European Review for Medical and Pharmacological Sciences 2017; 21: 5056-5064

RG108 induces the apoptosis of endometrial cancer Ishikawa cell lines by inhibiting the expression of DNMT3B and demethylation of HMLH1

L. YANG, J. HOU, X.-H. CUI, L.-N. SUO, Y.-W. LV

Department of Obstetrics and Gynecology, PLA 463 hospital, Shenyang, China

Abstract. – OBJECTIVE: The effects of DNA methyltransferase (DNMT) inhibitor RG108 on the proliferation and apoptosis of endometrial cancer was investigated, and whether its mechanism was related to the inhibition of DNMT3B, thereby affecting the human mutL homolog 1 (hMLH1) methylation status and its expression, was further studied.

MATERIALS AND METHODS: Culture of human endometrial cancer Ishikawa cell lines: cells grew adhering to the wall in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (supplemented with 10% fetal bovine serum (FBS) and 2 mM L-glutamic acid). After the cells were treated with RG108, the changes in cell viability were detected via methyl thiazolyl tetrazolium (MTT) assay. The effect of RG108 on cell cycle was detected via flow cytometry, and its effect on cell apoptosis was detected via flow cytometry and TUNEL. Moreover, the methylation status of hMLH1gene in endometrial cancer cells was detected via methylation specific-PCR (MSP), and the changes in DNMT3Band hMLH1 expressions were detected via RT-PCR and Western blotting, respectively.

RESULTS: MTT results showed that RG108 inhibited the cell viability in a dose-dependent and time-dependent manner. Flow cytometry revealed that RG108 blocked the cell cycle in G2/M phase and promoted the apoptosis, and TUNEL assay further proved that RG108 promoted the apoptosis. It was found in the detection via MSP that the methylated hMLH1 gene was significantly reduced after 72 h of treatment with RG108. Besides, RT-PCR and Western blotting showed that RG108 inhibited the DNMT3B expression and activated the hMLH1 expression.

CONCLUSIONS: The demethylation drug RG108 can significantly inhibit the proliferation of endometrial cancer cells, block the cell cycle in the G2/M phase and induce the cell apoptosis, which is a new candidate drug in the treatment of endometrial cancer. RG108 realizes the hMLH1 demethylation and increases the hMLH1 expression through inhibiting the expression of DNMT3B.

Key Words:
RG108, Endometrial cancer cell, Apoptosis, DNMT3B, hMLH1.

Introduction

Endometrial cancer is one of the common malignant tumors in the female reproductive system[1,2]. It can be divided into type I endometrial cancer and type II endometrial cancer according to its pathogenesis and histopathological features, the former of which accounts for about 80-90% in endometrial cancer and is mostly derived from the proliferative endometrium; it is related to the over-stimulation of estrogen, and the estrogen and progesterone receptor expressions are often positive[3]. Recent papers have shown that the changes in epigenetic information play important roles in the occurrence and development of type I endometrial cancer[4,5].

Epigenetic changes mainly include loss and acquisition of DNA methylation, the changes in chromatin structure characterized by histone modification, etc[6,7]. These epigenetic changes, especially the gene silencing caused by the hypermethylation of promoter, affect each stage of tumor development[8]. DNA methylation is involved in the temporal and spatial expression of controlling genes, which plays an important role in maintaining the structure of chromosomes, inactivation of X chromosome, gene imprinting, and occurrence and development of many human gene diseases (such as cancer, cardiovascular disease[9,10] and diabetes mellitus[11,12]). Moreover, DNA methylation is a mechanism that

Corresponding Author: Li Yang, MD; e-mail: yanglee2000@126.com

has been studied more profoundly in epigenetics, as well as an enzyme-mediated chemical modification process. In mammals, there are three types of DNA-methyltransferase (DNMT) associated with methylation: DNMT1, DNMT3A and DNMT3B. A variety of experimental results suggest that the three types of DNMT can lead to abnormal DNA methylation in tumor cells through direct or synergistic effect. Thus, they play important roles in the initiation and maintenance of DNA methylation in tumor cells. Studies have found that DNMT3B is overexpressed in endometrial cancer[13], and its expression level in well-differentiated endometrial cancer cell lines is lower than that in poorly-differentiated endometrial cancer cell lines.

DNA mismatch repair system plays a key role in maintaining the DNA replication fidelity and genomic stability. The loss of mismatch repair gene function is due to the absence of expression caused by the promoter methylation of mismatch repair gene, such as human MutL homolog 1 (hMLH1). HMLH1 promoter methylation can be seen in the endometrial hyperplasia and carcinoma-adjacent normal endometria, suggesting that the mismatch repair gene promoter methylation is an early event in the occurrence of endometrial cancer[14]. It is currently thought that the extensive methylation leads to the decreased expressions of some tumor suppressor genes and DNA mismatch repair genes, which may be the characteristics of some endometrial cancers, especially the type I endometrial cancer. The loss of DNA mismatch repair function will accelerate the mutation of microsatellite sequence in malignant cell transformation-associated genes, thereby accelerating the malignant transformation of tumors. Studies have shown that the mismatch repair gene hMLH1 promoter methylation in endometrial cancer is associated with the overexpression of DNMT3B[15]. With the deepening of study on methylase crystal structure, inhibitors with high specificity can be screened using the three-dimensional structure of enzymes.

