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MBNL2 enhances cisplatin resistance by regulating apoptosis in ovarian cancer cells

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Although cisplatin is an effective anticancer agent for treating ovarian cancer, it encounters significant resistance. A full understanding of the mechanisms behind cisplatin resistance has not been achieved. This study identifies MBNL2 as a crucial regulator of cellular responses to cisplatin, examining variations in gene expression and methylation profiles between cisplatin-sensitive and -resistant ovarian cancer cells. Cells resistant to cisplatin exhibited increased *MBNL2* mRNA expression and significant demethylation at promoter CpG sites. Treating ovarian cancer cell lines with a DNA demethylating agent significantly raised *MBNL2* mRNA expression, indicating that epigenetic mechanisms involving DNA methylation control *MBNL2* expression. Modulating MBNL2 levels altered the response to cisplatin through survival pathways that shield cells from cisplatin-induced apoptosis. Overexpressing MBNL2 enhanced resistance, while its depletion heightened cisplatin sensitivity. Furthermore, *MBNL2* mRNA levels differed among patients based on their response to platinum-based chemotherapeutics. Patients resistant to these drugs had higher *MBNL2* mRNA levels, effectively distinguishing them from those who were sensitive (AUC = 0.89, P = 0.0308). A meta-analysis of seventeen datasets confirmed that lower *MBNL2* expression levels are associated with a better chemotherapy response and longer relapse-free survival. Conversely, higher *MBNL2* expression levels correlated with increased recurrence rates and reduced survival. Thus, MBNL2 may serve as a promising prognostic and therapeutic target for overcoming cisplatin resistance. [BMB Reports 2025; 58(5): 224-231]

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INTRODUCTION

Ovarian cancer poses a significant health risk to women with a high mortality rate. Its main cause is the development of resistance to chemotherapy. The standard treatment for ovarian cancer involves a platinum-based combination chemotherapy regimen, typically consisting of cisplatin or carboplatin combined with paclitaxel. However, the effectiveness of this treatment strategy is often compromised by the development of resistance to these chemotherapeutic agents (1, 2). Despite extensive research, the full understanding of the mechanisms underlying cisplatin resistance remains unclear. Yet, it is known that these mechanisms involve a complex interplay of various molecular and cellular processes. A major factor contributing to resistance is the change in drug accumulation within cancer cells, due to either increased active efflux or reduced influx, resulting in diminished cytotoxic effects. Additionally, the activation of detoxification pathways, such as those involving glutathione and metallothioneins, can neutralize the drug before it can deliver its therapeutic effect. The increased DNA repair capacity of tumor cells is a key mechanism through which they circumvent cisplatin-induced cell death, as it enables rapid repair of drug-induced DNA lesions. Moreover, changes in the function and expression of various transporters can affect the intracellular distribution of cisplatin and its interaction with DNA, thus contributing to resistance development. Furthermore, the deregulation of apoptosis, the process of programmed cell death, is a notable factor in this resistance. Alterations in the expression and activation of genes that govern apoptosis can support the survival of cancer cells under cisplatin exposure (3-5). Recent observations indicate that abnormal DNA methylation at cytosines followed by guanine (CpG) residues in promoter regions occurs in cells resistant to cisplatin. These methylation changes, crucial for the efficacy of anticancer treatments, are associated with the development of resistance to these drugs, presenting a significant challenge in cancer therapy (6, 7).

The Muscleblind Like Splicing Regulator 2 (MBNL2), a member of the Muscleblind-like protein (MBNL) family, is implicated in the regulation of mRNA metabolism in mammals. This protein plays a crucial role in the pathogenesis of myotonic dystrophy

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type 1 by binding to aberrant RNA repeats, which alters splicing and results in symptoms like muscle wasting and impaired heart function (8, 9). In addition, MBNL2 has been identified as possibly contributing to cancer tumorigenesis and progression. It modulates the cellular response to DNA damage by stabilizing CDKN1A/p21 expression, independent of the p53 pathway, and is upregulated under hypoxic conditions to aid cancer cell adaptation by regulating hypoxia-associated genes. Its suppression enhances DNA damage-induced apoptosis, underscoring its potential in cellular survival mechanisms (10). Conversely, MBNL2 acts as a tumor suppressor in hepatocellular carcinoma, where its overexpression inhibits proliferation, migration, and invasive potential of liver cancer cells, while downregulation has the opposite effect (11).

