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Original Article

miR-142-5p Suppresses Ovarian Cancer Metastasis by Inhibiting the Epithelial-Mesenchymal Transition via DNMT1

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ABSTRACT

Aim/Background: Ovarian Cancer (OC) accounts for the highest number of deaths among gynecological cancers. Our research is focused on investigating the therapeutic potential and the fundamental mechanism by which miR-142-5p exerts its effects in the treatment of OC. **Materials and Methods:** The GSE53829 and GSE83693 data sets were collected for targeted miRNA identification. RT-qPCR was conducted to evaluate the expression levels of miRNA, N-cadherin, ZO-1, Claudin-1, E-cadherin, and DNMT1 mRNA expressions. Additionally, the protein expressions of these mentioned molecules were quantified using western blot analysis. The invasion and migratory abilities of OC cells were assessed through transwell and wound healing assays. Additionally, the possible interaction between miR-142-5p and DNMT1 was identified and confirmed using the Targetscan database in conjunction with a luciferase assay. **Results:** The mRNA levels of miR-142-5p showed a notable reduction in both OC cell lines and metastatic tumors, as compared to their counterparts of normal ovarian cancer cells and non-metastatic tumors, respectively. Besides, the inhibition or overexpression of miR-142-5p had a significant impact on the migration, invasion ability, and Epithelial-Mesenchymal Transition (EMT) process of OC cells. The levels of DNMT1 were significantly increased in metastatic tumors and were notably affected by the expression of miR-142-5p. Moreover, interaction between DNMT1 mRNA and miR-142-5p was confirmed, and the knockdown of DNMT1 effectively counteracted the significant reversal in OC cell migration, invasion, and EMT caused by miR-142-5p suppression. **Conclusion:** The role of miR-142-5p on OC metastasis is attributed to its ability to suppress EMT through DNMT1, indicating the promising therapeutic potential of miR-142-5p in the treatment of OC.

Keywords: DNMT1, EMT, Metastasis, miR-142-5p, Ovarian Cancer.

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INTRODUCTION

OC is a severe gynecologic cancer with high mortality and poor prognosis worldwide. It is reported that OC accounts for nearly 3.7% of cases and 4.7% of cancer deaths in 2020.[1] Besides, the mortality rate of OC increased by 84.2% between 1990 and 2017, partly due to the remarkable decrease in the number of pregnancies and the popularity of Westernized lifestyles.[2,3]

The high mortality of OC is partly explained by the delayed diagnosis.[4] OC is usually asymptomatic clinically. Despite the development in screening technology and surgical operation, most OC patients are confirmed at advanced stages (III or IV), while the advanced stages of OC are linked with descending survival and poor prognosis compared with the early stage (I).[5] The metastasis of OC within the peritoneal cavity is one of the key reasons accounting for the treatment failure of advanced OC. Besides, OC metastasis seems unique and easier to occur compared with most other cancers transferred in a hematogenous way.[6] Once the OC cells have separated as clusters or single cells from the primary cancer tissues, they metastasize via peritoneal fluid without undergoing normal intra- or extravasation. Several studies have proved that preventing OC metastasis is beneficial for OC treatment.[7,8] For example, multi-drug combination strategy and nanoscience have brought new opportunities in treating metastatic OC.[9] However, more effective therapeutics should be developed to inhibit OC metastasis among patients.

Before OC cells separate from the original position and start spreading, Epithelial-Mesenchymal Transition (EMT) is a special procedure that is widely observed in various cancer cells. EMT usually represents the biological progress of epithelial cells converting into mesenchymal phenotype cells via a definite

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program, which mitigates the adhesion of epithelial cells to the basilar membrane and confers great migration potential to metastatic diseases, especially epithelial cancer, like OC.[10] E-cadherin, one of the membrane glycoproteins, is located at cell adherence colligations, and the loss of E-cadherin is one of the hallmarks of the procurance of a migratory phenotype in OC cells. After the down-regulation of E-cadherin along with various other tight junction proteins, such as Zonula Occludes I (ZO-1) and claudin-1, the increased N-cadherin and aggressive phenotype can be observed in metastatic OC cells. Wang *et al.* have found that cinnamaldehyde can suppress OC progression by inhibiting the EMT process *in vivo*.[11] Silibinin is also been proven to exert anti-tumor activity by suppressing EMT in OC cells according to Narges Maleki's research.[12] Therefore, targeting EMT will be a promising therapy against OC metastasis.

MicroRNAs (miRNAs) are non-coding RNAs, typically around 20-24 nucleotides in length, that play a role in regulating various biological processes, including the growth and spread of cancers. After transcription via RNA polymerase II/III, pri-miRNA cleavage and export from the nucleus as pre-miRNA. Pre-miRNA cleavage through RNase Dicer as well as TRBP protein in the cytoplasm then participates in the formation of RNA-induced silencing complex, where it prompts the production of mature miRNA.[13] After recognizing the 5' Untranslated Region (UTR) in the seed region, the mature miRNA binds to the open reading frame or 3' UTR of its target mRNA, thus inhibiting or enhancing the following gene expression and further making a difference on cancer-related signaling pathways. To illustrate, Liu *et al.* discovered that the CXCL1 pathway can be suppressed by miR-27b-5p, leading to a reduction in the advancement and dissemination of OC cells.[14]

miR-142-5p, a miRNA generated from the miR-142 hairpin found on chromosome 17q22, is reported as a suppressor in numerous cancers.[15-17] Li *et al.* have also verified that miR-142-5p demonstrates the capability to prevent the emergence of cisplatin resistance in OC cells through its interaction with several anti-apoptotic genes.[18] Besides, in this study, compared with normal tissue or ovarian cells, significant low expressions were also observed in the OC tissue and cells, especially the metastatic one. These outcomes revealed the therapeutic avenues of miR-142-5p in treating OC, although its impact on inhibiting OC metastasis remains largely unexplored. Thus, in this research, our objective is to examine the inhibitory impact of miR-142-5p on the spread of OC and delve deeper into the mechanisms that drive it.

