

Smad7 drives cisplatin resistance in ovarian cancer via a PRMT5-dependent mechanism

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ABSTRACT

Acquired cisplatin resistance poses a major challenge in ovarian cancer management. This study investigated the role of protein arginine methyltransferase 5 (PRMT5) in this context. Using cisplatin-resistant ovarian cancer cell lines (A2780/DDP and SKOV3/DDP), we found that PRMT5 knockdown significantly inhibited cell proliferation, colony formation, migration, and invasion, while promoting apoptosis. Mechanistically, co-immunoprecipitation assays revealed that PRMT5, in complex with MEP50, interacts with and specifically methylates Smad7 at the R57 site in vitro. This methylation event was essential for activating the STAT3 signaling pathway and driving the observed malignant phenotypes. Consistent with a key oncogenic role for Smad7, in vivo knockdown of Smad7 in a xenograft mouse model markedly suppressed tumor growth and downregulated markers of proliferation (Ki67), invasion (MMP9, N-cadherin), while upregulating the tumor suppressor E-cadherin. In conclusion, our work identifies Smad7 as a critical driver of cisplatin resistance in vivo and delineates a novel in vitro mechanism whereby PRMT5 promotes oncogenic signaling through R57 methylation of Smad7. This PRMT5-Smad7 axis presents a promising therapeutic target for overcoming cisplatin resistance in ovarian cancer.

1. Introduction

Ovarian cancer is the eighth most common malignant tumor in women worldwide, accounting for 3.7 % of morbidity and 4.7 % of cancer-related mortality in 2020 (Webb and Jordan, 2024). The initial symptoms of ovarian cancer are vague and imperceptible, resulting in difficulty in early diagnosis (Thull and Kempton, 2024). Thus, more than 50 % of patients are diagnosed at an advanced stage of ovarian cancer, and the 5-year overall survival rate is approximately 45 % in patients with ovarian cancer (Thull and Kempton, 2024; Morand et al., 2021). Debulking surgery and platinum-taxane chemotherapy are the standard first-line treatments, which are effective in most patients (Morand et al., 2021). However, most patients still experience recurrence and metastasis, even after receiving standard treatment (Wang et al., 2024a). Acquired drug resistance remains a challenge in the management of ovarian cancer.

Cisplatin resistance is common and contributes to the increased mortality of ovarian cancer (Yang et al., 2022). The mechanisms are complex and include various biological dysregulations of cancer-related signaling pathways, such as DNA methylation, epigenetic alterations,

cell metabolism, oxidative stress, and histone modifications (Wang et al., 2024b). In the past decade, many discussions have been held to overcome cisplatin resistance (Song et al., 2022). Novel strategies have been proposed, and new therapeutic targets have been identified to facilitate new drug discovery to overcome cisplatin resistance and improve the prognosis of patients with ovarian cancer (Kapper et al., 2024; Schweer et al., 2021).

Protein arginine methyltransferases (PRMTs) are a family of enzymes that catalyze arginine methylation (Hwang et al., 2021). To date, nine PRMTs have been discovered, and their biological functions include cell cycle regulation, DNA damage response, transcription regulation, and histone modifications (Hwang et al., 2021), indicating that PRMTs are promising targets for cancer therapy. In ovarian cancer, PRMT1 promotes tumor growth through the methylation and phosphorylation of bromodomain-containing protein 4 (Liu et al., 2023). Inhibition of PRMT1 prevented clonal growth of ovarian cancer cells at low doses of cisplatin, indicating that PRMT1 inhibition could overcome cisplatin resistance in ovarian cancer (Musiani et al., 2020). PRMT5 is overexpressed and associated with poor prognosis in epithelial ovarian cancer (Bao et al., 2013). Inhibition of PRMT5 promotes DNA damage and

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increases the antitumor activity of niraparib in breast and ovarian cancers (O'Brien et al., 2023; Huang et al., 2022). However, the role of PRMT5 in cisplatin-resistant ovarian cancer remains unknown. This study explored the mechanism of PRMT5 in cisplatin-resistant ovarian cancer to improve the prognosis of patients with ovarian cancer.

2. Methods

2.1. Cell culture and treatment

Human cisplatin-resistant ovarian cancer cell lines (A2780/DDP and SKOV3/DDP) were provided by Xiamen Immocell Biotechnology Co., Ltd. (Immocell, China). The cells were cultured using Dulbecco's Modified Eagle's medium (DMEM, Sigma-Aldrich) containing 10 % fetal bovine serum (FBS, Biosharp), 1 % Penicillin-Streptomycin Solution (100 ×, Procell, China), and 0.5 µg/ml cisplatin (Beyotime, China) at 37°C with 5 % CO₂.

The short hairpin RNA of PRMT5 (sh-PRMT5), Smad7 (sh-Smad7), and its negative control (sh-NC), and sh-Smad7, and the plasmids containing the coding sequence of Smad7 (oe-Smad7) were provided by Guangzhou Ribobio Biotechnology Co., Ltd. (Ribobio, China). Cell transfection was performed using the Hieff Trans® Universal Transfection Reagent (Yeasen, China) following the manufacturer's instructions. Cells were typically harvested for subsequent analyses at 48 h post-transfection unless otherwise specified.

2.2. In vivo xenograft tumor model

An in vivo xenograft tumor model was constructed in female BALB/c nude mice (Cyagen, China) aged 9–10 weeks old. A2780/DDP cells were transfected with sh-Smad7 or its NC. After 24 h of transfection, 5×10^6 transfected cells/mouse were subcutaneously injected into the abdomen of each mouse (n = 6 mice/group). The mice were raised with standard animal food (Biopike, China) and drinking water under a 12 h light-dark cycle at $23 \pm 2^\circ\text{C}$ and $40 \pm 5\%$ humidity for 4 weeks. The mice were euthanized with an overdose of KCl (1 mM/kg). The tumors were dissected, and their volume and weight were recorded. All animal experiments were executed in accordance with the Care and Use of Laboratory Animals published by the US National Institutes of Health (Eighth Edition, 2011) (National Research Council Committee for the Update of the Guide for the, C. and A. Use of Laboratory, 2011).