A compound with good properties and inhibitory activity, RG108, was screened by the German Cancer Institute using the three-dimensional structure of enzymes, namely 2-(1,3-dioxo-1,3-dihydro-2-hydrogen-isoindole)-3-(1-hydrogen-indole) propionic acid. Studies have shown that RG108 has a good demethylation effect on colon cancer cells and leukemia cells[16], but whether RG108 has a good demethylation effect on endometrial cancer cells is unclear.

In this work, the effects of DNMT inhibitor N-phthaloyl-l-tryptophan 1 (RG108) on the proliferation and apoptosis of endometrial cancer were investigated, and whether its mechanism was related to the inhibition of endometrial cancer cell DNMT3B, thereby affecting the hMLH1 methylation status and its expression, was further studied.

Materials and Methods

Ishikawa Cell Culture and Passage

After cells were resuscitated, they were blown and beat to prepare the cell suspension, inoculated in a culture dish with 10 cm in diameter, and incubated with 5% CO_2 at 37°C, followed by subculture when the cell growth reached 80-100%. The original culture solution was absorbed using the sterile pipette and discarded, and the culture dish was washed with the preheated D-Hands liquid at 37°C twice and added with 2 mL trypsin. The culture dish was gently inclined to make all cells soaked in the digestive fluid, and placed at 37°C for 2-3 min for digestion. The culture dish was observed under the microscope until there was cytoplasm retraction and intercellular space dilatation, and the serum-containing liquid was added to stop digestion, and the single-cell suspension was mixed and prepared. Then, the suspension was transferred to the centrifuge tube, trimmed and centrifuged at 1000 rpm for 5 min. The supernatant was discarded and the cell sediments were resuspended in RPMI1640 medium. The cells were inoculated at 5×10^5/mL after counting.

Detection of Cell Viability Via Methyl Thiazolyltetrazolium (MTT) Assay

Cells adhering to the wall were digested with trypsin, and after termination, the cell density was adjusted to 5×10^4/mL. Next, cells were inoculated in the 96-well plate (100 μl/well) and divided into five groups: control group (CTR group), 5 μM RG108 group, 10 μM RG108 group, 20 μM RG108 group and 40 μM RG108 group. 5 repeated wells were set for each group, and the blank control group was set at the same time. The cells were incubated for 72 h and added with 5 mg/mL MTT into each well at 4 h before termination. The culture plate was incubated in an incubator containing CO_2 at 37°C for another 4 h. The yellow MTT was reduced to purple blue crystals by cells under the action of SDH. 150 μL dimethyl sulfoxide (DMSO) was added into each well and shaken at low speed for 10 min on a shaking table to dissolve the crystals fully. The optical density at 490 nm of each well was measured using the enzyme-linked immunosorbent detector.

Detection of Cell Cycle and Apoptosis Via Flow Cytometry

The effects of drug on cell cycle and apoptosis after 72 h were detected *via* flow cytometry. After cell digestion, the cell suspension was prepared, and 100 μL cells were taken from each group for propidium iodide (PI) labeling. After labeling, the cells were incubated at 4°C for 30 min. 100 μL cell suspension was placed in 5 mL flow tube and added with 5 μL Annexin V/FITC and 10 μL 20 μg/mL propidium iodide solution, and the mixture was incubated at room temperature in a dark place for 15 min, followed by detection on the machine.

Detection of Cell Apoptosis Via TUNEL Assay

Section preparation: the cells were inoculated on the cover glass of plate with appropriate cell density, and they could be dropped onto it without overflowing from the four sides. After the cells adhered to the wall, the liquid or treatment factors were supplemented. After washing, the liquid in the hole was absorbed, and 4% paraformaldehyde prepared freshly was added, followed by fixation at room temperature for 60 min. 3% hydrogen peroxide - methanol was added for reaction at room temperature for 10 min. The plate was placed on the ice, added with 0.2% Triton-x100, covered with the plate cover buried in ice for 5 min. Then TUNEL reaction solution was added for incubation at 37°C for 60 min, followed by observation *via* fluorescence microscope in a dark room.

Detection of mRNA Level in Cells Via RT-PCR

The cells were divided into two groups in the experiment: Group A (control) and Group B (20 uM RG108). After drug administration for 72 h, the mRNA levels of hMLH1 and DNMT3B were detected *via* RT-PCR.

