Am J Transl Res 2024;16(11):7145-7154
www.ajtr.org /ISSN:1943-8141/AJTR0159160

Original Article

Capecitabine regulates proliferation and apoptosis of ovarian cancer SKOV3 cells via the miR-29b-3p/MMP16 molecular axis

Zhuangzhuang Xu[1], Hongyan Li[2], Cheng Qian[3], Boliang Chu[1], Yan Yin[4], Shijie Yuan[5], Zeqiu Wan[6]

[1]Department of Obstetrics and Gynecology, Huzhou Maternity and Child Health Care Hospital, Huzhou 313000, Zhejiang, China; [2]Department of Obstetrics and Gynecology, Huzhou Central Hospital, Huzhou 313099, Zhejiang, China; [3]Department of Surgery, Huzhou Maternity and Child Health Care Hospital, Huzhou 313000, Zhejiang, China; [4]Department of General Surgery, Huzhou Maternity and Child Health Care Hospital, Huzhou 313000, Zhejiang, China; [5]Concorde East Stem Cell Gene Engineering Co., Ltd., Huzhou 313000, Zhejiang, China; [6]Department of Women's Health, Huzhou Maternity and Child Health Care Hospital, Huzhou 313000, Zhejiang, China

Received July 13, 2024; Accepted October 26, 2024; Epub November 15, 2024; Published November 30, 2024

Abstract: Objective: To investigate the molecular mechanism by which capecitabine regulates the proliferation and apoptosis of ovarian cancer SKOV3 cells through the miR-29b-3p/MMP16 axis. Methods: SKOV3 ovarian cancer cells were treated with capecitabine, miR-29b-3p mimics, miR-29b-3p inhibitor, and MMP16 siRNA. Cell proliferation was measured using the CCK-8 assay, and apoptosis was assessed by flow cytometry. Changes in miR-29b-3p and MMP16 mRNA levels were analyzed via qRT-PCR, while protein expression of MMP16, Ki67, Caspase-3, and Bcl-2 were evaluated by Western blot. Target genes of miR-29b-3p were predicted using bioinformatics tools, and their interaction was validated through a luciferase reporter assay. Transfection of SKOV3 cells with a miR-29b-3p inhibitor or pcDNA-MMP16 was followed by capecitabine treatment, with subsequent analysis of cell proliferation and apoptosis. Results: Capecitabine treatment reduced the viability of SKOV3 cells and promoted apoptosis, accompanied by increased miR-29b-3p expression and decreased MMP16 expression. Transfection with miR-29b-3p mimics or MMP16 siRNA also inhibited cell viability and enhanced apoptosis. Western blot analysis showed an increase in Ki67 and Caspase-3 expression and a decrease in Bcl-2 expression. Conversely, inhibition of miR-29b-3p or overexpression of pcDNA-MMP16 counteracted the effects of capecitabine, reversing the reduction in proliferation and the increase in apoptosis. Western blotting confirmed decreased Ki67 and Caspase-3 levels and increased Bcl-2 expression in these conditions. Conclusion: Capecitabine enhances miR-29b-3p expression, leading to the downregulation of MMP16, thereby inhibiting proliferation and promoting apoptosis in ovarian cancer cells.

Keywords: Capecitabine, miR-29b-3p, MMP16, ovarian cancer, apoptosis

Introduction

Ovarian cancer, known for its insidious onset and poor prognosis, is one of the most common malignant tumors affecting the female reproductive system. Current treatment strategies for ovarian cancer mainly include surgical resection, radiotherapy, and chemotherapy [1-3]. Capecitabine, a widely used chemotherapeutic agent, has been shown to induce tumor cell apoptosis and inhibit cell proliferation [4-6]. Studies have reported that capecitabine achieves a higher control rate in advanced recurrent ovarian cancer with minimal adverse effects, making it a promising option for clinical use [7-9]. However, the molecular mechanisms underlying its anti-cancer effects in ovarian cancer remain largely unexplored.

Research has indicated significant changes in serum miRNA levels in patients undergoing capecitabine chemotherapy, with these changes correlating with treatment outcomes [10]. Thus, capecitabine-induced variations in miRNA levels within tumor cells may trigger biological effects that contribute to its antitumor mechanisms. miRNAs are small, non-coding RNA molecules that regulate gene expression by binding complementarily to the 3'UTR of target mRNAs, thereby influencing protein expression [11-13].

https://doi.org/10.62347/YGPG5971

miRNAs play key roles in the development and progression of various cancers, including liver and breast cancer [13-15].

Preliminary experiments suggest that capecitabine affects the expression of miR-29b-3p in ovarian cancer cells. miR-29b-3p can bind to the 3'UTR of MMP16, indicating a possible link between capecitabine's mechanism of action and the miR-29b-3p/MMP16 axis. miR-29b-3p, typically downregulated in ovarian cancer, has been found to suppress the malignant phenotype of ovarian cancer when overexpressed [10]. MMP16, on the other hand, promotes ovarian cancer progression and acts as an oncogene by enhancing cell proliferation [16].

This study aims to elucidate how capecitabine regulates the miR-29b-3p/MMP16 axis, affecting the proliferation and apoptosis of ovarian cancer cells. The findings are expected to provide insights into the molecular mechanisms underlying capecitabine's antitumor effects, offering a foundation for future research.

Materials and methods

Materials

The Transcriptor First Strand cDNA Synthesis Kit and FastStart Universal SYBR Green Master (ROX) were purchased from Roche, Switzerland. Rabbit anti-MMP16 antibody was obtained from Wuhan Fei'en Biotechnology Co., Ltd. The miRcute Plus miRNA cDNA First Strand Synthesis Kit and miRcute Enhanced miRNA Fluorescence Quantitative Detection Reagent were sourced from TianGen Biotech (Beijing) Co., Ltd. MMP16 siRNA and the corresponding control siRNA were synthesized by Beijing Huaxia Xiongfeng Technology Co., Ltd. The miR-29b-3p inhibitor, inhibitor control, miR-29b-3p mimics, and mimics control were procured from Shanghai Gima Pharmaceutical Technology Co., Ltd. Rabbit anti-Ki67 antibody was purchased from Santa Cruz Biotechnology, USA. Capecitabine (0.5 g) was obtained from Shanghai Roche Pharmaceuticals Co., Ltd. Rabbit anti-Caspase-3 antibody was acquired from Trevigen, USA. MMP16-WT and MMP16-MUT constructs were prepared by Shanghai Jikai Gene Medical Technology Co., Ltd. Rabbit anti-Bcl2 antibody was sourced from Abcam, USA. pcDNA and pcDNA-MMP16 constructs were produced by PureGen Bio (Wuhan) Technology Co., Ltd. SKOV3 ovarian cancer cells were obtained from Shanghai Fuxiang Biotechnology Co., Ltd.