2.3. Cell viability measurement

Cell viability was examined using the Cell Counting Kit-8 (CCK-8; Beyotime, China). A2780/DDP or SKOV3/DDP cells (100 µL/well; 30 cells/µl) were cultured in a 96-well plate and treated according to the protocol. The CCK-8 assay was performed at 48 h after the initiation of treatment or transfection to generate growth curves. After treatment, 10 µL/well of CCK-8 solution was added to each well, and the cells were cultured for 2 h at 37°C. Optical density was measured at 450 nm using a SpectraMax Paradigm microplate reader (Molecular Devices, China).

2.4. Flow cytometry

At 48 h post-transfection or after the indicated drug treatment duration, the cells were detached using Gibco Trypsin-EDTA (Thermo Fisher) and resuspended in Phosphate-Buffered Saline (PBS; Procell, China). The cell suspension was stained using the Annexin V-FITC/PI Apoptosis Detection Kit (MeilunBio, China). A total of 10,000 cells were collected for apoptosis measurement using an Attune NxT Flow Cytometer (Thermo Fisher). The results were analyzed using the FlowJo software.

2.5. Colony formation assay

The cell culture medium was pre-mixed with soft agar (Yuduo Bio, China). The treated cells were cultured in BeyoGold™ 60 mm Cell Culture Dishes (Beyotime). Cells were cultured for 14 days to allow colony formation, with the medium refreshed every 3 days. The cells were fixed using Immunostaining Permeabilization Buffer with Triton X-100 (Beyotime) and stained with Crystal Violet Staining Solution (Beyotime). The number of cell colonies were counted under an inverted microscope (Keyence, China).

2.6. Transwell assay

For cell migration measurements, FBS-free MEM and FBS-supplemented MEM were added to the upper and lower layers of Transwell chambers (Corning, USA), respectively. A total of 5×10^4 treated cells were seeded in the upper chamber and allowed to migrate for 24 h. Non-adherent cells were removed. The cells were fixed using Immunostaining Permeabilization Buffer with Triton X-100 and stained with Crystal Violet Staining Solution. The stained cells were observed and recorded using an inverted microscope.

For cell invasion measurement, the upper layer of the Transwell chambers was pre-coated with Matrigel matrix (Beyotime). A total of 1×10^5 treated cells were seeded and allowed to invade for 48 h. The protocols were the same as those used for the cell migration assay.

2.7. Quantitative real-time polymerase chain reaction (qRT-PCR)

At 48 h post-transfection, total RNAs were extracted using GenElute™ Total RNA Extraction Kit (Sigma-Aldrich) and the concentration was measured using Qubit Fluorometers (Thermo Fisher).

cDNA synthesis was performed using the QuantiTect Reverse Transcription Kit (QIAGEN, Germany). The synthesized cDNAs were amplified using the BeyoFast™ SYBR Green One-Step qRT-PCR Kit (Beyotime) through CFX Real-Time PCR Detection Systems (BioRad, USA). The relative expression of RNA was quantified using the $2^{-\Delta\Delta\text{CT}}$ method. The primers (Ribobio) used in this study were as follows: PRMT5, forward 5'-CTGTCTCCATCCGCGTTTCA-3' and reverse 5'-GCAGTAGGCTC-GATCGTGTCTG-3'; GAPDH, forward 5'-ACAACTTTGGTATCGTG-GAAGG-3' and reverse 5'-GCCATCAGCCACAGTTTC-3'.

2.8. Western blotting

At 48 h post-transfection or after the indicated treatment, total proteins were extracted using RIPA lysis buffer (Yeasen), and the protein concentration was measured using a BCA Protein Assay Kit (Beyotime). Protein (10 µg) was separated using BeyoGel™ Plus Precast PAGE Gel for Tris-Gly System (Beyotime) and then transferred from the gel to PVDF membranes (BioRad). The membranes were blocked with StartingBlock™ Blocking Buffer (Thermo Fisher) for 15 min at room temperature. The membranes were probed with primary antibodies at 4°C overnight and then incubated with secondary antibodies for 1 h at room temperature. Clarity™ Western ECL Substrate (BioRad) was added to the membranes to visualize the probed proteins. The results were imaged using an iBright Imaging System (Thermo Fisher). The primary antibodies (Cell Signaling, USA) used were: PRMT5 (1:2000, #2252), GAPDH (1:5000, #2118), E-cadherin (1:500, #3195), N-cadherin (1:500, #3195), MMP9 (1:2000, #13667), Smad7 (1:1000, #25840-1-AP, Proteintech, China), p-STAT (1:2000, #9914), STAT (1:1000, #9939), MEK50 (1:2000, #2018) and Myc-Tag (1:1000, #2278). The secondary antibody used was an anti-rabbit IgG antibody (1:1000, #7074).

2.9. Immunoprecipitation (Co-IP)

The magnetic beads were pre-coated with protein A for rabbit IgG or

Protein G for mouse IgG (Beyotime). The extracted proteins were incubated with pre-coated magnetic beads for 20 min at room temperature to pull down the interacting proteins. The beads were gathered by centrifugation and heated at 95°C for 5 min. The proteins were eluted from the beads using Pierce™ IgG Elution Buffer (Thermo Fisher). The samples were analyzed using western blotting. Four groups were used to investigate the interaction between PRMT5 and Smad7: 1) HA-PRMT5, 2) FLAG-Smad7, 3) HA-PRMT5 + FLAG-Smad7, 4) HA-PRMT5 + FLAG-Smad7 + MYC-MEP50. To validate the interaction between PRMT5 and Smad7, vectors containing the full sequence of Smad7 (WT) and the R57k mutant sequence of Smad7 were transfected into A2780/DDP cells for Co-IP analysis.

