is an important miRNA molecule (12), which promotes benign and malignant tumor cell invasion and transformation, indicating the significance of miR-182 in ovarian cancer. It is conducive to ovarian cancer prevention and treatment through effective regulation.

Materials and methods

Patients and tissue samples. The present study was approved by the Ethics Review Committee of Liaocheng People's Hospital and informed consent for the use of tissues was obtained for all individuals. Twelve freshly resected ovarian cancer and tissues from 8 ovariectomized patients were collected from Liaocheng People's Hospital, Shandong, China. Adjacent and cancer tissue samples were collected from 12 freshly resected ovarian cancer patients, and normal ovarian tissue samples were collected from 8 ovariectomized patients, in accordance with the institutional guidelines and immediately frozen in liquid nitrogen for further analysis.

Cell culture. Human ovarian cancer Caov-3, OV-1063, OVCAR-3 and Caov-4 cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS) (both from HyClone, Thermo Scientific, Waltham, MA, USA) and 100 units penicillin-streptomycin at 37°C in a 5% CO₂ atmosphere in a humidified incubator.

Quantitative RT-PCR assay. RNA was extracted from frozen tissues or cell samples using TRIzol reagent (Life Technologies) according to the manufacturer's instructions. RNA (1 μ g) was used to compound cDNA using a SuperScript™ One-Step RT-PCR kit (Toyobo, Tokyo, Japan) according to the manufacturer's instructions. qRT-PCR was performed using SYBR-Green Mix reagent (Takara, Dalian, China) and the primer sequences of miR-182 are listed in Table I.

Transfection. Human ovarian cancer Caov-3 cells (2×10^5) were plated onto 6-well plates and were transfected with the miR-182 plasmid (miR-182 overexpression), the DNMT3 plasmid (DNMT3a overexpression), DNMT3a siRNA (down-regulation of DNMT3a expression) or the negative control using transfection reagent Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

Flow cytometric analysis of apoptosis. Human ovarian cancer Caov-3 cells (2×10^5) transfected with the different plasmids were plated onto 6-well plates. The apoptosis rate was assessed to detect early stage apoptosis by analysis of Annexin V-FITC binding (Becton-Dickinson, San Jose, CA, USA). Annexin V-FITC (10 μ l) and 5 μ l of propidium iodide (PI) were added into every well and the cells were cultured for 30 min. The apoptosis rate was detected using a flow cytometer (BD Biosciences, Oxford, UK).

Western blotting. Caov-3 cells transfected with the different plasmids were directly lysed in Laemmli's sample buffer and boiled for 30 min on ice. After removal of the insoluble fraction by centrifugation, total protein expression was quantitated using the BCA method (Life Technologies Co., Shanghai, China). Total protein extracts (50 μ g) were resolved on 10% SDS-PAGE and transferred to nitrocellulose membranes for western blotting. The membranes were incubated with the primary antibodies: anti-DNMT3a, anti-p53, anti-c-Myc (all from Bethyl Laboratories, Cambridge Biosciences, Cambridge, UK) and β -actin (Sangon Biotech) at a dilution of 1:1,000, at room temperature for 1 h. After washing with 5% bovine serum albumin (BSA) dissolved in Tris-buffered saline (TBS), containing 0.05% Tween-20 (TBST), the membranes were incubated with goat anti-rabbit secondary antibody conjugated to horseradish peroxidase (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) at a 1:2,000 dilution at room temperature for 1 h. Positive band intensities were detected using a gel documentation system (LAS-3000; Fujifilm, Tokyo, Japan).

Caspase-3 and -9 activity. Human ovarian cancer Caov-3 cells (1×10^3) transfected with the different plasmids were plated onto 96-well plates. Ac-DEVD-pNA (caspase-3) and Ac-LEHD-pNA (caspase-9) were added into every well and the cells were cultured for 30 min. Caspase-3 and -9 activity was detected using a BioTek Synergy plate reader (BioTek, Pottom, UK) at 450 nm.

Statistical analysis. The data are expressed as the mean \pm SD. The Student's t-test was used to determine the statistically significant differences in numbers with two significant levels ($p < 0.05$).

