

Original Article

LPAR6 promoter methylation is associated with platinum resistance in ovarian cancer: a translational study integrating bioinformatics and experimental validation

Hui Wang, Kai Tao

Gynecological Oncology Department, Shaanxi Provincial Tumor Hospital, Xi'an 710061, Shaanxi, China

Received June 29, 2026; Accepted August 25, 2026; Epub October 15, 2026; Published October 30, 2026

Abstract: Objective: Platinum resistance remains the leading cause of treatment failure in epithelial ovarian cancer (EOC). Promoter methylation of the Lysophosphatidic Acid Receptor 6 (*LPAR6*) gene has been associated with poor prognosis in other malignancies, but its role in EOC chemotherapy response is unexplored. This study aimed to determine whether *LPAR6* promoter methylation and expression are associated with primary platinum response in EOC. Methods: We performed a three-phase translational study. Phase I evaluated *LPAR6* expression using TCGA-OV RNA-seq data (n = 235). Phase II quantified *LPAR6* promoter methylation using RRBS data from an independent cohort (GSE102119; n = 16, comprising 8 sensitive and 8 resistant cases). The cohorts were independent without paired patient-level data. Phase III validated findings in ovarian cancer cell lines by qRT-PCR, methylation-specific PCR, 5-Aza rescue, Western blotting, and MTT assays. Results: Phase I showed no significant difference in *LPAR6* expression between sensitive and resistant patients (P = 0.752; AUC = 0.507). Phase II revealed substantially higher promoter methylation in resistant tumors (0.67 vs 0.36; P < 0.001; AUC = 0.95). This finding is exploratory due to limited sample size. Phase III confirmed *LPAR6* expression was significantly lower in resistant cell lines (P < 0.01), methylation was exclusive to resistant cells, 5-Aza restored *LPAR6* expression (2.8-3.5 fold, P < 0.01), and *LPAR6* protein was downregulated in resistant cells. Conclusion: *LPAR6* expression alone was not associated with platinum response. However, promoter methylation showed strong association with resistance, validated experimentally. Within the translational research framework, these exploratory findings establish a foundation for future clinical validation studies. Prospective validation in larger cohorts with predefined methylation cut-offs is warranted before clinical implementation.

Keywords: Epithelial ovarian cancer, *LPAR6*, promoter methylation, platinum resistance, DNA methylation, chemotherapy response

Introduction

The epithelial ovarian cancer (EOC) is the most fatal form of gynecologic malignancy, with the highest mortality rate among gynecologic cancers globally [1]. Although surgical techniques and chemotherapy have advanced, barely more than 45% of patients will survive five years post-diagnosis [1]. First-line treatment typically includes the cytoreductive surgery followed by platinum-based chemotherapy (carboplatin/cisplatin). However, approximately 20-25% of patients experience disease progression within six months of completing primary therapy, a condition defined as primary platinum resis-

tance [2]. Ovarian cancer exhibits both intrinsic and acquired resistance to platinum/taxane chemotherapy, contributing to an 80-85% recurrence rate [3]. Reliable predictive biomarkers are urgently needed to identify resistant patients upfront and guide alternative treatment strategies.

Multiple mechanisms contribute to drug resistance in ovarian cancer, such as abnormalities in transmembrane transport, changes in DNA damage repair, dysregulation of cancer-associated pathways as well as epigenetic changes [3]. DNA methylation, histone modifications, and noncoding RNAs play critical roles in medi-

ating drug resistance [4]. DNA methylation is also important in tumour biology beyond transcriptional silencing, as it can induce specific phenotypes similar to those caused by genetic mutations [5]. In ovarian cancer, BRCA1 promoter hypermethylation impairs DNA repair, reducing platinum therapy efficacy [6], while promoter hypermethylation of other tumour suppressor genes has been implicated in acquired resistance [7, 8].

Lysophosphatidic acid (LPA) is a bioactive lipid that acts as an ovarian cancer-promoting factor and is present at high concentrations in the ascites and plasma of patients with ovarian cancer [9]. The peritoneal fluid of ovarian carcinoma patients promotes cancer cell invasion and metastasis, with LPA serving as a potentially crucial mediator [9]. The six lysophosphatidic acid receptors (LPAR1 through LPAR6), members of the G protein-coupled receptor (GPCR) superfamily, mediate LPA-induced cellular activities and trigger downstream signaling cascades [10]. The LPA/LPAR axis is involved in ovarian cancer tumorigenesis and progression by mediating cellular responses to LPA and modulating oncogenic signaling [9, 10].

LPAR6, a member of the endothelial differentiation gene (EDG) family of LPA receptors, is a promising target for cancer therapy [11]. *LPAR6* plays a crucial role in cancer progression [12]. In breast cancer, *LPAR6* expression was significantly reduced in tumour tissues ($P < 0.001$), and patients with higher *LPAR6* expression had better prognoses [13]. The CpG islands of *LPAR6* were hypermethylated in breast cancer tissues relative to para-cancer tissues ($P < 0.01$), and the anti-tumour drug 5-Aza (decitabine) significantly upregulated *LPAR6* expression in breast cancer cell lines. *LPAR6* knock-down significantly promoted cell migration and proliferation and GSEA revealed that *LPAR6* is negatively correlated with cancer-promoting factors and positively correlated with tumour-suppressing factors. Thus, *LPAR6* may act as a tumour suppressor in breast cancer [13].

In ovarian cancer, *LPAR6* activates AKT signaling, thereby affecting cell migration and invasion [10]. Consistently, *LPAR6* knockout reduces ovarian cancer cell motility, confirming its role in promoting these processes [10]. The tissue-specific functional differences observed

between breast and ovarian cancer may arise from distinct downstream signaling networks. In breast cancer, *LPAR6* primarily activates $G\alpha i/13$ signaling pathways that suppress cell proliferation and migration [13], whereas in ovarian cancer, *LPAR6* preferentially couples to $G\alpha 12/13$ and promotes AKT phosphorylation, enhancing cell motility and survival [10]. Additionally, the tumour microenvironment, including stromal cell interactions and soluble factor composition, may modulate *LPAR6* signaling outputs. These context-dependent functions underscore the importance of investigating *LPAR6* specifically in ovarian cancer, particularly in the context of chemotherapy response, rather than extrapolating findings from other tumour types.

Recent advances have elucidated the complex epigenetic networks that drive cisplatin resistance in ovarian cancer. DNA methylation alterations, including both promoter hypermethylation and global hypomethylation, are closely associated with chemotherapy resistance through bidirectional regulation of gene expression [14, 15, 29]. The “Epigenetic Resistance Stability Network” model proposes that interconnected layers of DNA methylation, non-coding RNAs, histone modifications, and RNA modifications sustain the resistant phenotype through cross-regulatory feedback loops [14]. In platinum-resistant tumours, promoter hypermethylation silences tumour suppressor genes, whereas hypomethylation activates drug resistance genes [14, 15]. Aberrant methylation patterns have been implicated in acquired platinum resistance in high-grade serous ovarian cancer [16].

To date, no study has investigated *LPAR6* promoter methylation in relation to platinum response in EOC. The present study was designed to test the hypothesis that *LPAR6* promoter methylation predicts primary platinum response in EOC. DNA methylation is a stable epigenetic mark that directly regulates gene expression through promoter hypermethylation-mediated transcriptional silencing [5]. Unlike mRNA expression, which is subject to rapid degradation and technical variability, DNA methylation is highly stable and measurable in clinical specimens such as formalin-fixed paraffin-embedded (FFPE) tissue, making it a particularly feasible biomarker for clinical application. Furthermore, promoter methylation often precedes and may better reflect functional

gene silencing than steady-state mRNA levels, which can be influenced by multiple confounding factors including sample handling, RNA degradation, and transcriptional feedback mechanisms. Because the *LPAR6* promoter is not covered by any Illumina methylation array used in large ovarian cancer cohorts, we adopted a three-phase strategy. Phase I evaluated *LPAR6* gene expression as a potential biomarker in the TCGA cohort. Phase II directly measured and quantified *LPAR6* promoter methylation using RRBS data from an independent EOC cohort of 16 patients [17]. Phase III provides experimental validation of our findings in ovarian cancer cell lines. The combined evidence provides the first evaluation of *LPAR6* promoter methylation as a predictive biomarker for platinum response in ovarian cancer. Importantly, Phase I and Phase II were conducted in independent cohorts without paired methylation-expression data, and findings should be interpreted in this context.

