

Promoter Methylation of the *MGRN1* Gene Response to Chemotherapy of Epithelial Ovarian Cancer Patients

Xiaofei Li

Hebei Medical University Fourth Affiliated Hospital and Hebei Provincial Tumor Hospital

Haiyan Sun

Hebei Medical University Fourth Affiliated Hospital and Hebei Provincial Tumor Hospital

Hua Tian

Hebei Medical University Fourth Affiliated Hospital and Hebei Provincial Tumor Hospital

Haibo Zhang

Hebei Medical University Fourth Affiliated Hospital and Hebei Provincial Tumor Hospital

Yunjie Tian

Hebei Medical University Fourth Affiliated Hospital and Hebei Provincial Tumor Hospital

Yan Li

Hebei Medical University Fourth Affiliated Hospital and Hebei Provincial Tumor Hospital

Shan Kang (✉ kangshan202011@163.com)

Hebei Medical University Fourth Affiliated Hospital and Hebei Provincial Tumor Hospital

Research

Keywords: MGRN1, EOC, Methylation, Platinum resistance, EGR1

Posted Date: March 16th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-298962/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Objective: Aberrant DNA methylation is considered to play a critical role in the chemoresistance of epithelial ovarian cancer (EOC). In this study, we explored the relationship between hypermethylation of the Mahogunin Ring Finger 1 (*MGRN1*) gene promoter and primary chemoresistance in EOC patients.

Methods: Hypermethylation of the *MGRN1* promoter region in the cancer tissues of platinum-resistant EOC patients was observed by genome-wide methylation level analysis. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry was used to analyze the methylation level of the *MGRN1* promoter region. *MGRN1* mRNA and protein expression were examined using RT-qPCR and IHC assays, respectively. The effect of *MGRN1* methylation on the cellular response to cisplatin was detected by knockdown assays in SKOV3 cells. Additionally, we performed transcriptome analysis using RNA-seq and explored the possible mechanism by which *MGRN1* expression affects the resistance of ovarian cancer cells to platinum.

Results: The RRBS assay showed that the upstream region of *MGRN1* from -1148 to -1064 was significantly hypermethylated in chemoresistant EOC patients ($P=1.78\times 10^{-7}$). The MALDI-TOF mass spectrometry assays revealed a strong association between hypermethylation of the *MGRN1* upstream region and platinum resistance in patients with EOC. Spearman's correlation analysis revealed a significantly negative connection between the methylation level of *MGRN1* and its expression in EOC. In vitro analysis demonstrated that knockdown of *MGRN1* reduced the sensitivity of cells to cisplatin and that expression of *EGR1* was significantly decreased in SKOV3 cells with low levels of *MGRN1* expression. Similarly, *EGR1* mRNA expression was lower in platinum-resistant EOC patients and was positively correlated with *MGRN1* mRNA expression.

Conclusion: The hypermethylation of the *MGRN1* promoter region and low expression of *MGRN1* were associated with platinum resistance in EOC patients.

Introduction

Due to the lack of initial symptoms and sensitive screening methods, approximately 70% of women with epithelial ovarian cancer (EOC) are diagnosed at an advanced stage of disease [1, 2]; EOC is the most lethal gynecologic malignancy in China [3, 4]. Currently, the treatment strategy for patients with advanced EOC is platinum-based chemotherapy following primary debulking surgery [5–7]. Although the majority of EOC patients respond well to first-line chemotherapy, most of these patients relapse and develop platinum resistance within 2 years [7, 8]. In addition, nearly 20% of patients do not respond at the beginning of chemotherapy [9–11]. Therefore, chemotherapy resistance has become an important cause of high mortality among EOC patients.

Resistance to chemotherapeutics, including intrinsic and acquired resistance, is based on highly complex and individually variable biological mechanisms [12]. Abnormal methylation of DNA has been considered to play an important role during the development of acquired chemoresistance in EOC patients [13].

However, there are currently very few studies about the effect of DNA methylation on the development of intrinsic resistance in EOC patients. We observed abnormal hypermethylation in the promoter region of the Mahogunin Ring Finger 1 (*MGRN1*) gene in the ovarian cancer tissues of EOC patients with intrinsic resistance by reduced representation bisulfite sequencing (RRBS) (Tian et al. 2020). *MGRN1* is an intracellular C3HC4 RING finger domain protein that exhibits E3 ubiquitin ligase activity and plays critical roles in the control of protein degradation [14]. Ubiquitin-mediated proteolysis has played a crucial role in controlling protein level homeostasis and regulating the cell cycle, cell proliferation, apoptosis and DNA damage responses, which are involved in tumorigenesis, tumor development, prognosis and drug resistance [15, 16]. However, the role of *MGRN1* in tumorigenesis, tumor progression, and drug responses is not currently well understood. A study by Dugué et al suggests that hypomethylation of *MGRN1* CpG sites in peripheral blood DNA is associated with the development of sporadic and familial breast cancer [17].

Based on the results of RRBS, this study investigated the role of hypermethylation of the *MGRN1* upstream region in platinum resistance in EOC patients. First, we examined the level of *MGRN1* methylation and expression in platinum-resistant and platinum-sensitive EOC patients. Furthermore, we also investigated the possible role and mechanism of decreased *MGRN1* expression in ovarian cancer cells in the response to cisplatin in vitro. To the best of our knowledge, this is the first study to investigate the role of the methylation status of the *MGRN1* promoter region in platinum resistance in EOC patients.