Results

The protein expression of DNMT3a in normal, adjacent cancer and cancer tissues. Firstly, we assessed the DNMT3a protein expression in 12 freshly resected ovarian cancer and

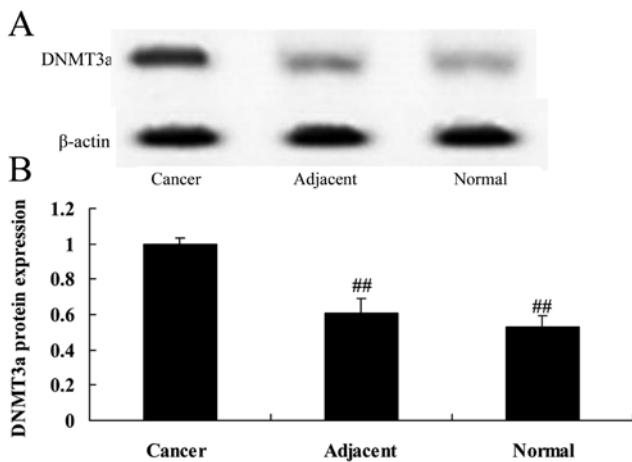


Figure 1. Protein expression of DNMT3a in normal, adjacent cancer and cancer tissue. Cancer, cancer tissue; adjacent, cancer adjacent tissue; normal, normal tissue; ^{##}p<0.01 compared with the cancer tissue group.

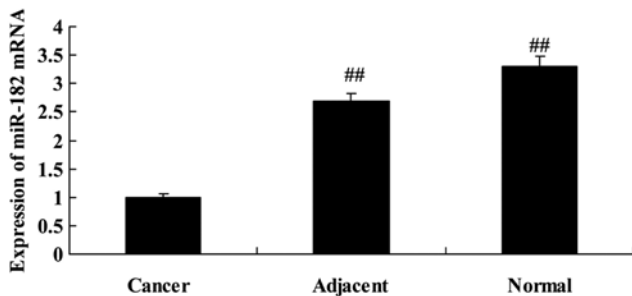


Figure 2. RT-PCR analysis of miR-182 in normal, adjacent cancer and cancer tissue. Cancer, cancer tissue; adjacent, cancer adjacent tissue; normal, normal tissue; ^{##}p<0.01 compared with the cancer tissue group.

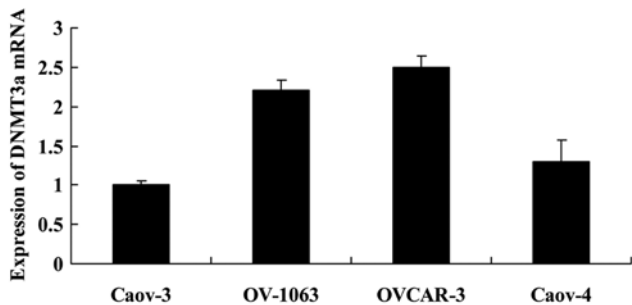


Figure 3. DNMT3a expression in human ovarian cancer cells.

8 ovariectomized patient tissues. We found that the protein expression of DNMT3a in the cancer tissues was much higher than that noted in the adjacent cancer or normal tissues (Fig. 1). However, the protein expression of DNMT3a in the adjacent cancer tissues was very similar to that noted in the normal tissues (Fig. 1).

RT-PCR analysis of miR-182 in normal, adjacent cancer and cancer tissues. Next, we found that the expression of miR-182 in cancer tissues was lower than that noted in the adjacent cancer or normal tissues (Fig. 2). However, the expression of

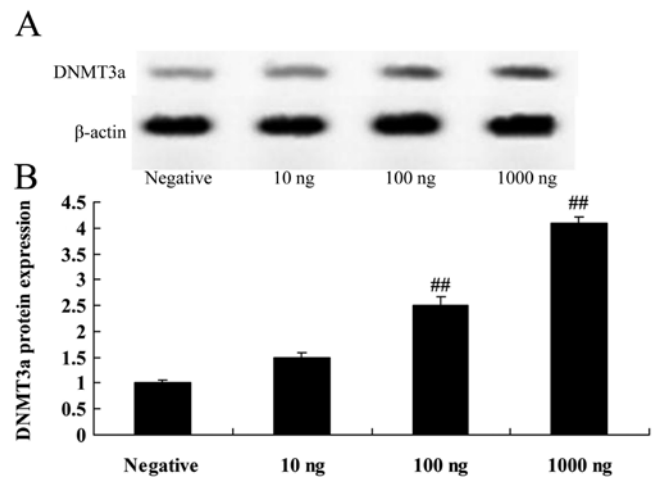


Figure 4. DNMT3a overexpression affects DNMT3a protein in human ovarian cancer. DNMT3a overexpression affects DNMT3a protein as detected by (A) western blotting, and (B) DNMT3a protein statistical analyses of human ovarian cancer. Negative, negative control group; 10 ng, cells transfected with 10 ng DNMT3 plasmid/well; 100 ng, cells transfected with 100 ng DNMT3 plasmid/well; 1,000 ng, cells transfected with 1,000 ng DNMT3 plasmid/well; ^{##}p<0.01 compared with the cancer tissue group.