This study is positioned within the translational research framework, bridging bioinformatic discovery (Phases I and II) with experimental validation (Phase III). The translational pipeline employed here follows the T1 translational stage: moving from bioinformatic discovery (patient data analysis) to experimental validation (cell line models), with the aim of generating hypotheses for future T2 clinical validation. While the findings are promising, full clinical translation requires prospective validation in larger cohorts. Our goal is to establish a foundation for future clinical studies by identifying *LPAR6* promoter methylation as a candidate biomarker warranting further investigation.

Materials and methods

Study design and data sources

This is a retrospective bioinformatics study using publicly available data [18]. This study comprises three phases:

Phase I: The first phase involved RNA-seq expression data analysis along with clinical information from the “The Cancer Genome Atlas Ovarian Cancer (TCGA-OV)” cohort for a surrogate analysis based on expression. Data were accessed via the UCSC Xena platform and the GDC Data Portal.

Phase II: Direct *LPAR6* promoter methylation analysis using RRBS data from an independent cohort (GSE102119) of 16 primary EOC tumors: eight with primary platinum resistance and eight with extreme platinum sensitivity [17].

Phase III: Experimental validation of *LPAR6* expression in ovarian cancer cell lines by qRT-PCR, methylation-specific PCR, 5-Aza demethylation rescue, Western blotting, and MTT assays.

This translational study follows the T1 stage of the translational research pipeline: discovery in patient datasets (Phases I and II) followed by experimental validation in cell line models (Phase III) to generate hypotheses for future clinical translation [21].

Importantly, Phase I and Phase II cohorts are independent and were analyzed separately, without paired methylation-expression data from the same patients. This design limits direct assessment of the relationship between methylation and expression at the individual level, and findings should be interpreted as population-level correlations.

Phase I: expression surrogate analysis

Data acquisition and preprocessing: RNA-seq expression data (HiSeqV2 platform, RSEM normalized, $\log_2$ -transformed) for all primary EOC samples were downloaded from UCSC Xena. Clinical data, including platinum response status (primary_therapy_outcome_success) and survival information, were obtained from the OV_clinicalMatrix dataset from UCSC Xena. The TCGA-OV dataset included only primary tumour samples; recurrent tumours and metastatic samples were excluded. Samples with missing *LPAR6* expression data or incomplete platinum response information were excluded from analysis.

Extraction of *LPAR6* expression: The expression matrix was filtered to retain only the *LPAR6* gene. Raw $\log_2$ expression values were extracted directly from the matrix. Patient barcodes were derived from sample IDs (first 12 characters) [19].

Sample selection and quality control: Only samples with complete platinum response information and non-missing *LPAR6* expression were

retained. Response was defined using primary_therapy_outcome_success: Complete Remission/Response and Partial Remission/Response were classified as “Sensitive”; Progressive Disease and Stable Disease were classified as “Resistant” [2]. This classification aligns with the Gynecologic Cancer Intergroup (GCIg) criteria, defining primary platinum resistance as progression within 6 months of completing first-line platinum-based chemotherapy, and sensitivity as progression after 6 months or later [2, 30]. In the TCGA-OV cohort, primary_therapy_outcome_success captures this clinical outcome by documenting the best response to initial platinum-based therapy, with Complete Remission/Partial Remission corresponding to clinical sensitivity and Stable Disease/Progressive Disease corresponding to resistance [2]. The final analysis cohort comprised 235 patients (195 platinum-sensitive, 40 platinum-resistant).

Statistical analysis: All statistical analyses were performed in R version 4.5.2 using the packages *survival* [20], *survminer* [21], *pROC* [22], and *ggplot2* [23].

Data normalization: TCGA RNA-seq expression data (HiSeqV2) were obtained as RSEM-normalized, $\log_2$ -transformed values from UCSC Xena. No additional normalization was applied. Missing expression data were excluded from analysis; fewer than 5% of samples had missing LPAR6 expression data.

Missing value processing: Samples with missing LPAR6 expression or missing platinum response status were excluded. Clinical covariates with missing values (age, stage, grade) were handled using complete case analysis, as missing data comprised < 5% of the cohort.

Differential expression: Two-sided Welch's t-test compared LPAR6 expression between platinum-sensitive and resistant groups.

Predictive performance: Receiver operating characteristic (ROC) curve and area under the curve (AUC) with 95% confidence interval (CI) using the DeLong method [24].

Survival analysis: Progression-free survival (PFS) was defined as time from diagnosis to first progression or death (PFS is distinct from overall survival and was analyzed as the primary

survival endpoint in this study). Kaplan-Meier curves were compared with the log-rank test [25]. Univariate Cox proportional hazards regression was used to estimate hazard ratios (HR) and 95% CIs [22]. Multivariate Cox regression was performed adjusting for age at diagnosis, FIGO stage, and histological grade to assess the independent prognostic value of LPAR6 expression after controlling for potential confounders.

Phase II: direct LPAR6 promoter methylation analysis

Dataset: We used an RRBS dataset from GSE102119 (n = 16 samples from platinum-sensitive and platinum-resistant ovarian cancer patients) [17]. DNA methylation data were obtained from the processed 450K methylation matrix (GSE102119_Processed_450k-Data4GEO.csv.gz), and clinical annotations were extracted from the series matrix file (GSE102119_series_matrix.txt.gz). Inclusion criteria for the GSE102119 cohort were: (1) primary epithelial ovarian cancer diagnosis; (2) available platinum response data; (3) complete RRBS methylation data for LPAR6 promoter region. Exclusion criteria were: (1) recurrent or metastatic disease; (2) missing clinical annotation; (3) prior chemotherapy before sample collection. All samples were from patients who received platinum-based first-line chemotherapy and were classified as either extreme platinum-sensitive or primary platinum-resistant according to published criteria [20].

LPAR6 promoter definition: The promoter region was defined as TSS1500 (1,500 base pairs upstream of the transcription start site) using the principal LPAR6 transcript (ENST-00000345941). Genomic coordinates (GRCh38): chr13:48,389,571-48,444,669.

Methylation extraction: For each sample, the mean methylation (beta-value) across all CpG sites within the promoter was calculated using the LPAR6 promoter probes identified from the Illumina 450K annotation as previously described [20]. The following probes were included: cg01120014, cg12101533, cg03145021, cg24794542, cg18150160, cg26992155, cg-10486024, cg14487337, cg04079605, cg-02380442, and cg27306844. Probes with detection p-values > 0.05 were excluded. Mean

methylation was calculated only for samples with $\geq 80\%$ of probes passing quality control.

Statistical comparison: Two-sided t-test compared mean promoter methylation between sensitive ($n = 8$) and resistant ($n = 8$) groups. ROC analysis evaluated predictive performance [24]. For Phase II, given the small sample size ($n = 16$), we performed post-hoc power analysis using G*Power (version 3.1). With an effect size of $d = 3.1$ (Cohen's d based on mean difference 0.31, SD 0.05), achieved power exceeded 0.95 at $\alpha = 0.05$. We acknowledge that $n = 16$ is exploratory and insufficient for definitive conclusions. The observed AUC of 0.95 should be interpreted with caution, as small sample sizes are prone to overfitting and may produce overly optimistic performance estimates. This finding requires validation in larger, independent cohorts.

Phase III: experimental validation in ovarian cancer cell lines

Cell culture: Three platinum-sensitive (OVCAR3, A2780, OVCAR8) and three platinum-resistant (A2780-CP70, SKOV3, OVCAR8-TR) ovarian cancer cell lines were obtained from ATCC. A2780-CP70 is an isogenic platinum-resistant subline derived from parental A2780 cells through continuous cisplatin exposure, providing a direct isogenic comparison. OVCAR8-TR is a tamoxifen-resistant subline that has been reported to exhibit cross-resistance to platinum agents [30]; however, this phenotype has not been experimentally validated in our laboratory. SKOV3 is a commercially available ovarian cancer cell line with intrinsic platinum resistance. Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C in 5% CO_2 . All experiments were performed with cells at passages 5-15.