MATERIALS AND METHODS

Cell culture and human tumor samples

SKOV3 and Caov-3 ovarian cancer cell lines were sourced from the Type Culture Collection of the Chinese Academy of Sciences' Cell Bank. Additionally, the immortalized human ovarian surface epithelial IOSE-80 cell line and OC cell lines, namely A2780 and OVCAR-5, were procured from the Cell Bank affiliated with the China Science Academy in Shanghai. These cell lines were cultivated in DMEM, which was purchased from Sigma and enriched with 10% FBS, along with antibiotics-streptomycin at a concentration of 100 µg/mL and penicillin at 100 U/mL. The cell cultures were kept at 37°C within a controlled environment that included a 5% CO_2 humidified atmosphere.

The collection of tumor and normal ovarian tissue was conducted from patients who underwent primary surgical treatment at our hospital. The tissues were quickly frozen by immersing them in cryogenic nitrogen and subsequently maintained at a temperature of -80°C for storage. The experimental procedure received ethical approval from Fujian Provincial Maternity and Children's Hospital (2022KYLLR01014), with all participants providing written informed consent.

Differential gene analysis

The GEO series (GSE53829 and GSE83693) were obtained from GEO Datasets (https://www.ncbi.nlm.nih.gov/geo/). GSE53829 included 15 normal tissue samples and 48 malignant OC tissue, while 8 primary OC tissue and 4 normal OC tissue were contained in GSE83693. To examine differential gene expression, the GEO2R tool was utilized to assess the expression levels of the genes of interest within these specified GEO datasets.

Real-Time (RT) PCR

Total RNA was extracted utilizing Tri-Reagent (TaKaRa, Tokyo, Japan). RT-PCR was conducted by SYBR master mix from Bio-Rad Laboratories Inc., located in Hercules, CA. The internal control for this study involved amplification of 18S. To determine the relative mRNA expression, we utilized the Comparative CT (Ct) method and quantified it as $2-\Delta\Delta Ct$. PCR was carried out at 95°C for 10 min, followed by 40 cycles at 95°C for 10 sec, 60°C for 30 sec and 72°C for 1 min. Table 1 provides the sequences of the primers utilized in our research.

Transwell assay

To investigate the regulation of miR-142-5p and DNMT1 regarding the invasion potential of OC cells, SKOV3 and OVCAR-5 cells (1×105 per cell) were cultured in DMEM with 0.1% bovine serum albumin. Then all cells were seeded in each insert on the Matrigel-coated membrane. After 24 hr-invasion, all cells that moved across the membrane were immobilized using a 4% paraformaldehyde fixative and subjected to crystal violet staining for subsequent analysis.

Wound healing assay

The SKOV3, as well as OVCAR5 cells, were cultured in 6-well plates and subjected to respective treatments. All cells underwent gentle scraping using a sterile pipette tip (200 µL) upon reaching

complete growth. After that, the cells were cultured in a medium devoid of serum. The growth status of each group was visually recorded at 0 and 24 hr post-scratching and quantified using ImageJ (version 1.8.0).

Western blotting

Simply put, the proteins underwent separation through Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) with a 10% gel Subsequently, the sample was moved onto a Polyvinylidene Fluoride (PVDF) membrane. After a 2 hr-blocking step with 5% skim milk, the membranes were treated overnight with primary antibodies specific to the target proteins. Later, the membranes were rinsed thrice with PBS (1 ×) followed by 1 hr-incubation of secondary antibodies. Ultimately, an ECL Kit (P0018S; Beyotime, Shanghai, China) was employed to detect the protein bands. Table 2 contains the antibodies in our study.

Transfection

For the process of introducing foreign genetic material into cells, RiboBio (Guangzhou, China) designed and supplied the miR-142-5p inhibitor, mimic, and a duplex serving as a Negative Control for microRNA (NC). DNMT1 siRNAs (si-DNMT1; F, 5′-UGUAUUUGGGGAUCAAAAGAG-3′; R, 5′-CUUUUGAUCCCCAAAUACAGC-3′) or NC siRNAs were provided by the Fu Gene HD transfection agent referring to the instruction. After a 48-hr transfection period, the validation of transfection efficiency was conducted using RT-qPCR and western blotting techniques.

Luciferase reporter assay

The segments of the 3'-UTR in DNMT1 that contain the predicted miRNA binding sites, were amplified through PCR and subsequently cloned into pGL3-DNMT1-wt, a luciferase reporter vector from Promega Corporation. Additionally, a plasmid with mutations in the complementary regions for miR-142-5p was generated with a MutanBEST Kit (TaKaRa Bio Inc., Tokyo, Japan) and designated as pGL3-DNMT1-mut. After a 48-hr co-culture with the specific pGL3-DNMT1, the luciferase activity was evaluated with a dual-luciferase reporter assay kit (Promega, Madison, WI, USA). The Renilla luciferase was utilized as a normalization control.

Table 1: Sequences of PCR primers used in this study.

Primers	Sequences	
hsa-miR-142-5p	Forward(5'-3')	CATAAAGTAGAAAGCACTAC
	Reverse(5'-3')	GAACATGTCTGCGTATCTC
hsa-miR-182-3p	Forward(5'-3')	GCTGTCGTGGTTCTAGACTTGC
	Reverse(5'-3')	GTGCAGGGTCCGAGGT
hsa-miR-200a-3p	Forward(5'-3')	GCGCGTAACACTGTCTGGTAA
	Reverse(5'-3')	AGTGCAGGGTCCGAGGTATT
hsa-miR-214-3p	Forward(5'-3')	CAATACTGACAGCAGGCACA
	Reverse(5'-3')	TATGGTTGTTCACGACTCCTTCAC
hsa-miR-429	Forward(5'-3')	GGGGGTAATACTGTCTGGT
	Reverse(5'-3')	TGCGTGTCGTGGAGTC
N-cadherin	Forward(5'-3')	TCAGGCGTCTGTAGAGGCTT
	Reverse(5'-3')	ATGCACATCCTTCGATAAGACTG
E-cadherin	Forward(5'-3')	CGAGAGCTACACGTTCACGG
	Reverse(5'-3')	GGGTGTCGAGGGAAAAATAGG
ZO-1	Forward(5'-3')	CAACATACAGTGACGCTTCACA
	Reverse(5'-3')	CACTATTGACGTTTCCCCACTC
Claudin-1	Forward(5'-3')	CCTCCTGGGAGTGATAGCAAT
	Reverse(5'-3')	GGCAACTAAAATAGCCAGACCT
DNMT1	Forward(5'-3')	AGGCGGCTCAAAGATTTGGAA
	Reverse(5'-3')	GCAGAAATTCGTGCAAGAGATTC
U6	Forward(5'-3')	AAAGCAAATCATCGGACGACC
	Reverse(5'-3')	GTACAACACATTGTTTCCTCGGA
GAPDH	Forward(5'-3')	GGAGCGAGATCCCTCCAAAAT
	Reverse(5'-3')	GGCTGTTGTCATACTTCTCATGG