In a previous study, we conducted genome-wide transcriptome and DNA methylome profiling to explore changes in gene expression and DNA methylation in ovarian cancer cell lines resistant to cisplatin compared to sensitive ones. This analysis pinpointed MBNL2 as a key gene linked to cisplatin resistance. The current study focuses on the cellular functions of MBNL2 and its role in developing cisplatin resistance *in vitro*. Additionally, it utilizes a meta-analysis of seventeen publicly available datasets to examine the correlation between *MBNL2* transcriptional expression and both the response to chemotherapy and prognosis in patients with serous ovarian cancer.

RESULTS

Increased transcriptional expression of *MBNL2* in cisplatin-resistant cell lines

A previous study conducted by our research team analyzed the cytotoxic effects of cisplatin on 11 human ovarian cancer cell lines, categorizing these effects into three sensitivity levels: sensitive, intermediate, and resistant (6). The mRNA expression profiles of five cisplatin-sensitive cell lines and three resistant cell lines were analyzed using microarray technology (6). Employing moderated t-statistics with an empirical Bayesian technique (12), we identified differential gene expression between the two groups of cell lines. This differential expression was established based on a minimum 1.5-fold change in expression levels, after adjustment for multiple testing using the Benjamin-Hoecker-Lehmakoff procedure (BH FDR-adjusted $P < 0.05$) (13). *MBNL2* was identified as a gene with increased expression in ovarian cancer cell lines that exhibited resistance compared to those sensitive to cisplatin (Fig. 1A). These results were corroborated by RT-qPCR findings, which showed significant upregulation of *MBNL2* mRNA expression in all resistant cell lines (Fig. 1B).

Enhanced MBNL2 expression is associated with reduced promoter DNA methylation in cisplatin-resistant cells

In a previous study, we explored changes in DNA methylation between cell lines sensitive and resistant to cisplatin using genome-wide methylation profiling with the Illumina Human-

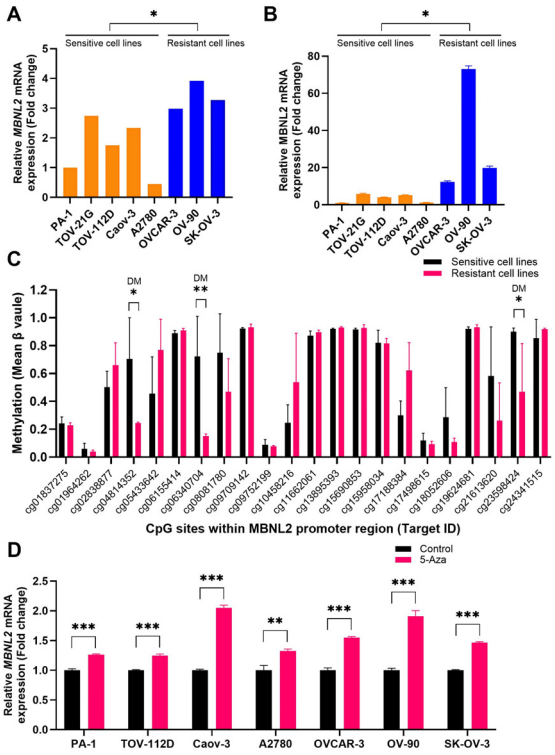

Fig. 1. Cisplatin-resistant cell lines exhibit increased *MBNL2* mRNA expression and hypomethylation at *MBNL2* promoter CpG sites. Quantifying *MBNL2* mRNA levels was conducted on five sensitive and three resistant ovarian cancer cell lines using gene expression microarray (A) and RT-qPCR (B). Triplicate measurements were used to calculate the standard deviation (SD) indicated by the error bars. A t-test assessed the statistical significance of the differences observed (*$P < 0.05$). (C) DNA methylation analysis of the *MBNL2* promoter region was performed with the Illumina HumanMethylation450 BeadChip, spanning 22 specific CpG sites located from 97,873,877 to 97,928,270 on chromosome 13 according to human GRCh37/hg19 assembly. Bayesian t-statistics were used for statistical analysis, presenting the data as mean ± SD (*$P < 0.05$, **$P < 0.01$). (D) *MBNL2* mRNA levels were measured using RT-qPCR after 5-aza-2′-deoxycytidine treatment. The *MBNL2* mRNA expression levels relative to dimethyl sulfoxide-treated controls are shown for both cisplatin-sensitive and -resistant cell lines. Data are presented as the mean value with the standard deviation from three separate experiments. A t-test was used for statistical comparisons (**$P < 0.01$, ***$P < 0.001$). 5-Aza, 5-aza-2′-deoxycytidine; DM, differentially methylated CpG site.