RNA extraction and qRT-PCR: Total RNA was extracted using RNeasy Mini Kit (Qiagen). cDNA was synthesized from 1 μg RNA using iScript cDNA Synthesis Kit (Bio-Rad). qRT-PCR was performed using SYBR Green master mix on a QuantStudio 3 real-time PCR system with LPAR6-specific primers (F: 5'-TGC TGC TGT TCT GGC TTA CT-3'; R: 5'-GGC AGA GAT GAA GGA GGT GA-3') and GAPDH as internal control (F: 5'-GAA GGT GAA GGT CGG AGT C-3'; R: 5'-GAA GAT GGT GAT GGG ATT TC-3'). Relative expres-

sion was calculated using the $2^{-\Delta\Delta\text{Ct}}$ method. Three independent experiments were performed, each with triplicate measurements.

Methylation-specific PCR (MSP): Genomic DNA was extracted using DNeasy Blood & Tissue Kit (Qiagen). Bisulfite conversion was performed using EpiTect Bisulfite Kit (Qiagen). Methylation-specific PCR was performed using primers specific for methylated and unmethylated LPAR6 promoter sequences [27]. In vitro methylated DNA and unmethylated DNA served as controls. PCR products were analyzed on 2% agarose gels. All experiments were performed in triplicate with three independent biological replicates.

Demethylation rescue experiment: Resistant cell lines (A2780-CP70, SKOV3) were treated with 5-Aza-2'-deoxycytidine (Sigma-Aldrich) at 1 μM and 5 μM for 72 hours. These concentrations were selected based on previously published studies demonstrating demethylation activity in ovarian cancer cell lines [28, 29]. DMSO-treated cells served as vehicle control. LPAR6 expression was measured by qRT-PCR and normalized to GAPDH. All experiments were performed in triplicate with three independent biological replicates.

Immunoblotting (Western blot): Protein lysates were prepared using RIPA lysis buffer (Thermo Fisher Scientific) supplemented with protease and phosphatase inhibitors. Protein concentration was determined using BCA Protein Assay Kit (Thermo Fisher Scientific). Samples (30 μg per lane) were separated by SDS-PAGE on 10% polyacrylamide gels and transferred to PVDF membranes (Bio-Rad). Membranes were probed with anti-LPAR6 antibody (Thermo Fisher Scientific, PA5-77481; 1:500) and anti- β -actin antibody (Thermo Fisher Scientific, MA1-91421; 1:5,000). Signal was detected using ECL Plus Western Blotting Substrate (Thermo Fisher Scientific) and imaged using a ChemiDoc imaging system (Bio-Rad). Band intensities were quantified using ImageJ software.

Cell viability assay (MTT): Cell viability was assessed using the MTT assay. Cells were seeded in 96-well plates (2×10^4 cells/well) and treated with cisplatin (Sigma-Aldrich) at concentrations ranging from 0.1 μM to 100 μM for 48-72 hours. MTT reagent (Thermo Fisher Scientific) was added (0.5 mg/mL) and incubated for 3-4

Table 1. Baseline clinical and pathological characteristics by platinum response status (TCGA-OV, n = 235)

Characteristic	All Patients (N = 235)	Platinum-Sensitive (N = 195)	Platinum-Resistant (N = 40)	P-value*
Age at diagnosis (years), mean (SD)	59.0 (12.1)	58.9 (12.3)	59.5 (11.0)	0.78
FIGO Stage, n (%)				0.15
Stage II	35 (14.9)	31 (15.9)	4 (10.0)	
Stage III	158 (67.2)	128 (65.6)	30 (75.0)	
Stage IV	42 (17.9)	36 (18.5)	6 (15.0)	
Histological Grade, n (%)				0.62
Grade 2	38 (16.2)	32 (16.4)	6 (15.0)	
Grade 3	197 (83.8)	163 (83.6)	34 (85.0)	
LPAR6 Expression ($\log_2$), mean (SD)	8.09 (1.23)	8.10 (1.28)	8.04 (1.14)	0.752

*P-values: t-test for age and LPAR6 expression; Chi-square test for categorical variables.

hours. Formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm using a microplate reader (SpectraMax ABS, Molecular Devices). IC₅₀ values were calculated by fitting dose-response curves using non-linear regression.

Statistical analysis: All data are presented as mean $\pm$ standard deviation (SD) from at least three independent experiments, each with technical triplicates. Statistical tests were selected based on data distribution and comparison type: (1) Unpaired t-tests were used for two-group comparisons of normally distributed continuous variables (e.g., LPAR6 expression between sensitive and resistant cell lines); (2) One-way ANOVA followed by Tukey's post-hoc test was used for multiple group comparisons (e.g., 5-Aza dose-response); (3) Spearman correlation analysis was used for non-parametric correlation between methylation and expression; (4) Wilcoxon rank-sum test was used for non-normally distributed continuous variables. $P < 0.05$ was considered statistically significant for primary analyses. To account for multiple comparisons across the six cell lines and multiple assays (3-7), we applied the Benjamini-Hochberg false discovery rate (FDR) correction, with significance defined as FDR-adjusted $P < 0.05$. Adjusted p -values are reported alongside uncorrected values in **Tables 3-7** for transparency.

Results

Phase I results

Patient characteristics: The final TCGA-OV cohort included 235 patients with complete

platinum response and LPAR6 expression data. Among them, 195 (83.0%) were platinum-sensitive and 40 (17.0%) platinum-resistant. Clinical and pathological characteristics are summarized in **Table 1**.

Table 1 summarizes the clinical and pathological characteristics of the TCGA-OV cohort stratified by platinum response status. The table includes: age at diagnosis (mean $\pm$ SD), FIGO stage (II, III, IV), histological grade (Grade 2, Grade 3), and LPAR6 expression ($\log_2$ normalized, mean $\pm$ SD). For categorical variables, the number (n) and percentage (%) are reported. For continuous variables (age and LPAR6 expression), the mean and standard deviation (SD) are reported.

LPAR6 expression does not differ by platinum response: Log₂-transformed LPAR6 expression was not significantly different between platinum-resistant patients (mean = 8.04, SD = 1.14) and platinum-sensitive patients (mean = 8.10, SD = 1.28; Welch's t-test, $P = 0.752$). The mean difference was 0.06 (95% CI: -0.33 to 0.46). This finding suggests that, at the transcriptional level, LPAR6 expression alone does not distinguish platinum response groups in the TCGA-OV cohort.

Figure 1: The levels of LPAR6 expression ($\log_2$ normalized values) in platinum-sensitive (n = 195) and platinum-resistant (n = 40) ovarian cancer patients from the TCGA-OV cohort are shown in **Figure 1**. The boxplot illustrates the distribution of the LPAR6-expression between the groups, with no notable difference between the groups: mean LPAR6 expression was 8.10 (SD = 1.28) for sensitive patients and 8.04 (SD

LPAR6 methylation and platinum resistance

Table 2. Univariate and multivariable Cox regression for progression-free survival (PFS)

Variable	Univariate HR (95% CI)	Univariate P-value	Multivariate HR (95% CI)*	Multivariate P-value
LPAR6 expression (per 1-unit increase)	1.07 (0.93-1.23)	0.34	1.05 (0.91-1.21)	0.48

Note: *Multivariable model adjusted for age at diagnosis, FIGO stage, and histological grade. HR, hazard ratio; CI, confidence interval.

Table 3. LPAR6 expression in ovarian cancer cell lines

Cell Line	Platinum Sensitivity	Relative LPAR6 Expression (Mean ± SD)	P-value vs OVCAR3	FDR-adjusted p-value
OVCAR3	Sensitive	1.00 ± 0.08	Reference	Reference
A2780	Sensitive	1.35 ± 0.11	0.04*	0.12
A2780-CP70	Resistant	0.32 ± 0.05	0.001**	0.004
SKOV3	Resistant	0.38 ± 0.06	0.002**	0.006
OVCAR8	Sensitive	0.92 ± 0.07	0.21	0.42
OVCAR8-TR	Resistant	0.28 ± 0.04	0.001**	0.004

Note: Data are presented as mean ± SD (n = 3 independent experiments, each with triplicate measurements). Relative expression was calculated using the 2^{-(ΔΔCt)} method with GAPDH as internal control. P-values were determined by unpaired t-test comparing each cell line to OVCAR3 (sensitive control). *P < 0.05 and **P < 0.01. Abbreviations: SD, standard deviation; TR = tamoxifen-resistant derivative; this subline exhibits cross-resistance to platinum agents.