2.10. Immunohistochemistry (IHC)

Tumor tissues were isolated from the abdomen of mice and fixed with 4 % Paraformaldehyde Fix Solution (Beyotime). The fixed tissues were embedded in paraffin wax (Merck KGaA, Darmstadt, Germany) and cut into 5 µm sections for further experiments. The paraffin sections were deparaffinized and dehydrated using xylene. The sections were boiled for 20 min using Antigen Retrieval Buffer (Abcam, UK) to retrieve the antigen and blocked using Immunostaining Blocking Buffer (Yeasten) for 1 h at room temperature. Primary antibodies were used to probe the target proteins at 4°C overnight. HRP-conjugated secondary antibodies and a DAB Peroxidase Substrate Kit for IHC (Yeasten) were used to visualize the target proteins. The proteins were visualized and photographed using an inverted microscope.

2.11. Statistical analysis

Data are shown as mean ± standard deviation (SD). Statistical analyses were performed using IBM SPSS Statistics V22.0 (IBM, USA). Student's *t*-test was used to analyze the statistical differences between the two groups. One-factor analysis of variance (one-way ANOVA) was performed to analyze the statistical differences between more than two groups. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Knockdown of PRMT5 inhibited cell proliferation in cisplatin-resistant ovarian cancer cells

PRMT5 is overexpressed in cisplatin-resistant ovarian cancer cells and drives their malignant phenotype. Western blot analysis confirmed that PRMT5 protein levels were significantly higher in cisplatin-resistant cell lines (A2780/DDP, SKOV3/DDP) compared to their sensitive parental counterparts (A2780, SKOV3) (Fig S1A). Given this overexpression, we next asked whether PRMT5 is functionally essential. Knockdown of PRMT5 (sh-PRMT5) successfully reduced its expression in the resistant cells (Figs. 1A and 1B). this knockdown profoundly sensitized the cells to cisplatin, lowering the IC50 values from 11.60 µM to 3.457 µM (A2780/DDP) and from 25.16 µM to 9.838 µM (SKOV3/DDP). Conversely, PRMT5 overexpression increased resistance (Fig S1B), establishing PRMT5 as a key determinant of cisplatin resistance. Beyond chemosensitivity, PRMT5 was essential for core oncogenic phenotypes: its knockdown significantly reduced cell viability (Fig. 1C) and colony-forming capacity (Fig. 1D), while promoting apoptosis (Fig. 1E). Collectively, these results demonstrate that the elevated PRMT5 in resistant cells is not a passive marker but an active driver of both cisplatin resistance and proliferative survival.

3.2. Knockdown of PRMT5 prevented cell migration and invasion in cisplatin-resistant ovarian cancer cells

The number of migrated and invasive cells was reduced by PRMT5 knockdown in A2780/DDP and SKOV3/DDP cells (Figs. 2A and 2B). E-

cadherin was overexpressed by sh-PRMT5 in A2780/DDP and SKOV3/DDP cells (Fig. 2C). The protein expression of N-cadherin and matrix metalloproteinase-9 (MMP9) was suppressed by the downregulation of PRMT5 in A2780/DDP and SKOV3/DDP cells (Fig. 2C). These results indicate that PRMT5 promotes cell migration and invasion in cisplatin-resistant ovarian cancer cells.

3.3. PRMT5 mediated arginine methylation of Smad7 on R57 site in cisplatin-resistant ovarian cancer cells

The expression of Smad7 and phosphorylated signal transducer and activator of transcription (STAT) was inhibited in A2780/DDP and SKOV3/DDP cells transfected with sh-PRMT5 compared to those transfected with sh-NC (Fig. 3A). Co-immunoprecipitation (Co-IP) revealed that the interaction between PRMT5 and Smad7 strictly required the adaptor protein MEP50 (Fig. 3B). To definitively establish the enzymatic mechanism, we performed critical rescue and methylation-dependency experiments. To definitively establish the enzymatic mechanism, we performed critical rescue and methylation-dependency experiments. Rescue experiments confirmed the specificity of PRMT5 knockdown phenotypes, as re-expression of a wild-type, shRNA-resistant PRMT5 (PRMT5r), but not a catalytically dead mutant (PRMT5r G367A/R368A), restored Smad7 and downstream signaling levels (Fig S1C). To confirm the enzymatic dependence, we used a catalytic-dead PRMT5 mutant (PRMT5-G367A/R368A). In a streptavidin pull-down assay, methylation of Smad7 (SDMA signal) occurred only with wild-type PRMT5, and was completely abolished with the PRMT5 mutant, despite equal binding (Fig. S1D). This proves that Smad7 methylation is directly catalyzed by PRMT5. Consistently, the PRMT5-MEP50 complex showed stronger interaction and arginine methylation with wild-type Smad7 than with the R57K mutant (Fig. 3C). Functionally, Smad7 overexpression enhanced STAT phosphorylation, while its knockdown repressed it (Fig. 3D). Collectively, these data conclusively demonstrate that PRMT5, in complex with MEP50, directly and enzymatically methylates Smad7 at the R57 site, and that this specific modification is essential for downstream STAT pathway activation in cisplatin-resistant ovarian cancer cells.

3.4. Arginine methylation of SMAD7 promoted proliferation and migration in cisplatin-resistant ovarian cancer cells

Cell viability was increased by oe-Smad7 and decreased by sh-Smad7 in A2780/DDP and SKOV3/DDP cells (Fig. 4A). The number of cell colonies increased by oe-Smad7 and decreased by sh-Smad7 in A2780/DDP and SKOV3/DDP cells (Fig. 4B). Cell migration and invasion were promoted by Smad7 overexpression and prevented by Smad7 knockdown in A2780/DDP and SKOV3/DDP cells (Fig. 4C). Cell apoptosis was inhibited by overexpression of Smad7 and accelerated by knockdown of Smad7 in A2780/DDP and SKOV3/DDP cells (Fig. 4D). Thus, arginine methylation of SMAD7 promotes cell proliferation and migration in cisplatin-resistant ovarian cancer cells.