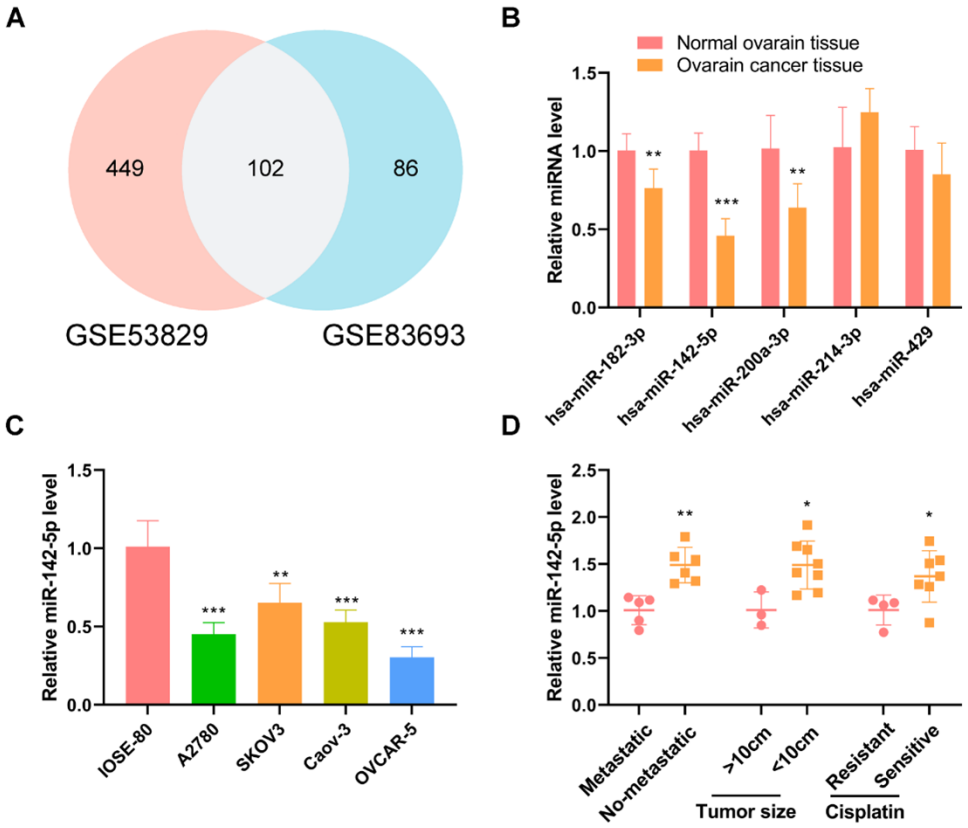

Figure 1: Low expression of miR-142-5p is associated with OC metastasis. The common miRNAs regulated by OC between GSE53829 and GSE83693 were shown (A). The relative mRNA of hsa-miR-182-3p, hsa-miR-142-5p, hsa-miR-200a-3p, hsa-miR-214-3p, and hsa-miR-429 were measured by RT-qPCR assays (B). The mRNA of miR-142-5p in different OC cell lines including IOSE-80, A2780, SKOV3, Caov-3, and OVCAR-5 were also quantified via RT-qPCR assays (C). The differences in the miR-142-5p expression in specific tumor groupings were measured by RT-qPCR assays. (No Significance (NS) exists when $p>0.05$; *$p<0.05$, **$p<0.01$, and ***$p<0.001$ versus the designative group including normal ovarian tissue in (B), IOSE-80 in (C), metastatic or tumor size >10 cm or cisplatin-resistant in (D)).

Binding sites prediction

The Targetscan database (http://www.targetscan.org) was utilized to forecast the potential binding locations of miR-142-5p on DNMT1.

Statistical analysis

In this study, GraphPad Prism software (version 10.1.0; GraphPad Software, La Jolla, CA, USA) was used for analysis. The normality of the data was tested using the Shapiro-Wilk test, with a $p<0.05$ being considered normally distributed. Homogeneity of variance was tested using Levene's test, with $p>0.05$ considered homogenous. All data are presented as "mean ± SEM" and were analyzed using one-way ANOVA followed by Tukey's *post hoc* test. $p<0.05$ was deemed statistically significant.

RESULTS

Low expression of miR-142-5p is correlated with ovarian cancer metastasis.

Table 2: **WB antibodies used in this study.**

Antibodies	Source	Cat log #
N-cadherin	CST	CST#4061
E-cadherin	CST	CST#8834
ZO-1	beyotime	AF8394
Claudin-1	abcam	ab211737
DNMT1	abcam	ab188453
GAPDH	beyotime	AF0006

Considering the large number of miRNAs involved in OC metastasis, we first screened the potential miRNA via the GEO database for the OC treatment. GSE53829 is a dataset containing the RT-PCR results of 15 normal tissue samples and 48 malignant ovarian tissues, while GSE83693 includes 8 primary OC tissues and 4 normal ovarian tissues. After differential gene analysis of the abovementioned GSE datasets, 102 common miRNAs were considered to be essential for the OC treatment (Figure 1A). Wherein, five notable differential miRNAs (miR-182-3p, miR-142-5p, miR-200a-3p, miR-214-3p, and miR-429) based

