Received: 27 August 2022 | Revised: 25 October 2022 | Accepted: 26 October 2022

DOI: 10.1002/jcla.24761

RESEARCH ARTICLE

WILEY

METTL3-mediated m6A modification of lnc RNA RHPN1-AS1 enhances cisplatin resistance in ovarian cancer by activating PI3K/AKT pathway

Shoubin Cui

Department of Gynaecology and Obstetrics, Yantai Affiliated Hospital of Binzhou Medical University, Yantai, Shandong, China

Correspondence
Shoubin Cui, Department of Gynaecology and Obstetrics, Yantai Affiliated Hospital of Binzhou Medical University, No. 717 Jinbu Street, Muping District, Yantai 264100, Shandong, China.
Email: cuishoubin6@163.com

Funding information
Projects of Medical and Health Technology Development Program in Shandong Province, Grant/Award Number: 202105010886

Abstract

Background: Cisplatin resistance is a big challenge for ovarian cancer (OC) therapy. The abnormal expression of long noncoding RNAs (lncRNAs) regulated by N6-methyladenosine (m6A) modification has been confirmed to play the crucial roles in OC. The aim of this study is to explore the regulatory mechanism of lncRNA RHPN1-AS1 on OC with cisplatin resistance.

Methods: The real-time reverse transcription-polymerase chain reaction was carried out to confirm the expression of RHPN1-AS1 and methyltransferase-like 3 (METTL3) in OC. The effects of RHPN1-AS1 on cisplatin-resistant OC cells were identified by cell functional experiments and animal experiment. Western blotting was performed to detect the effect of RHPN1-AS1 on PI3K/AKT pathway. Moreover, methylated RNA immunoprecipitation and RNA stability assays confirmed the interaction between RHPN1-AS1 and METTL3.

Results: RHPN1-AS1 and METTL3 were confirmed to be overexpressed in OC. After transfecting RHPN1-AS1 overexpression or RHPN1-AS1 knockdown vectors into cisplatin-resistant OC cells, it was found that upregulating RHPN1-AS1 contributed to cell viability, migration, invasion, and tumor growth in vivo. In addition, RHPN1-AS1 could enhance the protein levels of PI3K and phosphorylated AKT in cisplatin-resistant OC cells, and METTL3 could enhance the stability of RHPN1-AS1 by the m6A modification.

Conclusion: Overall, this study revealed that METTL3-mediated m6A modification of RHPN1-AS1 accelerates cisplatin resistance in OC by activating PI3K/AKT pathway.

KEYWORDS
cisplatin, METTL3, ovarian cancer, PI3K/AKT, RHPN1-AS1

1 | INTRODUCTION

Ovarian cancer (OC) with high incidence of recurrence and mortality rate becomes the most lethal gynecological malignancy, which threats to women's health.[1] The cisplatin as a cytotoxic-based platinum compound has been used in OC therapy for many years, since almost 70% of OC patients are sensitive to platinum.[2,3] However, cisplatin resistance becomes a big challenge for OC therapy owing to a 5-year survival rate <30%.[3,4] Therefore, fully investigating the mechanism of cisplatin resistance can remove the obstacle to the successful OC therapy caused by cisplatin resistance.

Long noncoding RNAs (lncRNAs) are identified to participate in drug resistance, thereby becoming key biomarkers for assessing therapeutic effects.[5-8] For example, lncRNA WDFY3-AS2,[9] lncRNA CHRF[10] and lncRNA MALAT1[11] contributed to cisplatin resistance in OV cells, whereas LINC01508,[12] LINC00312,[13] and lncRNA GAS5[14] enhanced cisplatin sensitivity in OV cells. RHPN1-AS1 affiliated with lncRNAs class has been found to be a tumor promoter in nasopharyngeal carcinoma,[15] glioma,[16] gastric cancer,[17] etc. In my previous studies,[18,19] I proved the facilitating influence of RHPN1-AS1 on OC. However, the function of RHPN1-AS1 in cisplatin resistance of OC have not been investigated.

N6-methyladenosine (m6A) modification occurring in eukaryotic cells has attracted the attention of researches in recent years.[20-22] It is reported that m6A modification depends on methyltransferases (writer), demethylases (erasers), and readers.[23] Methyltransferase-like 3 (METTL3) as a classical complex of writers has been widely investigated in human cancers. For instance, METTL3 induced the upregulation of lncRNA ABHD11-AS1 to promote the proliferation and Warburg effect of lung cancer.[24] METTL3 enhanced the stability of FOXD2-AS1 by m6A modification, thereby promoting cervical cancer progression.[25] In OC, the oncogenic function of METTL3 has been widely reported.[26-29] However, whether METTL3 can regulate lncRNA to affect OC progression is still unknown.

Phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) pathway has been confirmed to be an important pathway, which participating in multiple human diseases including cancers.[30,31] Increasing evidence shows the aberrant activation of PI3K/AKT pathway in human cancers, and phosphorylated AKT could mediate proapoptotic factors to regulate the apoptosis of tumor cells.[32,33] In OC, vast clinical data identified that PI3K/AKT pathway was activated and using the inhibitors of PI3K/AKT pathway was an effective antitumor treatment for OC.[34] Another study revealed that the inactivated PI3K/AKT pathway could alleviate cisplatin resistance by inhibiting epithelial–mesenchymal transition.[35] Based on the results from my previous studies,[18,19] I confirmed that RHPN1-AS1 could regulate TPX2 and TOP2A to exert tumorigenic role in OC. Meanwhile, TPX2 and TOP2A were reported to regulate PI3K/AKT pathway in cancers.[36,37] Therefore, I put forward a hypothesis that RHPN1-AS1 might be associated with cisplatin resistance of OC cells by affecting PI3K/AKT pathway.

In this research, I aim to prove the role and regulatory mechanism of RHPN1-AS1 in OC cells with cisplatin resistance by using clinical data, cell function experiment, and animal experiment. My findings may put forward a novel idea on removing the obstacle of OC therapy caused by cisplatin resistance.

2 | MATERIALS AND METHODS

2.1 | Bioinformatics analysis

Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/index.html), an online database, was applied to confirm the RHPN1-AS1 expression in multiple cancer types especially in OC. Another online database TNM plot (https://tnmplot.com/analysis/) was used to identify the RHPN1-AS1 expression in OC.