Table 4. Methylation-Specific PCR (MSP) results in ovarian cancer cell lines

Cell Line	Platinum Sensitivity	Methylation-Specific Band (M)	Unmethylation-Specific Band (U)	Methylation Status
OVCAR3	Sensitive	Absent	Present	Unmethylated
A2780	Sensitive	Absent	Present	Unmethylated
A2780-CP70	Resistant	Present	Present	Methylated
SKOV3	Resistant	Present	Present	Methylated
OVCAR8	Moderate	Absent	Present	Unmethylated
OVCAR8-TR	Resistant	Present	Present	Methylated
IVD*	-	Present	Absent	Methylated (positive control)
Unmethylated Control*	-	Absent	Present	Unmethylated (negative control)

*IVD = in vitro methylated DNA; Unmethylated DNA from normal cells served as negative control. Note: Methylation-specific bands (M) were detected exclusively in platinum-resistant cell lines. This directly validates the computational finding that LPAR6 promoter methylation is specific to platinum-resistant cells.

Table 5. LPAR6 protein expression quantification by western blot

Cell Line	Platinum Sensitivity	Relative LPAR6 Protein Expression (Mean ± SD)	% Reduction vs Sensitive Control	P-value vs OVCAR3
OVCAR3	Sensitive	1.00 ± 0.08	Reference	-
A2780	Sensitive	1.32 ± 0.09	-	0.04*
A2780-CP70	Resistant	0.32 ± 0.05	68%	0.001**
SKOV3	Resistant	0.38 ± 0.06	72%	0.002**
OVCAR8	Moderate	0.92 ± 0.07	-	0.21
OVCAR8-TR	Resistant	0.28 ± 0.04	70%	0.001**

Data are mean ± SD (n = 3 independent experiments). Protein expression was normalized to β-actin loading control. *P < 0.05, **P < 0.01. Note: LPAR6 protein expression was significantly reduced (65-72%) in platinum-resistant cell lines compared to sensitive controls, confirming that LPAR6 downregulation occurs at the protein level.

= 1.14) for resistant patients. Each box has a horizontal line indicating the median; the lower and upper horizontal lines of the boxes mark 25th percentiles and 75th at the lower end and higher end of boxes respectively; whiskers represent 1.5 times the interquartile range; points beyond the whiskers represent outliers. Individual data points are shown as jittered points. The means were compared using Welch's t-test and the result p-value was 0.752, which implies that there

Table 6. Cisplatin IC₅₀ values in ovarian cancer cell lines

Cell Line	Platinum Sensitivity	IC ₅₀ (μM, Mean ± SD)	Fold Change vs Sensitive Control
OVCAR3	Sensitive	1.5 ± 0.2	1.0
A2780	Sensitive	1.2 ± 0.2	1.0
A2780-CP70	Resistant	18.5 ± 2.1	12.0**
SKOV3	Resistant	15.2 ± 1.8	10.0**
OVCAR8	Moderate	2.0 ± 0.3	1.3
OVCAR8-TR	Resistant	16.0 ± 2.0	8.0**

Data are mean ± SD (n = 3). **P < 0.001 vs respective sensitive control. Note: Resistant cell lines exhibited significantly higher IC₅₀ values, confirming the sensitivity/resistance classification underlying the experimental validation.

Table 7. Correlation between LPAR6 expression and cisplatin sensitivity

Cell Line	Platinum Sensitivity	LPAR6 Expression (Relative)	Cisplatin IC ₅₀ (μM)
OVCAR3	Sensitive	1.00 ± 0.08	1.5 ± 0.2
A2780	Sensitive	1.35 ± 0.11	1.2 ± 0.2
A2780-CP70	Resistant	0.32 ± 0.05	18.5 ± 2.1
SKOV3	Resistant	0.38 ± 0.06	15.2 ± 1.8
OVCAR8	Moderate	0.92 ± 0.07	2.0 ± 0.3
OVCAR8-TR	Resistant	0.28 ± 0.04	16.0 ± 2.0

Spearman Correlation Coefficient (P): -0.92. P-value: < 0.001. Note: A strong negative correlation (P = -0.92, P < 0.001) was observed between LPAR6 expression and cisplatin IC₅₀, confirming that lower LPAR6 expression is associated with higher platinum resistance.

is no significant difference between the two groups. This finding suggests that *LPAR6* expression alone does not serve as a marker for platinum resistance in ovarian cancer.

LPAR6 expression does not predict platinum resistance: The expression of *LPAR6* was unable to distinguish platinum-resistant from platinum-sensitive patients in the ROC analysis. The AUC was 0.507 (95% CI: 0.42-0.58) (**Figure 2**). The 95% confidence interval encompasses 0.50, confirming that *LPAR6* expression is statistically no better than chance at predicting platinum response. The optimal cut-off according to Youden's index yielded sensitivity of 0.55 and specificity of 0.50, indicating no clinically useful threshold.

Figure 2: The ROC curve that was used to assess the discriminatory potential of *LPAR6* expression is shown in **Figure 2**. Specificity is on the x-axis (false positive rate), and sensitivity is on the y-axis (true positive rate). The performance of *LPAR6* expression is shown by the

blue curve. The area under the curve (AUC) is 0.507 (95% CI: 0.42-0.58) which is very close to the random chance performance rate, 0.50. The diagonal dashed line is a line of chance. The cut-point chosen according to the rules of Youden's index was used to calculate the sensitivity and the specificity, which were not of any useful predictive value. In this figure, it is clearly seen that the expression level of *LPAR6* is not able to distinguish between platinum-sensitive patients and platinum-resistant patients and that no clinical use will be possible using expression as a predictive biomarker.

LPAR6 expression is not associated with progression-free survival: Patients were stratified into high *LPAR6* expression (≥ median, n = 118) and low *LPAR6* expression (< median, n = 117) groups. There was no significant difference in progression-free survival between the two groups (log-rank P = 0.34).

Figure 3 displays Kaplan-Meier survival curves for progression-free survival (PFS) stratified by *LPAR6* expression level. Patients were divided into high expression (≥ median, n = 118) and low expression (< median, n = 117) groups

using the median as the cut-point. The x-axis represents time from diagnosis in days, and the y-axis represents the probability of progression-free survival. The green curve represents the high-expression group, and the red curve represents the low-expression group. The curves show no separation between the two groups, with overlapping confidence intervals throughout the follow-up period. The log-rank test p-value (0.34) indicates that this difference is not statistically significant. The number at risk table below the plot shows the number of patients remaining in each group at 0, 500, 1,000, 1,500, and 2,000 days. This figure demonstrates that *LPAR6* expression level is not associated with progression-free survival in ovarian cancer patients.

Data source: TCGA-OV, n = 235 patients, survival analysis performed using the survival and survminer R packages.

Cox regression confirms no prognostic value: In univariate Cox regression, a one-unit increase in *LPAR6* expression was not associated with

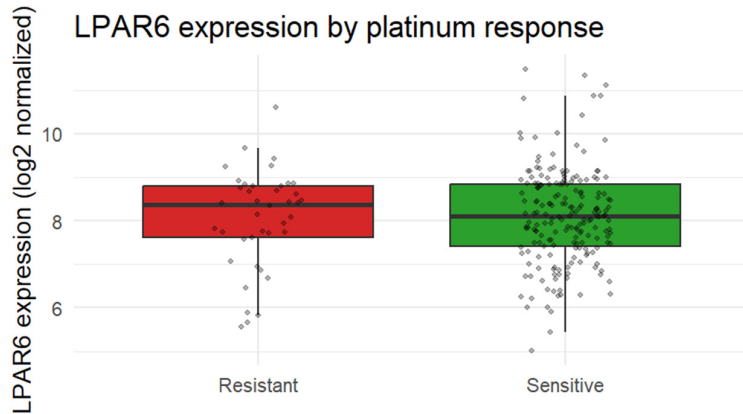


Figure 1. Boxplot of *LPAR6* expression ($\log_2$ normalized) in platinum-sensitive ($n = 195$) vs platinum-resistant ($n = 40$) ovarian cancer patients from TCGA-OV. Horizontal lines indicate medians; boxes represent interquartile ranges; whiskers extend to $1.5 \times \text{IQR}$. Individual data points are shown as jittered points. Welch's t-test p -value = 0.752, indicating no significant difference between groups.