Materials And Methods

1.1 Tissue Samples

A total of 96 epithelial ovarian cancer tissues were obtained from the Department of Gynecology at the Fourth Hospital, Hebei Medical University, China (November 2011–June 2015). The informed consent of each subject was obtained, and this study was approved by the Medical Ethics Committee of the Fourth Affiliated Hospital of Hebei Medical University. The detailed clinicopathological features of the patients are summarized in Table 1. The inclusion criterion for cases was histologically confirmed primary EOC. The exclusion criteria was a history of chemotherapy therapy before surgery. According to the NCCN guidelines, recurrent disease was identified clinically (i.e., pelvic pain and weight loss), biochemically (i.e., elevated CA-125 levels), and/or with imaging [18]. Based on the platinum-free interval (PFI), which was calculated from the date of the last platinum compound treatment to the date of disease progression, all the study participants were divided into a platinum-sensitive group (n=92) and a platinum-resistant group (n=62). PFI of less than 6 months is widely used to clinically define platinum-resistant disease, whereas a PFI greater than 6 months is often used to define platinum-sensitive disease [19]. The participants were regularly followed-up for 5 years.

Table 1

Clinical characteristics of 96 EOC patients

Characteristics	Stage	Patients(n)	Median	Percentage/Range
Age	<50 Years	34	58Years	35.4%
	≥50 Years	62	58Years	64.6%
Histology	Serous	60		62.5%
	Endometrioid	21		21.9%
	Mucinous	6		6.25%
	Clear cell	3		3.1%
	Mixed type	6		6.25%
FIGO stage	I-II	21		21.9%
	III-IV	75		78.1%
Grade	1	24		25.0%
	2	42		43.8%
	3	30		31.2%
tumor residual size	0	25		26.0%
	<1cm	48		50.0%
	>1cm	23		24.0%
Platinum-based	Cisplatin	26		27.1%
	Carboplatin	70		72.9%
Follow-up time		96	37.2months	2-60 months

1.2 Genomic DNA extraction and MALDI-TOF mass spectrometry

High-quality DNA was isolated from 40 EOC tissue samples using the Wizard Genomic DNA Purification Kit (Promega, Madison, Wisconsin), as described by the manufacturers. MALDI-TOF mass spectrometry (Sequenom, San Diego, California, U.S.), described by Breitling et al. [20], was used to detect the methylation level of the *MGRN1* promoter region. This experiment was conducted at CapitalBio Co., Ltd. (Beijing, China).

1.3 RNA extraction and quantitative real-time reverse transcriptase-PCR (RT-qPCR)

Total RNA was isolated from 96 epithelial ovarian cancer tissue samples using the TRIzol-chloroform extraction method (Generay Biotech Co., Ltd., Shanghai, China), as described by the manufacturers. The total cDNA was reverse-transcribed using the Revert Aid First-Strand cDNA Synthesis Kit (Thermo Scientific, USA). The specific primers for the target genes that were used in RT-qPCR were designed using Primer Premier 5.0 and produced by Sangon Biotech Co., Ltd. (Shanghai, China). GAPDH was used as the housekeeping gene. The primer sequences for PCR amplification were as follows: *MGRN1* forward, 5'-TACAAAGACGATG CCGACAG-3'; *MGRN1* reverse, 5'-GCCTGGCAGTAGATGGTGAT-3'; *GAPDH* forward, 5'-AATCCCATCACCATCTTCCA-3'; and *GAPDH* reverse, 5'-TGGACTCCACGACGTA C-3'. The reactions were run with the QuantiNova TMSYBR® Green PCR Kit (Qiagen, Hilden, Germany) in an Mx3005P instrument. The comparative quantification of each target gene was performed based on the cycle threshold (Ct) and normalized to GAPDH using the $2^{-\Delta Ct}$ method.

1.4 MGRN1 immunohistochemical (IHC) study of the clinical samples

Of the 96 epithelial ovarian cancer samples, 86 paraffin-embedded epithelial ovarian cancer tissue samples collected in the pathology department of the Fourth Hospital of Hebei Medical University were used for immunohistochemical (IHC) staining of MGRN1. MGRN1 immunostaining was performed using a primary antibody, namely rabbit antihuman MGRN1 (RNF156, 1:500 dilution; Proteintech, China). Briefly, 4- μ m thick sections were dewaxed in xylene and dehydrated through a graded series of ethanol. After blocking endogenous peroxidase and non-specific binding, the sections were incubated overnight at 4°C with primary antibody and then with biotinylated secondary antibody and streptavidin-peroxidase complex. After the sections were washed in PBS, they were incubated with DAB reagent and counterstained with haematoxylin. Negative control sections were incubated with PBS instead of primary antibody. The immunohistochemical staining was evaluated using a previously reported scoring method [21]. The immunoreactivity of MGRN1 was considered to be positive in tumor cells showing cytoplasmic staining without nuclear staining. The sections were independently examined by two pathologists, who were blinded to the clinicopathological information.

2.1 Cell culture

The human serous ovarian cancer SKOV3 cell line was purchased from the iCell Bioscience Inc. (Shanghai, China). The SKOV3 cell line was cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco; Thermo Fisher Scientific, Inc.) The medium was always supplemented with 10% (w/v) fetal bovine serum, 100 U penicillin, and 100 μ g/L streptomycin (Gibco; Thermo Fisher Scientific, Inc.). The cells were maintained in a 95% humidified and 5% CO₂ atmosphere at 37°C. All the experiments were performed in triplicate.