Methylation450 BeadChip (6). This analysis includes 22 CpG sites within the MBNL2 promoter region, located from 97,873,877 to 97,928,270 on chromosome 13 according to the human GRCh37/hg19 assembly. We identified three CpG sites in the MBNL2 promoter that exhibited differential methylation. All three sites showed significant hypomethylation in cell lines resistant to cisplatin compared to those sensitive to the drug, based on criteria including an absolute mean β-value difference > 3.5 and a statistical significance of $P < 0.05$ (Fig.

1C). We then investigated whether epigenetic mechanisms regulate *MBNL2* expression by using a DNA methyltransferase inhibitor. The DNA methyltransferase inactivator, 5-aza-2′-deoxycytidine (5-aza-dc), was tested in seven ovarian cancer cell lines, comprising four cisplatin-sensitive and three resistant lines. Subsequently, RT-qPCR was used to measure *MBNL2* mRNA expression after treatment. A significant increase in *MBNL2* mRNA expression was observed in all treated ovarian cancer cell lines, with fold changes ranging from 1.25 to 2.05 compared to untreated controls. This suggests that DNA methylation had epigenetically repressed *MBNL2* expression (Fig. 1D).

Enhanced cisplatin resistance due to MBNL2 overexpression

The aim of this study was to assess whether upregulating MBNL2 expression could enhance ovarian cancer cells' resistance to cisplatin. Transient transfection of MBNL2 expression plasmids or empty vector plasmids was carried out on SK-OV-3 cells. Subsequently, MBNL2 expression was quantified using RT-qPCR after a 24-hour transfection period. In MBNL2-transfected SK-OV-3 cells, *MBNL2* mRNA expression increased significantly, approximately 52-fold, compared to cells transfected with empty vectors, as demonstrated in Fig. 2A. The cytotoxicity of cisplatin in cells transfected with either empty vector or MBNL2 expression plasmid was evaluated using an MTT assay. The IC_{50} of cisplatin was found to increase significantly by about 85% in MBNL2-transfected SK-OV-3 cells compared to empty vector-transfected cells (mock) (Fig. 2C). Increased MBNL2 expression has been shown to contribute to enhanced cisplatin resistance in human ovarian cancer cells. MBNL2 knockdown was induced by siRNA to further explore the relationship between MBNL2 expression levels and ovarian cancer cells' response to cisplatin. A small interfering RNA (siRNA) targeting MBNL2 (siMBNL2) or a non-targeting control siRNA (siNC) was transiently transfected into cisplatin-resistant SK-OV-3 cells. The effectiveness of the MBNL2 knockdown was confirmed via RT-qPCR after a 24-hour transfection period. Following transfection with siMBNL2, *MBNL2* expression was significantly reduced by 74% in SK-OV-3 cells compared to siNC-transfected cells (Fig. 2B). An MTT assay was used to assess the response of cells transfected with siNC or siMBNL2 to cisplatin. In SK-OV-3 cells transfected with siMBNL2, suppression of MBNL2 expression significantly decreased the cisplatin IC_{50} levels by 28% compared to siNC-transfected control cells (Fig. 2D).

Previous studies have demonstrated that MBNL2 regulates the expression of *Cyclin Dependent Kinase Inhibitor 1A* (*CDKN1A*)/p21 by binding to its mRNA and enhancing its stability (10). CDKN1A/p21 acts as a crucial cell cycle inhibitor, pausing progression to facilitate DNA repair, thus averting apoptosis. Additionally, CDKN1A/p21 inhibits Cyclin-dependent kinase (CDK) activity, necessary for caspase activation, thereby safeguarding cells from stress-induced apoptosis (14, 15). To further investigate MBNL2's role in CDKN1A/p21 regulation, we analyzed the impacts of MBNL2 overexpression and knockdown on *CDKN1A*/p21 mRNA levels. As depicted in Fig. 2E, MBNL2