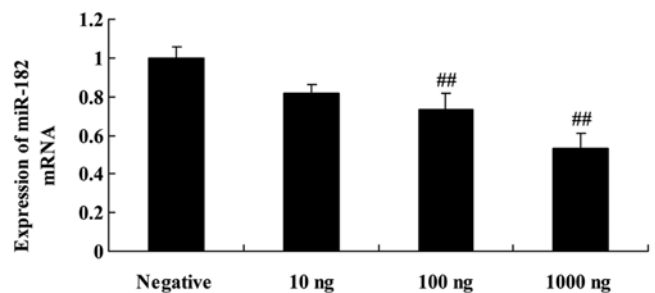


Figure 5. DNMT3a overexpression affects miR-182 in human ovarian cancer. Negative, negative control group; 10 ng, cells transfected with 10 ng DNMT3 plasmid/well; 100 ng, cells transfected with 100 ng DNMT3 plasmid/well; 1,000 ng, cells transfected with 1,000 ng DNMT3 plasmid/well; ^{##}p<0.01 compared with the cancer tissue group.

miR-182 in the adjacent cancer tissues was very similar to that noted in the normal tissues (Fig. 2).

Expression of DNMT3a in human ovarian cancer cells. We used Caov-3, OV-1063, OVCAR-3 and Caov-4 cells to analyze the expression of DNMT3a mRNA. As shown in Fig. 3, the expression of DNMT3a in the Caov-3 cells was the lowest. Thus, we selected Caov-3 cells to serve as the main cells used in the present study.

Overexpression of DNMT3a affects the DNMT3a protein in human ovarian cancer. The DNMT3 plasmid was transfected into Caov-3 cells, in which we induced expression of DNMT3a. As shown in Fig. 4, the DNMT3 plasmid induced the DNMT3a protein expression in the Caov-3 cells in a dose-dependent manner. Particularly, 100 or 1,000 ng DNMT3 plasmid significantly activated the protein expression of DNMT3a in the Caov-3 cells, compared with the negative control group (Fig. 4).

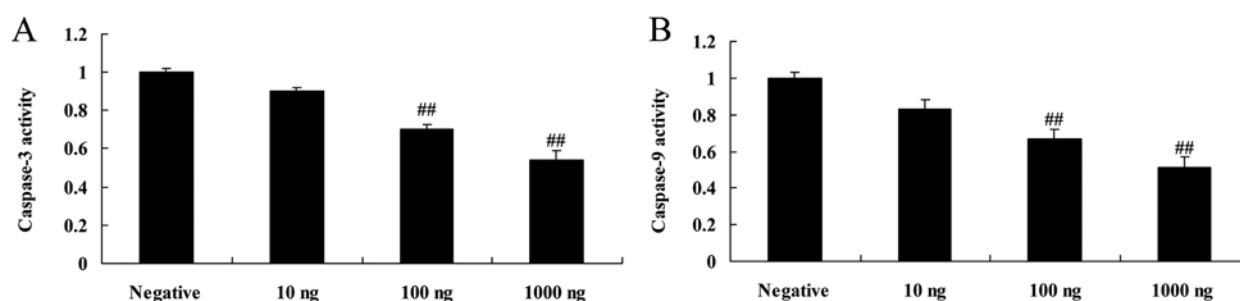


Figure 6. DNMT3a overexpression affects caspase-3 and -9 activity in human ovarian cancer. DNMT3a overexpression affects (A) caspase-3 and (B) caspase-9 activity in human ovarian cancer. Negative, negative control group; 10 ng, cells transfected with 10 ng DNMT3 plasmid/well; 100 ng, cells transfected with 100 ng DNMT3 plasmid/well; 1,000 ng, cells transfected with 1,000 ng DNMT3 plasmid/well; ^{##} $p < 0.01$ compared with the cancer tissue group.