2.2 | Clinical samples collection

The OC and corresponding adjacent non-tumor tissues were achieved from 50 patients diagnosed with OC between May 2020 to December 2021 in Yantai Affiliated Hospital of Binzhou Medical University. All patients joining in this study gave the written informed consent, as well as the Ethics Committee of Yantai Affiliated Hospital of Binzhou Medical University approved my study (Approval number: 20200909002). Table 1 listed the clinical characteristics of 50 OC patients.

2.3 | Real-time reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA from tissues or cells was isolated using Trizol (Invitrogen) with the instruction of manufacturer. The reverse transcription and qRT-PCR were performed by Reverse Transcription Kit (Vazyme,

TABLE 1 Clinical characteristics of 36 ovarian cancer patients in this study

Variables	Case, No. (%)
Age	
<60	20 (40)
≥60	30 (60)
FIGO stage	
I and II	19 (38)
III and IV	31 (62)
Grade stage	
G1 and G2	17 (34)
G3 and G4	33 (66)
Cisplatin	
Resistant	25 (50.00)
Sensitive	25 (50.00)

Abbreviation: FIGO, Federation International of Gynecology and Obstetrics.

TABLE 2 qRT-PCR primers used in this study

Gene	Primer type	Sequence
RHPN1-AS1	Forward	5′-TGTGAGTCCTCCGACAATGC-3′
	Reverse	5′-AACTTGATGACCAGGAGCCG-3′
METTL3	Forward	5′-CAAGCTGCACTTCAGACGAA-3′
	Reverse	5′-GCTTGGCGTGTGGTCTTT-3′
GAPDH	Forward	5′-GGAGCGAGATCCCTCCAAAAT-3′
	Reverse	5′-GGCTGTTGTCATACTTCTCATGG-3′

Nanjing, China) and SYBR Green PCR Kit (Takara), respectively. $2^{-\Delta\Delta Cq}$ method was applied for the relative expression with GAPDH as internal reference of RHPN1-AS1 and METTL3. Table 2 listed all sequences of primers.

2.4 | Cell culture

Two OC cell lines (SKOV3 and OVCAR3) and cisplatin-resistant cell lines (SKOV3/DDP and OVCAR3/DDP) were purchased from Chuang Qiu Biotechnology. SKOV3 cells were cultured in McCoy's medium with 10% FBS, whereas OVCAR3 cells were cultured in RPMI-1640 medium with 20% FBS and 0.01 mg/ml insulin. SKOV3/DDP cells and OVCAR3/DDP cells were cultured in RPMI-1640 medium with 10% FBS, as well as culturing SKOV3/DDP and OVCAR3/DDP cells need to add 0.2 μM DDP and 0.5 μM DDP, respectively. All cells were incubated in an incubator at 37°C and 5% CO_2.

2.5 | Cell transfection

RHPN1-AS1 or METTL3 overexpression vectors (RHPN1-AS1-OE or METTL3-OE), empty vector (negative control of overexpression vectors), RHPN1-AS1 or METTL3 knockdown vectors (si-RHPN1-AS1 or si-METTL3), and si-NC (negative control of knockdown vectors) were provided by GenePharma (China). The cisplatin-resistant cells were transfected with aforementioned vectors at 50 nM concentration using lipofectamine 3000 (Invitrogen, USA) per manufacturer's protocol. After 48 h, the transfected cells were collected to perform qRT-PCR for the detection of transfection efficiency.

2.6 | cell counting kit-8 (CCK8) assay

Cell viability was detected using CCK8 assay according to previous study. Briefly, 2×10^4 cells/well cells were seeded in 96-well plates with −0.5, −0.25, 0, 0.25, 0.5, 0.75, 1, 1.25, and 1.5 logDDP (μg/ml) of DDP for 48 h. Next, the cells were added 10 μl CCK8 solution (Beyotime) to incubate for 2 h. Finally, the cell viability was detected relative to that of non-DDP cells or negative control group by a microplate reader.

2.7 | Migration and invasion assays

Transwell was used for detecting cell migration and invasion with the similar experimental procedure except that Matrigel (BD biosciences) was used to cover the upper chamber of transwell in invasion assay. 2×10^4 cells were seeded into the upper chamber without FBS, at the same time, 600 μl medium with 10% FBS were added into the lower chamber. After incubating cells for 24 h, the migrated or invaded cells at the lower surface of membrane were fixed, stained, and finally observed under a light microscope.

2.8 | Animal experiment

Ethics Committee of Yantai affiliated hospital of Binzhou Medical University (Approval number: 20200909001) approved the animal experiment. Ten BALB/c female nude mice from Charles River were randomly divided into Lv-si-NC group and Lv-si-RHPN1-AS1 group (n = 5/group). RHPN1-AS1 knockdown lentiviral vector (Lv-si-RHPN1-AS1) and its negative control (Lv-si-NC) were constructed by Genomeditech. 2×10^5 SKOV3/DDP cells transfected with Lv-si-NC and Lv-si-RHPN1-AS1 vectors were subcutaneously injected into mice per different groups. The tumor volume was detected every five days. At 25 days after injection, the tumor tissues were removed after mice were sacrificed, and the tumor weight was measured.

2.9 | Western blotting

Western blotting was carried out to detect the PI3K/AKT pathway-associated proteins using RIPA buffer (Beyotime) to isolate protein and BCA kit (Beyotime, China) to detect protein concentration. 20 μg protein was separated with 10% SDS-PAGE, and then transferred to PVDF membranes. Using 5% fat-free milk to block membranes, the primary antibodies including PI3K, Akt, phosphorylated AKT (p-AKT), and GAPDH were added to incubate membranes overnight in a refrigerator with 4°C. Next, the membranes were incubated with fluorescent antibody (LI-COR Biosciences) for 3 h. The protein was measured by Odyssey 3.2 (LI-COR Biosciences). All primary antibodies were purchased from Abcam.

2.10 | Methylated RNA immunoprecipitation (MeRIP) assay

The cells transfected with si-METTL3 or METTL3-OE were performed using Magna MeRIP m6A Kit (Merck Millipore, USA). Briefly, the isolated RNA was fragmented by fragmentation buffer. The m6A/IgG-conjugated magnetic beads and MeRIP reaction mixture were added into fragmented RNA to incubate at 4°C for 2 h. After washing with RIP wash buffer, the RNA enrichment was detected by qRT-PCR.

2.11 | RNA stability assay

The transfected cells seeded in 12-well plates were treated with 5 μg actinomycin D (Shanghai YSRIBIO industrial co., China) for 0, 4, 8, and 12 h. Then, the total RNA was isolated and performed qRT-PCR to confirm the stability of RHPN1-AS1.