Experimental groups

The ovarian cancer cells were divided into the following groups: control group, capecitabine group, miR-NC group, miR-29b-3p group, si-NC group, si-MMP16 group, anti-miR-NC group, anti-miR-29b-3p group, capecitabine + anti-miR-NC group, capecitabine + anti-miR-29b-3p group, capecitabine + pcDNA-NC group, and capecitabine + pcDNA-MMP16 group.

Control group: Treated with 0 μmol/L capecitabine. Capecitabine group: Treated with varying concentrations of capecitabine (0.25, 0.5, 1.0, 1.5, 2.0 μmol/L), depending on experimental requirements. miR-NC group: Transfected with mimics control 24 hours prior to the experiment. miR-29b-3p group: Transfected with miR-29b-3p mimics 24 hours before the experiment. si-NC group: Transfected with siRNA control 24 hours before starting the experiment. si-MMP16 group: Transfected with MMP16 siRNA 24 hours prior to the experiment. Anti-miR-NC group: Transfected with inhibitor control 24 hours before the experiment. Anti-miR-29b-3p group: Transfected with miR-29b-3p inhibitor 24 hours before the experiment. Capecitabine + anti-miR-NC group: Transfected with inhibitor control 24 hours prior, then treated with 1.0 μmol/L capecitabine at the start of the experiment. Capecitabine + anti-miR-29b-3p group: Transfected with miR-29b-3p inhibitor 24 hours prior, followed by treatment with 1.0 μmol/L capecitabine at the experiment's onset. Capecitabine + pcDNA-NC group: Transfected with the pcDNA control vector 24 hours before the experiment, then treated with 1.0 μmol/L capecitabine. Capecitabine + pcDNA-MMP16 group: Transfected with pcDNA-MMP16 24 hours before the experiment and treated with 1.0 μmol/L capecitabine.

Transfections were performed according to the manufacturer's instructions for the Lipofectamine 2000 transfection reagent.

CCK-8 assay for cell proliferation

Ovarian cancer cells (5 × 10^4) were seeded in a 96-well plate and treated as outlined in section 1.2. Following incubation at 37°C with 5% CO_2 for 48 hours, the cells were collected, resuspended, and incubated with 100 μL of culture medium and 10 μL of CCK-8 solution for an additional 4 hours. Cell viability was determined by measuring the optical density (OD) at 450 nm.

Table 1. PCR primer sequences

U6-F	5'-CTCGCTTCGGCAGCACA-3'
U6-R	5'-AACGCTTCACGAATTTGCGT-3'
miR-29b-3p-F	5'-TGCGGTAGCACCATTTGAAAT-3'
miR-29b-3p-R	5'-CCAGTGCAGGGTCCGAGGT-3'
MMP16-F	5'-AGCACGTTGTTTCCCTTCC-3'
MMP16-R	5'-CCCGAGCTGTTTATCCATCA-3'
β-actin-F	5'-AAGTCCTCACCCTCCCAAAAG-3'
β-actin-R	5'-AAGCAATGCTGTCACCTTCCC-3'

Flow cytometry for apoptosis detection

Ovarian cancer cells (5 × 10^5) were seeded in a 12-well plate and treated according to the experimental requirements in section 1.2. After 48 hours of incubation at 37°C with 5% CO_2, cells were harvested by digestion with 0.25% trypsin, washed twice with PBS, and resuspended in 400 μL of Binding Buffer. Subsequently, 5 μL each of Annexin V-FITC and PI solution were added to the suspension and incubated for 15 minutes in the dark at room temperature. Then, 100 μL of Binding Buffer was added, and the proportion of apoptotic cells was analyzed using flow cytometry.

Western blot for Ki67, caspase-3, Bcl2, and MMP16 expression

Ovarian cancer cells (5 × 10^5) were seeded in a 12-well plate and treated as described in section 1.2. Total protein was extracted from each group and quantified using the BCA assay. Protein samples (20 μg) were separated via SDS-PAGE and transferred to PVDF membranes. Membranes were cut according to the molecular weights of the target proteins and blocked with 5% skim milk at room temperature for 1 hour. Primary antibodies for Ki67 (1:1,000), Bcl2 (1:800), MMP16 (1:800), and β-actin (1:1,000) were added and incubated overnight at 4°C. After washing three times with PBS, membranes were incubated with HRP-conjugated secondary antibodies (1:2,000) at room temperature for 30 minutes. Protein bands were visualized using an ECL detection reagent, imaged, and analyzed with Image J for grayscale quantification.

qRT-PCR for miR-29b-3p and MMP16 mRNA expression

Ovarian cancer cells (5 × 10^5) were seeded in a 12-well plate and treated according to section 1.2. After 48 hours of incubation at 37°C with 5% CO_2, total RNA was extracted from each group. Reverse transcription was performed using the miRcute Plus miRNA cDNA First Strand Synthesis Kit and the Transcriptor First Strand cDNA Synthesis Kit. Quantitative PCR was conducted using the miRcute Enhanced miRNA Fluorescence Quantitative Detection Kit and FastStart Universal SYBR Green Master (ROX). The PCR program was as follows: 50°C for 2 minutes, 95°C for 2 minutes; 40 cycles of 95°C for 10 seconds, 60°C for 10 seconds, and 72°C for 30 seconds with fluorescence detection. The relative expression levels of miR-29b-3p and MMP16 mRNA were calculated using the 2^-ΔΔCt method, with U6 and β-actin as internal controls (See **Table 1**).