2.2 Stable cell lines

MGRN1 expression plasmids and lentiviral packaging reagents and shRNA were purchased from Genecopoeia Inc. (MD, USA). The designed three target sequences in the MGRN1 gene were 5'-GGAACTACTTTGCTTCGCAC-3' (shRNAa); 5'-GCGTGTTCAGTAGTCATC C-3' (shRNAb) and 5'-

GGCATTGAGAACAAGAACAAC-3' (shRNAc). The most effective construct, recombinant plasmid inserted with MGRN1 gene shRNA expression vector shRNAa was selected for the study. A random sequence of shRNA (shNC) was used as the negative control. SKOV3 cells were seeded in a six-well plate at a density of 4×10^5 cells/mL in a volume of 2mL/well. When the SKOV3 cells reached 70–80% confluence, they were transfected with shRNA. Transfection of the SKOV3 cell line was performed according to the manufacturer's protocol.

2.3 Detection of changes in MGRN1 via Western blotting

Proteins were isolated using RIPA lysis buffer. The total proteins were extracted, and a BCA protein assay kit (Thermo) was used to quantify protein concentration. Rabbit anti-human MGRN1 antibody (RNF156, Proteintech, China) and β -actin (ab8226, Abcam, Cambridge, UK) were used as the primary antibody. Anti-rabbit IgG was used as the secondary antibody (diluent ratio of 1:5000; Proteintech, China). The antigen-antibody reaction was visualized by detection with an odyssey assay (ECL, Millipore, Billerica, MA).

2.4 Cell viability assays

The cells were inoculated in 96-well microplates in medium containing 10% fetal bovine serum and penicillin/streptomycin. After overnight incubation, the cells were treated with cisplatin (Pfizer), returned to the incubator for 24 h, and then analyzed. Cell Counting Kit-8 (CCK-8) was used to measure cell activity. Ten microliters of CCK-8 was added to each well and incubated for 3 h (37°C; 5% carbon dioxide). Then, the absorbance was measured at 492 nm with a microplate reader. Each experiment was repeated three times.

2.5 apoptosis assays

The cells were collected after 24h of cisplatin treatment. The Annexin V Apoptosis Detection kit I (BD Biosciences, Franklin Lakes, NJ, USA) was used to analyze the apoptosis of the SKOV3 cells. Briefly, cells were seeded into 6-well plates. After treatment with drugs, the adherent cells were trypsinized without EDTA and collected by centrifugation. After washing with PBS two times, the cells were resuspended in 100 μ L of 1 \times binding buffer and were subsequently incubated with 5 μ L of Annexin V staining solution at room temperature for 30min in the dark. Then, 400 μ L of 1 \times binding buffer was added, and the fluorescence intensity was evaluated on a FACS Aria™ (BD Biosciences) flow cytometer. Each assay was performed in triplicate.

2.6 RNA sequencing

This experiment was conducted at Differential Gene Technology Co., Ltd. (Anhui, China). EdgeR was used to identify the top ten enriched annotation terms among the differentially expressed genes (1.5-fold in either direction, $P < 0.05$) between the SKOV3 sh-NC group and the SKOV3 sh-MGRN1 group.

3. Statistical analysis

The statistical analyses were performed using SPSS 21.0 (Chicago, IL, USA). The Wilcoxon rank sum test was used to compare the methylation level and mRNA expression of *MGRN1* between the two groups. The χ^2 test was used to compare the protein expression of *MGRN1* in each group. Spearman correlation analysis was performed to analyze the correlation between *MGRN1* expression and methylation status. A t test was used to analyze the cell activity and apoptosis data.

Results

***MGRN1* promoter methylation levels in the platinum-resistant group and platinum-sensitive group**

In our previous study, we used the RRBS assay to compare the differences in the genome-wide methylation patterns between 8 platinum-resistant EOC patients and 8 platinum-sensitive EOC patients. The results showed that a region from -1148 to -1064 within the promoter of *MGRN1* was significantly hypermethylated in the platinum-resistant group compared to the platinum-sensitive group (Fig. 1A). To further confirm the results of the RRBS assay, MALDI-TOF mass spectrometry was used to examine the methylation levels of this region in 18 platinum-resistant EOC patients and 22 platinum-sensitive EOC patients. In the present study, we tested the methylation levels of five CpG sites (-1148, -1118, -1107, -1097 and -1064 from the transcription start site) within this region. The analysis revealed that methylation levels of two CpG sites (-1107 and -1097) were significantly higher in the tumor tissues of platinum-resistant EOC patients than in those of platinum-sensitive EOC patients ($P=0.01, 0.04$, Fig. 1B).

***MGRN1* expression in the platinum-resistant group and platinum-sensitive group**

RT-qPCR was used to determine the mRNA levels of *MGRN1* in the tumor tissues from 41 platinum-resistant EOC patients and 55 platinum-sensitive EOC patients. The results showed that the mRNA level of *MGRN1* in platinum-resistant EOC patients was 1.20-fold lower than that in platinum-sensitive EOC patients ($P<0.01$, Fig. 1C). Furthermore, IHC analysis was conducted to examine the protein expression of *MGRN1* in 36 platinum-resistant EOC patients and 50 platinum-sensitive EOC patients. The analysis results showed that the frequency of positive *MGRN1* expression in platinum-resistant EOC patients was significantly lower than that in platinum-sensitive EOC patients ($P=0.02$, Table 2). IHC staining showed that the *MGRN1* protein was mainly expressed in the cytoplasm of EOC tissues (Fig. 1D).