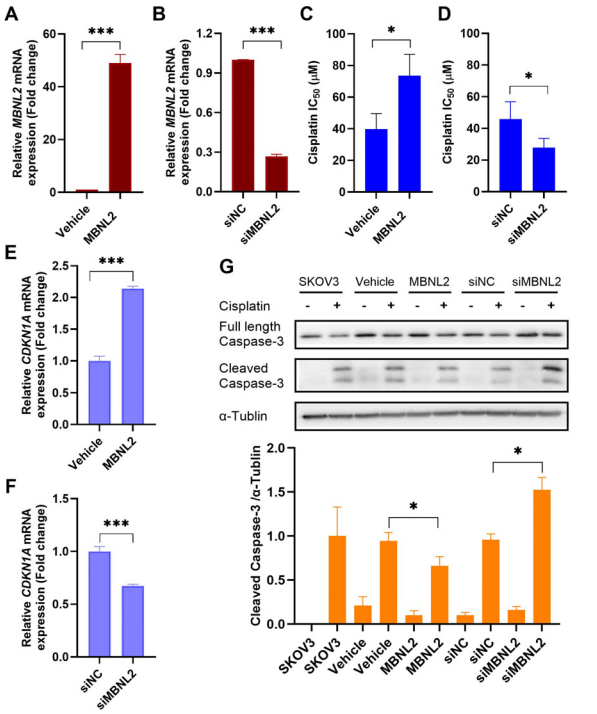

Fig. 2. MBNL2 expression confers tumor resistance to cisplatin by inhibiting apoptosis. SK-OV-3 cells were transiently transfected with either vehicle control and MBNL2 expression constructs or with non-targeting control siRNA (siNC) and siRNA targeting MBNL2 (siMBNL2). (A, B) After a 24-hour transfection period, *MBNL2* mRNA expression in the transfected SK-OV-3 cells was confirmed using RT-qPCR. (C, D) The cytotoxic effects of cisplatin on the transiently transfected SK-OV-3 cells were assessed using an MTT assay after a 48-hour treatment period. (E, F) Following a 24-hour transfection period, *CDKN1A* mRNA expression in the transfected SK-OV-3 cells was confirmed using RT-qPCR. (G) The culture medium was exchanged for fresh medium containing 100 μM cisplatin 24 hours post-transfection. Caspase-3 activation was analyzed via western blot in cells harvested after 24 hours of cisplatin exposure. Triplicate measurements were used to compute the mean ± standard deviation for all data, with statistical significance evaluated using a t-test (*P < 0.05, ***P < 0.001). siNC, non-targeting control siRNA; siMBNL2, siRNA targeting *MBNL2*; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; SD, standard deviation.

overexpression markedly increased *CDKN1A*/p21 mRNA levels by approximately 2.1-fold compared to the vehicle control group (Fig. 2E), supporting the hypothesis that MBNL2 amplifies *CDKN1A*/p21 expression through mRNA stabilization. Conversely, as illustrated in Fig. 2F, MBNL2 knockdown using siRNA (siMBNL2) led to a significant decrease in *CDKN1A*/p21 mRNA levels to about 33% of those in siNC-transfected control cells (Fig. 2F), confirming that MBNL2 positively regulates *CDKN1A*/p21 expression at the mRNA level.

To determine if MBNL2-mediated regulation of CDKN1A/p21 influences apoptosis, caspase 3 cleavage was analyzed in

SK-OV-3 cells with modified MBNL2 expression following cisplatin treatment (100 μM, 24 hours). Caspase 3 cleavage was significantly reduced in SK-OV-3 cells transfected with MBNL2 expression plasmids compared to those transfected with empty vectors (Fig. 2G). This finding is consistent with the increased IC_{50} for cisplatin seen in the MTT assay, indicating decreased apoptosis. Conversely, caspase 3 cleavage was increased in cells transfected with siMBNL2 compared to siNC-transfected control cells (Fig. 2G). Quantitative analysis of cleaved caspase 3 protein levels (normalized to α-tubulin) revealed that MBNL2 overexpression inhibits apoptosis, whereas MBNL2 knockdown promotes apoptosis in response to cisplatin treatment (Fig. 2G). Similarly, A2780 cells, which are sensitive to cisplatin, showed heightened *CDKN1A*/p21 expression and decreased caspase 3 cleavage activity under MBNL2-overexpressing conditions. However, the impact of MBNL2 knockdown on *CDKN1A*/p21 expression and caspase 3 cleavage activity was less marked, likely due to the inherently low endogenous expression of MBNL2 in A2780 cells (Supplementary Fig. 1).