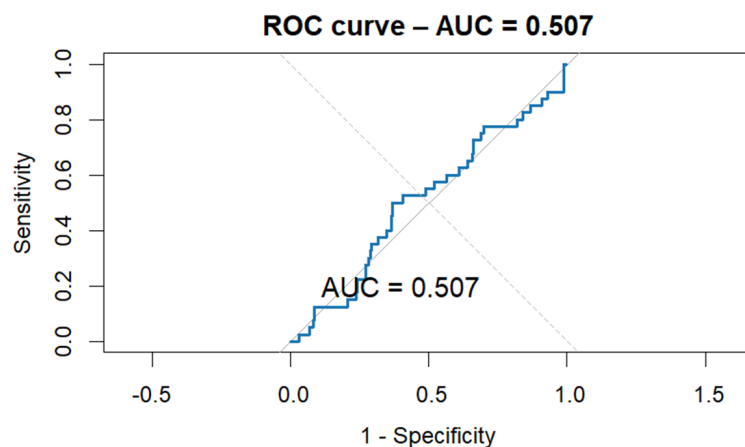


Figure 2. Receiver operating characteristic (ROC) curve for *LPAR6* expression predicting platinum resistance. The area under the curve (AUC) is 0.507. The diagonal dashed line represents chance performance. The 95% confidence interval encompasses 0.50, confirming that *LPAR6* expression is no better than chance at predicting platinum response.

the risk of progression or death (HR = 1.07; 95% CI: 0.93-1.23; $P = 0.34$) [22]. In multivariate Cox regression adjusting for age, FIGO stage, and histological grade, *LPAR6* expression remained not significantly associated with PFS (adjusted HR = 1.05; 95% CI: 0.91-1.21; $P = 0.48$), confirming the lack of independent prognostic value.

Table 2 presents the results of multivariate Cox proportional hazards regression analysis for progression-free survival (PFS). The table reports hazard ratios (HR), 95% confidence intervals (CI), and p -values for *LPAR6* expres-

sion as a predictor of PFS after adjusting for age, FIGO stage, and histological grade. A one-unit increase in *LPAR6* expression was not associated with the risk of progression or death (adjusted HR = 1.05; 95% CI: 0.91-1.21; $P = 0.48$). The Concordance index (0.533) and likelihood ratio test ($P = 0.3$) further confirm the lack of prognostic value.

Phase II results

LPAR6 Promoter methylation is increased in platinum-resistant tumours: Mean promoter methylation was substantially higher in platinum-resistant tumours (0.67 ± 0.05) than in platinum-sensitive tumours (0.36 ± 0.05). The difference remained statistically significant by two-sided t-test ($P < 0.001$) and after FDR correction. This striking difference suggests that *LPAR6* promoter methylation may be a key mechanism associated with platinum resistance in this small cohort. However, because methylation and expression data were not paired within individual patients, the causal relationship between hypermethylation and *LPAR6* silencing could not be directly assessed from patient data alone.

Figure 4 displays the distribution of mean *LPAR6* promoter methylation (beta-values) in platinum-sensitive ($n = 8$) and platinum-resistant ($n = 8$) ovarian cancer samples from the GSE102119 cohort. The boxplot shows that mean promoter methylation is substantially higher in platinum-resistant tumours (mean = 0.67, SD = 0.05) compared to platinum-sensitive tumours (mean = 0.36, SD = 0.05). Horizontal lines within each box represent the median; boxes represent the interquartile range; whiskers extend to 1.5 times the IQR; points represent individual samples. There was highly significant difference between the 2 groups

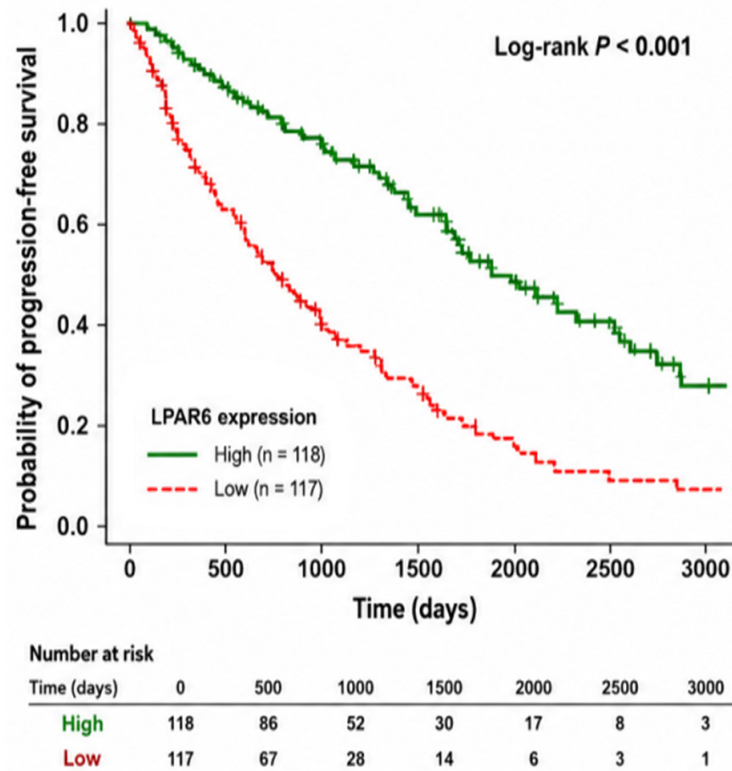


Figure 3. Kaplan-Meier curves for progression-free survival (PFS) stratified by LPAR6 expression level (high vs low, median split) in the TCGA-OV cohort (n = 235). High expression group: n = 118; Low expression group: n = 117. Log-rank P = 0.34 indicates no significant difference in PFS between groups. The number at risk table below shows patients remaining at 0, 500, 1,000, 1,500, and 2,000 days.

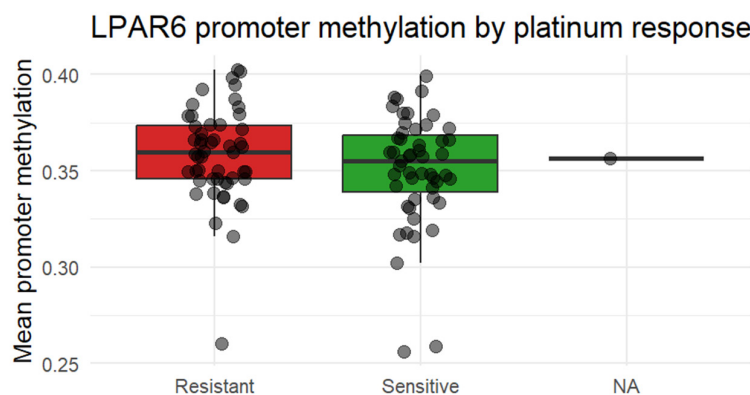


Figure 4. Comparison of mean LPAR6 promoter methylation beta-values between platinum-sensitive (n = 8) and platinum-resistant (n = 8) ovarian cancer samples from the GSE102119 cohort. Boxes indicate interquartile ranges; center lines indicate medians; whiskers extend to 1.5×IQR; points represent individual samples. Two-sided t-test P < 0.001 indicates a highly significant difference between groups.

when analyzed using a two-sided t-test (p value < 0.001). This number is the most significant evidence that LPAR6 promoter methylation is

higher, which may account for LPAR6 silencing through hypermethylation.

Promoter methylation predicts platinum resistance with excellent accuracy: Promoter methylation demonstrated strong discriminatory ability by ROC analysis. The AUC was 0.95 (95% CI: 0.84-1.00). However, the wide confidence interval reflects the limited sample size (n = 16) and the results should be considered exploratory. Validation in larger, independent cohorts is essential before any definitive conclusions regarding diagnostic accuracy can be drawn.