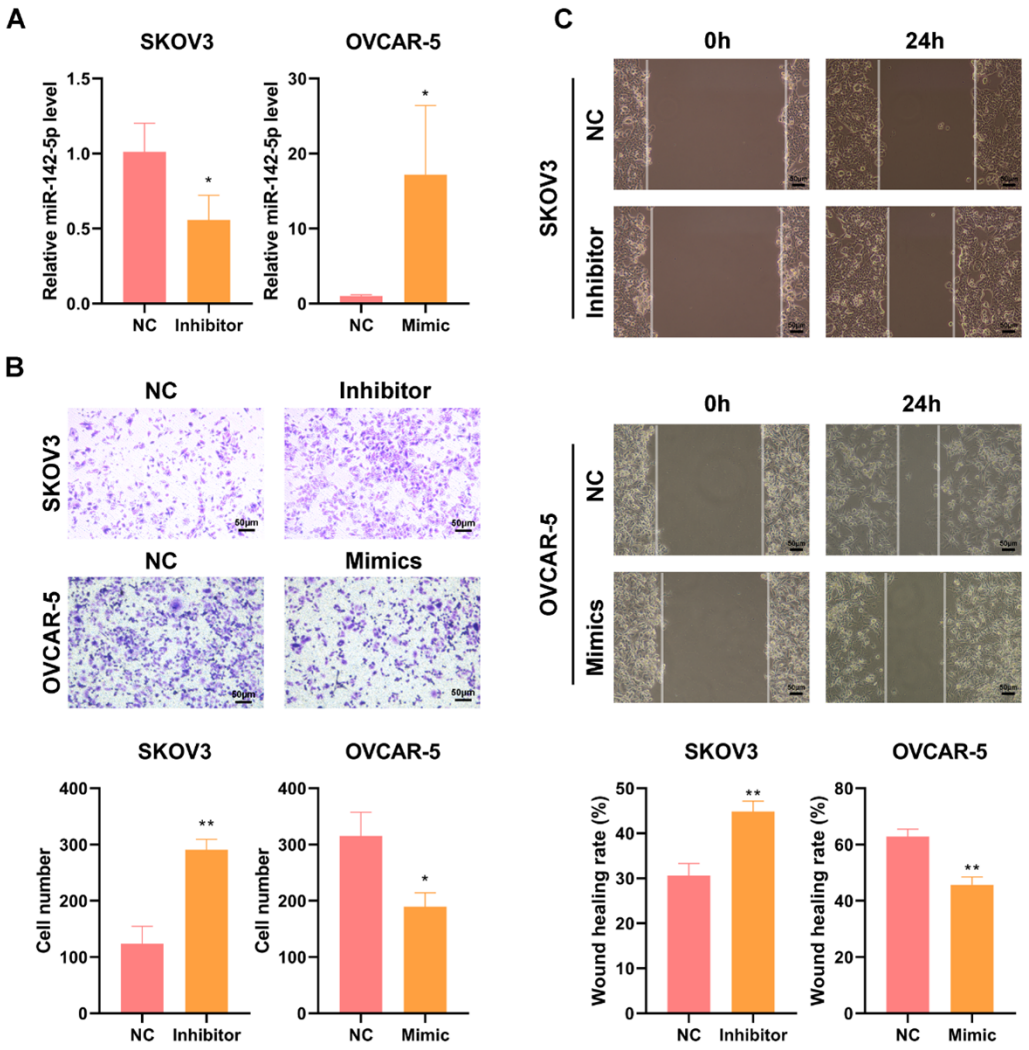

Figure 2: miR-142-5p inhibits the EMT phenomenon in OC cells. The effect of miR-142-5p on the mRNA expression of N-cadherin, Claudin-1, ZO-1, and E-cadherin was measured via RT-qPCR assays, while the protein expressions of the abovementioned genes were measured via western blotting. (*p<0.05, **p<0.01, and ***p<0.001 versus the NC group).

on relevant literature references. These selected miRNAs were subsequently confirmed through RT-qPCR assays (Figure 1B). According to the results, it is the miR-142-5p that with the greatest expression difference between normal ovarian tissue and OC tissues. This suggests that miR-142-5p may potentially regulate OC cells, prompting further investigation in this area. As expected, the mRNA expression of miR-142-5p was notably reduced in four OC cell lines (A2780, SKOV3, Caov-3, and OVCAR-5) when compared to normal ovarian epithelial cell lines like IOSE-80 (Figure 1C). After confirming the downregulation in ovarian cancer cells, our research additionally analyzed the expression profile of miR-142-5p in tumor samples collected from OC patients with diverse clinical conditions, including metastasis, tumor growth, and resistance to cisplatin. The most significant expression difference of miR-142-5p was obtained through the grouping of metastatic and non-metastatic cancer tissues, which suggests the therapeutic target for OC metastasis (Figure 1D). Taken together, the preliminary results indicate a possible link between lower levels of miR-142-5p and the metastasis of OC.

miR-142-5p suppresses OC cell migration

Given the significant inhibition of miR-142-5p in metastatic OC tissues, we aim to investigate how miR-142-5p might diminish the ability of ovarian cancer cells to migrate. Based on the results of Figure 1C, the modulation of miR-142-5p involves both its downregulation and upregulation were conducted by corresponding inhibitors and mimics in SKOV3 and OVCAR-5 cell lines respectively, and the validation of transfection efficiency was conducted through RT-qPCR analysis (Figure 2A). Compared to the control group, a notable rise in the quantity of