Overall, these findings identify MBNL2 as a crucial regulator of cisplatin resistance in human ovarian cancer cells by stabilizing *CDKN1A*/p21 mRNA and modulating apoptotic pathways. This previously unrecognized role of MBNL2 in chemoresistance provides fresh insights into the molecular mechanisms of cisplatin resistance in ovarian cancer.

Enhanced transcriptional expression of *MBNL2* has been observed in ovarian cancer patients resistant to chemotherapy

Patients who relapsed within 12 months of their last platinum-containing chemotherapy regimen were considered resistant to cisplatin, while those whose relapse occurred beyond 12 months were considered sensitive to cisplatin (Table 1). The aim of this study was to determine if there were differences in the expression levels of *MBNL2* mRNA in primary ovarian cancer tissue between the two groups: those sensitive and those resistant to chemotherapy. The expression of *MBNL2* mRNA was statistically significant, exhibiting an approximately 2.35-fold increase in patients resistant to chemotherapy compared to those sensitive to the treatment (Fig. 3A). ROC analysis showed moderate effectiveness in distinguishing between patients resistant and sensitive to chemotherapy, with an AUC of 0.89 (Fig. 3B). A post hoc power analysis was conducted using G*power 3.1.9.2 software (http://www.gpower.hhu.de) to determine the power needed to detect a significant mean difference given the sample size, using a two-tailed test and a 5% significance level. This analysis revealed a 76% ability to detect a significant difference in *MBNL2* mRNA expression

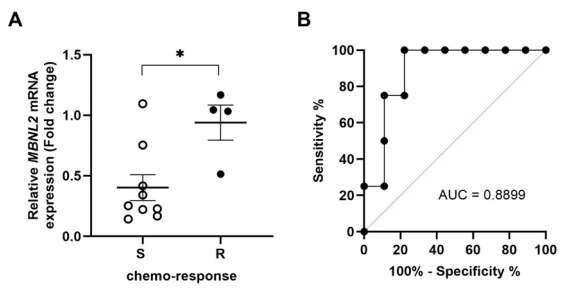

Fig. 3. *MBNL2* expression correlates with the cisplatin response. (A) *MBNL2* mRNA levels were quantified using RT-qPCR in ovarian cancer specimens from nine cisplatin-sensitive patients and four cisplatin-resistant patients. Data were subjected to a t-test and presented as mean ± standard error of the mean (SEM) (*$P < 0.05$). (B) A receiver operating characteristic (ROC) curve analysis was conducted to evaluate the relationship between *MBNL2* mRNA expression levels and cisplatin response in ovarian cancer patients. S, ovarian tumor tissues from cisplatin-sensitive patients; R, ovarian tumor tissues from cisplatin-resistant patients; AUC, area under the curve.

Table 1. Characteristics of patients diagnosed with ovarian cancer exhibiting sensitivity or resistance to first-line chemotherapy

Patient No.	Chemo-response	Histology	Stage[a]	Grade[b]
1	R	Endometrioid adenocarcinoma	IIIC	G3
2	R	Endometrioid adenocarcinoma	IVA	G2
3	S	Serous papillary carcinoma	IIIC	G3
4	S	Serous papillary carcinoma	IIIC	G3
5	R	Serous carcinoma	IIIC	G3
6	S	Serous papillary carcinoma	IV	G3
7	S	Serous papillary carcinoma	IVB	G3
8	S	Serous carcinoma	IIIC	G3
9	R	Serous carcinoma	IIIC	G3
10	S	Endometrioid adenocarcinoma	IA	G2
11	S	Mixed endometrioid adenocarcinoma and serous adenocarcinoma	IIIB	G3
12	S	Clear cell carcinoma	IIA	GX
13	S	Clear cell carcinoma	IA	GX

[a]International Federation of Gynecology and Obstetrics (FIGO) stage, [b]Histological grade.
R, ovarian tumor tissues from cisplatin-resistant patients; S, ovarian tumor tissues from cisplatin-sensitive patients.

levels between the two groups (Supplementary Fig. 2).