Methylation Specific PCR (MSP)

After DNA extraction and purification, and treatment with sodium bisulfite, two pairs of methylated PCR primers were added. The TapDNA polymerase and dNTPs were added for PCR amplification. Finally, the product received the agarose gel electrophoresis, followed by observation and photograph in UV monitor.

Detection of Protein Expression Level in Cells Via Western Blotting

The total protein was extracted from cells according to the standard procedure and quantified. The quantified protein was transferred into the new Eppendorf (EP) tube, and 1/4 volume of 5× loading buffer was added for denaturation at 100°C for 10 min. Each well was loaded with 50 μg protein, one of which was loaded with the marker. After electrophoresis and membrane transfer, the sealing solution was added for sealing at room temperature for 1 h. Then, primary antibodies were incubated at 4°C overnight, and secondary antibodies were incubated on the next day, followed by color development and observation.

Statistical Analysis

Statistical Product and Service Solutions (SPSS19.0, Armonk, NY, USA) software was used for statistical analysis. The homogeneity test of variance and one-way analysis of variance were used for the comparisons of means among groups. Student Newman Keuls-q (SNK-q) test was used for the pairwise comparisons among groups. The *t*-test was used for the comparison between the two groups. Quantitative data were presented as mean ± standard deviation; $p<0.05$ suggested that the difference was statistically significant.

Results

Detection of Cell Viability

The results of MTT assay showed that RG108 inhibited the cell viability in a concentration-dependent manner. Compared with normal control group, 20 μM RG108 group could significantly inhibit the cell proliferation ($p < 0.05$). 40 μM RG108 group could also significantly inhibit the cell proliferation compared with control group ($p < 0.05$), but there was no significant difference compared with 20 μM RG108 group. Therefore, the action concentration of RG108 was determined as 20 μM. After action of 20 μM RG108 for 72 h, it was found that its inhibition of cell viability was in a time-dependent manner; in other words, the inhibitory effect of demethylation drug RG108 on cell viability was in a concentration- and time-dependent manner (Figure 1).

Effect of RG108 on Endometrial Cancer Cell Cycle

The cells were divided into five groups in the experiment. After drug action for 72 h, the cell cycle distribution was detected *via* flow cytometry, and it was found that the cell cycle was blocked in the G2/M phase. Compared with that in control group, the proportion of cells in G2/M