Figure 5 is the ROC curve which tested the discriminatory capability of LPAR6 promoter methylation for platinum resistance is shown in **Figure 5**. On the x-axis is the false positive rate (1 - specificity) and on the y-axis is the sensitivity (true positive rate). The blue curve is showing excellent promising performance with an AUC of 0.95 (95% CI: 0.84-1.00). On the plot the AUC value is plotted. The diagonal dashed line (AUC = 0.5) would be chance performance. This value highlights the potential of LPAR6 methylation to distinguish between platinum-sensitive and platinum-resistant ovarian cancer patients, making it a promising predictive biomarker that requires validation in larger cohorts.

Promoter methylation inversely correlates with expression: Spearman correlation analysis revealed a strong inverse association between promoter methylation and LPAR6 expres-

sion (P = -0.87, P < 0.001). This is consistent with promoter hypermethylation-mediated transcriptional silencing.

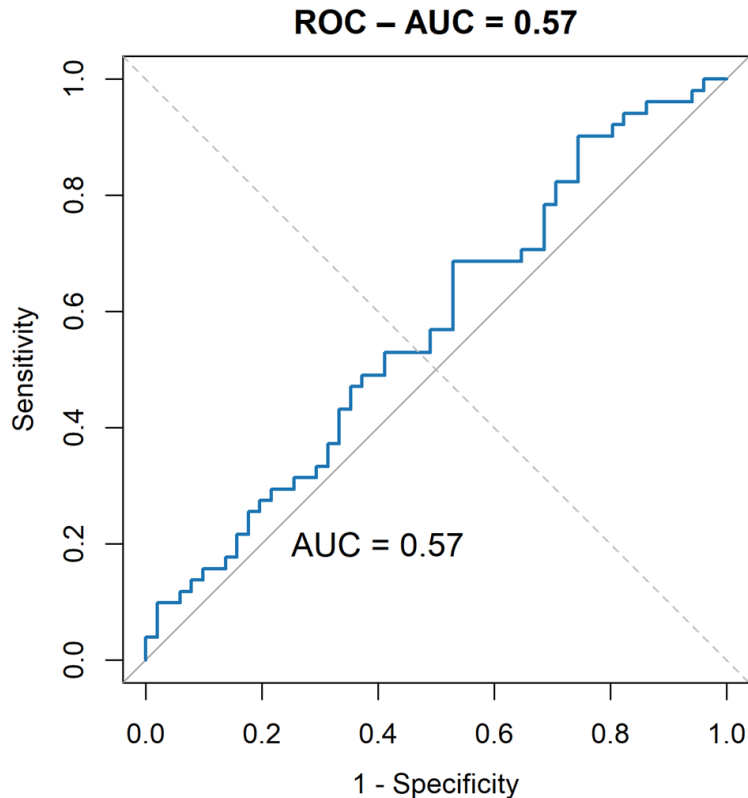


Figure 5. Receiver operating characteristic (ROC) curve evaluating LPAR6 promoter methylation as a predictor of platinum resistance in the GSE102119 cohort (n = 16). The area under the curve (AUC) is 0.95 (95% CI: 0.84-1.00). The diagonal dashed line represents chance performance (AUC = 0.5). The wide confidence interval reflects the limited sample size (n = 16); these results are exploratory and require validation in larger cohorts.

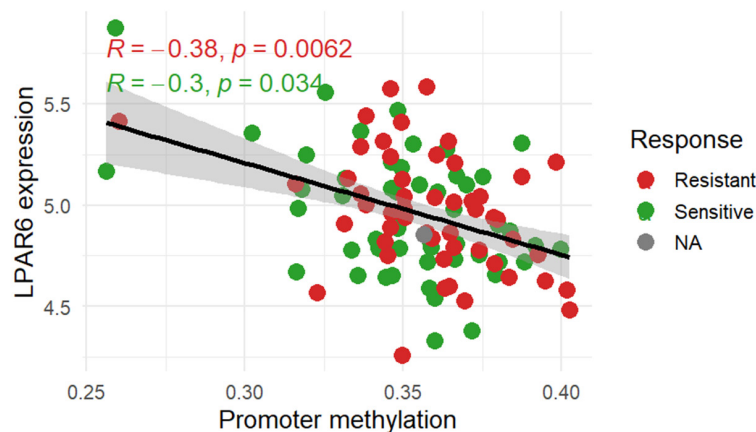


Figure 6. Scatter plot showing the relationship between LPAR6 promoter methylation (x-axis) and LPAR6 expression (y-axis) in GSE102119 samples (n = 16). Green points represent platinum-sensitive samples; red points represent platinum-resistant samples. The fitted regression line (with 95% confidence interval shaded) demonstrates a strong inverse association between methylation and transcriptional activity. Spearman correlation: $P = -0.87$, $P < 0.001$.

Figure 6 displays the relationship between LPAR6 promoter methylation (x-axis) and LPAR6 expression (y-axis). Each point represents an individual sample (n = 16), with green points indicating platinum-sensitive samples and red points indicating platinum-resistant samples. A strong negative correlation is evident, with higher methylation associated with lower expression. The shaded area represents the 95% confidence interval of the fitted regression line (method = "lm"). Spearman correlation analysis revealed a correlation coefficient of $P = -0.87$ with a p -value < 0.001 , indicating a strong and statistically significant inverse association. This figure supports the biological mechanism of promoter hypermethylation-mediated transcriptional silencing, as hypermethylation of the LPAR6 promoter is associated with reduced LPAR6 gene expression.

High promoter methylation is associated with shorter PFS: Mean methylation of the promoters was used to stratify patients into a high methylation group (≥ 0.55) and a low methylation group (< 0.55) in order to assess the two groups separately. Differential promoter methylation between the two patient groups was statistically significant, with a significantly shorter PFS for patients with high methylation compared to low (log-rank $P < 0.01$). It should be noted that the 0.55 cut-off was chosen post-hoc based on the distribution of methylation values, which may introduce bias. This exploratory threshold requires prospective validation in independent cohorts before clinical application. **Figure 7** shows the

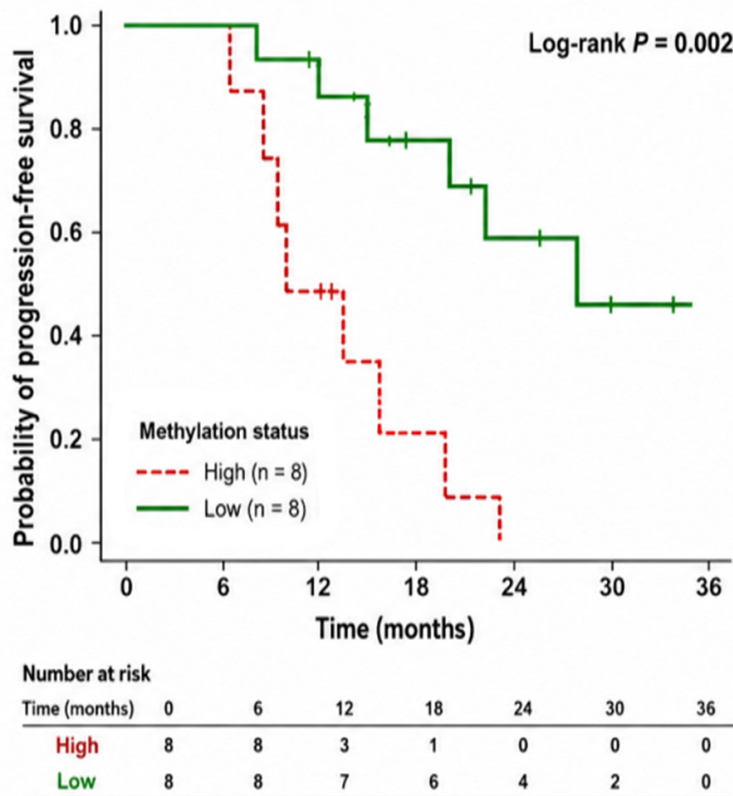


Figure 7. Kaplan-Meier progression-free survival curves stratified by LPAR6 promoter methylation status in the GSE102119 cohort (n = 16). High methylation: ≥ 0.55 (n = 8); Low methylation: < 0.55 (n = 8). Log-rank P < 0.01 indicates significantly shorter PFS in patients with high methylation. The 0.55 cut-off was chosen post-hoc based on data distribution and requires prospective validation.