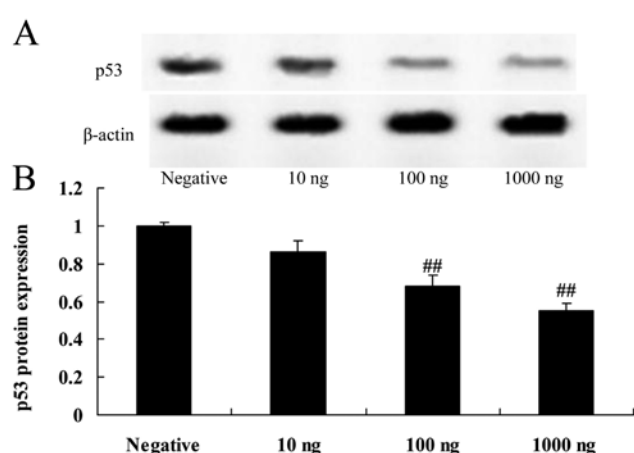


Figure 7. DNMT3a overexpression affects p53 protein expression in human ovarian cancer. DNMT3a overexpression affects p53 protein as detected by (A) western blotting and (B) p53 protein statistical analyses in human ovarian cancer. Negative, negative control group; 10 ng, cells transfected with 10 ng DNMT3 plasmid/well; 100 ng, cells transfected with 100 ng DNMT3 plasmid/well; 1,000 ng, cells transfected with 1,000 ng DNMT3 plasmid/well; ^{##} $p < 0.01$ compared with the cancer tissue group.

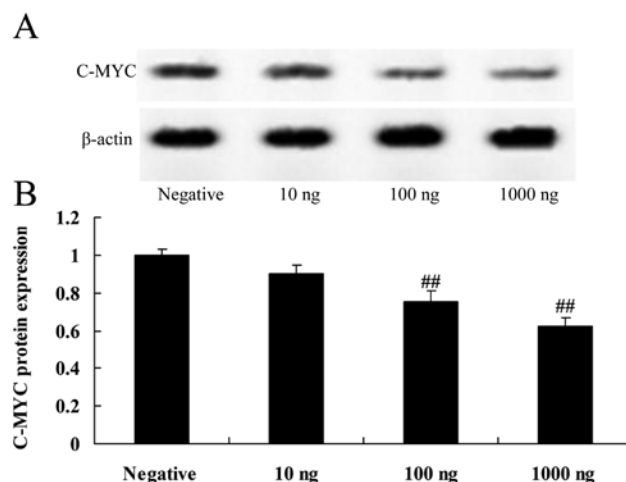


Figure 8. DNMT3a overexpression affects c-Myc protein expression in human ovarian cancer. DNMT3a overexpression affects c-Myc protein as detected by (A) western blotting and (B) c-Myc protein statistical analyses in human ovarian cancer. Negative, negative control group; 10 ng, cells transfected with 10 ng DNMT3 plasmid/well; 100 ng, cells transfected with 100 ng DNMT3 plasmid/well; 1,000 ng, cells transfected with 1,000 ng DNMT3 plasmid/well; ^{##} $p < 0.01$ compared with the cancer tissue group.

Overexpression of DNMT3a affects miR-182 in human ovarian cancer. When the DNMT3 plasmid was transfected into the Caov-3 cells, miR-182 expression was assessed using quantitative RT-PCR assay. As shown in Fig. 5, the DNMT3 plasmid suppressed miR-182 expression in the Caov-3 cells in a dose-dependent manner. The DNMT3 plasmid (100 or 1,000 ng) significantly suppressed miR-182 expression in the Caov-3 cells, compared with that in the negative control group (Fig. 5).

Overexpression of DNMT3a affects caspase-3 and -9 activity in human ovarian cancer. Overexpression of DNMT3a inhibited caspase-3 and -9 activity in the Caov-3 cells in a dose-dependent manner. As shown in Fig. 6, the DNMT3 plasmid (100 or 1,000 ng) significantly inhibited caspase-3 and -9 activity in the Caov-3 cells, as compared with the negative control group.

Overexpression of DNMT3a affects p53 protein expression in human ovarian cancer. Overexpression of DNMT3a suppressed p53 protein expression in the Caov-3 cells in a dose-dependent manner. As shown in Fig. 7, 100 or 1,000 ng of the DNMT3 plasmid significantly suppressed p53 protein expression in the Caov-3 cells, as compared with the negative control group.