3.5. Knockdown of Smad7 prevented tumor progression in cisplatin-resistant ovarian cancer

Tumor growth was significantly suppressed by targeting the PRMT5-Smad7 axis in vivo. Knockdown of Smad7 reduced tumor volume and weight in a xenograft model (Fig. 5A). To directly assess the therapeutic potential of targeting the upstream regulator PRMT5, we extended our in vivo validation in two key ways. First, genetic knockdown of PRMT5 itself also demonstrated potent antitumor efficacy (Fig S1E). Second, and more translatable, treatment with the selective PRMT5 inhibitor DW14761 (60 mg/kg) similarly resulted in marked suppression of tumor progression (Fig S1E). At the molecular level, both genetic perturbation (sh-Smad7) and pharmacological inhibition (DW14761) led to consistent phenotypic changes: upregulation of the tumor suppressor E-

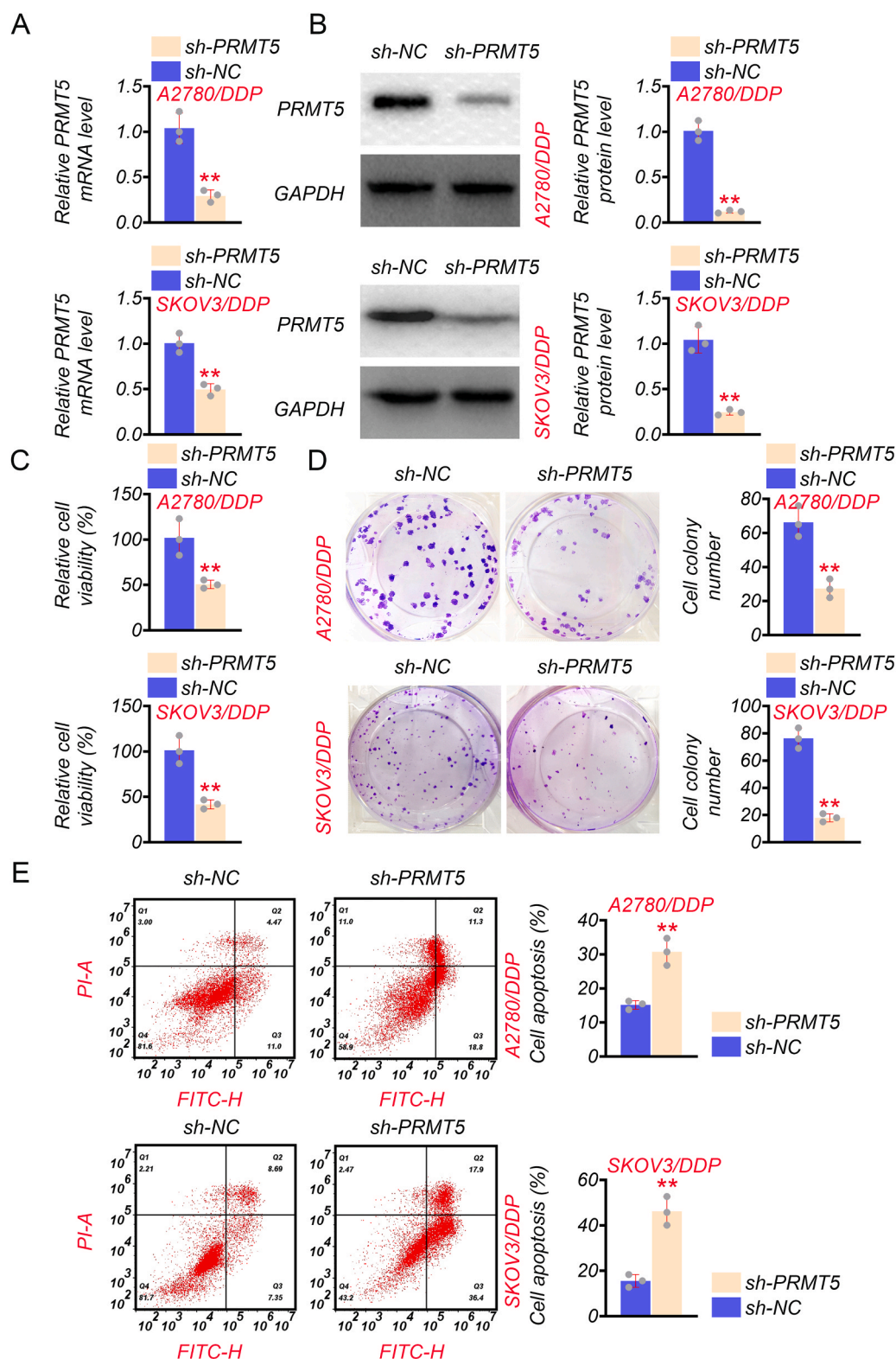


Fig. 1. Knockdown of PRMT5 inhibited cell proliferation in cisplatin-resistant ovarian cancer cells. (A) PRMT5 mRNA levels were measured by qRT-PCR 48 h post-transfection with sh-PRMT5 or sh-NC. (B) PRMT5 protein levels were analyzed by Western blot 48 h post-transfection. GAPDH: loading control. (C) Cell viability was assessed by CCK-8 assay at 24 h post-transfection (n = 3, with 5 replicates each). (D) Colony formation was assessed 14 days post-transfection. (E) Apoptosis was analyzed by Annexin V/PI staining and flow cytometry 48 h post-transfection. Data: n = 3, mean \pm SD. $p < 0.01$ vs. sh-NC (two-tailed unpaired *t*-test). PRMT5, protein arginine methyltransferase 5; sh-PRMT5, short hairpin RNA of PRMT5; sh-NC, negative control of sh-PRMT5.