In the next step, we explored the correlations between transcriptional expression of *MBNL2* and chemoresponse or prognosis in patients with serous ovarian cancer, using publicly available datasets. Although the P-values were marginally significant or not significant, the mRNA expression levels of *MBNL2* were generally lower in responders, those sensitive to treatment, or those without disease recurrence, across five public datasets (Fig. 4A). Furthermore, expression levels of *MBNL2* were higher in 274 patients who experienced cancer recurrence compared to 198 who did not, in the TCGAOVARIAN dataset (Fig. 4B). Additionally, the group with lower MBNL2 expression showed a significantly prolonged recurrence-free interval (Fig. 4C). A meta-analysis of nine datasets, which included progression-free survival data of ovarian cancer patients receiving chemotherapy, revealed a significant association between high *MBNL2* expression and an increased risk of disease progression (Fig. 4D). Furthermore, elevated MBNL2 expression was strongly correlated with reduced overall survival rates (Fig. 4E, left). This correlation persisted even after adjusting for tumor stage (Fig. 4E, right), suggesting that increased transcriptional expression of MBNL2 might be a prognostic marker for ovarian cancer, independent of tumor stage.

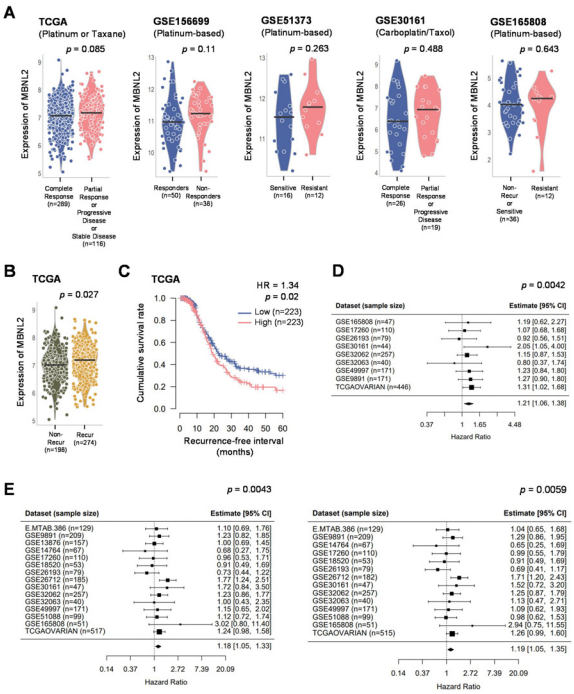

Fig. 4. Associations between *MBNL2* mRNA expression and chemoresponsiveness or prognosis in serous type ovarian cancer patients. (A) Violin plots of *MBNL2* expression with median expression and P-value from Welch's t-test across different chemoresponsive groups in five public datasets containing chemoresponse information. (B) Violin plot of *MBNL2* expression with median expression and P-value from Welch's t-test comparing recurred or non-recurred patient groups post-chemotherapy in the TCGAOVARIAN dataset. (C) Kaplan-Meier curves for progression-free survival (or interval) in the high- or low-expression group of *MBNL2* in the TCGAOVARIAN dataset. (D) Forest plot of meta-analysis for the progression-free survival (or interval) of ovarian cancer patients across nine datasets containing chemotherapy and progression-free survival information. (E) Forest plots of meta-analysis for overall survival of ovarian cancer patients in fifteen datasets without tumor stage adjustment (left), and in fourteen datasets with tumor stage adjustment (left).

DISCUSSION

MBNL2, a protein belonging to the muscleblind-like family, regulates the metabolism of precursor and mature mRNAs in mammals. In mammals, this gene is located on the same chromosome as MBNL1 and MBNL3 and shares structural features, including zinc finger domains crucial for RNA binding and splicing regulation (16, 17). MBNL1 and MBNL2 are widely expressed across multiple tissues, while MBNL3 is primarily found in the placenta (18). MBNL proteins are essential in the pathogenesis of myotonic dystrophy type 1 (DM1), an inherited disease characterized by expanded CTG trinucleotide repeats in the 3'-untranslated region of the myotonic dystrophy protein kinase (DMPK) gene. The resulting protein accumulation leads to a range of clinical symptoms, including myotonia, muscle wasting, cardiac dysfunction, cataract formation in the eyes, and neuropathy (8, 9, 19). In myotonic dystrophy, interactions between the CUG or CCUG repeat expansions and the MBNL proteins result in altered splicing patterns favoring fetal isoforms, detaching the proteins from their regular RNA targets (20, 21). Recent research has unveiled MBNL2's complex and context-dependent roles in cancer biology. The protein exhibits remarkable versatility, acting as either a tumor promoter or suppressor depending on the cellular context and type of cancer. In some cancers, MBNL2 promotes tumor survival and progression by regulating the cellular response to DNA damage through stabilizing the expression of CDKN1A/p21 cyclin-dependent kinase inhibitor, a key protein in cell cycle progression, apoptosis, and DNA repair via a pathway independent of p53, a major tumor suppressor and regulator of *CDKN1A*/p21 transcription (10). In human lung and breast cancer cells, MBNL2 is upregulated in hypoxic conditions and plays a vital role in cancer cell adaptation by regulating genes associated with the hypoxia response, including vascular endothelial growth factor A (VEGFA). Inhibiting MBNL2 prevents cancer cell proliferation and migration, underscoring its role in cancer progression. This function is unique to MBNL2, as MBNL1 does not influence hypoxia adaptation (22). Conversely, MBNL2 acts as a tumor suppressor gene in hepatocarcinogenesis, highlighting the context-dependent nature of its functions. In liver cancer, higher MBNL2 levels correlate with smaller, less advanced tumors and marginally better patient survival rates. The protein exerts its tumor-suppressive effects through various mechanisms: during liver regeneration, the increased expression of MBNL2 inhibits invasion, migration,