Table 2
Comparison of *MGRN1* protein expression between platinum-resistant tissues and platinum-sensitive tissues

MGRN1 expression	Resistant group	Sensitive group	P
	n (%)	n (%)	
High	15(60.0)	23(85.2)	0.02
Low	10(40.0)	4(14.8)	

Association between *MGRN1* mRNA expression and its methylation levels in EOC

Spearman's correlation analysis showed that *MGRN1* mRNA expression was significantly negatively correlated with the methylation level of the *MGRN1* promoter region (average of -1107/-1097 CpGs: $r = -0.511$, $P = 0.01$). The results indicated that the hypermethylation of the *MGRN1* promoter may be responsible for the downregulation of *MGRN1* mRNA expression in EOC tissues.

Silencing of *MGRN1* expression in serous ovarian cancer cells by shRNA

To investigate the role of *MGRN1* expression in the sensitivity of ovarian cancer cells to cisplatin, SKOV3 cells were transfected with shRNAa-*MGRN1*, shRNAb-*MGRN1*, shRNAc-*MGRN1* plasmid or shNC plasmid, respectively. After transfection for 48 hours, the expression of *MGRN1* was confirmed by RT-qPCR. As shown in Fig. 2A, shRNAa-*MGRN1* could effectively decrease *MGRN1* expression in SKOV3 cells, as compared with shNC groups. We also confirmed the expression of *MGRN1* in shRNAa-*MGRN1* group by Western blot. Therefore, we established *MGRN1* stable knockdown cell lines using shRNAa-*MGRN1* ($P < 0.05$, Fig. 2A, B).

Effect of *MGRN1* knockdown on the cellular response to cisplatin

CCK-8 assays were used to compare the cell proliferation of the shRNA-*MGRN1* group and shNC group. The proliferation rate was significantly higher in the shRNA-*MGRN1* group than in the shNC group after treatment with cisplatin at several concentrations for 24 h ($P < 0.05$, Fig. 2C). In addition, flow cytometry analysis demonstrated that the percentage of apoptotic cells in the shRNA-*MGRN1* group was significantly lower than that in the shNC group after exposure to 10 μ M cisplatin ($P = 0.03$, Fig. 2D).

RNA-seq analysis reveals that *EGR1* expression is differentially regulated by *MGRN1* in ovarian cancer cells

To gain a better understanding of the differential regulation of transcription between the shNC transfection group and shRNA-*MGRN1* transfection group, we performed an RNA-seq analysis of the total RNA harvested from the shNC group and shRNA-*MGRN1* group. The top ten annotated, protein-coding genes that were differentially regulated in the shNC group compared to the shRNA-*MGRN1* group are shown in Table 3 ($P < 0.05$). Of these genes, *EGR1* was the most differentially expressed, with 4.26-fold lower expression in the shRNA-*MGRN1* group than in the shNC group ($P = 8.65E^{-07}$). *EGR1* is a key gene involved in regulating cell proliferation and apoptosis in a variety of cancer tissues, and knockdown of *EGR1* has been shown to promote resistance to cisplatin. Thus, we further validated the mRNA levels of *EGR1* in cells and tissues by quantitative reverse-transcription PCR (RT-qPCR). The results showed that *EGR1* mRNA expression was reduced by 72% in the SKOV3 shRNA-*MGRN1* group compared with the SKOV3 shNC group ($P < 0.01$, Fig. 3A). The mRNA level of *EGR1* in 41 platinum-resistant EOC patients was 1.15-fold lower than that in 55 platinum-sensitive EOC patients ($P = 0.02$, Fig. 3B). Spearman's correlation analysis showed that *MGRN1* mRNA expression was significantly positively correlated with *EGR1* mRNA expression ($r = 0.379$, $P = 0.01$).

Table 3
Genes differentially expressed between shRNA-*MGRN1* group and shNC group.

Gene symbol	Description	Fold change	p
FOSB	FosB Proto-Oncogene, AP-1 Transcription Factor Subunit	-6.51	5.00E-08
EGR1	Early Growth Response 1	-4.26	8.65E-07
NGFR	Nerve Growth Factor Receptor	-5.53	0.000002
ADM	Adrenomedullin	-3.25	0.00009
KRT5	Keratin 5	-4.44	0.00010
HK2	Hexokinase 2	-3.23	0.00011
CES1P2	Carboxylesterase 1 Pseudogene 2	8.9	0.00015
DEPP1	DEPP1 Autophagy Regulator	-4.26	0.00024
STC1	Stanniocalcin 1	-3.6	0.00026
EDIL3	EGF Like Repeats And Discoidin Domains 3	-4.71	0.00033

Discussion

In this study, we confirmed that *MGRN1* gene promoter hypermethylation is associated with platinum resistance in patients with EOC based on the following findings: 1) the upstream region of *MGRN1* was significantly hypermethylated and lower expression in the cancer tissues of platinum-resistant patients with EOC, 2) *MGRN1* mRNA expression was significantly negatively correlated with the methylation level of the *MGRN1* promoter region, 3) knockdown of *MGRN1* expression could desensitize SKOV3 ovarian cancer cells to cisplatin, 4) knockdown of *MGRN1* expression in SKOV3 cells could significantly reduce *EGR1* mRNA expression, which significantly correlated with the resistance in platinum-treated cancer patients.