Target gene prediction and verification

The starBase software was used to predict the binding sites between miR-29b-3p and the 3'UTR of MMP16. A luciferase reporter assay was then performed to verify this interaction. Ovarian cancer cells were co-transfected with MMP16-WT (wild-type luciferase reporter vector containing the predicted binding sites in the 3'UTR of MMP16) or MMP16-MUT (mutant luciferase reporter vector with altered binding sites in the 3'UTR of MMP16), along with miR-29b-3p mimics or mimics control. After 48 hours of incubation, luciferase activity was measured using a luciferase detection kit.

Statistical analysis

Data were analyzed using SPSS 21.0 software and were expressed as mean ± standard deviation (mean ± SD). Comparisons between two groups were made using the t-test, while one-way analysis of variance (ANOVA) was employed for comparisons among multiple groups. A *P*-value of less than 0.05 was considered statistically significant.

Results

Capecitabine inhibits ovarian cancer cell proliferation and promotes apoptosis

Capecitabine treatment at concentrations of 0.25, 0.5, 1.0, 1.5, and 2.0 μmol/L significantly reduced ovarian cancer cell survival ($P<0.05$). The half-maximal inhibitory concentration (IC50) of capecitabine was approximately 1.0 μmol/L, which was used for subse-

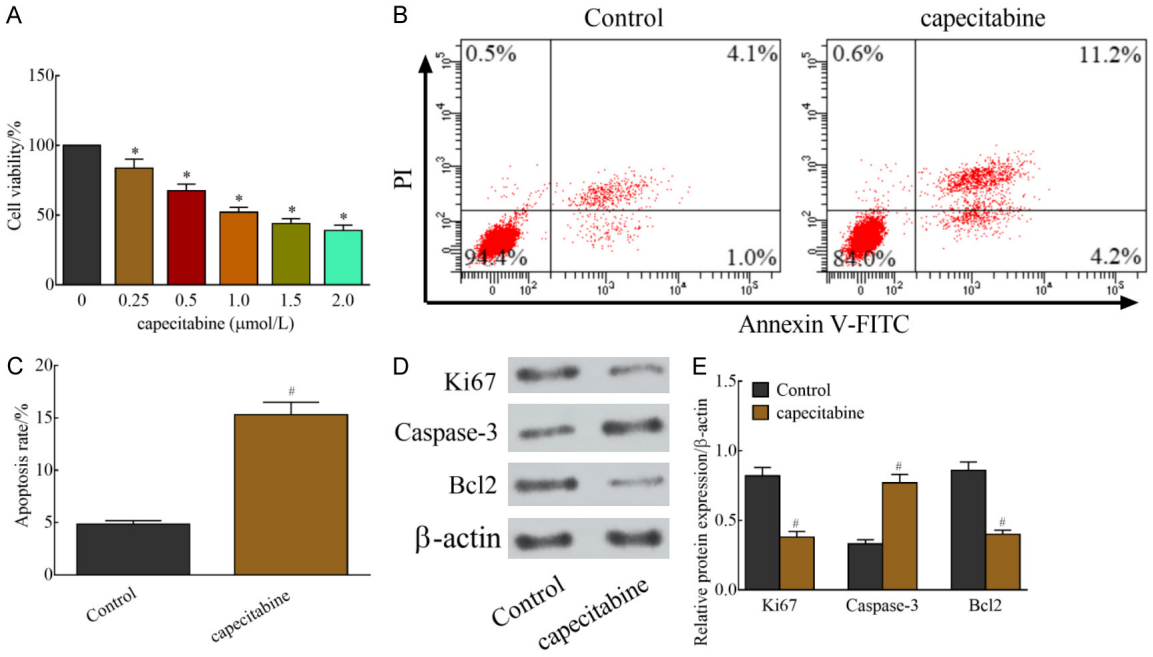

Figure 1. Capecitabine inhibits ovarian cancer cell proliferation and promotes apoptosis. A: Changes in survival rate of ovarian cancer cells after treatment with different concentrations of capecitabine; B, C: Changes in cell apoptosis; D, E: Changes in Ki67, Caspase-3, and Bcl2 protein expression in cells. N=6, *P<0.05 compared to 0 μmol/L capecitabine; #P<0.05 compared to the control group.

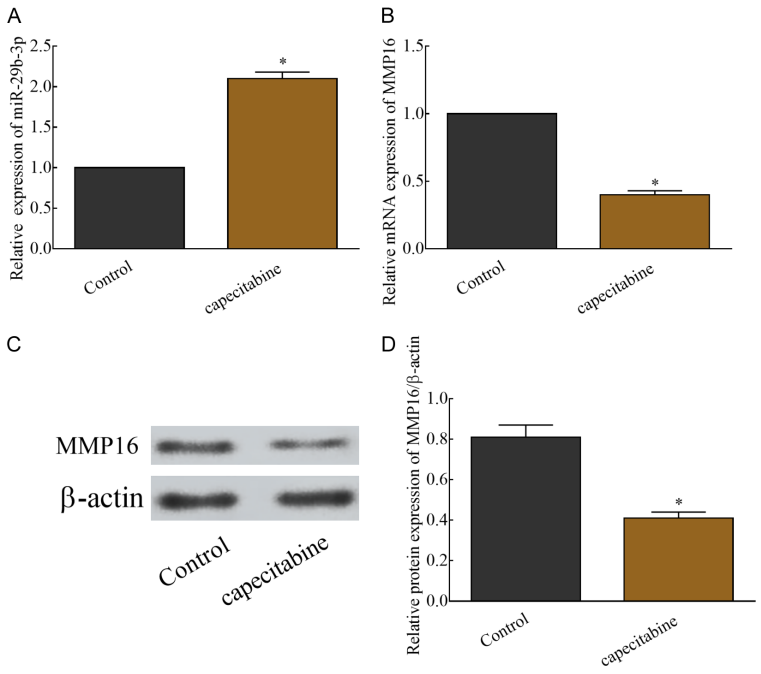

Figure 2. Changes in miR-29b-3p and MMP6 expression levels in ovarian cancer cells after capecitabine treatment. A: Changes in miR-29b-3p expression; B: Changes in MMP6 mRNA expression; C, D: Changes in MMP6 protein expression. N=6, *P<0.05 compared to the control group.

quent experiments. Compared to the control group, treatment with 1.0 μmol/L capecitabine significantly increased the apoptosis rate, decreased Ki67 and Bcl2 protein expression, and elevated Caspase-3 protein levels in ovarian cancer cells (P<0.05) (**Figure 1**).