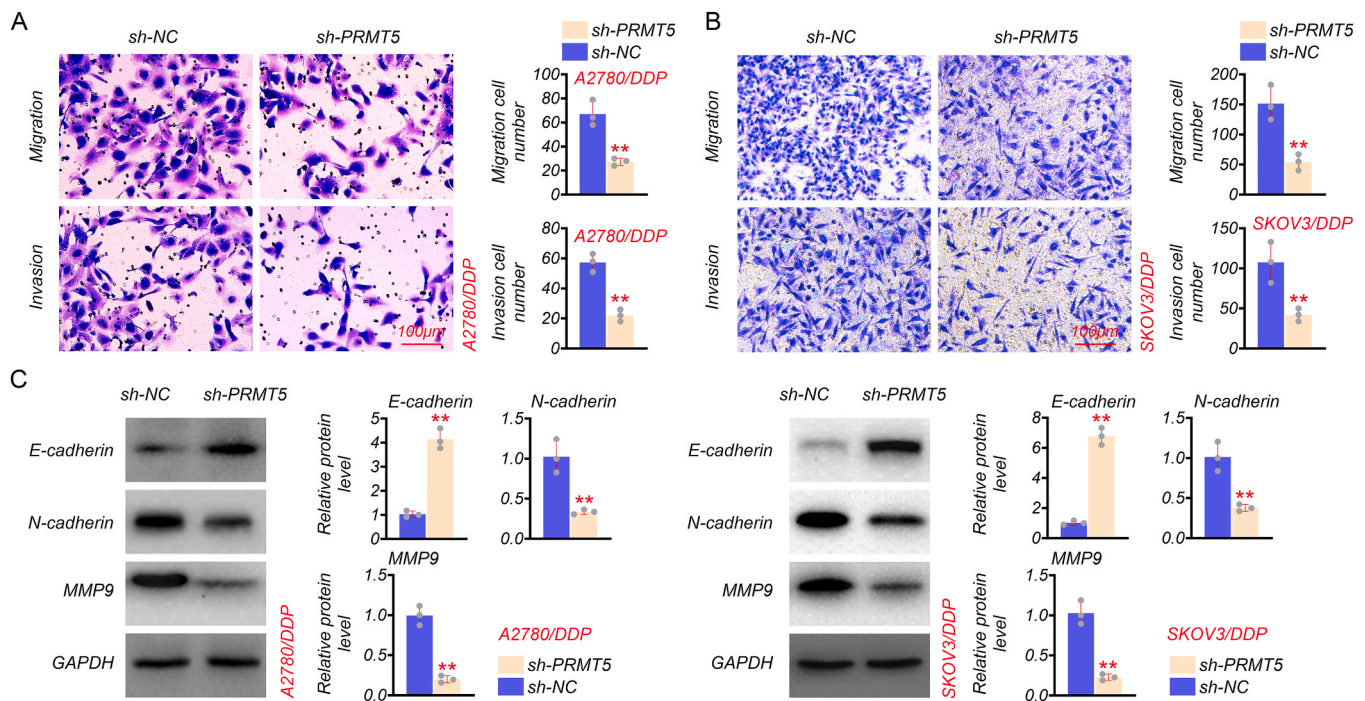


Fig. 2. Knockdown of PRMT5 prevented cell migration and invasion in cisplatin-resistant ovarian cancer cells. (A, B) Transwell migration (24 h) and invasion (48 h, Matrigel-coated) assays in A2780/DDP (A) and SKOV3/DDP (B) cells post-transfection. Quantification of migrated/invaded cells is shown. (C) Protein levels of E-cadherin, N-cadherin, and MMP9 were analyzed by Western blot 48 h post-transfection. Data: $n = 3$, mean \pm SD. $p < 0.01$ vs. sh-NC (two-tailed unpaired t -test). MMP9: matrix metalloproteinase-9.

cadherin and downregulation of the proliferation and invasion markers Ki67, MMP9, and N-cadherin in tumor tissues (Fig. 5B). These complementary genetic and pharmacological data converge to strongly suggest that the PRMT5-Smad7 axis accelerates tumor progression in cisplatin-resistant ovarian cancer and represents a promising therapeutic target for intervention.

4. Discussion

As mentioned above, PRMT5 is a member of the arginine methyltransferase family and is highly expressed in many tumors, such as colon cancer, breast cancer, melanoma, and lung cancer (Kim and Ronai, 2020). As an oncogene, PRMT5 overexpression has been reported to promote tumor growth and is associated with poor prognosis in ovarian cancer (Xie et al., 2023). In this study, PRMT5 knockdown significantly inhibited the proliferation, migration, and invasion of cisplatin-resistant ovarian cancer cells. Mechanistic experiments revealed that PRMT5 mediates arginine methylation of Smad7, enhancing cell growth and mobility in cisplatin-resistant ovarian cancer cells. *In vivo* experiments demonstrated that downregulation of Smad7 significantly suppressed tumor progression, reinforcing the critical role of PRMT5 in ovarian cancer. Thus, targeting PRMT5 may offer a promising therapeutic strategy for improving treatment outcomes in patients with cisplatin-resistant ovarian cancer. The development of PRMT5 inhibitors could provide additional clinical benefits for cisplatin-resistant ovarian cancer cells.

E. cadherin is downregulated in tumors and acts as a tumor suppressor (Semb and Christofori, 1998). N-cadherin is overexpressed in the most invasive cancer cells (Hazan et al., 2004). MMP9 is a biosensor of tumor invasion and metastasis in several cancers (Huang, 2018). In this study, sh-PRMT5 upregulated E-cadherin and downregulated N-cadherin and MMP9, indicating that PRMT5 promotes tumor progression, including tumor growth, invasion, and metastasis, which is consistent with previous findings (Shailesh et al., 2018).

Therefore, PRMT5 is a potential therapeutic target for ovarian cancer, and the inhibition of PRMT5 could prevent tumor growth and metastasis, offering a new treatment option for patients with cisplatin-resistant ovarian cancer.

Smad7 is a critical regulator of the transforming growth factor β (TGF- β) signaling pathway and positively contributes to malignancy development in various organs (Christner and Jimenez, 2004; Luo et al., 2014). Methylosome protein 50 (MEP50) is a critical cofactor of PRMT5 that enhances the histone methyltransferase ability of PRMT5 and increases its affinity for protein substrates by directly binding to PRMT5 (Stopa et al., 2015). PRMT5-MEP50 complex has been reported to be involved in the regulation of gene expression and epithelial-to-mesenchymal transition (EMT) and is highly correlated with poor outcomes in many cancers (Chen et al., 2017). A previous study identified R57 as the methylation site on Smad7 by the PRMT5-MEP50 complex and linked this modification to STAT3 activation in lung cancer (Cai et al., 2021). Our work significantly extends these findings by defining a distinct and therapeutically critical role for this identical molecular mechanism in a different malignancy. We demonstrate that in ovarian cancer, PRMT5-mediated methylation of Smad7 at the known R57 site is specifically co-opted to drive cisplatin resistance—a phenotype of paramount clinical importance that was not explored in the lung cancer context. Thus, while the core biochemical event is conserved, its biological consequence is context-dependent: it promotes general tumor progression in lung cancer but confers lethal therapy resistance in ovarian cancer. This recontextualization of the PRMT5-Smad7-STAT3 axis not only confirms its relevance across cancer types but also reveals its direct implication in treatment failure, thereby offering a novel therapeutic rationale for targeting this pathway to overcome chemoresistance in ovarian cancer.