In the analysis of genome-wide methylation levels in tissue samples from 8 platinum-resistant and 8 platinum-sensitive EOC patients, we found that the methylation level of the *MGRN1* upstream region (-1148 to -1064) was significantly higher in the platinum-resistant group. Mass spectrometry analysis of an expanded EOC sample size showed that hypermethylation of the *MGRN1* promoter region was associated with platinum resistance in EOC patients. We also discovered that the expression levels of *MGRN1* mRNA and protein in platinum-resistant EOC patients were significantly lower than those in platinum-sensitive EOC patients. Correlation analysis indicated that the methylation level of the *MGRN1* promoter region was associated with *MGRN1* mRNA expression. Furthermore, knockdown of *MGRN1* expression could increase proliferation and decrease apoptosis in SKOV3 cells challenged with cisplatin. These findings suggested that lower *MGRN1* expression due to hypermethylation of its promoter region might induce platinum resistance in EOC.

MGRN1, an E3 ubiquitin ligase of the Really Interesting New Gene (RING) finger family, is involved in many biological and cellular mechanisms [22]. However, there is no study of the role of *MGRN1* in chemotherapy resistance in cancer patients to date. In the current study, microarray analysis of total RNA showed that knockdown of *MGRN1* expression in SKOV3 cells resulted in significant downregulation of multiple genes, including early growth response protein 1 (*EGR1*). *EGR1* is a transcription factor that can be induced by a variety of stimuli or stressors, including growth factors, hormones, ionizing radiation, and chemotherapy drugs [23–25], and plays essential roles in cell proliferation and apoptosis [26–28]. Knockdown of *EGR1* expression can decrease cisplatin-induced apoptosis in a variety of cancer cells [29–31], while Kim overexpression of this gene sensitizes ovarian cancer cells to cisplatin-induced apoptosis [30]. He et al found that *EGR1* expression levels were significantly higher in ovarian cancer tissues with low *ERCC1* expression than in ovarian cancer tissues with high *ERCC1* expression, suggesting that *EGR1* expression is positively correlated with potential cisplatin-sensitive ovarian cancer, since *ERCC1* is widely accepted as a biomarker of platinum resistance [30]. In our study, it was also observed that the expression of *EGR1* in EOC patients with platinum resistance was significantly downregulated and was positively correlated with the expression of *MGRN1*. Therefore, we speculate that *MGRN1* may affect the platinum resistance of ovarian cancer by regulating the expression of *EGR1*. Of course, the molecular mechanism by which *MGRN1* regulates *EGR1* requires further study.

In summary, our study demonstrated that the hypermethylation of *MGRN1* may be predictive of platinum resistance in EOC patients. Considering that DNA methylation may be used as a molecular marker for ovarian cancer chemotherapy, we believe that our findings warrant confirmation in a larger patient cohort and could facilitate patient selection for chemotherapy.

Declarations

Acknowledgments

We greatly acknowledge several doctors in the Department of Obstetrics and Gynecology, the Fourth Affiliated Hospital of Hebei Medical University, China, for their assistance in recruiting the study subjects.

Author contributions

Yan Li, Shan Kang, and Xiao-fei Li designed the study and carried out the experiments. Xiao-fei Li, Hai-yan Sun, and Tian Hua recruited the patients and collected the data. Xiao-fei Li, Hai-bo Zhang and Yun-jie Tian analysed the data and prepared draft figures and tables. All authors were involved in writing the paper and provided final approval of the submitted and published versions.

Funding

Not applicable.

Availability of data and materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The present study was approved by the Medical Ethics Committee of the Fourth Affiliated Hospital of Hebei Medical University. All patients and healthy volunteers provided written informed consent prior to their inclusion within the study.

Consent for publication

Not Applicable.

Conflict of interest

All authors declare that they have no conflict of interest.

References

1. Sato S, Itamochi H. Neoadjuvant chemotherapy in advanced ovarian cancer: latest results and place in therapy. *Therapeutic Advances in Medical Oncology*. 2014.
2. Buono NL, Morone S, Parrotta R, et al. Ectoenzymes in epithelial ovarian carcinoma: potential diagnostic markers and therapeutic targets: InTech; 2012.
3. Jia J, Wang Z, Cai J, et al. PMS2 expression in epithelial ovarian cancer is posttranslationally regulated by Akt and essential for platinum-induced apoptosis. *Tumor Biology*. 2016; 37(3):1-11.
4. Chen J, Wang L, Matyunina LV, et al. Overexpression of miR-429 induces mesenchymal-to-epithelial transition (MET) in metastatic ovarian cancer cells. *Gynecologic Oncology*. 2011.
5. Borley J, Wilhelm-Benartzi C, Brown R, et al. Does tumour biology determine surgical success in the treatment of epithelial ovarian cancer? A systematic literature review. *British Journal of Cancer*. 2012; 107(7):1069-74.
6. Leary A, Cowan R, Chi D, et al. Primary Surgery or Neoadjuvant Chemotherapy in Advanced Ovarian Cancer: The Debate Continues.... *Am Soc Clin Oncol Educ Book*. 2016; 35:153-62.
7. Mueller JJ, Zhou QC, Iasonos A, et al. Neoadjuvant chemotherapy and primary debulking surgery utilization for advanced-stage ovarian cancer at a comprehensive cancer center. *Gynecologic Oncology*. 2016.
8. Rochet N, Kieser M, Sterzing F, et al. Phase II study evaluating consolidation whole abdominal intensity-modulated radiotherapy (IMRT) in patients with advanced ovarian cancer stage FIGO III - The OVAR-IMRT-02 Study. *BMC Cancer*, 11, 1(2011-01-28). 2011; 11(1):1-7.
9. Mantia-Smaldone GM, Edwards RP, Vlad AM. Targeted treatment of recurrent platinum-resistant ovarian cancer: current and emerging therapies. *Cancer Management & Research*. 2011; 3(default):25-38.