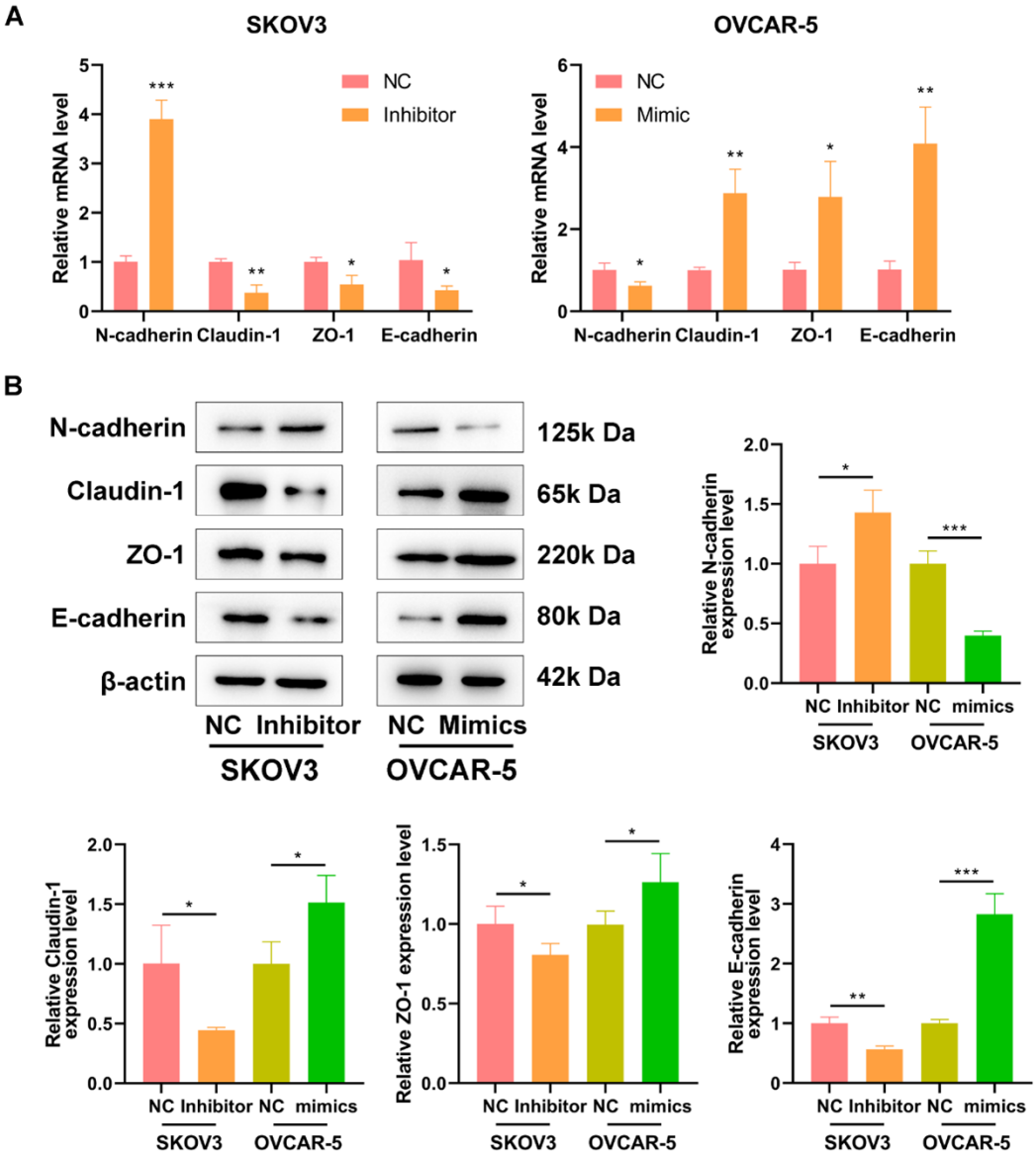

Figure 3: miR-142-5p inhibits the EMT phenomenon in OC cells. The effect of miR-142-5p on the mRNA expression of N-cadherin, Claudin-1, ZO-1, and E-cadherin was measured via RT-qPCR assays, while the protein expressions of the abovementioned genes were measured via western blotting. (*p<0.05, **p<0.01, and ***p<0.001 versus the NC group).

migrating cells was noted in the groups that received inhibitor treatment. Conversely, an increase in miR-142-5p levels resulted in a reduction in the number of cells that migrated (Figure 2B). These results suggest that miR-142-5p possesses the potential to suppress the invasive ability of OC cells. Similarly, the wound healing assays demonstrated that changes in miR-142-5p expression levels had a significant impact on the rate of wound closure in OC cells. These findings provide strong evidence that miR-142-5p is essential for regulating the migratory ability of OC cells (Figure 2C).

miR-142-5p inhibits the EMT phenomenon in OC cells

Since the EMT phenomenon is closely related to OC metastasis, the effect of miR-142-5p on the EMT process was subsequently examined in ovarian cancer cells. The loss of E-cadherin and up-regulation of N-cadherin are two hallmarks of the EMT occurrence. Based on the findings from RT-qPCR analysis, the levels of E-cadherin mRNA in OC cells were found to be either upregulated or downregulated upon overexpression or suppression of miR-142-5p. In contrast, the modulation of miR-142-5p had a significant impact on the expression levels of N-cadherin, resulting in both increases and decreases (Figure 3A). The expression levels of ZO-1 and Claudin-1, which are