Capecitabine promotes miR-29b-3p expression and suppresses MMP16 expression in ovarian cancer cells

The capecitabine-treated group showed a significant increase in miR-29b-3p expression and a decrease in MMP16 mRNA and protein levels compared to the control group (P< 0.05) (**Figure 2**).

Overexpression of miR-29b-3p inhibits ovarian cancer cell proliferation and promotes apoptosis

Compared to the control and miR-NC groups, the miR-29b-3p overexpression group exhibited higher miR-29b-3p levels, reduced cell viability, an increased apoptosis rate, lower

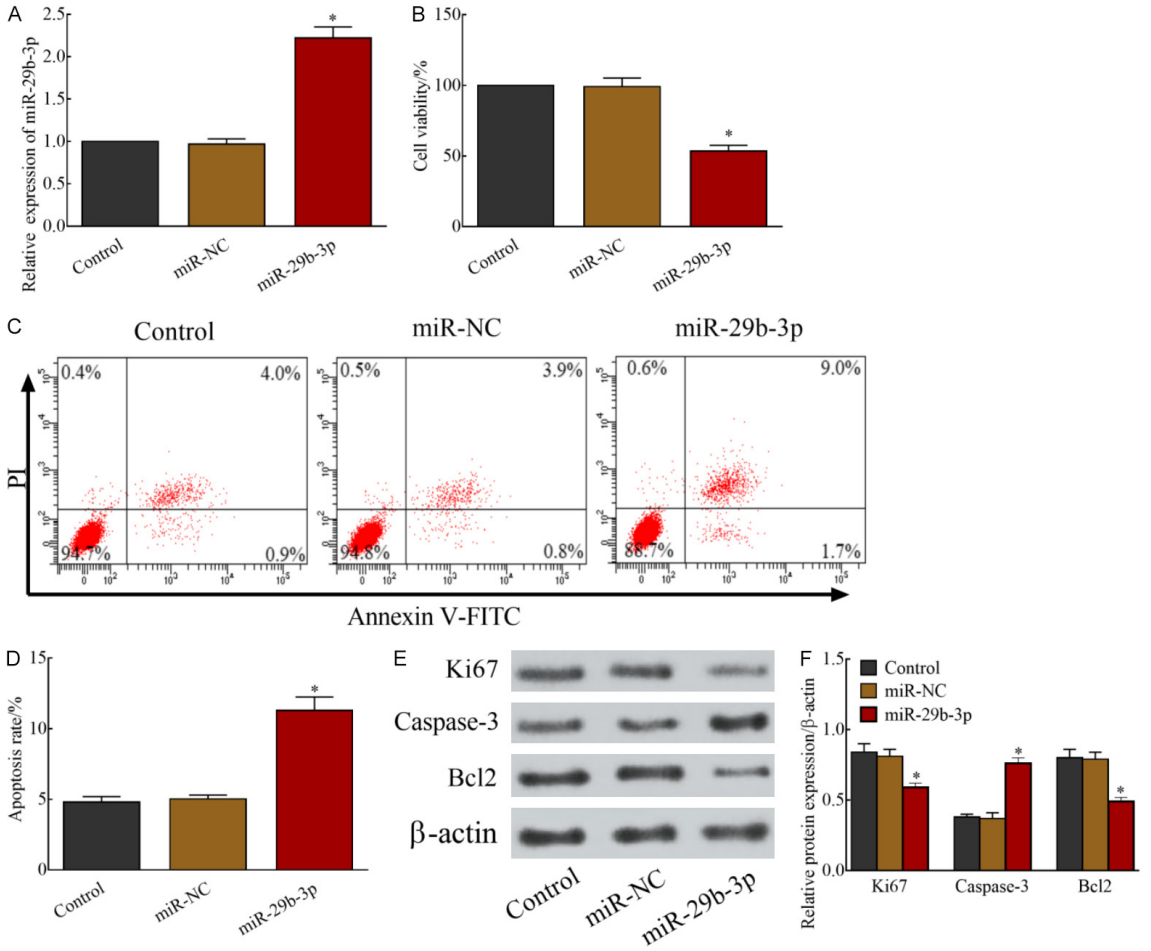

Figure 3. Effects of miR-29b-3p overexpression on apoptosis, proliferation, and expression of Ki67, Bcl2, and Caspase-3 proteins in ovarian cancer cells. A: miR-29b-3p expression; B: Changes in cell viability; C, D: Apoptosis in cells; E, F: Expression of Ki67, Bcl2, and Caspase-3 proteins in cells. N=6, *P<0.05 compared to the control group.

expression of Ki67 and Bcl2 proteins, and higher Caspase-3 protein expression (P<0.05) (**Figure 3**).

Knockdown of MMP16 suppresses ovarian cancer cell proliferation and promotes apoptosis

Compared to the control and miR-NC groups, MMP16 knockdown resulted in significantly lower MMP16 mRNA and protein levels in ovarian cancer cells. This was associated with reduced cell viability, increased apoptosis, decreased Ki67 and Bcl2 protein expression, and increased Caspase-3 protein levels (P< 0.05) (**Figure 4**).

miR-29b-3p negatively regulates MMP16

The starBase software predicted binding sites for miR-29b-3p in the 3'UTR of MMP16 (**Figure 5**). Following co-transfection with MMP16-WT and miR-29b-3p mimics, a significant decrease in luciferase activity was observed in ovarian cancer cells (P<0.05), indicating a direct interaction. In contrast, the anti-miR-29b-3p group exhibited significantly higher MMP16 mRNA and protein expression compared to the control and anti-miR-NC groups (P<0.05) (**Figure 5**).