proliferation, and tumor sphere formation by reducing the levels of crucial stem cell transcription factors like SOX2, NANOG, and OCT4. Moreover, it has been shown to reduce tumor growth in mice that lack a functional immune system. The reduction in MBNL2 expression has been observed to promote migration and invasion in liver cancer cells without affecting tumor size (11). These diverse effects of MBNL2 can be ascribed to multiple factors, including tissue-specific molecular settings, epigenetic modifications, cellular stress conditions, and interactions with different signaling pathways. The protein's role appears to be particularly significant under specific cellular stressors, such as hypoxia or DNA damage, which may differ among cancer types and stages. The significance of epigenetic regulation cannot be overstated, as evidenced in our study where the expression of MBNL2 is substantially influenced by DNA methylation. This intricate interaction of factors leads to MBNL2 having distinctly different impacts on cancer progression and the response to treatment across various tissue types.

In this study, we identified MBNL2 as a key regulator of response to cisplatin treatment by analyzing differential gene expression and methylation profiles between ovarian cancer cell lines that are either sensitive or resistant to cisplatin. Ovarian cancer cells resistant to cisplatin exhibited a marked increase in *MBNL2* mRNA expression and significant demethylation at promoter CpG sites. Treatment with a DNA methyltransferase inhibitor significantly elevated *MBNL2* mRNA expression in these cell lines, suggesting that the transcriptional activity of *MBNL2* is controlled by epigenetic mechanisms involving DNA methylation. Moreover, overexpressing *MBNL2* enhanced resistance to cisplatin, while silencing *MBNL2* expression increased the cytotoxic effects of cisplatin in SK-OV-3 cells. Previous research indicates a strong association between DNA methylation and resistance to cisplatin in ovarian cancer. Elevated TMEM88 protein expression coupled with DNA hypomethylation has been linked to resistance to platinum-based chemotherapy in ovarian cancer cells. Conversely, a reduction in TMEM88 expression affects cisplatin efficacy through the Wnt signaling pathway (23).

The development of resistance to cisplatin in cancer cells, often via CDKN1A/p21, results in reduced apoptosis, a critical process for eliminating damaged or superfluous cells. The mechanisms underlying this resistance include enhanced DNA repair capabilities, elevated detoxification processes, and alterations in the cellular pathways that govern apoptosis. Additionally, changes in the tumor microenvironment and the activation of survival pathways may attenuate the apoptotic effects of anticancer drugs (3, 4). It has been shown that MBNL2 influences the DNA damage response by stabilizing CDKN1A/p21 expression, crucial for cell cycle regulation and apoptosis. Moreover, MBNL2 depletion has been shown to augment apoptosis in DNA-damaged cells, highlighting its potential role in cellular survival mechanisms (10). According to earlier research, the caspase 3 cleavage assay indicated that MBNL2 knockdown in SK-OV-3 cells facilitated cisplatin-induced apoptosis, while MBNL2 overexpression suppressed it. These findings suggest that MBNL2 contributes to cisplatin resistance in human ovarian cancer cells by regulating CDKN1A/p21 expression and blocking apoptotic pathways. Our results uncover a previously unrecognized role of MBNL2 in promoting chemoresistance through the modulation of *CDKN1A/p21* mRNA stability and downstream apoptotic signaling, establishing it as a key molecular determinant in the development of cisplatin resistance.