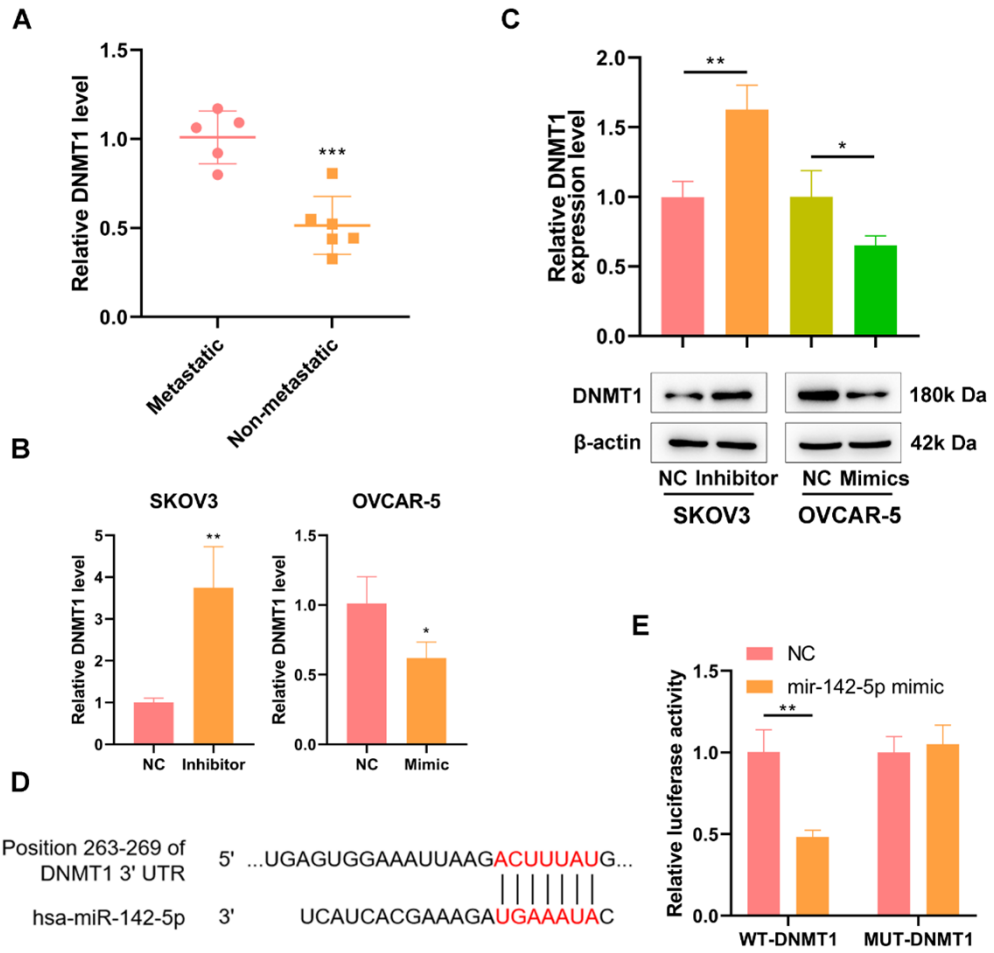

Figure 4: miR-142-5p inhibits DNMT1 expression via direct interaction. The mRNA expression of DNMT1 in metastatic and non-metastatic tumors was measured via RT-qPCR assays. The regulation of miR-142-5p on the DNMT1 expression was quantified at RNA and protein levels by RT-qPCR and western blotting correspondingly (B and C). Besides, the binding site for miR-142-5p and DNMT1 was predicted by the Targetscan (https://www.targetscan.org/vert_80/) (D), and the interaction between miR-142-5p and DNMT1 was further validated by luciferase assay (E). (*p<0.05, **p<0.01 and ***p<0.001 compared with the designative group including metastatic in (A) and NC in (B, C, E)).

essential for intercellular adhesion via tight junctions, were found to be modulated by inhibiting or overexpressing miR-142-5p in OC cells. These findings indicate that upregulating miR-142-p can suppress EMT, leading to improved intercellular adhesion and ultimately inhibiting OC metastasis. The abovementioned results were also reconfirmed at the protein levels by western blotting assays (Figure 3C).

miR-142-5p inhibits DNMT1 expression via direct interaction

Once the inhibition of miR-142-5p regarding OC metastasis and EMT was established, we aim to further uncover the targeted genes of miR-142-5p. DNMT1 is a crucial enzyme responsible for DNA methylation during mammalian DNA replication, which can significantly impact the progression and recurrence of various types of cancer through epigenetic modifications. Studies have indicated that by targeting DNMT1, miR-142-5p suppresses the proliferation and migration of gastric cancer cells.[19] However, the regulation of DNMT1 on OC metastatic remains for investigation. In this study, the mRNA of DNMT1 was notably decreased in non-metastatic tumor issues compared with the metastatic one, which indicates the potential regulation of DNMT1 in OC metastasis (Figure 4A). Besides, the levels of DNMT1 expression were significantly altered in reaction to either miR-142-5p inhibition or overexpression, as evidenced by changes in both RNA and protein levels (Figure 4B and C), which uncover the crosstalk between miR-142-5p and DNMT1. Thus, our study aimed to investigate the interaction between miR-142-5p and DNMT1 mRNA. The Targetscan database predicted that miR-142-5p interacts with the 3'UTR region of DNMT1 mRNA (Figure 4D). In the luciferase assay, the miR-142-5p mimics significantly suppressed the luciferase activity in the WT-DNMT1 group, while no effect was observed on luciferase activity in the MUT-DNMT1 group (Figure 4E), demonstrating that direct interaction between miR-142-5p and DNMT1 leads to the suppression of DNMT1 expression.