Capecitabine regulates MMP16 via miR-29b-3p to mediate ovarian cancer cell proliferation and apoptosis

Compared to the capecitabine and capecitabine + anti-miR-NC groups, the capecitabine + anti-miR-29b-3p group showed significantly lower miR-29b-3p expression, higher MMP16 mRNA and protein levels, increased cell viability, decreased apoptosis, elevated Ki67 and

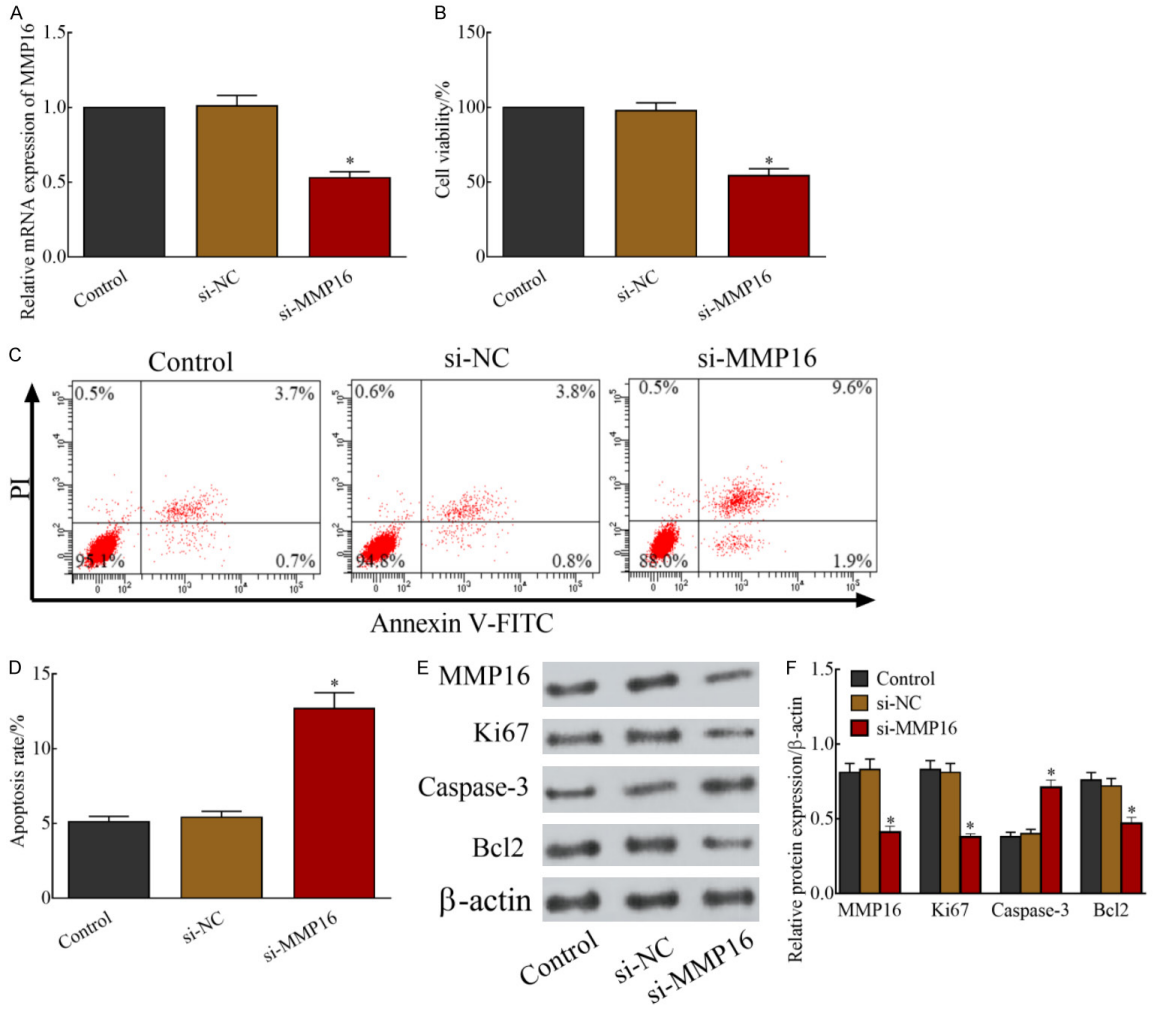

Figure 4. Effect of MMP16 knockdown on apoptosis and expression of Ki67, Bcl2, Caspase-3 proteins in ovarian cancer cells. A: Relative expression of MMP16 mRNA; B: Changes in cell viability; C, D: Apoptosis in cells; E, F: Expression of MMP16, Ki67, Caspase-3, and Bcl2 proteins. N=6, *P<0.05 compared to the control group.

Bcl2 protein expression, and reduced Caspase-3 protein expression (P<0.05). Similarly, the capecitabine + pcDNA-MMP16 group, compared to the capecitabine and capecitabine + pcDNA-NC groups, exhibited higher MMP16 expression, increased cell viability, lower apoptosis rate, and higher Ki67 and Bcl2 levels, with reduced Caspase-3 expression (P<0.05) (**Figure 6**).

Discussion

Capecitabine, a commonly used chemotherapeutic agent, has demonstrated anti-tumor effects by inhibiting cell proliferation and inducing apoptosis, thereby improving the prognosis of ovarian cancer patients. This study confirms, at the in vitro level, that capecitabine exerts anti-tumor activity by upregulating miR-29b-3p expression, leading to the inhibition of MMP16 protein expression. Ki67 is a well-known marker for assessing cellular proliferation [17-19], while Caspase-3, an executor in the apoptotic cascade, promotes irreversible apoptosis, and Bcl2 functions as an anti-apoptotic protein [20]. The results show that capecitabine treatment reduces Bcl2 and Ki67 expression, increases Caspase-3 expression, decreases cell proliferation, and enhances apoptosis in ovarian cancer cells. These findings align with previous studies, confirming the anti-tumor efficacy of capecitabine [21, 22].