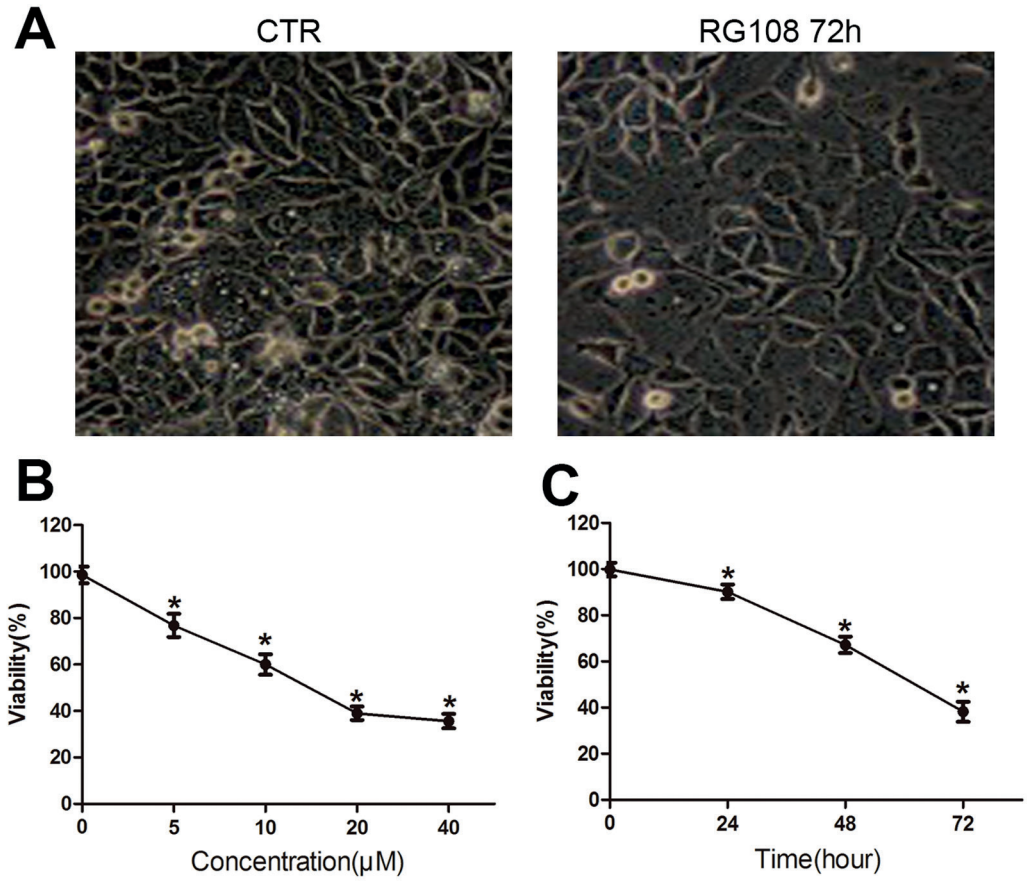

Figure 1. RG108 inhibited the cell viability in a concentration-dependent and time-dependent manner. ***A,*** The representative images of cell culture. ***B,*** The analysis of MTT assay show RG108 inhibited the cell viability in a concentration-dependent manner. ***C,*** The analysis of MTT assay show RG108 inhibited the cell viability in a time-dependent manner. $^{*}p < 0.05$ *vs.* CTR group.

phase in drug group was increased by about three times. The proportion of cells in G2/M phase was gradually increased with the time (Figure 2).

Effect of RG108 on Apoptosis

It was found *via* flow cytometry and TUNEL assay that RG108 could significantly induce the apoptosis. The results revealed that the percentage of apoptotic cells in drug group was significantly higher than that of necrotic cells, and also significantly higher than that in control group, proving that the decreased cell viability is caused by the apoptosis, rather than necrosis. The results of TUNEL assay showed that the number of apoptotic cells was increased gradually with the extension of action time. Compared with that in control group, the number of apoptotic cells was increased significantly after action of 20 μM RG108 for 24 h, further indicating that RG108 can significantly induce the apoptosis (Figure 3).

Effect of RG108 on hMLH1 Methylation Status in Endometrial Cancer Cell

The hMLH1 methylation status in endometrial cancer cells was detected *via* MSP, and it was found that the number of methylated hMLH1 gene was obviously reduced after treatment with RG108 for 72 h, while the methylated hMLH1 gene had no significant change in control group, suggesting that RG108 demethylates the hMLH1 gene, thus activating the hMLH1 gene expression (Figure 4).

Effects of RG108 on mRNA and Protein Expression of DNMT3B and hMLH1 in Endometrial Cancer Cells

After drug action for 72 h, the expressions of DNMT3B and hMLH1 were detected *via* RT-PCR and Western blotting. The results of RT-PCR revealed that RG108 could significantly inhibit the mRNA expression of hMH3 and increase the

mRNA expression of hMLH1, suggesting that RG108 may demethylate the hMLH1 gene through inhibiting DNMT3B, thus activating the hMLH1 gene expression. The results of Western blotting showed that RG108 could obviously inhibit the protein expression of DNMT3B and increase the protein expression of hMLH1, further confirming the above results (Figure 4B and C).

Discussion

Endometrial cancer is one of the common malignant tumors of the female reproductive tract, and its incidence rate is increased year by year[1,17]. At present, its exact pathogenesis is still unclear, which may be related to the excessive stimulation of estrogen. With the rise of epigenetics, the role of disordered epigenetic regulation in cells in the occurrence and development has been gradually revealed. DNA methylation is the most important form of epigenetic modification, which is studied the most profoundly. Many studies have shown that the abnormal DNA methylation plays an important role in the development and progression of endometrial cancer[18,19], and it is also another important pathway of tumor suppressor gene inactivation following gene mutation and gene deletion. DNA methylation leads to the inactivation of a large number of genes, which plays an important role in the tumorigenesis, so inhibiting the methylation or re-activating the corresponding genes can treat the cancer, which opens up a new way for cancer treatment. These genes are not regulated by DNA methylation in non-cancer cells, so the toxicity of methylation inhibitors against non-cancer cells is much less than that of traditional anticancer drugs. In this respect, the prospect of this therapy is promising.

At present, the anti-DNA methylation therapies include the antisense oligonucleotides, RNA interference, drug therapy, etc[20,21]. The most important two major drugs are methyltransferase inhibitors and histone deacetylase inhibitors, such as butyrate and hydroxamic acids. The former is mostly the cytosine analogues modified on the pyrimidine ring, forming the irreversible complex through the covalent bond and DNMT to competitively inhibit its activity, so the DNA methylation is progressively lost with cell division and the malignant phenotype of tumors is partially reversed. In this study, RG108 belongs to the transmethylase inhibitor, a highly specific inhibitor screened by the three-dimensional structure of enzymes. It was found in this study that RG108 could significantly inhibit the proliferation of endometrial cancer cells, block the cell cycle in the G2/M phase and induce the cell apoptosis, providing a new candidate drug for the treatment of endometrial cancer.