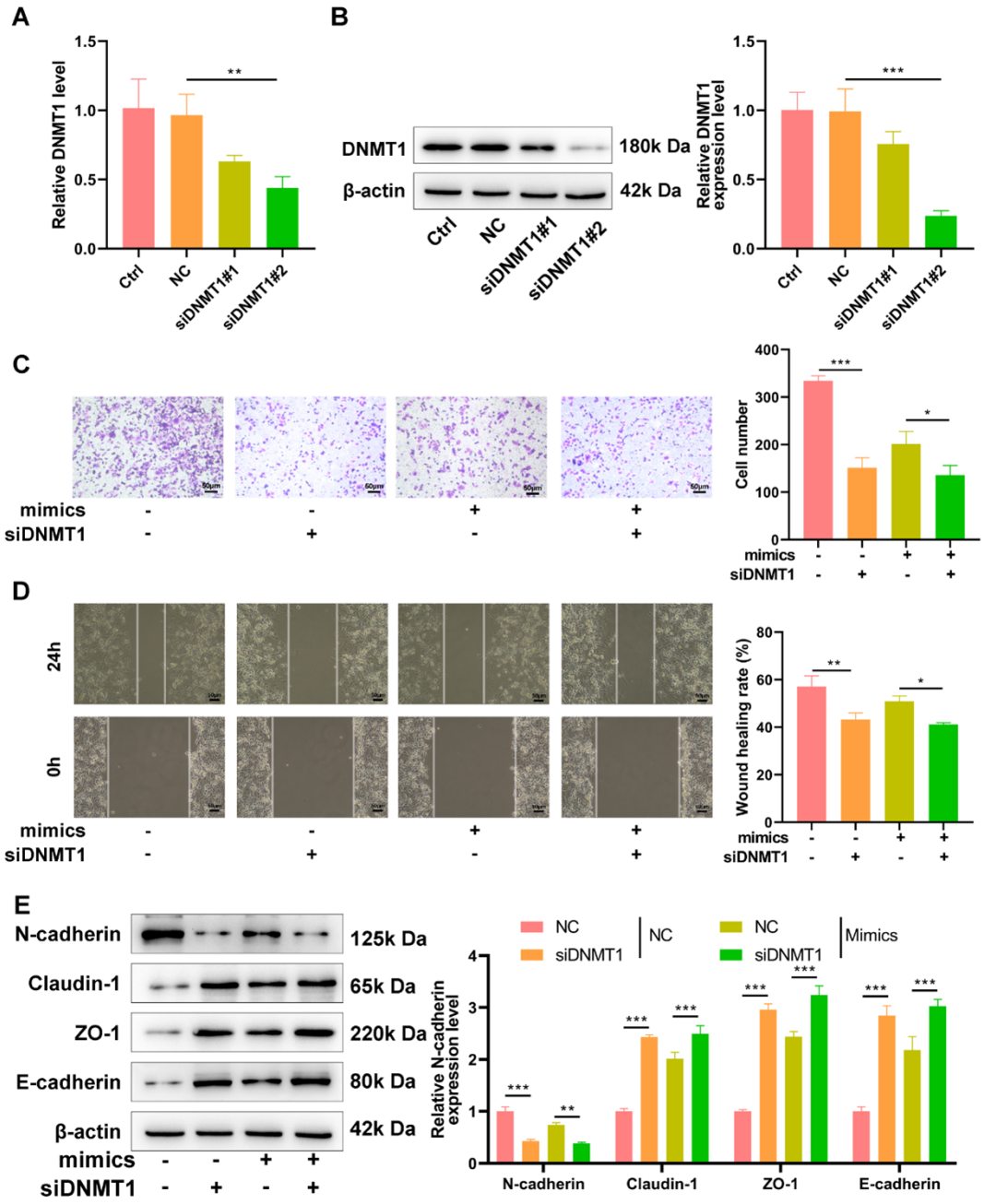

Figure 5: Knockdown DNMT1 reverses the suppression effect of mir-142-5p on metastasis and EMT phenomenon in OVCAR-5 cells. The knockdown efficiency of DNMT1 was measured at mRNA and protein levels (A and B) by RT-qPCR and western blotting correspondingly. The effects of DNMT1 in the mir-142-5p regulation on the invasion and migration capacity were measured via transwell and wound healing assays in OVCAR-5 cells (scale bar=50 μm) (C and D). The effects of DNMT1 in the mir-142-5p regulation on the expressions of EMT-related genes, including N-cadherin, Claudin-1, ZO-1, and E-cadherin, were analyzed by western blotting assays (E). (*p<0.05, **p<0.01 and ***p<0.001 versus the designative group).

Knockdown DNMT1 reverses the suppression of mir-142-5p on metastasis and EMT phenomenon in OVCAR5 cells

To further confirm the contribution of DNMT1 within the inhibition of miR-142-5p on OC metastasis, knockdown DNMT1 was applied in our study. The effectiveness of DNMT1 knockdown was measured at the levels of both RNA and protein, and based on the outcome, siDNMT1#2 was chosen for the subsequent experiment (Figure 5A and B). In the transwell assays, the number of migrated cells was notably down-regulated after the application of siDNMT1, which suggests the promotion of DNMT1 on the invasion capacity of OC cells (Figure 5C). However, no measurable difference exists after miR-142-5p mimics treatment in siDNMT1 groups, which means that knockdown siDNMT1 reverses the suppression of miR-142-5p on the invasion capacity of OC cells. Comparable outcomes can likewise be witnessed in the experiments assessing wound

healing (Figure 5D). These outcomes suggest the role of DNMT1 as a downstream factor of the regulation of miR-142-5p on OC metastasis. Besides, the N-cadherin was evidently suppressed by siDNMT1, while Claudin-1, ZO-1, and E-cadherin was notably elevated by siDNMT1 (Figure 5E), which indicates that DNMT1 can enhance EMT in OC cells and thus further promote OC metastasis. It is unlikely that any noticeable variation in the levels of EMT-related proteins can be detected following treatment with miR-142-5p mimics in the siDNMT1 groups. In conclusion, miR-142-5p was proven to suppress OC metastasis by inhibiting the EMT phenomenon via DNMT1 protein.

DISCUSSION

OC is a globally prevalent and lethal form of gynecologic malignancy. However, since OC is usually asymptomatic in the early stage, a definite diagnosis is made until the advanced stage in many cases of OC, thus highlighting the importance of effective therapy against OC metastasis.[19] Cui *et al.* systematically reviewed 2667 subjects and 8 single miRNAs in 22 studies and found that miR-200c, miR-200a, and miR-200b were potential biomarkers with diagnostic value for OC.[20] miRNA is a type of RNA that does not code for proteins regulating multiple biological pathways in mammals at the level of post-transcriptional regulation. The miR-142-/5p has been extensively studied and established as a key regulator in various malignancies since it could target the cancer-related signaling pathway and epigenetic factor.[21] According to the research of Tsang *et al.*, the levels of miR-142-5p expression are reduced in hepatocellular carcinoma, and increasing the levels of miR-142-5p has been shown to suppress migration in cancer cells.[22] Similarly, the current investigation observed a notable reduction in miR-142-5p levels in both OC tissue samples and cell lines. Besides, miR-142-5p was found to correlate with tumor growth, resistance to cisplatin treatment, and particularly metastasis when grouped differently. The suppression or overexpression of miR-142-5p markedly increased or reduced the invasion and migration abilities of OC cells respectively, suggesting the possible therapeutic value of targeting miR-142-5p in preventing OC metastasis.