While capecitabine's anti-ovarian cancer effects are established, the precise molecular mechanisms remain unclear. Previous studies [23,

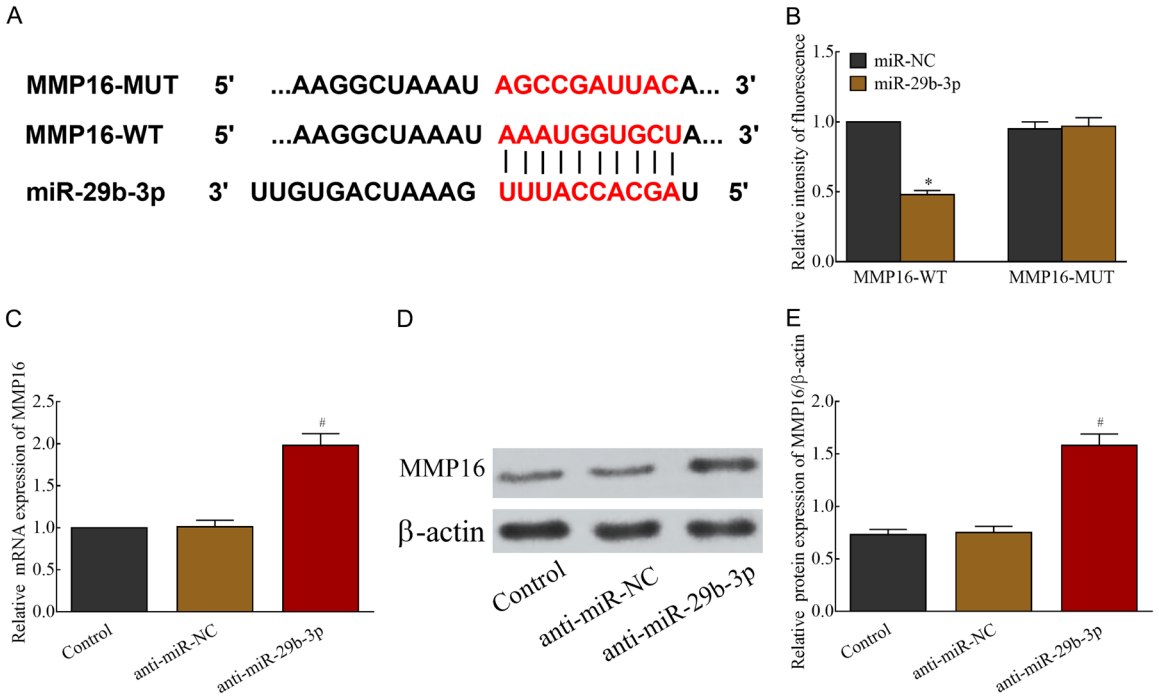

Figure 5. miR-29b-3p directly regulates MMP16 expression. A: Prediction of binding sites between miR-29b-3p and MMP16 from StarBase; B: Luciferase reporter assay detecting miR-29b-3p regulation of MMP16 expression; C: Changes in MMP16 mRNA expression; D, E: MMP16 protein expression. N=6, *P<0.05 compared to miR-NC group; #P<0.05 compared to the control group.

24] have suggested that capecitabine can alter miRNA expression in patients undergoing cancer treatment, implicating miRNAs in its mechanism of action. miRNAs, approximately 20 nucleotides in length, do not encode proteins but regulate physiological and pathological processes by influencing the expression of target genes [25, 26]. miR-29b-3p, a member of the miR-29 family, has shown tumor-suppressive effects by inhibiting cell proliferation in cancers such as esophageal cancer [27] and cholangiocarcinoma [28]. In ovarian cancer, miR-29b-3p is typically downregulated, and its overexpression can reduce the malignancy of ovarian cancer cells [29].

This study found that capecitabine elevates miR-29b-3p levels in ovarian cancer cells, leading to increased Caspase-3 expression and decreased Bcl2 expression, thereby inducing apoptosis. Further experiments showed that downregulating miR-29b-3p could reverse capecitabine's anti-cancer effects, indicating that the downstream mechanisms activated by miR-29b-3p are critical for capecitabine's therapeutic action against ovarian cancer.

miRNAs bind to the 3'UTR of target genes through base complementarity, thereby influencing their expression. The target genes of miRNAs may vary across different physiological and pathological conditions [30, 31]. Identified miR-29b-3p target genes involved in tumor growth regulation include ANO1 and MDM2, among others [32, 33]. MMP16, a member of the matrix metalloproteinase family, is often overexpressed in various tumors and can promote tumor growth. Previous studies have shown that downregulation of MMP16 reduces ovarian cancer cell proliferation and induces apoptosis [34].

In this study, the starBase software predicted binding sites between miR-29b-3p and MMP16, which were further confirmed by luciferase reporter assays and Western blot analysis, demonstrating that miR-29b-3p negatively regulates MMP16. This suggests that the mechanism of action of miR-29b-3p may be related to its inhibitory effect on MMP16 expression. Our experiments showed that knocking down MMP16 enhances apoptosis, decreases cell proliferation, and reduces the expression