2.12 | Statistical analysis

Statistical analysis in my study was carried out via GraphPad Prism 7 with paired student's t-test for two groups, Fisher's exact test for the correlation between RHPN1-AS1 and clinical characteristics, and ANOVA for more than two groups. The data from three repeated experiments were shown as means ± standard deviation (SD). The difference was considered significant if p value (p) <0.05.

3 | RESULTS

3.1 | Bioinformatics analysis predicts the upregulation of RHPN1-AS1 in OC

Based on the data of GEPIA, RHPN1-AS1 was upregulated in most human cancer types (Figure 1A). Then, I used GEPIA and TNM plot to identify the level of RHPN1-AS1 in OC samples. The results from GEPIA (Figure 1B) and TNM plot (Figure 1C) displayed the upregulation of RHPN1-AS1 in OC samples. The bioinformatic analysis identified the RHPN1-AS1 overexpression in OC samples.

3.2 | RHPN1-AS1 was upregulated in OC with cisplatin resistance

After performing qRT-PCR in my collected clinical samples, the RHPN1-AS1 expression was increased by 3.6-fold in OC samples compared with para-tumor samples (Figure 2A). After analyzing the correlation between RHPN1-AS1 expression and clinical

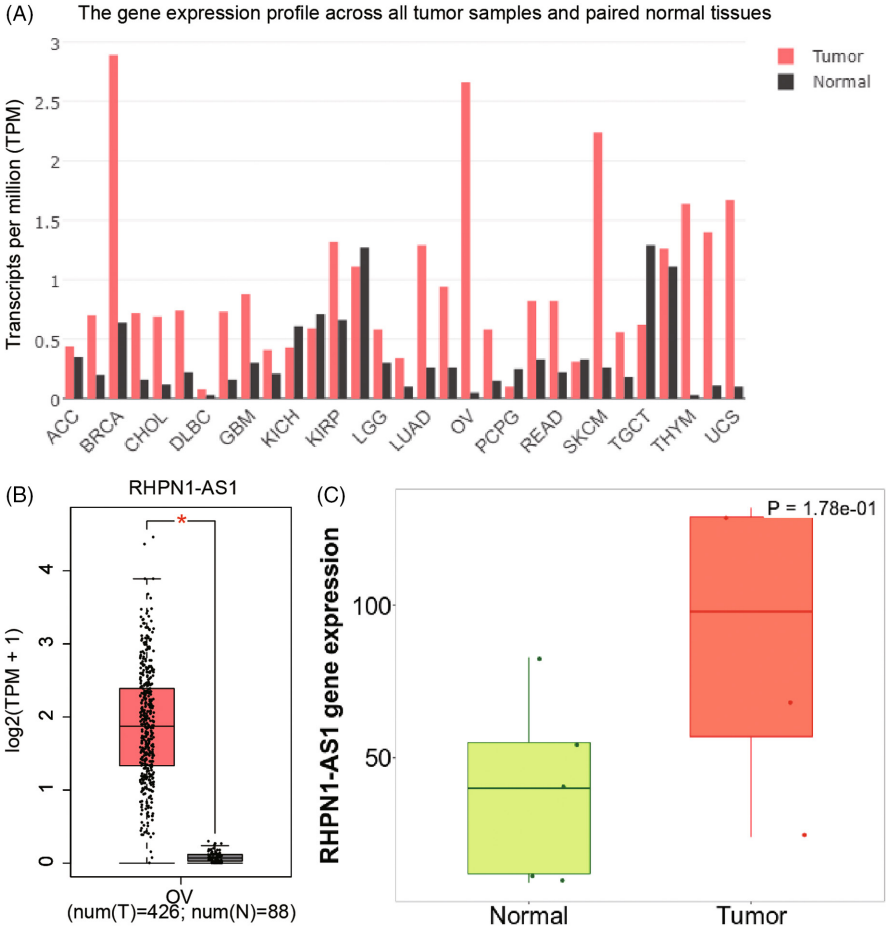


FIGURE 1 Bioinformatics analysis predicts the upregulation of RHPN1-AS1 in OC. (A) The upregulation of RHPN1-AS1 in most cancer types according to the prediction of GEPIA. (B) GEPIA showed the RHPN1-AS1 overexpression in OC tumor samples. T, tumor. N, normal. OV, ovarian cancer. *p <0.01. (C) TNM plot displayed the upregulation of RHPN1-AS1 in OC tumor samples.