The *in vivo* xenograft tumor model is a useful tool for validating the findings of *in vitro* cell experiments (Yoshida, 2020). The results of this study confirmed that tumor size and weight were reduced by the inhibition of Smad7, indicating that Smad7 could promote tumor growth in

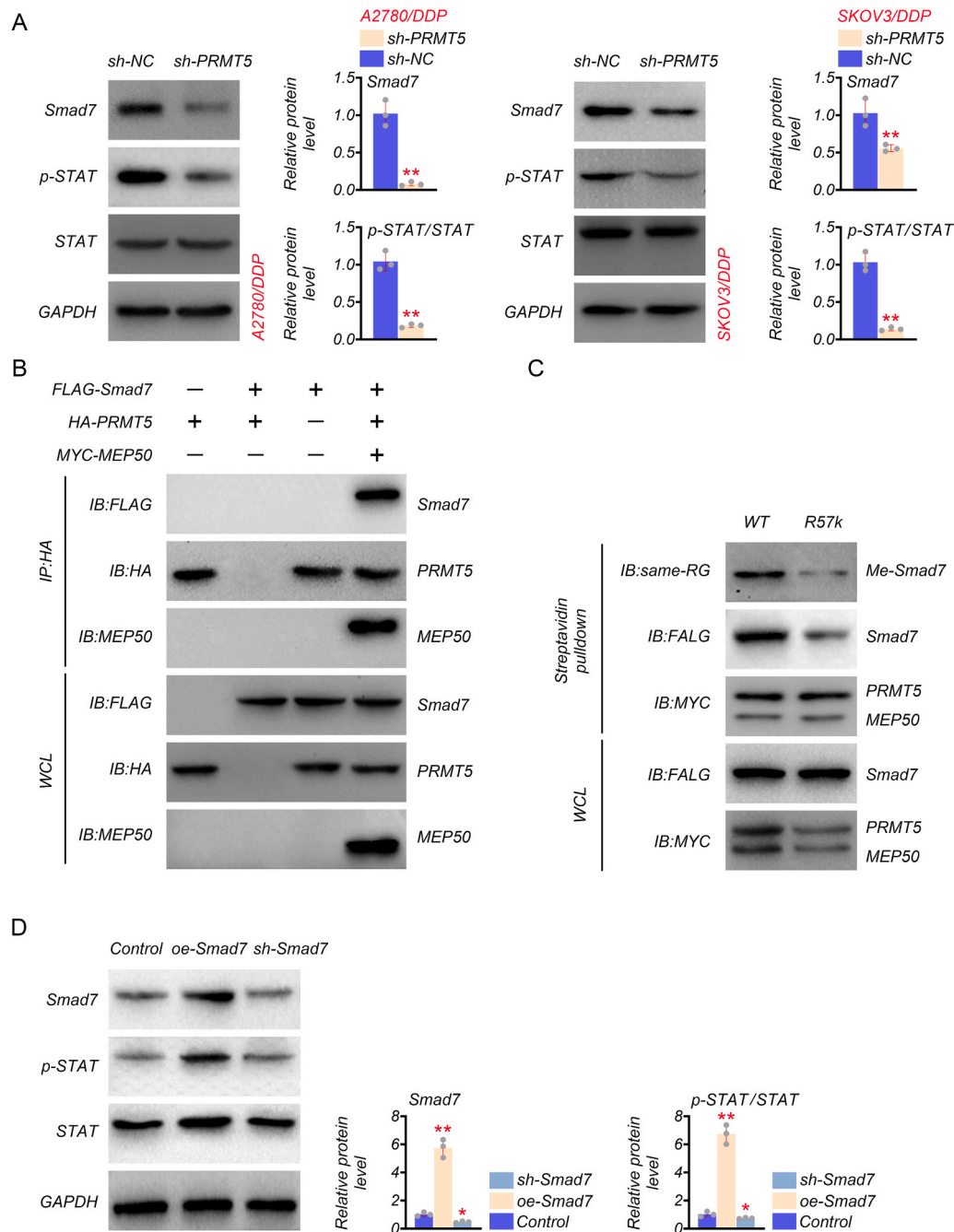
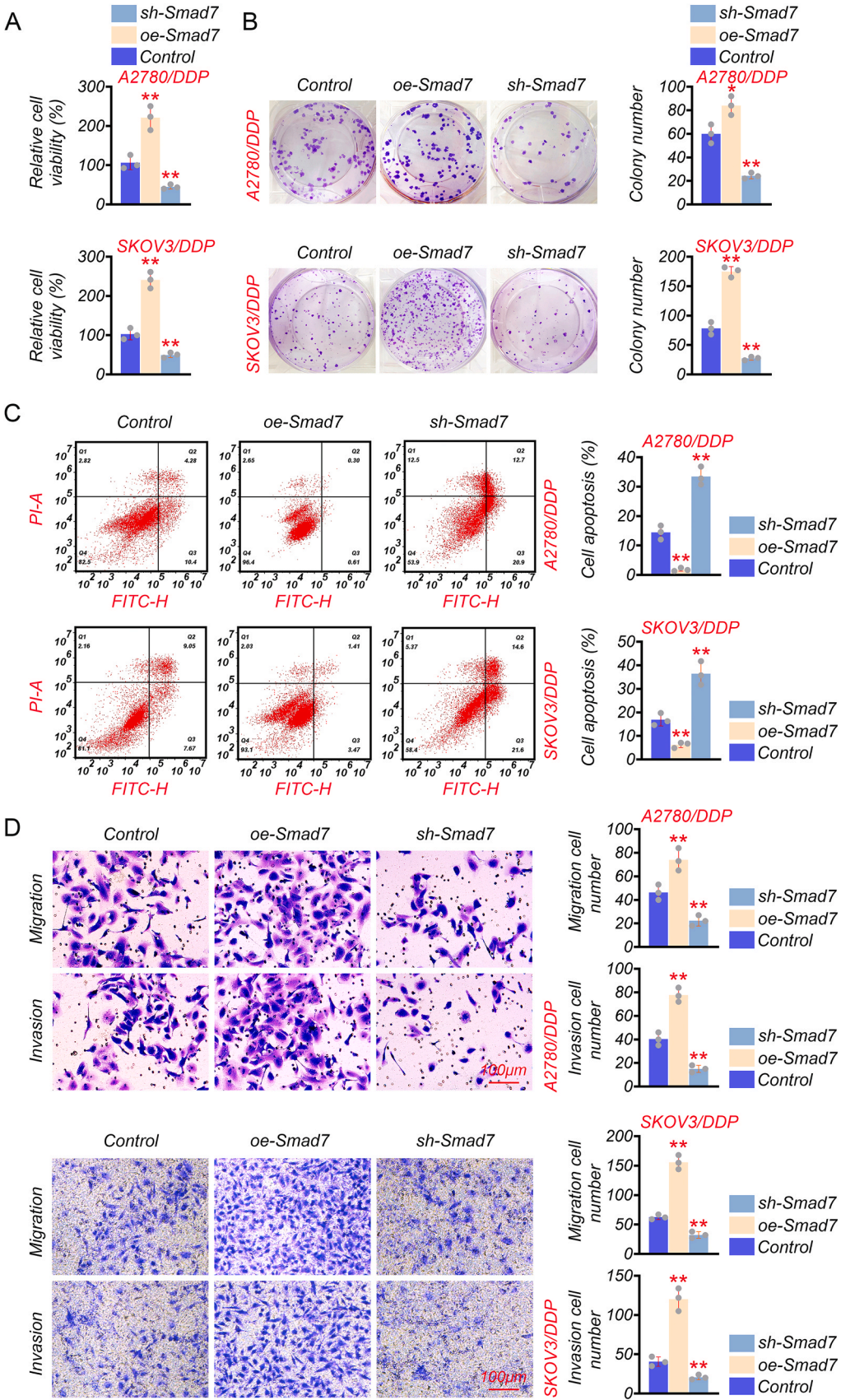