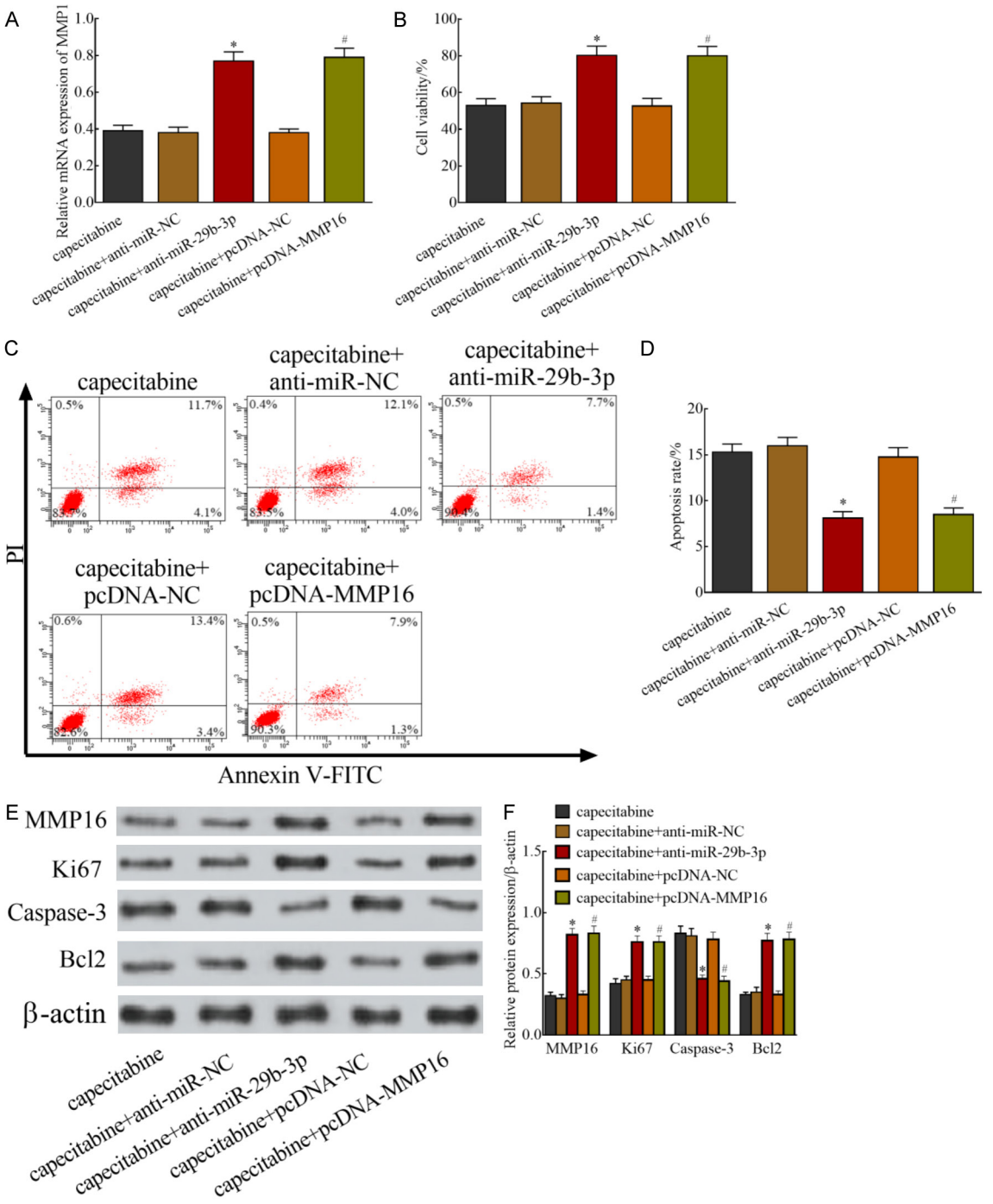

Figure 6. Capecitabine regulates MMP16 via miR-29b-3p to modulate apoptosis and expression of Ki67, Caspase-3, Bcl2 proteins in ovarian cancer cells. A: MMP mRNA expression; B: Changes in cell viability; C, D: Apoptosis in cells; E, F: Expression of MMP16, Ki67, Caspase-3, and Bcl2 proteins. N=6, *P<0.05 compared to capecitabine+anti-miR-NC group; #P<0.05 compared to capecitabine+pcDNA-NC group.

of the proliferation marker Ki67 in ovarian cancer cells. Conversely, overexpression of MMP16 can reverse the effects of capecitabine on inhibiting cell proliferation and promoting apoptosis, indicating that the impact of MMP16 knockdown is similar to that of capecitabine treatment or miR-29b-3p overexpression. These findings suggest that capecitabine may exert its effects on ovarian cancer progression by targeting MMP16 via miR-29b-3p.

In summary, capecitabine regulates the miR-29b-3p/MMP16 axis to inhibit ovarian cancer cell proliferation and promote apoptosis, providing a foundation for understanding the molecular mechanisms behind its anti-tumor effects. However, the specific mechanisms by which capecitabine affects the miR-29b-3p/MMP16 axis remain unclear and warrant further investigation in future studies.

Disclosure of conflict of interest

None.

Address correspondence to: Zeqiu Wan, Department of Women's Health, Huzhou Maternity and Child Health Care Hospital, No. 2 East Street, Wuxing District, Huzhou 313000, Zhejiang, China. Tel: +86-0572-2030181; E-mail: 15053932607@163.com