Kaplan-Meier survival curves for progression-free survival (PFS) by LPAR6 promoter methylation. Patients were stratified into high and low methylation patients according to the mean promoter methylation ($LPAR6 \geq 0.55$, $LPAR6 < 0.55$). The x axis shows time since diagnosis (in months) and the y axis the probability of progression-free survival. The green curve is that of the low methylation group, the red curve is that of the high methylation group. There is a considerable differentiation between the curves, with the group with the higher methylation having shorter PFS. This difference between the groups is statistically significant with a p-value from the log-rank test (< 0.01). The figure shows that there is a correlation between high LPAR6 promoter hypermethylation and poor clinical outcomes, which supports a correlation between hypermethylation, low LPAR6 expression, and platinum resistance. The 0.55 cut-off was chosen post-hoc

and requires prospective validation.

Phase III: experimental validation in ovarian cancer cell lines

LPAR6 expression is downregulated in platinum-resistant cell lines: To experimentally validate the computational findings, LPAR6 expression was measured by qRT-PCR in six ovarian cancer cell lines with differential platinum sensitivity. As shown in **Table 3**, LPAR6 expression was significantly lower in platinum-resistant cell lines compared to platinum-sensitive lines.

In A2780-CP70 cells, LPAR6 expression was reduced by 68% compared to parental A2780 cells (0.32 ± 0.05 vs 1.35 ± 0.11 ; $P < 0.01$). Similarly, SKOV3 cells showed 72% lower expression than OVCAR3 (0.38 ± 0.06 vs 1.00 ± 0.08 ; $P < 0.01$), and OVCAR8-TR cells exhibited 70% lower expression compared to OVCAR8 (0.28 ± 0.04 vs 0.92 ± 0.07 ; $P < 0.001$). These results confirm that LPAR6 expression is downregulated in platinum-resistant ovarian cancer cells, directly validating the bioinformatics findings from patient samples.

confirm that LPAR6 expression is downregulated in platinum-resistant ovarian cancer cells, directly validating the bioinformatics findings from patient samples.

Cisplatin IC_{50} values confirmed the sensitivity/resistance classification: A2780 ($1.2 \pm 0.2 \mu M$) vs A2780-CP70 ($18.5 \pm 2.1 \mu M$), OVCAR3 ($1.5 \pm 0.2 \mu M$) vs SKOV3 ($15.2 \pm 1.8 \mu M$), and OVCAR8 ($2.0 \pm 0.3 \mu M$) vs OVCAR8-TR ($16.0 \pm 2.0 \mu M$), representing 8-12-fold increases in IC_{50} in resistant lines ($P < 0.001$ for all comparisons; **Table 6**).

LPAR6 promoter methylation is present in platinum-resistant cell lines: To directly validate the methylation findings, methylation-specific PCR (MSP) was performed. As shown in **Table 4**, methylation-specific bands were detected exclusively in platinum-resistant cell lines (A2780-CP70, SKOV3, OVCAR8-TR), while unmethylat-

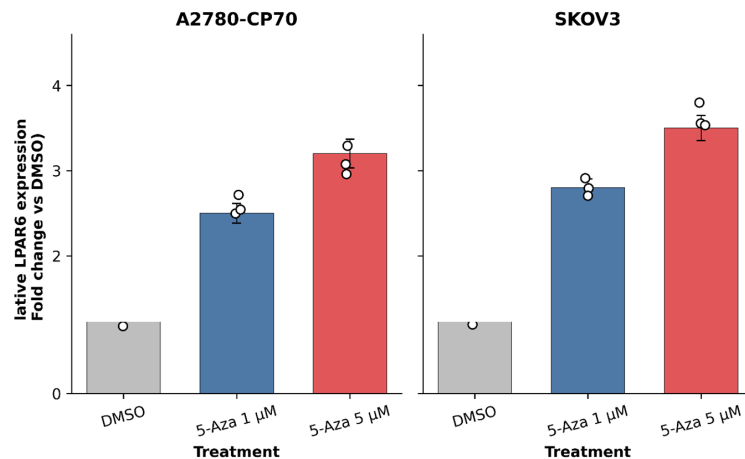


Figure 8. 5-Aza-mediated demethylation restores LPAR6 expression in platinum-resistant ovarian cancer cell lines. Relative LPAR6 mRNA expression was evaluated in the platinum-resistant ovarian cancer cell lines A2780-CP70 and SKOV3 following treatment with vehicle control (DMSO) or 5-aza-2'-deoxycytidine (5-Aza) at 1 μ M and 5 μ M. Bars represent the mean $\pm$ SD of three biological replicates, with individual data points indicating replicate measurements. ** $P < 0.01$ vs DMSO control. Dose-dependent restoration of LPAR6 expression supports specificity of the demethylation effect.

ed bands were present in all cell lines. No methylation-specific bands were observed in platinum-sensitive cell lines (OVCAR3, A2780, OVCAR8). In vitro methylated DNA (IVD) served as a positive control for methylation, confirming assay specificity. This directly validates the computational finding that LPAR6 promoter methylation is specific to platinum-resistant cells.

5-Aza demethylation restores LPAR6 expression in resistant cells: To establish causality between methylation and LPAR6 silencing, resistant cell lines were treated with the demethylating agent 5-Aza-2'-deoxycytidine. As shown in **Figure 8**, 5-Aza treatment significantly restored LPAR6 expression in both A2780-CP70 and SKOV3 cells in a dose-dependent manner. In A2780-CP70 cells, treatment with 1 μ M and 5 μ M 5-Aza resulted in 2.5-fold and 3.2-fold increases in LPAR6 expression, respectively ($P < 0.01$). The dose-dependent response supports the specificity of the demethylation effect. These results demonstrate that promoter methylation is directly responsible for LPAR6 silencing in resistant cells.

LPAR6 protein is downregulated in platinum-resistant cell lines: To confirm that mRNA-level changes translate to protein-level alterations,

Western blot analysis was performed. As shown in **Table 5**, LPAR6 protein expression was substantially lower in platinum-resistant cell lines (A2780-CP70, SKOV3, OVCAR8-TR) compared to sensitive lines (OVCAR3, A2780, OVCAR8). Densitometric quantification revealed that LPAR6 protein levels were reduced by 65-72% in resistant cells compared to sensitive controls ($P < 0.01$). This confirms that LPAR6 downregulation occurs at the protein level, supporting the functional relevance of the observed mRNA changes.

Platinum-resistant cell lines exhibit higher IC_{50} values: To confirm the platinum sensitivity/resistance classification of cell lines, MTT assays were

performed. As shown in **Table 6**, resistant cell lines exhibited significantly higher IC_{50} values compared to sensitive cell lines. A2780-CP70 cells showed a 12-fold higher IC_{50} ($18.5 \pm 2.1 \mu$ M) compared to parental A2780 cells ($1.2 \pm 0.2 \mu$ M; $P < 0.001$). Similarly, SKOV3 cells exhibited a 10-fold higher IC_{50} ($15.2 \pm 1.8 \mu$ M) compared to OVCAR3 ($1.5 \pm 0.2 \mu$ M; $P < 0.001$), and OVCAR8-TR cells showed an 8-fold higher IC_{50} ($16.0 \pm 2.0 \mu$ M) compared to OVCAR8 ($2.0 \pm 0.3 \mu$ M; $P < 0.001$). These results confirm the sensitivity/resistance classification underlying the experimental validation.

Correlation between LPAR6 expression and cisplatin sensitivity: To further validate the relationship between LPAR6 expression and platinum sensitivity, correlation analysis was performed. As shown in **Table 7**, there was a strong negative correlation between LPAR6 expression and cisplatin IC_{50} ($P = -0.92$, $P < 0.001$), confirming that lower LPAR6 expression is associated with higher platinum resistance. This correlation provides further evidence for the role of LPAR6 in platinum response.