Overexpression of DNMT3a affects c-Myc protein expression in human ovarian cancer. Overexpression of DNMT3a inhibited c-Myc protein expression in the Caov-3 cells in a dose-dependent manner (Fig. 8). As shown in Fig. 8, 100 or 1,000 ng of the DNMT3 plasmid significantly inhibited c-Myc protein expression in the Caov-3 cells, as compared with the negative control group.

DNMT3a regulates the effect of miR-182 on the apoptotic rate of human ovarian cancer. We analyzed the DNMT3a-regulated miR-182 effects on the apoptotic rate of human ovarian cancer. As shown in Fig. 9, overexpression of miR-182 significantly increased the apoptotic rate in the Caov-3 cells compared with the negative control group. The DNMT3 plasmid (100 ng) significantly reduced the apoptotic rate of the Caov-3 cells induced by the miR-182 plasmid, compared with the miR-182 plasmid group.

Downregulation of DNMT3a expression affects the DNMT3a protein in human ovarian cancer. To research the effects of the downregulation of DNMT3a expression on the DNMT3a

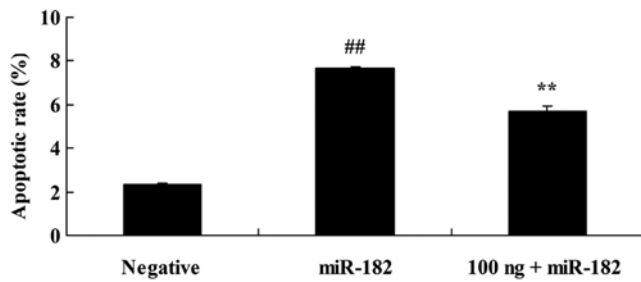


Figure 9. DNMT3a regulation of miR-182 affects the apoptotic rate of human ovarian cancer. Negative, negative control group; miR-182, cells transfected with the miR-182 plasmid; 100 ng + miR-182, cells transfected with 100 ng DNMT3 plasmid + miR-182 plasmid/well; ^{##}p<0.01 compared with cancer tissue group, ^{**}p<0.01 compared with the cancer tissue group.

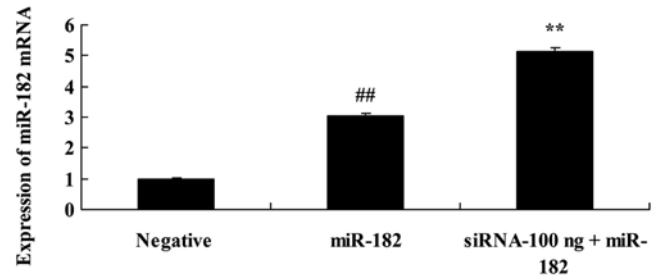


Figure 11. Downregulation of DNMT3a expression affects miR-182 in human ovarian cancer. Negative, negative control group; miR-182, cells transfected with the miR-182 plasmid; siRNA-100 ng + miR-182, cells transfected with 100 ng siRNA DNMT3a plasmid + miR-182 plasmid/well; ^{##}p<0.01 compared with the cancer tissue group, ^{**}p<0.01 compared with the cancer tissue group.

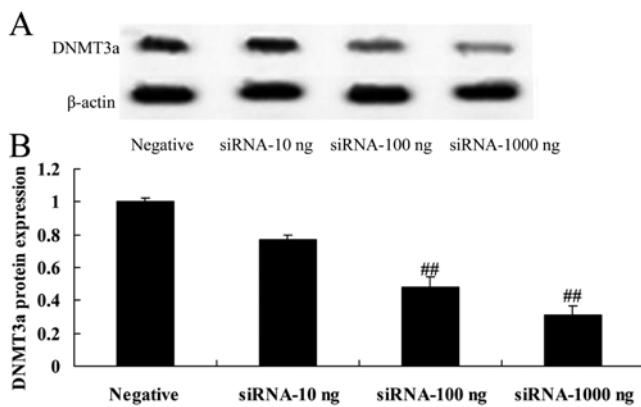


Figure 10. Downregulation of DNMT3a expression affects DNMT3a protein in human ovarian cancer. Downregulation of DNMT3a expression affects DNMT3a protein as detected by (A) western blotting and (B) DNMT3a protein statistical analyses of human ovarian cancer. Negative, negative control group; siRNA-10 ng, cells transfected with 10 ng siRNA DNMT3a/well; siRNA-100 ng, cells transfected with 100 ng siRNA DNMT3a/well; siRNA-1,000 ng, cells transfected with 1,000 ng siRNA DNMT3a/well; ^{##}p<0.01 compared with the cancer tissue group.

protein in human ovarian cancer, DNMT3a protein expression was assessed using western blotting. As shown in Fig. 10, downregulation of DNMT3a expression inhibited the protein expression of DNMT3a in the Caov-3 cells. Particularly, 100 or 1,000 ng DNMT3 siRNA significantly inhibited the protein expression of DNMT3a in the Caov-3 cells, compared with the negative control group (Fig. 10).