In mammals, methylation mainly occurs in CpG island. Although the role of CpG island methylation in tumors is increasingly evident, its formation mechanism remains unclear yet. The possible cause is the increase in the expression of one or more DNMTs, and most studies support this claim, despite of different opinions[22,23]. In short, how much the effect of the increase in DNMT expression on

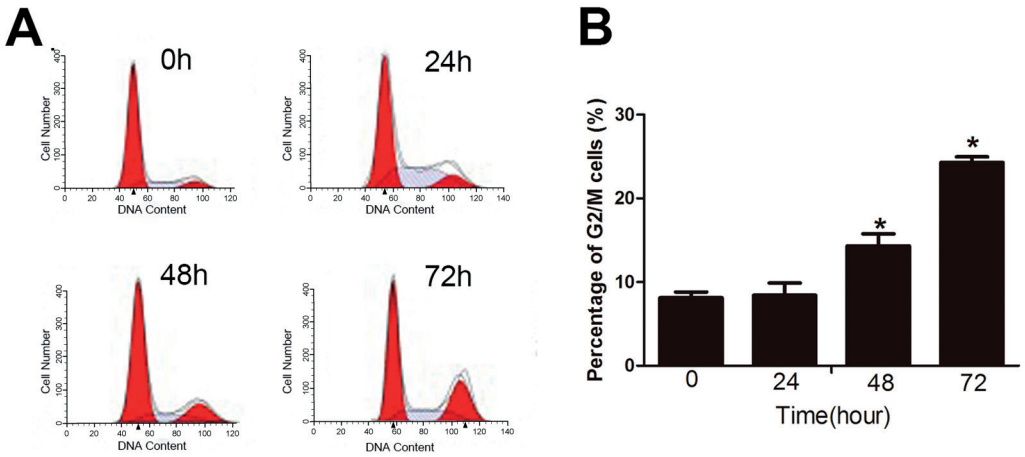

Figure 2. RG108 blocked cell cycle in the G2/M phase. ***A***, Cell cycle analysis by FACS. ***B***, The result show that cells arrested in G2/M phase was gradually increased with the time. *$p < 0.05$ *vs.* CRT group.

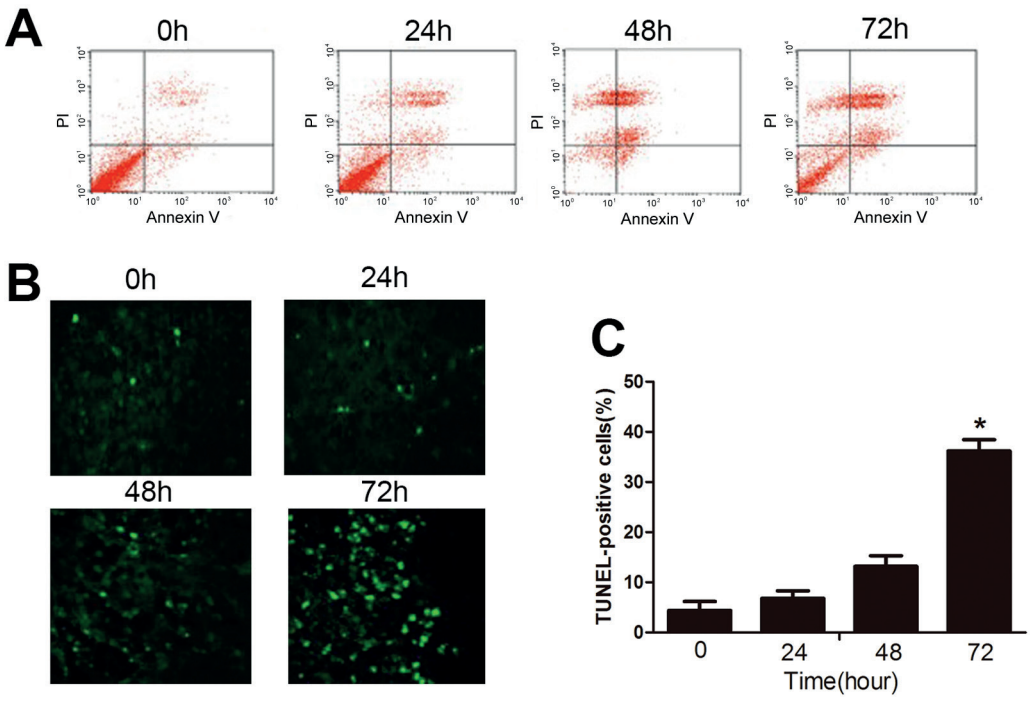

Figure 3. RG108 induced apoptosis of Ishikawa cell in a time-dependent manner. ***A,*** Flow cytometry analysis of apoptosis by Annexin V/PI staining. ***B,*** The representative images by TUNEL-histochemistry. ***C,*** The analysis of TUNEL-positive cells show RG108 induced apoptosis of Ishikawa cell in a time-dependent manner. $^{*}p < 0.05$ *vs.* CTR group.

abnormal tumor methylation is remains uncertain. Researches have found that the estrogen can increase the protein expression level in DNMT3B; therefore, it is speculated that DNMT3B plays an important role in the methylation process of tumor suppressor gene in endometrial cancer cells. In this study, it was found that the DNMT3B expression in endometrial cancer cells was increased, while the abnormal methylation of hMLH1 was also increased significantly; the conjecture was verified *via* experimental results.