References

[1] Konstantinopoulos PA and Matulonis UA. Clinical and translational advances in ovarian cancer therapy. Nat Cancer 2023; 4: 1239-1257.
[2] Ponti G, De Angelis C, Ponti R, Pongetti L, Losi L, Sticchi A, Tomasi A and Ozben T. Hereditary breast and ovarian cancer: from genes to molecular targeted therapies. Crit Rev Clin Lab Sci 2023; 60: 640-650.
[3] Webb PM and Jordan SJ. Global epidemiology of epithelial ovarian cancer. Nat Rev Clin Oncol 2024; 21: 389-400.
[4] Alzahrani SM, Al Doghaither HA, Al-Ghafari AB and Pushparaj PN. 5-Fluorouracil and capecitabine therapies for the treatment of colorectal cancer (Review). Oncol Rep 2023; 50: 175.
[5] Lang T, Zhu R, Zhu X, Yan W, Li Y, Zhai Y, Wu T, Huang X, Yin Q and Li Y. Combining gut microbiota modulation and chemotherapy by capecitabine-loaded prebiotic nanoparticle improves colorectal cancer therapy. Nat Commun 2023; 14: 4746.
[6] Santhosh A, Sharma A, Bakhshi S, Kumar A, Sharma V, Malik PS, Pramanik R, Gogia A, Prasad CP, Sehgal T, Gund S, Dev A, Cheung WY, Pandey RM, Kumar S, Gupta I and Batra A; D-TORCH Trial Investigators. Topical Diclofenac for prevention of capecitabine-associated hand-foot syndrome: a double-blind randomized controlled trial. J Clin Oncol 2024; 42: 1821-1829.
[7] Cioffi R, Fais ML, Bergamini A, Vanni VS, Pagliardini L, Papaleo E, Mangili G and Candiani M. Ovarian failure risk in post-pubertal patients with cancer: a prognostic model. Future Oncol 2022; 18: 2391-2400.
[8] Kerr AJ, Dodwell D, McGale P, Holt F, Duane F, Mannu G, Darby SC and Taylor CW. Adjuvant and neoadjuvant breast cancer treatments: a systematic review of their effects on mortality. Cancer Treat Rev 2022; 105: 102375.
[9] Kurnit KC and Frumovitz M. Primary mucinous ovarian cancer: options for surgery and chemotherapy. Int J Gynecol Cancer 2022; 32.
[10] Fan B, Shen C, Wu M, Zhao J, Guo Q and Luo Y. miR-17-92 cluster is connected with disease progression and oxaliplatin/capecitabine chemotherapy efficacy in advanced gastric cancer patients: a preliminary study. Medicine (Baltimore) 2018; 97: e12007.
[11] Azhar A, Khan WH, Al-Hosaini K and Kamal MA. miRNAs in SARS-CoV-2 infection: an update. Curr Drug Metab 2022; 23: 283-298.
[12] Iacomino G. miRNAs: the road from bench to bedside. Genes (Basel) 2023; 14: 314.
[13] Smolarz B, Durczyński A, Romanowicz H, Szyłło K and Hogendorf P. miRNAs in cancer (Review of Literature). Int J Mol Sci 2022; 23: 2805.
[14] Chakrabortty A, Patton DJ, Smith BF and Agarwal P. miRNAs: potential as biomarkers and therapeutic targets for cancer. Genes (Basel) 2023; 14: 1375.
[15] Zhu L, Zhao L, Wang Q, Zhong S, Guo X, Zhu Y, Bao J, Xu K and Liu S. Circulating exosomal miRNAs and cancer early diagnosis. Clin Transl Oncol 2022; 24: 393-406.
[16] Wu M, Wang G, Tian W, Deng Y and Xu Y. MiRNA-based therapeutics for lung cancer. Curr Pharm Des 2018; 23: 5989-5996.
[17] Dovnik A and Repše Fokter A. The role of p16/Ki67 dual staining in cervical cancer screening. Curr Issues Mol Biol 2023; 45: 8476-8491.
[18] Kreipe H, Harbeck N and Christgen M. Clinical validity and clinical utility of Ki67 in early breast cancer. Ther Adv Med Oncol 2022; 14: 17588359221122725.
[19] Lashen AG, Toss MS, Ghannam SF, Makhlouf S, Green A, Mongan NP and Rakha E. Expression, assessment and significance of Ki67 expression in breast cancer: an update. J Clin Pathol 2023; 76: 357-364.
[20] Lossi L, Castagna C and Merighi A. Caspase-3 mediated cell death in the normal development of the mammalian cerebellum. Int J Mol Sci 2018; 19: 3999.
[21] Garcia-Aguilar J, Patil S, Gollub MJ, Kim JK, Yuval JB, Thompson HM, Verheij FS, Omer DM, Lee M, Dunne RF, Marcet J, Cataldo P, Polite B, Herzig DO, Liska D, Oommen S, Friel CM, Ternent C, Coveler AL, Hunt S, Gregory A, Varma MG, Bello BL, Carmichael JC, Krauss J, Gleisner A, Paty PB, Weiser MR, Nash GM, Pappou E, Guillem JG, Temple L, Wei IH, Widmar M, Lin S, Segal NH, Cercek A, Yaeger R, Smith JJ, Goodman KA, Wu AJ and Saltz LB. Organ preservation in patients with rectal adenocarcinoma treated with total neoadjuvant therapy. J Clin Oncol 2022; 40: 2546-2556.

[22] Schrag D, Shi Q, Weiser MR, Gollub MJ, Saltz LB, Musher BL, Goldberg J, Al Baghdadi T, Goodman KA, McWilliams RR, Farma JM, George TJ, Kennecke HF, Shergill A, Montemurro M, Nelson GD, Colgrove B, Gordon V, Venook AP, O'Reilly EM, Meyerhardt JA, Dueck AC, Basch E, Chang GJ and Mamon HJ. Preoperative treatment of locally advanced rectal cancer. N Engl J Med 2023; 389: 322-334.

[23] Cao SH, Wang QQ and Zhang JR. Curative effect of sorafenib combined with capecitabine in treatment of hepatocellular carcinoma and its influence on serum miR-212 and miR-132 levels. Shanxi Medical 2020; 49: 615-618.

[24] Matuszyk J. MALAT1-miRNAs network regulate thymidylate synthase and affect 5FU-based chemotherapy. Mol Med 2022; 28: 89.

[25] Wu Y, Pu N, Su W, Yang X and Xing C. Down-regulation of miR-1 in colorectal cancer promotes radioresistance and aggressive phenotypes. J Cancer 2020; 11: 4832-4840.

[26] Zhang N, Hu X, Du Y and Du J. The role of miRNAs in colorectal cancer progression and chemoradiotherapy. Biomed Pharmacother 2021; 134: 111099.

[27] Zhao W, Huang Z, Liu H and Wang C. LncRNA GIHCG promotes the development of esophageal cancer by modulating miR-29b-3p/ANO1 axis. Onco Targets Ther 2020; 13: 13387-13400.

[28] Cao K, Sun L, Zhang Y, Wang T, Li H and Zuo S. Overpression of miR-29b suppresses the proliferation and induces apoptosis of cholangiocarcinoma cells. Nan Fang Yi Ke Da Xue Xue Bao 2018; 38: 1234-1238.

[29] Yang X, Xin N, Qu HJ, Wei L and Han Z. Long noncoding RNA TUG1 facilitates cell ovarian cancer progression through targeting MiR-29b-3p/MDM2 axis. Anat Rec (Hoboken) 2020; 303: 3024-3034.

[30] Diener C, Keller A and Meese E. Emerging concepts of miRNA therapeutics: from cells to clinic. Trends Genet 2022; 38: 613-626.

[31] Vrščaj LA, Marc J and Ostanek B. Interactome of PTH-regulated miRNAs and their predicted target genes for investigating the epigenetic effects of PTH (1-34) in bone metabolism. Genes (Basel) 2022; 13: 1443.

[32] Dinesen S, El-Faitarouni A and Dalgaard LT. Circulating microRNAs associated with gestational diabetes mellitus: useful biomarkers? J Endocrinol 2022; 256: e220170.

[33] Weng HR, Taing K, Chen L and Penney A. EZH2 methyltransferase regulates neuroinflammation and neuropathic pain. Cells 2023; 12: 1058.

[34] Wang H, Qi C and Wan D. MicroRNA-377-3p targeting MMP-16 inhibits ovarian cancer cell growth, invasion, and interstitial transition. Ann Transl Med 2021; 9: 124.