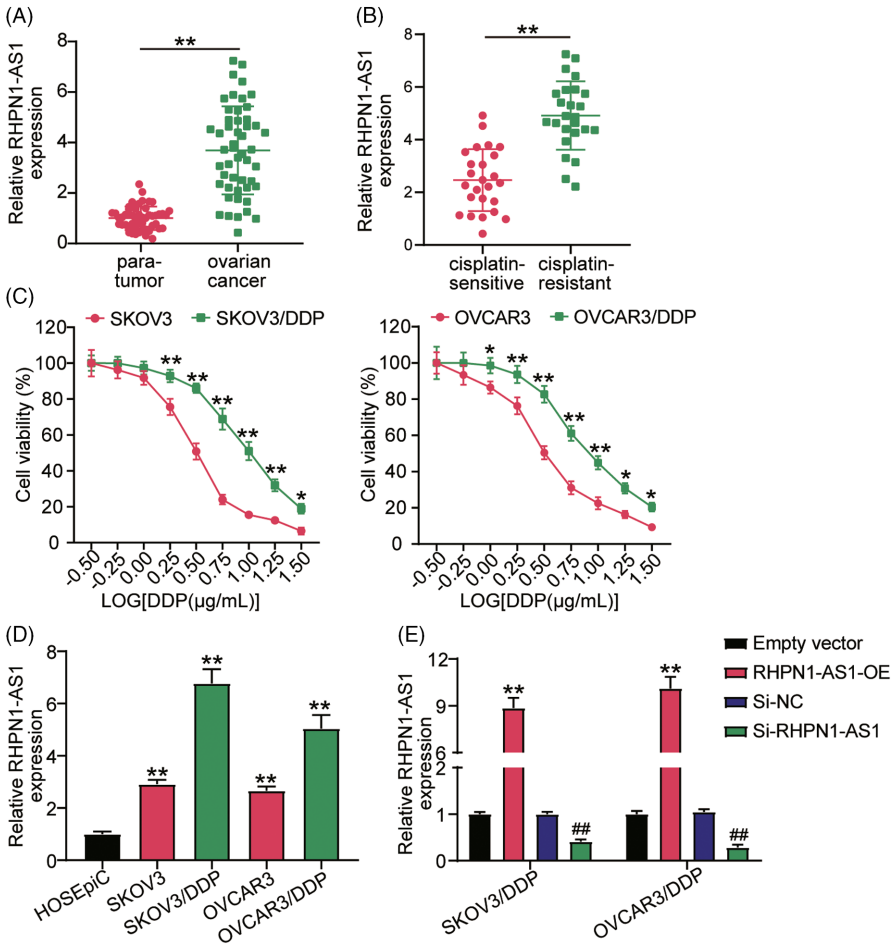


FIGURE 2 RHPN1-AS1 was upregulated in OC with cisplatin resistance. (A) qRT-PCR confirmed the upregulation of RHPN1-AS1 in OC tissues compared with para-tumor tissues. $^{**}p<0.001$. (B) qRT-PCR confirmed the upregulation of RHPN1-AS1 in cisplatin-resistant OC tissues compared with cisplatin-sensitive OC tissues. $^{**}p<0.001$. (C) The value of IC50 of cisplatin-resistant OC cells (SKOV3/DDP and OVCAR3/DDP) elevated compared with cisplatin-sensitive OC cells (SKOV3 and OVCAR3) by CCK8 assay. $^{*}p<0.05$, $^{**}p<0.001$. (D) The cisplatin-resistant OC cells showed the higher RHPN1-AS1 expression. $^{**}p<0.001$ vs. HOSEpiC. (E) RHPN1-AS1 expression vectors (RHPN1-AS1) and si-RHPN1-AS1 were transfected into cisplatin-resistant OC cells. $^{**}p<0.001$ vs. empty vector; $^{\#\#}p<0.001$ vs. si-NC.

characteristics using Fisher's exact test, the RHPN1-AS1 with high expression indicated the higher FIGO stage ($p = 0.0031$), higher Grade stage ($p = 0.0066$), and cisplatin resistance ($p<0.0001$, Table 3). Meanwhile, the results of Figure 2B showed that the RHPN1-AS1 expression in cisplatin-resistant tissues was elevated. The half maximal inhibitory concentration (IC50) value of cisplatin in SKOV3/DDP and OVCAR3/DDP cells was higher than that in SKOV3 and OVCAR3 cells, suggesting that we successfully cultured the cisplatin-resistant OC cells (Figure 2C). By qRT-PCR, RHPN1-AS1 expression was further increased by more than 5-fold in cisplatin-resistant OC cells (Figure 2D). After cell transfection in cisplatin-resistant OC cells, RHPN1-AS1 overexpression vectors induced almost 6-fold increase in RHPN1-AS1 expression, whereas si-RHPN1-AS1 caused 70% decrease of RHPN1-AS1 expression (Figure 2E). These results confirmed the upregulation of RHPN1-AS1 in OC under cisplatin-resistant.

TABLE 3 Correlation between RHPN1-AS1 and clinical characteristics of 36 ovarian cancer patients

Variables	Cases, *n*	RHPN1-AS1		*p* value
		Low, *n*	High, *n*	
Age				0.7790
<60	20	9	11	
≥60	30	15	15	
FIGO stage				0.0031
I and II	19	15	4	
III and IV	31	10	21	
Grade stage				0.0066
G1 and G2	17	13	4	
G3 and G4	33	11	22	
Cisplatin				<0.0001
Resistant	25	4	21	
Sensitive	25	20	5	

Note: *p* value was analyzed by Fisher's exact test.

3.3 | RHPN1-AS1 enhanced cisplatin resistance in vitro and in vivo

The data from CCK8 assay confirmed that upregulating RHPN1-AS1 elevated the IC50 value in cisplatin-resistant OC cells, whereas si-RHPN1-AS1 reduced the IC50 value (Figure 3A). The transwell migration assay proved that the ability of cell migration was boosted in RHPN1-AS1 overexpression group and impaired in si-RHPN1-AS1 group in cisplatin-resistant OC cells (Figure 3B). Similar to