HMLH1 is a gene that plays an important role in DNA repair signal pathway[24]. After hMLH1 hypermethylation, the base mismatch repair function will be lost, which often occurs in colon cancer[25], endometrial cancer[26] and gastric cancer[27], and it may be an important molecular basis of occurrence of these cancers. Approximately 5% endometrial cancer occurs in women with strong genetic predisposition, which is caused by germline mutations associated with hereditary nonpolyposis colorectal cancer (HNPCC) syndrome. The genetic mutations in DNA repair gene lead to a large number of mutations in microsatellite repetitive sequences in the entire genome, namely the microsatellite instability (MSI). HNPCC is manifested as the familial aggregation of early-onset colon cancer, and the incidence rates of some other types of cancers are also increased, among which endometrial cancer is the most significant in women. MSI also occurs in some sporadic endometrial cancers without DNA repair gene germline mutation. Studies have shown that the loss of mismatch repair gene function in these cases is due to the absence of expression caused by the promoter methylation in mismatch repair genes[28], such as hMLH1, while hMLH1 promoter methylation is also seen in the endometrial hyperplasia and carcinoma-adjacent normal endometria, suggesting that the promoter methylation of mismatch repair gene is an early event in the development of endometrial cancer. It is currently thought that the extensive methylation will reduce the expressions of some tumor suppressor genes and DNA mismatch repair genes, which may be the characteristics of some endometrial cancers, especially the type I endometrial cancer. The loss of DNA mismatch repair function will accelerate the mutation of microsatellite sequence in malignant transformation-associated genes, thereby accelerating the malignant transformation of tumors[29,30]. In this paper, the methylation status of hMLH1 gene in endometrial cancer cells was detected *via* MSP and it was found that the

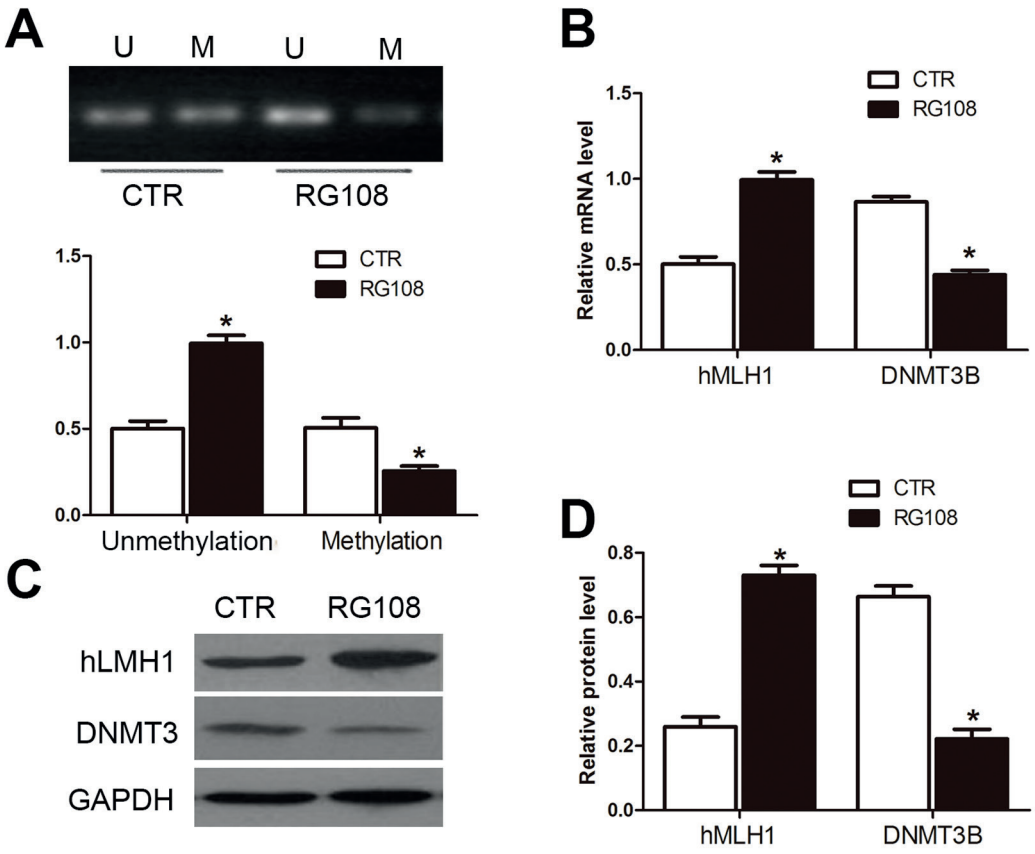

Figure 4. RG108 inhibited the expression of DNMT3B and increased the hMLH1 expression after RG108 treatment for 72 h. ***A,*** Methylation-specific PCR analysis of Ishikawa cell in different group, M = primers specific to methylated template DNA, U = primers specific to unmethylated template DNA. ***B,*** The analysis of mRNA level of hMLH1 and DNMT3B. ***C,*** Western blots detected the protein level of hMLH1 and DNMT3B. ***D,*** Semi-quantitative analysis of hMLH1 and DNMT3B. *$p < 0.05$ *vs.* CTR group.