Downregulation of DNMT3a expression affects miR-182 in human ovarian cancer. To further research the effects of the downregulation of DNMT3a expression on miR-182 expression in human ovarian cancer, miR-182 expression was assessed using quantitative RT-PCR assay. As shown in Fig. 11, 100 ng of DNMT3 siRNA significantly increased the miR-182 expression in the Caov-3 cells, compared with the miR-182 plasmid group.

Downregulation of DNMT3a expression affects the apoptotic rate of human ovarian cancer cells. We explored the effect of the downregulation of DNMT3a on the apoptotic rate of human ovarian cancer. As shown in Fig. 12, 100 ng of DNMT3

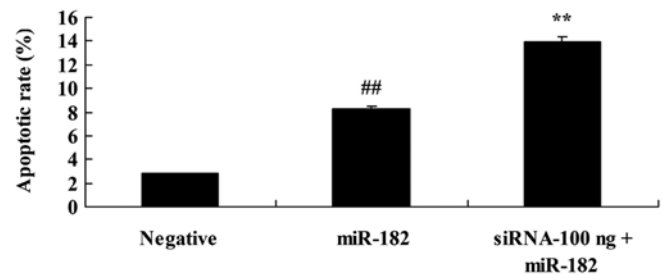


Figure 12. Downregulation of DNMT3a expression affects the apoptotic rate of human ovarian cancer. Negative, negative control group; cells transfected with the miR-182, miR-182 plasmid; siRNA-100 ng + miR-182, cells transfected with 100 ng siRNA DNMT3a plasmid + miR-182 plasmid/well; ^{##}p<0.01 compared with the cancer tissue group, ^{**}p<0.01 compared with the cancer tissue group.

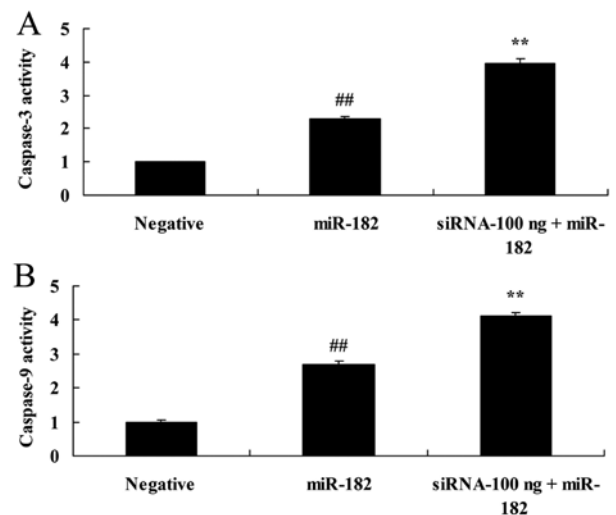


Figure 13. Downregulation of DNMT3a expression affects caspase-3 and -9 activity in human ovarian cancer. Downregulation of DNMT3a expression affects caspase-3 (A) and -9 activity (B) in human ovarian cancer. Negative, negative control group; miR-182, cells transfected with the miR-182 plasmid; siRNA-100 ng + miR-182, cells transfected with 100 ng siRNA DNMT3a plasmid + miR-182 plasmid/well cell; ^{##}p<0.01 compared with the cancer tissue group, ^{**}p<0.01 compared with the cancer tissue group.

siRNA significantly increased the apoptotic rate in the Caov-3 cells, compared with the miR-182 plasmid group.