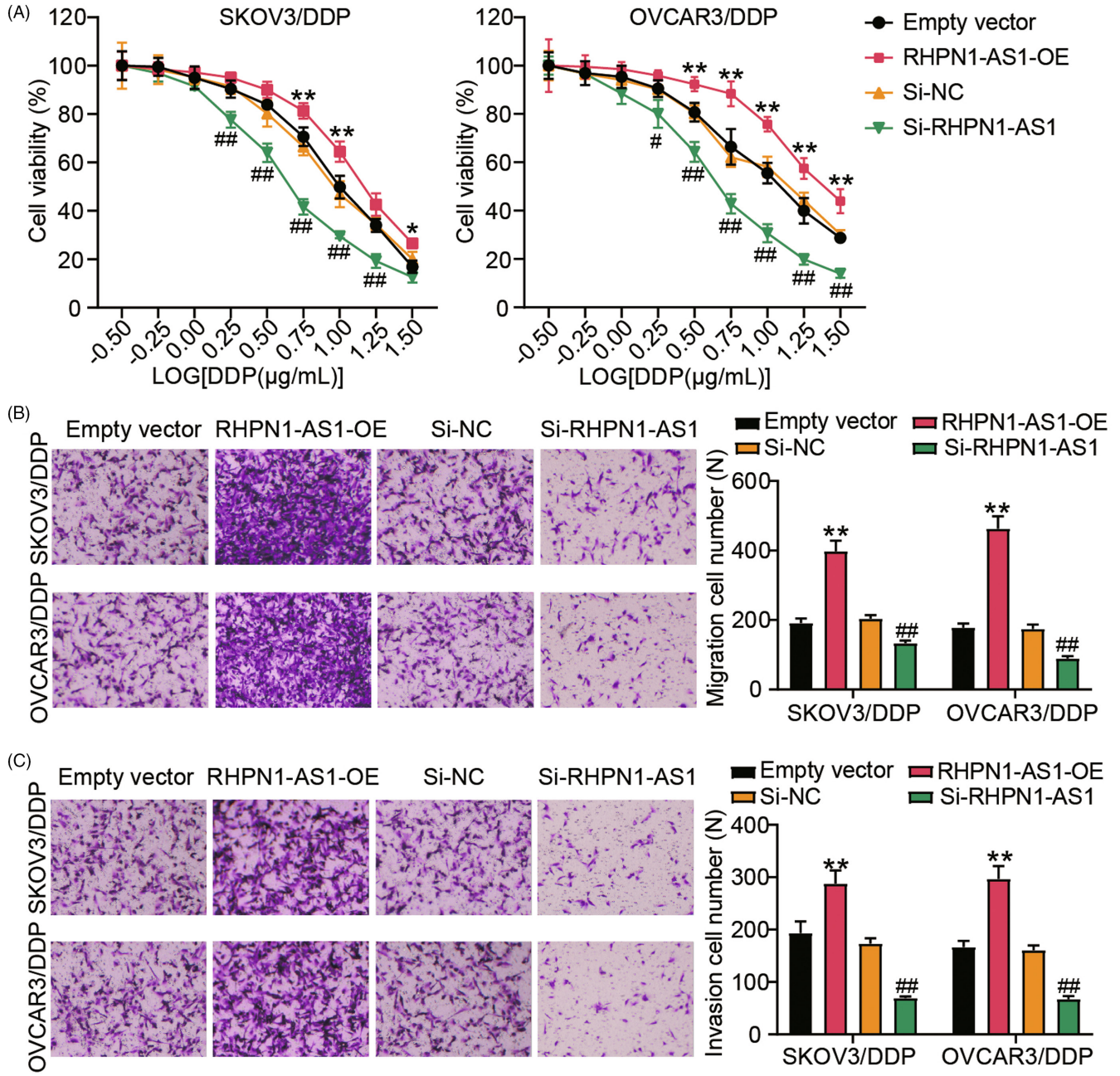


FIGURE 3 RHPN1-AS1 enhanced cisplatin resistance in vitro. (A) CCK8 assay proved the higher IC50 value in cisplatin-resistant OC cells (SKOV3/DDP and OVCAR3/DDP) with RHPN1-AS1 overexpression. (B, C) Transwell migration and invasion assays revealed that RHPN1-AS1 overexpression contributed to cell migration (B) and cell invasion (C) in cisplatin-resistant OC cells. $^{*}p<0.05$, $^{**}p<0.001$ vs. empty vector; $^{\#}p<0.05$, $^{\#\#}p<0.001$ vs. si-NC.

migration, RHPN1-AS1 overexpression contributed to cell invasion, but si-RHPN1-AS1 hindered cell invasion in cisplatin-resistant OC cells (Figure 3C). After infection of SKOV3/DDP into mice, Lv-si-RHPN1-AS1 group showed the smaller tumor volume than Lv-si-NC group (Figure 4A). Removing the tumor tissues, the lighter tumor weight in Lv-si-RHPN1-AS1 group was observed (Figure 4B,C). These data confirmed that RHPN1-AS1 enhanced cisplatin resistance in OC.

3.4 | RHPN1-AS1 enhanced cisplatin resistance in OC by regulating PI3K/AKT pathway

To deeply verify the mechanism of RHPN1-AS1 on cisplatin resistance in OC, western blotting was used to detect the key proteins of PI3K/Akt pathway including PI3K, AKT and p-AKT. In SKOV3/DDP cells, RHPN1-AS1 overexpression induced the increased levels of PI3K and p-AKT, whereas si-RHPN1-AS1 led to the decreased

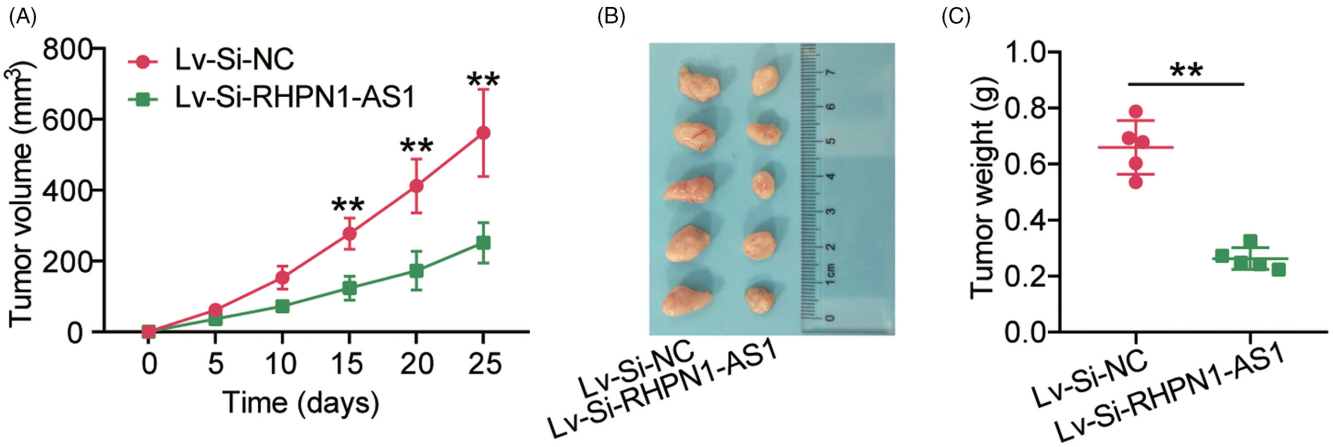
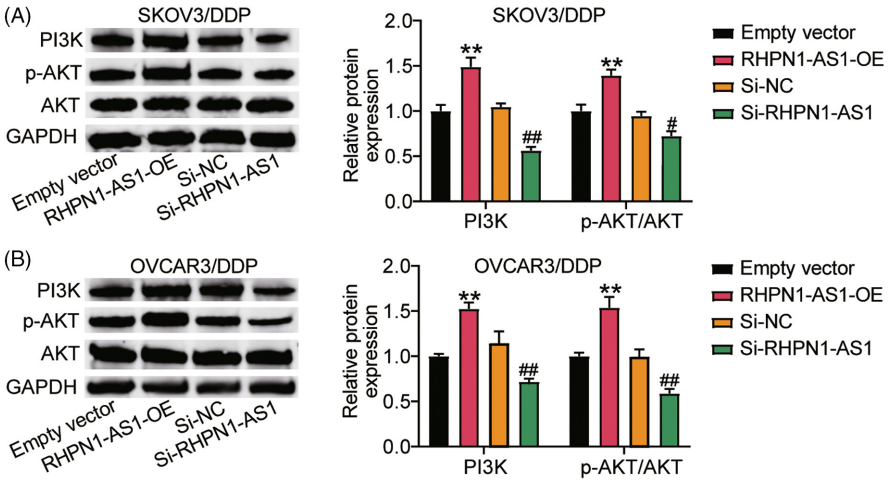


FIGURE 4 RHPN1-AS1 knockdown inhibited cisplatin resistance in vivo. (A) RHPN1-AS1 knockdown reduced the tumor volume after nude mice infected with transfected cisplatin-resistant OC cells. (B) Representative images of tumor from nude mice infected with transfected cisplatin-resistant OC cells. (C) Tumor weight reduced in RHPN1-AS1 knockdown group from nude mice infected with transfected cisplatin-resistant OC cells. Lv-si-RHPN1-AS1, RHPN1-AS1 knockdown lentiviral vector. Lv-si-NC, negative control of lentiviral vector. $^{**}p < 0.001$.