demethylation drug RG108 could demethylate the hMLH1 gene to activate the expression of hMLH1 gene, suggesting that there is methylation of mismatch repair genes in endometrial cancer cells. The results of this study showed that the expression level of DNMT3B was decreased after action of RG108, the hMLH1 gene was demethylated and activated, and the mRNA and protein expression levels of hMLH1 were increased; at the same time, RG108 could inhibit the proliferation of endometrial cancer cells and promote the apoptosis of cancer cells, further proving that the hMLH1 gene methylation is involved in the malignant transformation of endometrial cancer.

Conclusions

The demethylation drug RG108 can significantly inhibit the proliferation of endometrial cancer cells, block the cell cycle in the G2/M phase and induce the cell apoptosis, which is a new candidate drug in the treatment of endometrial cancer. RG108 realizes the hMLH1 demethylation and increases the hMLH1 expression through inhibiting the expression of DNMT3B.

Conflict of interest

The authors declare no conflicts of interest.

References

1) Gaber C, Meza R, Ruterbusch JJ, Cote ML. Endometrial cancer trends by race and histology in the USA: projecting the number of new cases from 2015 to 2040. J Racial Ethn Health Disparities 2016 Oct 17. [Epub ahead of print]
2) Koskas M, Huchon C, Amant F. Characteristics and prognosis of patients with early-stage endometrial cancer who refuse adjuvant radiotherapy. Gynecol Oncol 2016; 141: 428-433.

3) Malik TY, Chishti U, Aziz AB, Sheikh I. Comparison of risk factors and survival of Type 1 and Type II endometrial cancers. Pak J Med Sci 2016; 32: 886-890.

4) Pabalan N, Kunjantarachot A, Ruangpratheep C, Jarjanazi H, Christofolini DM, Barbosa CP, Bianco B. Potential of RASSF1A promoter methylation as biomarker for endometrial cancer: a systematic review and meta-analysis. Gynecol Oncol 2017; 146: 603-608.

5) Cosgrove CM, Cohn DE, Hampel H, Frankel WL, Jones D, McElroy JP, Suarez AA, Zhao W, Chen W, Salani R, Copeland LJ, O'Malley DM, Fowler JM, Yilmaz A, Chassen AS, Pearlman R, Goodfellow PJ, Backes FJ. Epigenetic silencing of MLH1 in endometrial cancers is associated with larger tumor volume, increased rate of lymph node positivity and reduced recurrence-free survival. Gynecol Oncol 2017; 146: 588-595.

6) Kimura T. Metal-mediated epigenetic regulation of gene expression. Yakugaku Zasshi 2017; 137: 273-279.

7) Li B, Feng ZH, Sun H, Zhao ZH, Yang SB, Yang P. The blood genome-wide DNA methylation analysis reveals novel epigenetic changes in human heart failure. Eur Rev Med Pharmacol Sci 2017; 21: 1828-1836.

8) Dong Z, Guo W, Guo Y, Kuang G, Yang Z. Concordant promoter methylation of transforming growth factor-beta receptor types I and II occurs early in esophageal squamous cell carcinoma. Am J Med Sci 2012; 343: 375-381.

9) Muka T, Koromani F, Portilla E, O'Connor A, Bramer WM, Troup J, Chowdhury R, Dehghan A, Franco OH. The role of epigenetic modifications in cardiovascular disease: a systematic review. Int J Cardiol 2016; 212: 174-183.

10) Guay SP, Legare C, Brisson D, Mathieu P, Bosse Y, Gaudet D, Bouchard L. Epigenetic and genetic variations at the TNNT1 gene locus are associated with HDL-C levels and coronary artery disease. Epigenomics 2016; 8: 359-371.

11) Gillberg L, Ling C. The potential use of DNA methylation biomarkers to identify risk and progression of type 2 diabetes. Front Endocrinol (Lausanne) 2015; 6: 43.

12) Kato M, Natarajan R. Diabetic nephropathy--emerging epigenetic mechanisms. Nat Rev Nephrol 2014; 10: 517-530.

13) Jin F, Dowdy SC, Xiong Y, Eberhardt NL, Podratz KC, Jiang SW. Up-regulation of DNA methyltransferase 3B expression in endometrial cancers. Gynecol Oncol 2005; 96: 531-538.