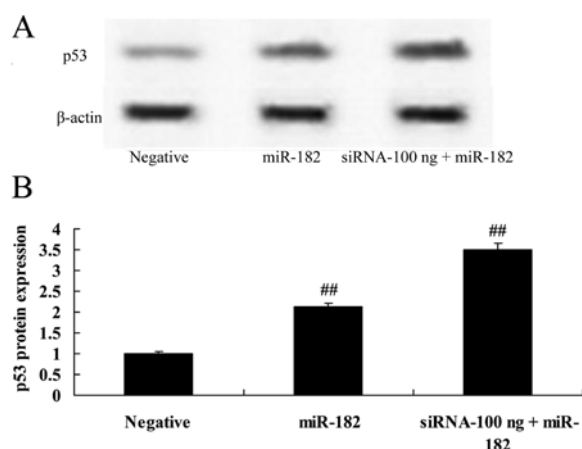


Figure 14. Downregulation of DNMT3a expression affects p53 protein expression in human ovarian cancer. Downregulation of DNMT3a expression affects p53 protein as detected by (A) western blotting and (B) p53 protein statistical analyses of human ovarian cancer. Negative, negative control group; miR-182, cells transfected with the miR-182 plasmid; siRNA-100 ng + miR-182, cells transfected with 100 ng siRNA DNMT3a plasmid + miR-182 plasmid/well; ^{##} $p < 0.01$ compared with the cancer tissue group, ^{*} $p < 0.01$ compared with the cancer tissue group.

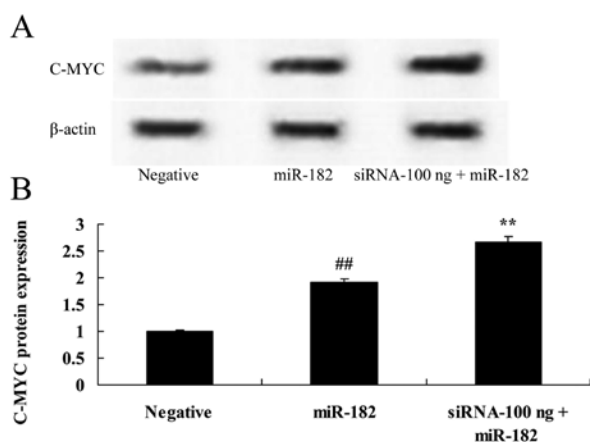


Figure 15. Downregulation of DNMT3a expression affects c-Myc protein expression in human ovarian cancer. Downregulation of DNMT3a expression affects c-Myc protein as detected by (A) western blotting and (B) c-Myc protein statistical analysis of human ovarian cancer. Negative, negative control group; miR-182, cells transfected with the miR-182 plasmid; siRNA-100 ng + miR-182, cells transfected with 100 ng siRNA DNMT3a plasmid + miR-182 plasmid/well; ^{##} $p < 0.01$ compared with the cancer tissue group, ^{**} $p < 0.01$ compared with the cancer tissue group.

Downregulation of DNMT3a expression affects caspase-3 and -9 activity in human ovarian cancer. We further explored the affect of the downregulation of DNMT3a expression on caspase-3 and -9 activity in human ovarian cancer. As shown in Fig. 13, 100 ng of DNMT3 siRNA significantly increased caspase-3 and -9 activity in the Caov-3 cells, compared with the miR-182 plasmid group.

Downregulation of DNMT3a expression affects p53 protein expression in human ovarian cancer. We investigated the affects of the downregulation of DNMT3a expression on p53 protein expression in human ovarian cancer. Compared with miR-182 plasmid group, 100 ng of siRNA DNMT3 plasmid

significantly enhanced p53 protein expression in the Caov-3 cells (Fig. 14).

Downregulation of DNMT3a expression affects c-Myc protein expression in human ovarian cancer. We investigated the affects of the downregulation of DNMT3a expression on c-Myc protein expression in human ovarian cancer. As shown in Fig. 15, 100 ng of siRNA DNMT3 plasmid significantly increased c-Myc protein expression in the Caov-3 cells, compared with the miR-182 plasmid group.

Discussion

Ovarian cancer is the fifth leading gynecologic malignant tumor resulting in the death of American women (3). The main reason for the high lethality is that ovarian cancer is frequently at the advanced stage at initial diagnosis (5). After surgery, only 30% of patients gain a 5-year survival rate after initial diagnosis, although good results can be achieved by chemotherapy (8). At present, the major issue associated with ovarian cancer is the lack of early diagnosis and distant metastasis at the advanced stage, accompanied by chemotherapy resistance (6). Therefore, accurate and early diagnosis, and effective and safe therapeutic strategies are key factors in ovarian cancer treatment (7). In the present study, DNMT3a protein expression was identified in the cancer tissue group, which was higher than that in the adjacent cancer or normal group. However, miR-182 expression in the adjacent cancer group was very similar to that of the normal group.