10. Siddiqui GK, Maclean AB, Elmasry K, et al. Immunohistochemical expression of VEGF predicts response to platinum based chemotherapy in patients with epithelial ovarian cancer. *Angiogenesis*. 2011; 14(2):155-61.
11. Colombo PE, Manoir SD, Orsett B, et al. Ovarian carcinoma patient derived xenografts reproduce their tumor of origin and preserve an oligoclonal structure. *Oncotarget*. 2015; 6(29).
12. Yongfei, Hu, Yuying, et al. Genomic insights into intrinsic and acquired drug resistance mechanisms in *Achromobacter xylosoxidans*. *Antimicrobial Agents & Chemotherapy*. 2015.
13. Wilting RH, Dannenberg JH. Epigenetic mechanisms in tumorigenesis, tumor cell heterogeneity and drug resistance. *Drug Resistance Updates*. 2012; 15(1-2):21-38.
14. Phan LK, Feng L, Leduc CA, et al. The mouse mahoganoid coat color mutation disrupts a novel C3HC4 RING domain protein. *Journal of Clinical Investigation*. 2002; 110(10):1449-59.
15. O'Brien P M, Davies M J, Scurry J P, et al. The E3 ubiquitin ligase EDD is an adverse prognostic factor for serous epithelial ovarian cancer and modulates cisplatin resistance in vitro [J]. *British Journal of Cancer*, 2008, 98(6):1085. The E3 ubiquitin ligase EDD is an adverse prognostic factor for serous epithelial ovarian cancer and modulates cisplatin resistance in vitro. *British Journal of Cancer*. 2008; 98(6):1085-93.
16. Yang L, Chen J, Huang X, et al. Novel insights into E3 ubiquitin ligase in cancer chemoresistance. *American Journal of the Medical Sciences*. 2017:S0002962917306900.
17. Dugué P-A, Milne RL, Southey MC. A prospective study of peripheral blood DNA methylation at RPTOR, MGRN1 and RAPSN and risk of breast cancer. *Breast Cancer Research & Treatment*. 2017; 161(1):181-3.
18. National Comprehensive Cancer Network. (NCCN) Clinical Practice Guidelines in Oncology. Ovarian Cancer, Version 1. 2019.
19. Bookman MA, Tyczynski JE, Espirito JL, et al. Impact of primary platinum-free interval and BRCA1/2 mutation status on treatment and survival in patients with recurrent ovarian cancer. *Gynecologic Oncology*. 2017.
20. Breitling LP, Yang R, Korn B, et al. Tobacco-smoking related differential DNA methylation: 27K discovery and replication. *Am J Hum Genet*. 2011; 88: 450-7.
21. Liu YB, Mei Y, Tian ZW, et al. Downregulation of RIF1 Enhances Sensitivity to Platinum-Based Chemotherapy in Epithelial Ovarian Cancer (EOC) by Regulating Nucleotide Excision Repair (NER) Pathway. *Cellular Physiology & Biochemistry*. 2018:1971-84.
22. Upadhyay A, Amanullah A, Chhangani D, et al. Mahogunin Ring Finger-1 (MGRN1), a Multifaceted Ubiquitin Ligase: Recent Unraveling of Neurobiological Mechanisms. *Molecular Neurobiology*. 2016; 53(7):4484-96.
23. Gregg J, Fraizer G. Transcriptional Regulation of EGR1 by EGF and the ERK Signaling Pathway in Prostate Cancer Cells. *Genes & Cancer*. 2011; 2(9):900-9.
24. Kim HR, Kim YS, Yoon JA, et al. Egr1 is rapidly and transiently induced by estrogen and bisphenol A via activation of nuclear estrogen receptor-dependent ERK1/2 pathway in the uterus. *Reproductive*

Toxicology. 2014; 50:60-7.

25. Ko H, Kim JM, Kim S, et al. Induction of apoptosis by genipin inhibits cell proliferation in AGS human gastric cancer cells via Egr1/p21 signaling pathway. *Bioorganic & Medicinal Chemistry Letters*. 2015.
26. Gabriella MA, Giulia P, Daniela T, et al. Coordinated sumoylation and ubiquitination modulate EGF induced EGR1 expression and stability. *Plos One*. 2011; 6(10):e25676.
27. Ning ZC, Shi JD, Zhou Y, et al. EGR1 Promotes Proliferation in Prostatic Hyperplasia Epithelial BPH-1 Cells. *Chinese Journal of Biochemistry & Molecular Biology*. 2013.
28. Wang L, Sun H, Wang X, et al. EGR1 mediates miR-203a suppress the hepatocellular carcinoma cells progression by targeting HOXD3 through EGFR signaling pathway. *Oncotarget*. 2016.
29. Zhao. GROβ and its downstream effector EGR1 regulate cisplatin-induced apoptosis in WHCO1 cells. *Oncology Reports*. 2011; 25(4).
30. He J, Yu JJ, Xu Q, et al. Downregulation of ATG14 by EGR1-MIR152 sensitizes ovarian cancer cells to cisplatin-induced apoptosis by inhibiting cyto-protective autophagy. *Autophagy*. 2015; 11(2):373-84.
31. Parra, Eduardo. Increased expression of p21Waf1/Cip1 and JNK with costimulation of prostate cancer cell activation by a siRNA Egr-1 inhibitor. *Oncology Reports*. 2013; 30(2).