The validation process assessed differential levels of *MBNL2* mRNA in primary ovarian tumor samples from nine cisplatin-sensitive patients and four cisplatin-resistant patients. The data revealed that patients resistant to cisplatin displayed significantly higher levels of *MBNL2* mRNA. The *MBNL2* mRNA levels accurately distinguished patients resistant to cisplatin from those sensitive to it (AUC = 0.89, P = 0.0308). Additionally, a meta-analysis using seventeen publicly available datasets evaluated the relationship between *MBNL2* transcriptional expression and chemotherapy response or prognosis in serous ovarian cancer patients. The results indicated that lower MBNL2 expression correlates with better chemotherapy response and longer relapse-free survival, whereas higher *MBNL2* expression levels correlate with increased recurrence rates and reduced survival probabilities. These findings suggest that MBNL2 could serve as an independent prognostic factor in ovarian cancer.

In conclusion, our results provide new evidence supporting MBNL2 as a gene linked to chemoresistance, contributing to increased recurrence and diminished survival outcomes for patients. Further exploration of the full molecular mechanisms behind MBNL2's role in cisplatin resistance is essential. Nevertheless, the current study highlights the potential use of MBNL2 as a prognostic and therapeutic target in combating cisplatin resistance.

MATERIALS AND METHODS

Tissue specimens from ovarian cancer patients

The study analyzed ovarian cancer specimens from nine patients with cisplatin sensitivity and four patients with cisplatin resistance. The primary solid tumor tissues were obtained from the Ewha Specimens Bank, along with comprehensive patient data, including chemoresponse, histology, International Federation of Gynecology and Obstetrics (FIGO) stage, and histological grade. The details of this information are provided in Supplementary Table 2. Approval for all procedures was granted by the ethics committee of Ewha Womans University Medical Center and the Korea National Institute of Health (permit number: EUMC 2014-05-004-001). Written consent was obtained from all patients.

Analysis of associations between transcriptional expression of MBNL2 and chemoresponse or prognosis in public datasets

To examine the association between transcriptional expression

of *MBNL2* and chemoresponse or prognosis in serous ovarian cancer patients, we used total seventeen publicly available datasets: twelve datasets (TCGAOVARIAN, E.MTAB.386, GSE13876, GSE14764, GSE17260, GSE18520, GSE26193, GSE26712, GSE30161, GSE49997, GSE51088, and GSE9891) were obtained using the MetaGxOvarian within MetaGxData R package (24), and five datasets (GSE156699, GSE165808, GSE32062, GSE32063, GSE51373) were downloaded from the Gene Expression Omnibus (GEO) database using the GEOquery R package (25). In all datasets, we selected only the data from serous ovarian cancer patients to eliminate histological heterogeneity in data analysis. The association between the mRNA expression of *MBNL2* and chemoresponse was assessed by two-sided Welch's t-test in five datasets, TCGAOVARIAN, GSE156699, GSE165808, GSE30161, and GSE51373, which contained chemoresponsiveness of ovarian cancer patients against platinum- and/or taxane-based chemotherapy. The difference of the expression of *MBNL2* between recurred and non-recurred ovarian cancer patients after chemotherapy was evaluated using two-sided Welch's t-test in the TCGAOVARIAN dataset. The difference of recurrence-free interval between high and low expression level of *MBNL2* was assessed using a Kaplan-Meier analysis with a logrank test in the TCGAOVARIAN dataset. With nine datasets containing progression-free survival (or interval) information in ovarian cancer patients who received adjuvant chemotherapy, meta-analysis was performed with a fixed effect model using the hazard ratios (HRs) that were calculated for progression-free survival (or interval) by Kaplan-Meier analysis between high (50%) vs. low (50%) expression group of *MBNL2* in each dataset. Finally, meta-analyses were performed for overall survival with or without adjustment for tumor stage using the hazard ratios (HRs) calculated by Kaplan-Meier analysis between high vs. low expression group of *MBNL2*.

The supplementary information provides detailed descriptions of the following methodologies: cell culture, reverse-transcription quantitative polymerase chain reaction (RT-qPCR), 5-aza-2′-deoxycytidine (5-aza-dc) treatment, transient transfection, cytotoxicity assay, western blot analyses and statistical analyses.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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