FIGURE 5 RHPN1-AS1 enhanced cisplatin resistance in OC by regulating PI3K/AKT pathway. (A) RHPN1-AS1 activated PI3K/AKT pathway in SKOV3/DDP cells. (B) RHPN1-AS1 activated PI3K/AKT pathway in OVCAR3/DDP cells. RHPN1-AS1-OE, RHPN1-AS1 overexpression vectors. $^{**}p < 0.001$ vs. empty vector; $^{\#}p < 0.05$, $^{\#\#}p < 0.001$ vs. si-NC.



levels of PI3K and p-AKT (Figure 5A). The results in OVCAR3/DDP were the similar to SKOV3/DDP. It showed that RHPN1-AS1 overexpression enhanced the levels of PI3K and p-AKT, and RHPN1-AS1 knockdown impaired that (Figure 5B). These data proved that PI3K/AKT pathway was activated by RHPN1-AS1 in cisplatin-resistant OC cells.

3.5 | METTL3 enhanced the stability of RHPN1-AS1 by m6A modification in cisplatin-resistant OC cells

By qRT-PCR, the upregulation of METTL3 in OC tissues was observed (Figure 6A), and its expression was positively correlated to RHPN1-AS1 expression by Pearson correlation analysis ($R = 0.7835$, Figure 6B). When SKOV3/DPP and OVCAR3/DPP cells were transfected with si-METTL3, the RHPN1-AS1 expression was attenuated by si-METTL3 (Figure 6C), and MeRIP assay confirmed that si-METTL3 impaired the m6A modification of RHPN1-AS1 (Figure 6D). However, METTL3 overexpression induced the upregulation of RHPN1-AS1 (Figure 6E) and enhanced the m6A modification of RHPN1-AS1 in cisplatin-resistant OC cells (Figure 6F). Furthermore, the stability of RHPN1-AS1 was enhanced and impaired by METTL3 overexpression and si-METTL3, respectively (Figure 6G). Overall, the results showed that the m6A modification of RHPN1-AS1 caused by METTL3 upregulated the stability of RHPN1-AS1 in cisplatin-resistant OC cells.

3.6 | METTL3 overexpression recovered the inhibitory effect of si-RHPN1-AS1 on cisplatin-resistant OC cells

After performing CCK8 assay, METTL3 overexpression induced the increase in IC50 value in cisplatin-resistant OC cells, and it also relieved the inhibitory effect of si-RHPN1-AS1 on IC50 value (Figure 7A). The transwell migration and invasion assays proved that METTL3 overexpression enhanced cell migration and invasion,