Figures

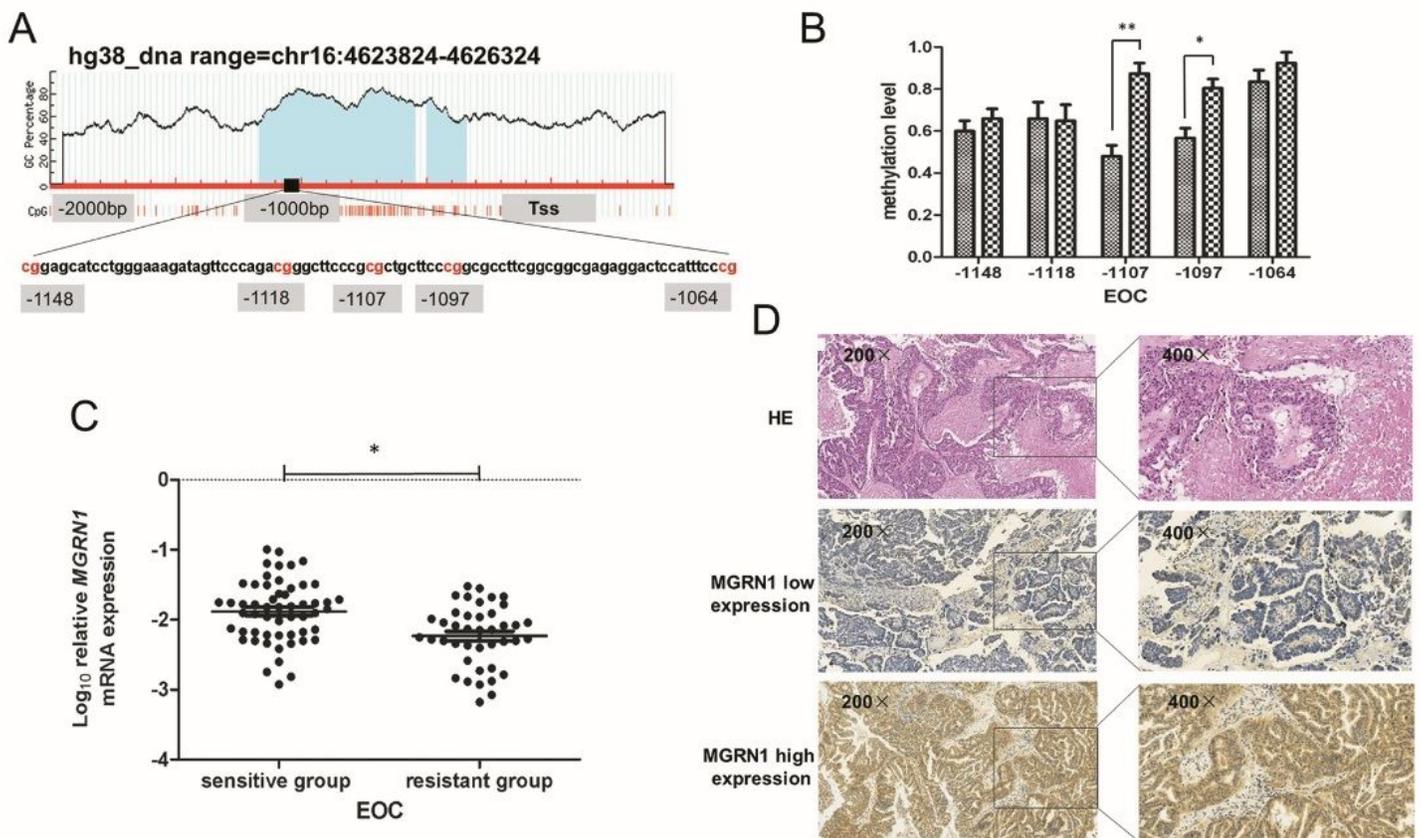


Figure 1

The methylation level of MGRN1 promoter was associated with platinum-resistant in EOC patients. (A) Reduced representation bisulfite sequencing (RRBS) assay showed the region (-1148 to -1064 upstream) within the promoter of MGRN1 was hypermethylated in platinum-resistant EOC tissues compared with platinum-sensitive tissues ($P=1.78\times 10^{-7}$). (B) The methylation of -1107 and -1097 CpG site were significantly hypermethylated in platinum-resistant EOC tissues compared with platinum-sensitive EOC tissues by MALDI-TOF Sequenom MassARRAY. (C) The mRNA expression of MGRN1 was lower in platinum-resistant EOC patients than platinum-sensitive EOC patients. (D) MGRN1 protein expression in EOC tissues. * $P < 0.05$; ** $P < 0.01$