Fig. 3. PRMT5 mediated arginine methylation of Smad7 in cisplatin-resistant ovarian cancer cells. (A) Protein levels of Smad7, p-STAT3, and STAT3 were analyzed by Western blot 48 h post-transfection. (B) Co-immunoprecipitation (Co-IP) was performed 48 h post-transfection in A2780/DDP cells expressing Myc-tagged Smad7. Lysates were immunoprecipitated with anti-Myc antibody and blotted for indicated proteins. (C) PRMT5 methylated Smad7 at the R57 site in A2780/DDP cells. Co-IP of Flag-PRMT5 from A2780/DDP cells co-expressing Myc-Smad7 (WT or R57K). Immunoblots show that both Smad7 variants bind the complex, but methylation (SDMA) is detected only on the WT. (D) p-STAT3 and STAT3 levels were analyzed by Western blot 48 h post-transfection with oe-Smad7 or sh-Smad7. Data (A, D): $n = 3$, mean \pm SD. * $p < 0.05$, $p < 0.01$ vs. control or sh-NC (two-tailed unpaired t -test). MEP50, methylome protein 50; STAT, signal transducer and activator of transcription; oe-Smad7, plasmids containing the coding sequence of Smad7; sh-Smad7, short hairpin RNA of Smad7.

cisplatin-resistant ovarian cancer. Furthermore, tumor growth and the invasive and metastatic markers Ki67, N-cadherin, and MMP9 were downregulated in the sh-Smad7 group, implying that Smad7 promotes tumor metastasis in cisplatin-resistant ovarian cancer. *In vivo* translational validation of the molecular mechanisms would accelerate the discovery of new treatment approaches for overcoming drug resistance in ovarian cancer, thus improving patient outcomes.

This study has some limitations. While our *in vitro* data provide compelling evidence that PRMT5 drives chemoresistance by

methylating Smad7 at R57, the direct contribution of this specific methylation event to tumor progression *in vivo* remains to be fully established. Future studies employing xenograft models reconstituted with methylation-deficient Smad7 (R57K) or catalytically dead PRMT5 mutants will be crucial to confirm the causal role of this enzymatic modification in the tumor microenvironment. Nonetheless, the potent antitumor effects observed with both Smad7 knockdown and PRMT5 pharmacological inhibition strongly support the PRMT5-Smad7 axis as a viable therapeutic target for overcoming cisplatin resistance.



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Fig. 4. Arginine methylation of SMAD7 promotes proliferation and migration in cisplatin-resistant ovarian cancer cells. (A) Cell viability was increased by oe-Smad7 and decreased by sh-Smad7 in A2780/DDP and SKOV3/DDP cells. Cell viability was assessed by CCK-8 assay at 48 h post-transfection ($n = 3$, with 5 replicates each). (B) The number of cell colonies was increased by oe-Smad7 and decreased by sh-Smad7 in A2780/DDP and SKOV3/DDP cells. Colony formation assay was performed. Cells were cultured for 14 days post-transfection. (C) Cell apoptosis was reduced by oe-Smad7 and induced by sh-Smad7 in A2780/DDP and SKOV3/DDP cells. Apoptosis was analyzed by flow cytometry using Annexin V/PI staining 48 h post-transfection. (D) Cell migration and invasion were increased by oe-Smad7 and decreased by sh-Smad7 in A2780/DDP and SKOV3/DDP cells. Data: $n = 3$, mean \pm SD. $p < 0.01$ vs. sh-NC (one-way ANOVA). * $p < 0.05$ vs control, ** $p < 0.01$ vs control.