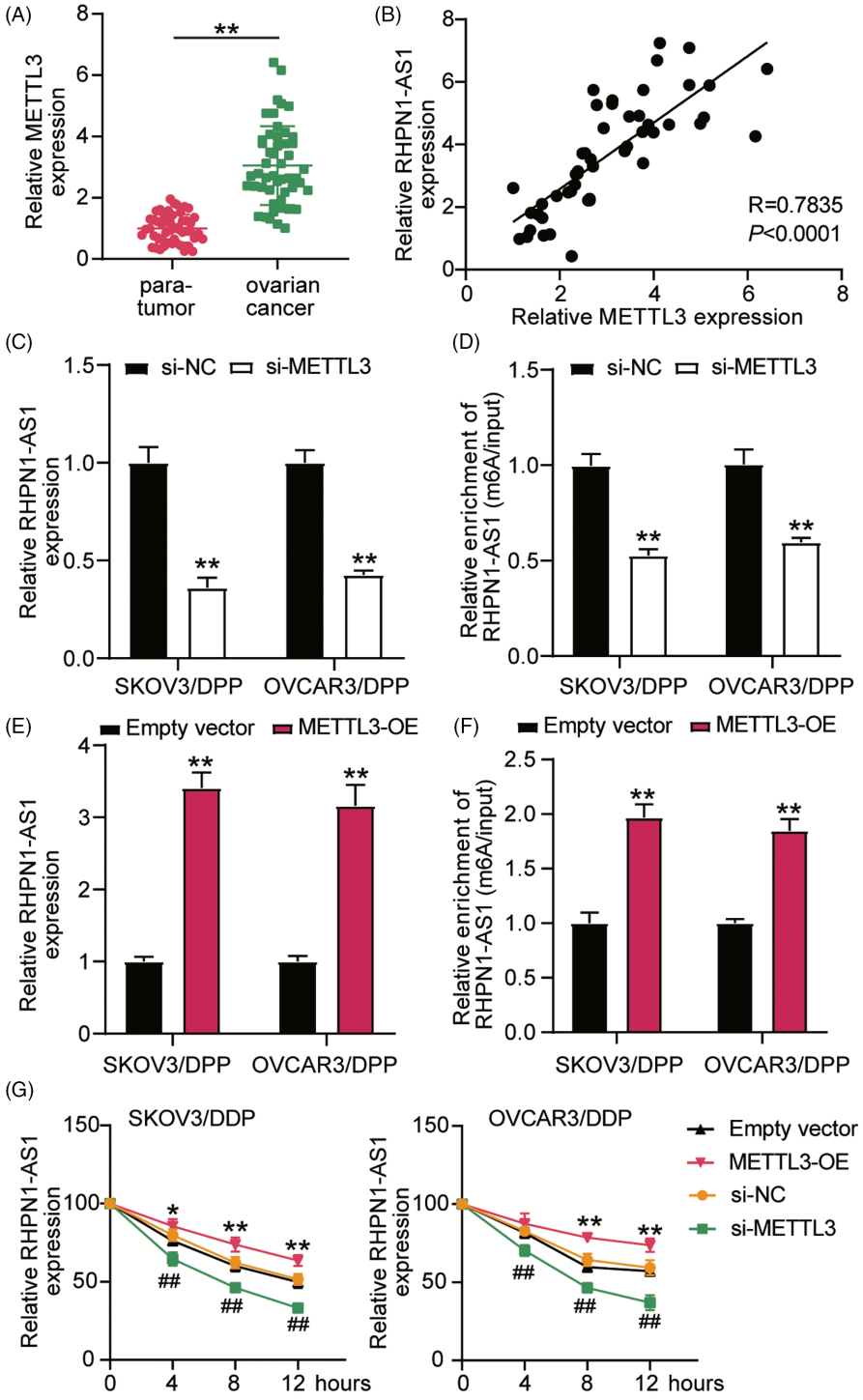


FIGURE 6 METTL3 enhanced the stability of RHPN1-AS1 by m6A modification in cisplatin-resistant OC cells. (A) qRT-PCR confirmed the upregulation of METTL3 in OC tissues compared with para-tumor tissues. $^{**}p<0.001$. (B) The positive correlation between METTL3 and RHPN1-AS1 in OC tissues. (C) METTL3 knockdown reduced RHPN1-AS1 expression in cisplatin-resistant OC cells. $^{**}p<0.001$. (D) MeRIP assay identified the m6A modification of RHPN1-AS1 in cisplatin-resistant OC cells with si-METTL3. $^{**}p<0.001$. (E) METTL3 overexpression enhanced RHPN1-AS1 expression in cisplatin-resistant OC cells. $^{**}p<0.001$. (F) MeRIP assay identified the m6A modification of RHPN1-AS1 in cisplatin-resistant OC cells with METTL3 overexpression transfection. $^{**}p<0.001$. (G) Actinomycin D (5 μg/ml) was used to confirm the stability of RHPN1-AS1 in cisplatin-resistant OC cells with si-METTL3 or METTL3 overexpression transfection. $^{*}p<0.05$, $^{**}p<0.001$ vs. empty vector; $^{\#\#}p<0.001$ vs. si-NC.

as well as the decrease of cell migration and invasion caused by si-RHPN1-AS1 was recovered by co-transfection of METTL3 overexpression in cisplatin-resistant OC cells (Figure 7B,C). These results revealed that METTL3 overexpression could offset the negative effect of si-RHPN1-AS1 on cisplatin-resistant OC cells.

4 | DISCUSSION

Cisplatin chemotherapy is a commonly used methods for OC therapy, but cisplatin resistance affects the therapeutic effect of OC.[38,39] A lot of studies have revealed that lncRNAs could act as the crucial roles in cisplatin resistance of OC. For instance, lncRNA UCA1 with high expression in OC could alleviate cisplatin-induced cell apoptosis and promote cell proliferation, thereby enhancing cisplatin resistance in OC.[40] This study revealed that RHPN1-AS1 was upregulated in OC with cisplatin resistance, as well as RHPN1-AS1 overexpression accelerated cisplatin resistance of OC cells. Besides, the further study verfied that RHPN1-AS1 aggravated the malignancy of cisplatin-resistant OC cells via activating PI3K/AKT pathway. My findings suggested that RHPN1-AS1 might become a target for preventing cisplatin resistance of OC cells.