The expression of DNMT3a in ovarian cancer ascites tumor cell strain SKOV3 was found to be high (9), while the expression of DNMT3b was weak. During the developmental process, the main function of DNMT1 is to maintain DNA methylation mode of the body. However, the main function of DNMT3a and 3b is to establish a new methylation mode. Generally, it has been recognized that DNMT1 mainly participates in and maintains methylation (13), whereas DNMT3a and 3b mainly participate in *de novo* methylation. However, recent research has shown that DNMT3a and 3b participate in and maintain methylation as well (13,14). In addition, they can enhance the fidelity of methylation reproduction mediated by DNMT1 which can participate in *de novo* methylation *in vitro* with lower activity (9). mRNAs encoding three types of DNA methyltransferase genes and the protein expressed by them are highly expressed (15). Furthermore, a large number of studies indicate that the three can lead to abnormal DNA methylation of suppressor genes in cancer cells and silencing through direct or synergistic effects (15,16). Consequently, DNA transmethylation plays an important role in methylation start-up and maintenance in tumor cells. DNMT3a upregulation was observed in human ovarian cancer Caov-3, OV-1063, OVCAR-3 and Caov-4 cells.

miRNAs are non-coding single-stranded RNAs, 17-28 bp in length, which play a vital role in the regulation of genetic transcription, and participate in the genesis and development of various tumors (17). The abnormal expression of miR-182 has been found in numerous tumors and is closely related to tumor metastasis (12,18). In ovarian cancer, melanoma, hepatocellular carcinoma and glioma, miR-182 expression has been shown to be upregulated, and to promote the genesis and development of tumors as an oncogene (10).

Our results of western blotting and quantitative RT-PCR assay showed that DNMT3a was highly expressed and miR-182 expression was downregulated in cancer tissues. Downregulation of DNMT3a expression significantly activated miR-182 expression in the Caov-3 cells. Our data indicate a role for DNMT3a in the regulation of miR-182 and elucidate the anticancer mechanism in ovarian cancer. Moreover, Sun *et al* reported that miR-182 induced cervical cancer cell apoptosis by suppressing DNMT3a expression (19).

COMT mRNA and protein expression has been shown to be increased in numerous cancer cells, and to lead to DNA methylation of suppressor genes in cancer cells through direct or synergistic effects (20). Consequently, DNA methylase plays a very significant role in methylation start-up and maintenance in tumor cells (21). In the present study, DNMT3a overexpression significantly inhibited caspase-3 and -9 activity in the Caov-3 cells, which was reversed by DNMT3a down-regulation.

The p53 gene is a cancer-suppressor gene and is associated with the incidence of human tumors (22). Known as a 'gene guard', p53 protein plays a vital role in the cell response to the environment and all types of stress, such as DNA damage, proto-oncogene activation and oxygen deprivation (23,24). Located at the center of such stress signals (23), p53 transfers signals to cells and promotes downstream gene transcription, so as to function as a transcription factor, which includes short or long term cell retardation, DNA duplication, rehabilitation and cell apoptosis thus maintaining the integrity of the cellular genome or preventing the early proliferation of cancer cells (25). In addition, our results showed that DNMT3a overexpression significantly suppressed Caov-3 cell p53 protein expression, which was reversed by DNMT3a downregulation. Ma *et al* indicated that p53 inhibits epigenetic reprogramming through HDAC1 and DNMT3a (26).

The c-Myc gene is located on the long arm of #8 chromosome (8q24), and belongs to protooncogenes of nuclear transcription factor type (27). The encoding protein is intranuclear combining on DNA chain, which is implementing regulation at transcription process (28). Presenting as a large number of gene amplification, the formation of c-Myc induction affects cell proliferation and transformation (28,29). It can directly regulate transcription of other genes through other genes and bypass systems. In the present study, the core finding is that DNMT3a overexpression significantly suppressed c-Myc protein expression in Caov-3 cells, which was altered by the downregulation of DNMT3a expression. Stewart *et al* suggested that DNMT3a mutates leukemia cells and induces apoptosis through p53 and c-Myc-independent manner (30).

In summary, further elucidation of the DNMT3a-miR-182 regulatory mechanism was presented. DNMT3a regulates miR-182 via caspase-3 and -9-mediated apoptosis and DNA damage response may contribute to the induction of apoptosis in ovarian cancer.

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