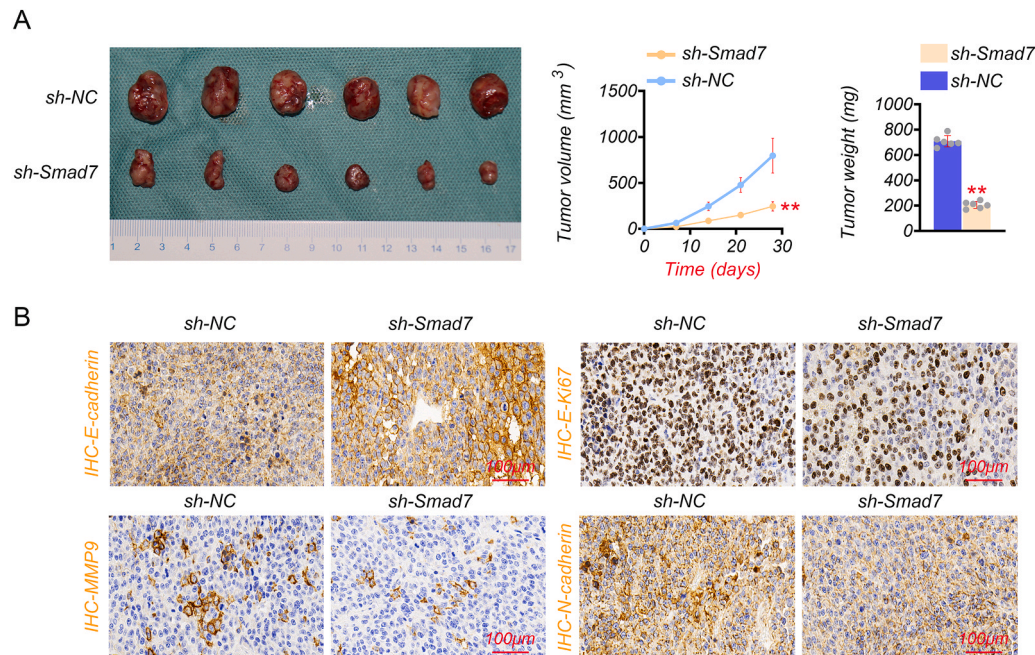


Fig. 5. Knockdown of Smad7 inhibits tumor progression in cisplatin-resistant ovarian cancer model. (A) Tumor growth curves (volume measured every 7 days) and final tumor weights 28 days post-injection of A2780/DDP cells stably expressing sh-Smad7 or sh-NC ($n = 6$ mice/group). (B) Representative immunohistochemistry (IHC) images and quantification of indicated markers (Ki67, MMP9, and N-cadherin) in tumor sections. Data: mean \pm SD. ** $p < 0.01$ vs sh-NC (two-tailed unpaired t-test).

In conclusion, our study establishes that PRMT5 is a key driver of malignancy in cisplatin-resistant ovarian cancer. In vitro, PRMT5 promotes cell proliferation, migration, and invasion. Mechanistically, this is achieved through its complex with MEP50, which catalyzes the arginine methylation of Smad7 at R57—a modification strictly dependent on PRMT5 enzymatic activity—leading to STAT3 activation. In vivo, the therapeutic relevance of this axis is strongly supported by two complementary findings: downregulation of Smad7 and pharmacological inhibition of PRMT5 both potently suppressed tumor progression. Collectively, our work delineates a novel methylation-dependent mechanism in vitro and validates the PRMT5-Smad7 signaling node as a promising therapeutic target for overcoming cisplatin resistance in ovarian cancer.

Ethics approval

Ethical approval was obtained from the Ethics Committee of Nantong First People's Hospital.

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CRediT authorship contribution statement

Yanli Zheng: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Yun Han:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Geshuyi Chen:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Kexin Wang:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Cong Wan:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Manman Zhao:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jinlong Ji:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Competing interests

The authors state that there are no conflicts of interest to disclose.

Contribution of authors

All authors contributed to the study conception and design. Material preparation and the experiments were performed by Jinlong Ji, Kexin Wang, Geshuyi Chen, Manman Zhao, Cong Wan. Data collection and analysis were performed by Jinlong Ji, Kexin Wang, Geshuyi Chen, Manman Zhao, Cong Wan. The first draft of the manuscript was written by Jinlong Ji, Yanli Zheng, Yun Han and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Fig S1 Supplementary validation of the PRMT5-Smad7 mechanism. (A) PRMT5 is upregulated in cisplatin-resistant ovarian cancer cells. Western blot assays of PRMT5 levels in cisplatin-sensitive (A2780, SKOV3) and -resistant (A2780/DDP, SKOV3/DDP) cell lines. (B) PRMT5 regulates cisplatin sensitivity. The half-maximal inhibitory concentration (IC₅₀) of cisplatin was determined in A2780/DDP and SKOV3/DDP cells following PRMT5 knockdown (sh-PRMT5). (C) Rescue of PRMT5 knockdown confirms specificity. Western blot of A2780/DDP cells treated as indicated. Knockdown of PRMT5 (sh-PRMT5) reduced Smad7 levels. This effect was rescued by re-expressing a wild-type, shRNA-resistant PRMT5 (PRMT5r-WT), but not by a catalytically dead mutant (PRMT5r-G367A/R368A). (D) Catalytic activity of PRMT5 is required for Smad7 methylation. Streptavidin pull-down assay. SFB-tagged wild-type (WT) or R57K mutant Smad7 was co-expressed with myc-tagged wild-type (WT) or catalytic-dead (G367A/R368A) PRMT5 and MEP50. Methylation (SDMA signal) was detected only when both WT Smad7 and WT PRMT5 were present (Lane 1). Input panels show expression levels. (E) Tumor growth in mice treated with the PRMT5 inhibitor DW14761 (60 mg/kg). Data are mean \pm SD, n = 6 per group. **p < 0.01 vs. A2780 or DMSO or shNC or shRNA (one-way ANOVA or two-tailed t-test). ###p < 0.01 vs. SKOV3 or shPRMT5 (one-way ANOVA). ~p < 0.01 vs. SKOV3 or shPRMT5 + PRMT5r (one-way ANOVA).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.tice.2025.103304](https://doi.org/10.1016/j.tice.2025.103304).

Data availability

Data will be made available on request.

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