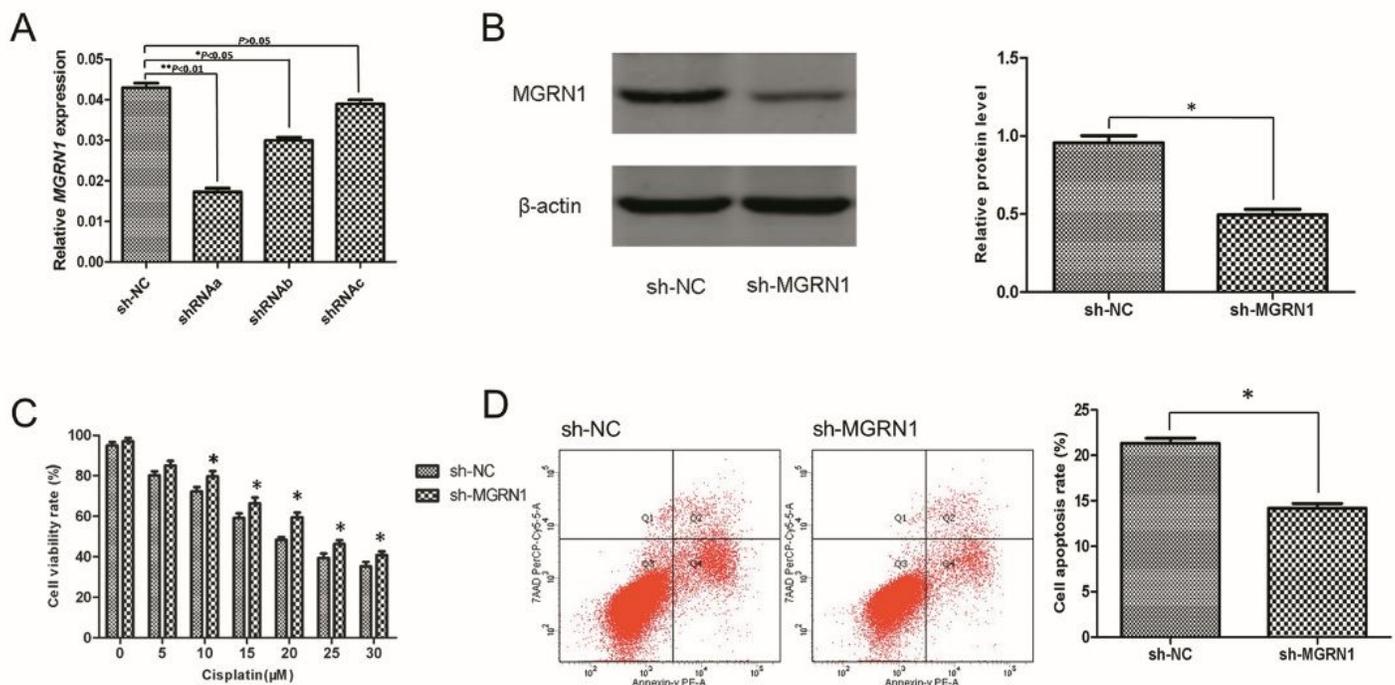


Figure 2

The alteration of the sensitivity to cisplatin after a knockdown of MGRN1 expression in SKOV3 cells. (A) RT-qPCR assay showed the reduced expression of MGRN1 in shRNAa-MGRN1, shRNAb-MGRN1 and shRNAc-MGRN1 cells compared to shNC cells. (B) Western blot assay showed the reduced expression of MGRN1 in shRNAa-MGRN1 cells compared to shNC cells. (C) CCK-8 assays showed a significant increase in the proliferation rates in the shRNA-MGRN1 cells compared with the shNC cells after cisplatin treatment at several concentrations for 24h. (D) Flow cytometry showed that the apoptosis rate in the shRNA-MGRN1 cells was significantly lower than that in the shNC cells after exposure to cisplatin at the 10 μ M concentration for 24h. * $P < 0.05$; ** $P < 0.01$. All the experiments were performed in triplicate.

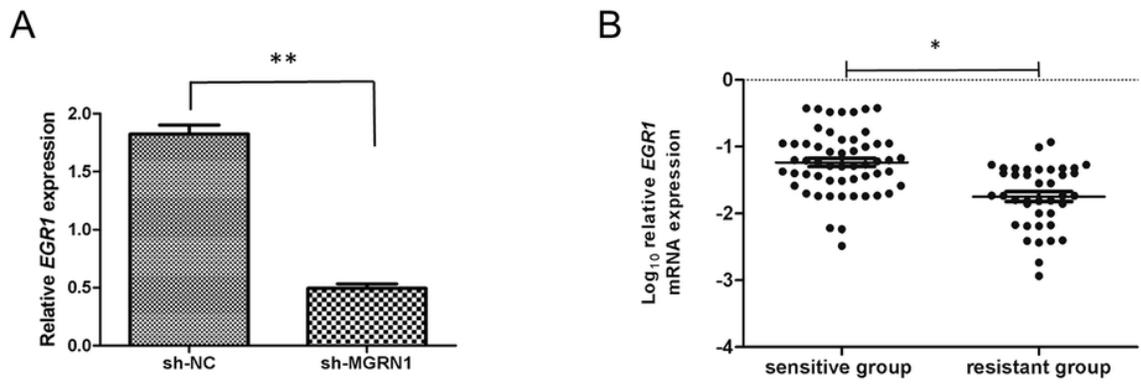


Figure 3

The low mRNA expression of EGR1 was associated with platinum-resistant in shRNA-MGRN1 cells and EOC patients. (A) RT-qPCR showed the reduced expression of EGR1 in sh-MGRN1 cells compared to shNC cells. (B) The mRNA expression of EGR1 in platinum-resistant EOC patients and platinum-sensitive EOC patients. **P < 0.01.