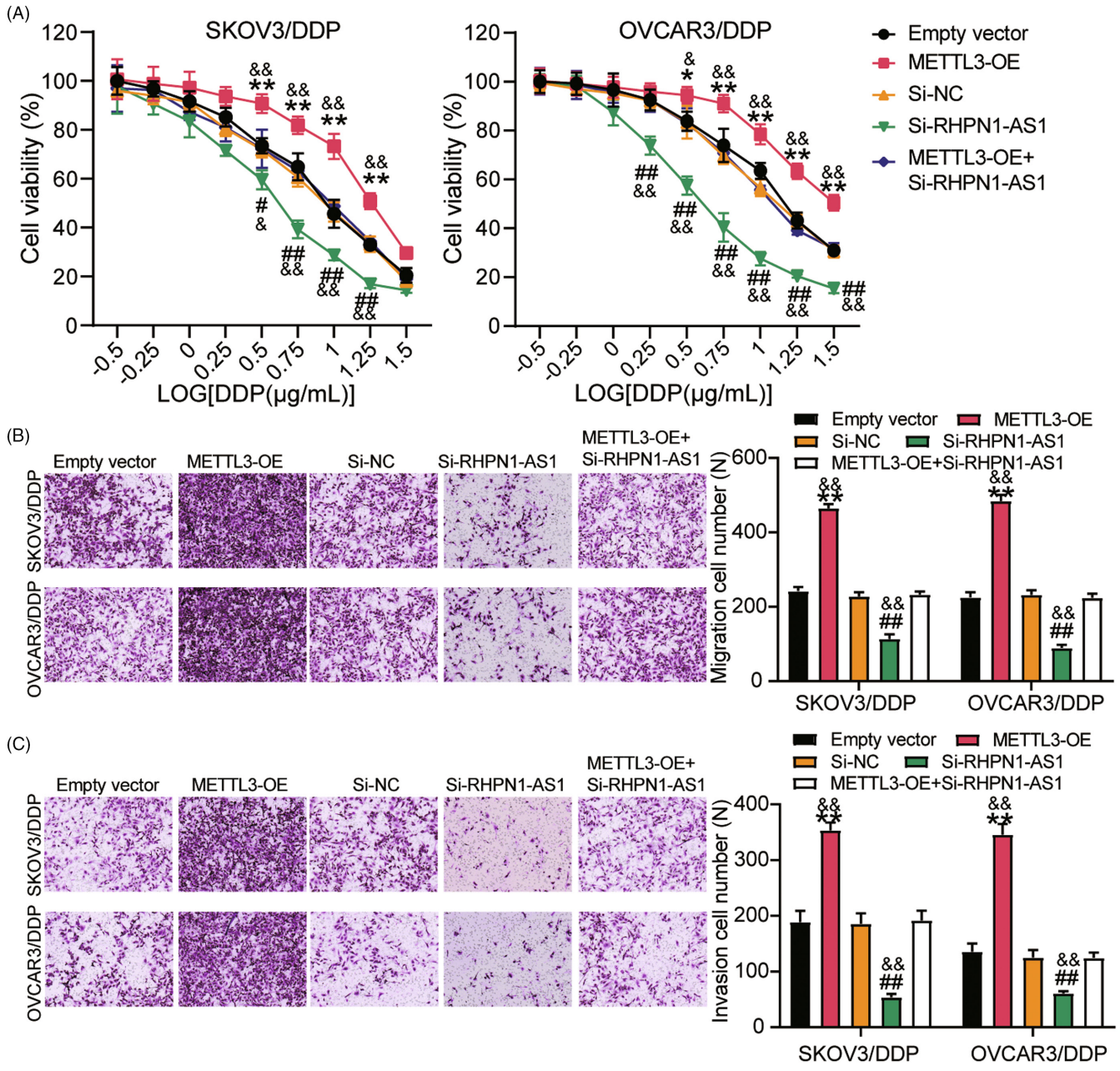


FIGURE 7 METTL3 overexpression recovered the inhibitory effect of si-RHPN1-AS1 on cisplatin-resistant OC cells. (A) CCK8 assay proved that the effect of si-RHPN1-AS1 on IC50 value in cisplatin-resistant OC cells (SKOV3/DDP and OVCAR3/DDP) was relieved by METTL3 overexpression. (B, C) Transwell migration and invasion assays revealed that METTL3 overexpression offset the effect of si-RHPN1-AS1 on cell migration (B) and cell invasion (C) in cisplatin-resistant OC cells. $^{*}p<0.05$, $^{**}p<0.001$ vs. empty vector; $^{\#}p<0.05$, $^{\#\#}p<0.001$ vs. si-NC; $^{\&}p<0.05$, $^{\&\&}p<0.001$ vs. METTL3-OE + si + RHPN1-AS1.

The promotive role of RHPN1-AS1 in cancer has been widely explored.[41,42] For example, RHPN1-AS1 targeting miR-7-5p/OGT axis was able to elevate cell migration and invasion abilities in colorectal cancer.[43] RHPN1-AS1 promoted tumorigenesis of cervical cancer by modulating miR-299-3p/FGF2 axis.[44] In my previous studies, I proved the positive function of RHPN1-AS1 in OC progression by sponging miR-6884-5p/TOP2A axis[19] or miR-485-5p/TPX2 axis.[18] However, I did not explore the relationship between RHPN1-AS1 and clinical characteristics. Moreover, identifying the role of RHPN1-AS1 in OC progression is not enough to solve the therapeutic problems in OC. Therefore, I continued to explore the influence of RHPN1-AS1 on clinical characteristics and cisplatin resistance in OC, revealing that RHPN1-AS1 with high expression indicated the higher FIGO stage, Grade stage, and cisplatin resistance. RHPN1-AS1 overexpression enhanced cisplatin resistance of OC cells.

PI3K/AKT pathway has been confirmed as a key pathway in tumorigenesis including thyroid carcinoma,[45] prostate cancer,[46] and breast cancer.[47] In OC, many researches prove the oncogenic role of PI3K/AKT pathway in OC tumorigenesis and chemotherapy

resistance.[48-50] Deleted in malignant brain tumors 1 (DMBT1) overexpression induced the inactivation of PI3K/AKT pathway, thereby suppressing cisplatin resistance in OC.[51] Wogonin enhanced cisplatin sensitivity in OC by inhibition of PI3K/AKT pathway.[52] As for the correlation between RHPN1-AS1 and PI3K/AKT pathway, only one article reported that RHPN1-AS1 could activate PI3K/AKT pathway to promote the malignancy of hepatocellular carcinoma.[53] Whether RHPN1-AS1 was able to regulate PI3K/AKT pathway to affect cisplatin resistance had not been reported in OC. Here, I found that RHPN1-AS1 could elevate the levels of PI3K and phosphorylated AKT in cisplatin-resistant OC cells, meaning that the PI3K/AKT pathway was activated in cisplatin-resistant OC cells. Therefore, RHPN1-AS1 participated in cisplatin resistance of OC cells by activating PI3K/AKT pathway.

The m6A modification of lncRNA has aroused interest in recent years.[54-56] lncRNA PACERR interacting with m6A reader IGF2BP2 played the pro-tumor function in pancreatic ductal adenocarcinoma.[57] lncRNA XIST regulated by m6A writer METTL14 contributed to proliferation and metastasis of colorectal cancer.[58] METTL3 as a m6A writer could regulated lncRNA ABHD11-AS1 in lung cancer,[24] lncRNA FOXD2-AS1 in cervical cancer,[25] lncRNA SNHG7 in prostate cancer,[59] etc. Nevertheless, no study revealed the regulatory effect of METTL3 on lncRNA in OC, although the oncogenic function of METTL3 in OC has been confirmed.[28] Here, my study proved that METTL3 was upregulated in OC tissues, and positive correlated with RHPN1-AS1 in OC tissues. Further investigation confirmed that METTL3 enhanced the stability of RHPN1-AS1 by m6A modification in cisplatin-resistant OC cells.

My study confirmed that RHPN1-AS1 enhanced cisplatin resistance of OC cells by activating PI3K/AKT pathway. However, this study still has some limitations. Cisplatin resistance in vivo is complex, which involves many regulators. Therefore, the regulatory network of RHPN1-AS1 in vivo needs to further explore by collecting more clinical OC samples and constructing animal model. In addition, the oncogenic role of PI3K/AKT pathway in OC cells with cisplatin resistance is also complex. Therefore, the changes in key genes or proteins at the downstream of PI3K/AKT pathway needs to deeply explored in OC cells with cisplatin resistance.

5 | CONCLUSION

To sum up, I am the first to prove that METTL3-mediated m6A modification of RHPN1-AS1 can enhance cisplatin resistance of OC cells by activating PI3K/AKT pathway. My findings indicate that RHPN1-AS1 may act as a target for solving cisplatin resistance of OC therapy.

AUTHOR CONTRIBUTIONS

SBC designed this study, performed the experiments and data analysis, and drafted the article. The author agreed to be accountable for all aspects of this study.

ACKNOWLEDGMENT

None.

FUNDING INFORMATION

This study was supported by Projects of Medical and Health Technology Development Program in Shandong Province (no. 202105010886).

CONFLICT OF INTEREST

There is no conflict of interest declared by the author.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONSENT FOR PUBLICATION

Consent for publication was obtained from the participants.

ORCID

Shoubin Cui https://orcid.org/0000-0002-4331-5163

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How to cite this article: Cui S. METTL3-mediated m6A modification of lnc RNA RHPN1-AS1 enhances cisplatin resistance in ovarian cancer by activating PI3K/AKT pathway. *J Clin Lab Anal*. 2022;36:e24761.
doi: 10.1002/jcla.24761