EMT is a widely existing phenomenon in multiple fundamental processes, such as cancer metastasis, embryonic evolution, and tissue fibrosis.[23] Generally speaking, the process of EMT refers to the acquisition of mesenchymal stem cell characteristics by epithelial cells at a biological level, thus conferring the puissant metastasis on various cancer cells.[24] In other words, this transition also contains many therapeutic targets for OC treatment. For example, the inhibition of Epithelial-Mesenchymal Transition (EMT) in prostate cancer through the COX2/STAT3 axis has been observed as a result of metformin administration.[25] Metformin is widely recognized as a hypoglycemic drug primarily used for cancer treatment. In this research, the upregulation of miR-142-5p significantly enhanced the levels of E-cadherin, ZO-1, and Claudin-1, markers of epithelial cell, while reducing the expression levels of mesenchymal cell markers such as N-cadherin. This outcome revealed that miR-142-5p has the ability to inhibit EMT in OC cells, thus further inhibiting the OC metastasis *in vitro*.

EMT not only reduces the cell-cell adhesion and promotes metastasis in most cancer cells, but also enables cancer cells to survive during separation from the original sites via anoikis resistance.[26] Anoikis is the programmed cell death that occurs when epithelial or endothelial cells from the extracellular matrix and basement membrane.[27] The significance of anoikis is to prevent these shedding cells from planting in other inappropriate places. However, studies have found that most tumor cells possess anti-anoikis properties, especially in metastatic malignancy, as cancer cells can be implanted and transferred in other areas after shedding from the original tumor issues.[28-30] There is evidence demonstrating that EMT-promoting proteins are one of the inducers of anoikis resistance in human cancers.[31] For instance, an increase in N-cadherin accompanied by a decrease in E-cadherin levels can improve resistance to anoikis and promote metastasis.[32,33] According to our research, the suppression of miR-142-5p led to a decrease in E-cadherin levels and elevated the N-cadherin in OC cells, which indicates that the inhibition of anti-anoikis may be other mechanisms accounting for the therapeutic effect of miR-142-5p against OC metastasis. Besides, several studies suggested that some EMT-related transcription factors such as Snail, can regulate anoikis resistance via E-cadherin and N-cadherin.[34,35] For example, Zhang *et al.* discovered that acacetin can inhibit the EMT of gastric cancer cells via the PI3K/Akt/Snail pathway.[36] However, more details about the underlying regulations on EMT by miR-142-5p and the crosstalk between EMT and anoikis resistance remain for further investigation.

miRNA regulates the biological process via directly binding to the 3'UTR of the specific mRNA at the posttranscription level. Once the specific role of miR-142-5p in targeting OC metastasis and EMT was established, our study further investigated the target mRNA of miR-142-5p. In recent years, epigenetic modifications have been shown to make a difference in cancer occurrence, metastasis, and miRNA expression.[37,38] DNA methylation is a common epigenetic change in mammals, and its hyperactivation in cancer cells is often associated with the suppression of tumor suppressor genes and resistance to apoptosis.[39] Significantly, DNA hypermethylation is induced by aberrant expression of DNA methyltransferases, such as DNMT1, DNMT3a, and DNMT3b, while the excessive up-regulation of DNMT1 was observed in various cancer cells, especially metastatic tumors.[40,41] Fu *et al.* revealed the increased expression of DNMT1 in breast cancer cells when compared with normal cells, and decitabine, a DNMT1 inhibitor, can notably suppress the development and metastasis of cancer cells.[42] In the present study, the DNMT1 was also notably increased in the metastasis tumor compared with the

non-metastatic one. More importantly, the expression of DNMT1 was proved to be down-regulated by miR-142-5p, thus suggesting that DNMT1 may be a potential target of miR-142-5p. Besides, the combination sites and the interactions between miR-142-5p and DNMT1 were explored in our study, and the downstream role of DNMT1 in the miR-142-5p was further confirmed via a knockdown experiment. Although we proved the therapeutic potential of DNMT1 for OC treatment as a downstream factor of miR-142-5p, the regulation of DNMT1 on OC metastatic remains quite complex nowadays. Runt-related transcription factor 3 has been reported to be hypermethylated by DNMT1 in the OC transformation and thus may account for the OC metastasis via the miR-142-5p/DNMT1 axis.[43] However, this hypothesis requires experimental support in the future.

CONCLUSION

In conclusion, our data show that miR-142-5p suppresses OC metastasis by suppressing EMT of OC cells via DNMT1, thus suggesting the therapeutic prospect of miR-142-5p against OC metastasis. However, there are still some deficiencies that need clarification in our research. Although miR-142-5p was proven to regulate the EMT phenomenon of OC cells, molecular mechanisms should also be uncovered. Besides, animal experiments should be conducted to verify the conclusion of this research. More investigations are still needed to further develop the promising therapy against OC metastasis by targeting miR-142-5p.

Our study determined the therapeutic activity of miR-142-5p for OC treatment and was the first to demonstrate that miR-142-5p can inhibit the migration of OC cells by targeting the EMT process through DNMT1. In conclusion, our research indicated the therapeutic promise of miR-142-5p against OC metastasis.

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AUTHORS' CONTRIBUTIONS

QQ and XQZ designed experiments. HY, JRS and HZ carried out experiments, analyzed experimental results. QQ wrote the manuscript. XQZ revised the manuscript. All authors approved the final manuscript.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

The experiment protocol was approved by Fujian Provincial Maternity and Children's Hospital (2022KYLLR01014) and all patients gave written informed consent.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

OC: Ovarian cancer; **EMT:** Epithelial-mesenchymal transition; **ZO-1:** Zonula occludes I; **miRNAs:** MicroRNAs; **UTR:** 5' Untranslated region; **TRBP:** Trans-activation-responsive RNA-binding protein; **DMEM:** Dulbecco's modified eagle medium; **FBS:** Fetal bovine serum; **RT:** Real-time; **PCR:** Polymerase chain reaction; **SDS-PAGE:** Sodium dodecyl sulphate-polyacrylamide gel electrophoresis; **PVDF:** Polyvinylidene fluoride; **PBS:** Phosphate buffered saline; **ECL:** Electrochemiluminescence**; ANOVA:** Analysis of variance; **DNMT1:** DNA Methyltransferase 1.

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