14) Gonzalez L, Ortiz AP, Suarez EL, Umpierre S, Billoch J, Marcos MJ, Joy L, Charneco E, Lacourt MY, Bernabe-Dones RD, Cruz-Correa MR. Case-case study of factors associated to hMLH1, hMSH2, and hMSH6 protein expression among endometrial cancer patients of the University District Hospital of San Juan, Puerto Rico. Int J Gynecol Cancer 2012; 22: 826-829.

15) Cui M, Wen Z, Chen J, Yang Z, Zhang H. 5-Aza-2'-deoxycytidine is a potent inhibitor of DNA methyltransferase 3B and induces apoptosis in human endometrial cancer cell lines with the up-regulation of hMLH1. Med Oncol 2010; 27: 278-285.

16) Brueckner B, Garcia BR, Siedlecki P, Musch T, Kliem HC, Zielenkiewicz P, Suhai S, Wiessler M, Lyko F. Epigenetic reactivation of tumor suppressor genes by a novel small-molecule inhibitor of human DNA methyltransferases. Cancer Res 2005; 65: 6305-6311.

17) Klil-Drori AJ, Yin H, Abenhaim HA, du Fort GG, Azoulay L. Prolactin-Elevating antipsychotics and the risk of endometrial cancer. J Clin Psychiatry 2017; 78: 714-719.

18) Caplakova V, Babusikova E, Blahovcova E, Balharek T, Zelieskova M, Hatok J. DNA methylation machinery in the endometrium and endometrial cancer. Anticancer Res 2016; 36: 4407-4420.

19) Jiao Y, Widschwendter M, Teschendorff AE. A systems-level integrative framework for genome-wide DNA methylation and gene expression data identifies differential gene expression modules under epigenetic control. Bioinformatics 2014; 30: 2360-2366.

20) Braun FK, Mathur R, Sehgal L, Wilkie-Grantham R, Chandra J, Berkova Z, Samaniego F. Inhibition of methyltransferases accelerates degradation of cFLIP and sensitizes B-cell lymphoma cells to TRAIL-induced apoptosis. PLoS One 2015; 10: e117994.

21) Berghauser PL, Kleijn A, Kloezeman JJ, van den Bosche W, Kaufmann JK, de Vrij J, Leenstra S, Dirven CM, Lamfers ML. The HDAC inhibitors scriptaid and LBH589 combined with the oncolytic virus Delta24-RGD exert enhanced anti-tumor efficacy in patient-derived glioblastoma cells. PLoS One 2015; 10: e127058.

22) Sharma D, Bhave S, Gregg E, Uht R. Dexamethasone induces a putative repressor complex and chromatin modifications in the CRH promoter. Mol Endocrinol 2013; 27: 1142-1152.

23) Horii T, Hatada I. Regulation of CpG methylation by Dnmt and Tet in pluripotent stem cells. J Reprod Dev 2016; 62: 331-335.

24) Fishel R. Mismatch repair. J Biol Chem 2015; 290: 26395-26403.

25) Liu L, Nakatsuru Y, Gerson SL. Base excision repair as a therapeutic target in colon cancer. Clin Cancer Res 2002; 8: 2985-2991.

26) Muraki Y, Banno K, Yanokura M, Kobayashi Y, Kawaguchi M, Nomura H, Hirasawa A, Susumu N, Aoki D. Epigenetic DNA hypermethylation: clinical applications in endometrial cancer (Review). Oncol Rep 2009; 22: 967-972.

27) Falchetti M, Saieva C, Lupi R, Masala G, Rizzolo P, Zanna I, Ceccarelli K, Sera F, Mariani-Costantini R, Nesi G, Palli D, Ottini L. Gastric cancer with high-level microsatellite instability: target gene mutations, clinicopathologic features, and long-term survival. Hum Pathol 2008; 39: 925-932.

28) Caldwell GM, Jones C, Gensberg K, Jan S, Hardy RG, Byrd P, Chughtai S, Wallis Y, Matthews GM, Morton DG. The Wnt antagonist sFRP1 in colorectal tumorigenesis. Cancer Res 2004; 64: 883-888.

29) Pinheiro M, Ahlquist T, Danielsen SA, Lind GE, Veiga I, Pinto C, Costa V, Afonso L, Sousa O, Fragoso M, Santos L, Henrique R, Lopes P, Lopes C, Lothe RA, Teixeira MR. Colorectal carcinomas with microsatellite instability display a different pattern of target gene mutations according to large bowel site of origin. BMC Cancer 2010; 10: 587.

30) Boyer JC, Hawk JD, Stefanovic L, Farber RA. Sequence-dependent effect of interruptions on microsatellite mutation rate in mismatch repair-deficient human cells. Mutat Res 2008; 640: 89-96.