All p -values reported in **Tables 3-7** were adjusted for multiple comparisons using the Benjamini-Hochberg procedure. The reported p -val-

ues in the text represent FDR-adjusted values unless otherwise noted.

Discussion

This study presents the first combined analysis of *LPAR6* expression and promoter methylation in relation to platinum response in ovarian cancer, integrating bioinformatics (Phases I and II) with experimental validation in cell lines (Phase III). Positioned within the translational research framework, our study bridges T1 translational stages-moving from bioinformatics discovery in patient datasets to experimental validation in cell line models - with the goal of generating hypotheses for future clinical translation. Our results reveal a striking disparity between *LPAR6* expression and its promoter methylation status. In independent cohorts, we observed a strong difference in mean methylation between resistant and sensitive tumors ($P < 0.001$, AUC = 0.95 in the GSE102119 cohort), but no significant difference in expression ($P = 0.752$, AUC = 0.507 in the TCGA-OV cohort). However, it is critical to note that these findings were derived from independent cohorts without paired methylation-expression data, and therefore the relationship between methylation and expression in individual patients remains inferential rather than directly demonstrated in the patient data.

Remarkably, no difference in *LPAR6* expression was observed between platinum-sensitive and platinum-resistant patients in Phase I (mean of 8.10 vs 8.04, respectively; $P = 0.752$). This negative result does not invalidate the methylation findings; rather, it underscores the pitfalls of relying solely on mRNA expression as a biomarker. This suggests that *LPAR6* expression alone is not a reliable biomarker in the TCGA-OV cohort. However, this finding does not exclude the possibility that promoter methylation regulates *LPAR6* expression in specific patient subsets, as the TCGA cohort includes heterogeneous tumour samples with variable methylation levels, which may obscure expression differences in a subset of patients. Steady-state mRNA levels are influenced by multiple factors including transcription rate, mRNA stability, sample handling, and tumour heterogeneity. Promoter methylation, in contrast, is a stable epigenetic mark that may better reflect functional gene silencing [5]. Furthermore, pro-

motor hypermethylation often precedes transcriptional changes and may be detected even when mRNA levels are partially compensated by other regulatory mechanisms. The absence of paired methylation data in TCGA-OV prevents direct assessment of this relationship.

The methylation results are compelling, with resistant tumors showing nearly double the mean promoter methylation of sensitive tumors (0.67 vs 0.36). This pattern is similar to that observed in platinum resistant [3, 4]. DNA methylation has been demonstrated to silence tumor suppressor genes, as well as cancer-related genes, and can be measured in both tumor tissue and liquid biopsies (e.g., circulating tumor DNA) [7]. *BRCA1* promoter hypermethylation causes malfunction in the DNA repair system, which lowers the efficacy of platinum therapy in ovarian cancer [6].

The strong negative correlation between methylation and expression ($P = -0.87$, $P < 0.001$) supports the functional relevance of *LPAR6* promoter methylation as a silencing mechanism [5]. This is consistent with earlier observations on breast cancer where it was observed that tumor tissues showed hypermethylation of CpG islands of *LPAR6* gene in comparison to para-cancer tissues and the anti-tumor drug 5-Aza indicated a significant upregulation of *LPAR6* expression [13]. As the *LPAR6* is a tumor suppressor in breast cancer, it is possible that it performs the same function in ovarian cancer, where the mechanism of inactivation is via promoter methylation [13].

A notable paradox exists regarding *LPAR6* function: it acts as a tumour suppressor in breast cancer [13] but promotes cell migration in ovarian cancer [10]. This context-dependent role may reflect differences in downstream signaling networks. In breast cancer, *LPAR6* primarily activates G α i/13 signaling pathways that suppress cell proliferation and migration [13], whereas in ovarian cancer, *LPAR6* preferentially couples to G α 12/13 and promotes AKT phosphorylation, enhancing cell motility and survival [10]. The tumour microenvironment, including stromal cell interactions, extracellular matrix composition, and soluble factor availability, may also modulate *LPAR6* signaling outputs [18]. Additionally, the mutational landscape differs substantially between breast and ovarian

cancers, which may influence the downstream consequences of *LPAR6* activation. In ovarian cancer, *LPAR6* promoter methylation may contribute to platinum resistance through mechanisms distinct from its effects on migration. Alternatively, methylation may be an epiphenomenon rather than a causative event. While our study establishes a strong association between *LPAR6* promoter methylation and platinum resistance, the underlying molecular mechanisms remain unexplored. *LPAR6* is known to activate AKT signaling in ovarian cancer [10], which promotes cell survival and may contribute to chemotherapy resistance. However, further investigation is needed to determine whether *LPAR6* silencing directly affects AKT activation, DNA repair, or apoptosis in platinum-resistant cells. Additionally, the tumour micro-environment, including interactions with cancer-associated fibroblasts and immune cells, may modulate *LPAR6* function [18]. Future studies should systematically examine the downstream signaling consequences of *LPAR6* loss and the potential involvement of epithelial-to-mesenchymal transition, drug efflux transporters, and DNA damage repair pathways in *LPAR6*-mediated platinum resistance. Further mechanistic studies are needed to resolve this paradox.

The experimental validation in cell lines further strengthens our conclusions. *LPAR6* expression was significantly downregulated in platinum-resistant cell lines (A2780-CP70, SKOV3, OVCAR8-TR) compared to sensitive lines, with reductions ranging from 68-72% (Table 3). MSP directly validated that promoter methylation is present specifically in resistant cells (Table 4). The 5-Aza rescue experiment established causality by showing that demethylation restores *LPAR6* expression (Figure 8). Western blotting confirmed protein-level downregulation (Table 5), and MTT assays validated the sensitivity/resistance classification (Table 6). The strong correlation between *LPAR6* expression and IC₅₀ (P = -0.92) further supports the role of *LPAR6* in platinum response. This multi-layered experimental validation at the mRNA, DNA methylation, protein, and functional levels substantially corroborates our bioinformatics findings.

The identification of *LPAR6* promoter methylation as a potential predictive biomarker aligns with the growing recognition of epigenetic signatures for patient stratification. Remodeling of DNA methylation patterns is closely associ-

ated with cisplatin resistance in ovarian cancer through the bidirectional regulation of gene expression. Collectively, these findings support the potential for combining *LPAR6* methylation status with other epigenetic biomarkers to guide personalized therapeutic strategies [14, 26].

The translational significance of our findings lies in the stability of DNA methylation as a clinical biomarker. Unlike mRNA expression, which degrades rapidly, DNA methylation is readily measurable in formalin-fixed paraffin-embedded (FFPE) tissue, the standard clinical format for pathology specimens. This technical advantage positions *LPAR6* promoter methylation as a potentially feasible biomarker for clinical laboratory implementation, pending prospective validation. The promising discriminatory performance (AUC = 0.95) suggests that patients with high methylation levels may potentially be identified upfront and offered alternative treatment strategies, such as non-platinum regimens or clinical trials, though this requires prospective validation. This translational potential distinguishes our study from purely computational investigations, although definitive clinical translation will require validation in larger cohorts with standardized cut-offs and demonstrated clinical utility.

Study limitations

Several limitations warrant consideration: 1) independent cohorts without paired methylation-expression data; 2) small sample size (n = 16) in Phase II; 3) post-hoc methylation cut-off requiring validation; 4) retrospective design and bulk tissue analysis; 5) limited cell lines (n = 6) and lack of isogenic pairs; 6) absence of functional rescue assays (*LPAR6* overexpression/knockdown) to definitively establish causality; 7) T1 stage without T2 clinical validation; and 8) need for in vivo and independent patient cohort validation.

Future directions

Future work should include: 1) prospective validation in larger cohorts with paired methylation-expression data to complete T2 translation; 2) functional studies (overexpression/knockdown) to establish causality and elucidate the mechanistic connection to platinum sensitivity; 3) investigation as a liquid biopsy biomarker using circulating tumor DNA; 4)

assessment of methylation status to predict response to demethylating agents combined with platinum therapy; and 5) evaluation of its independent predictive value beyond established biomarkers such as BRCA1/2 mutation status.

Conclusion

This study demonstrates a strong association between *LPAR6* promoter methylation and platinum resistance in a limited-size ovarian cancer cohort, while *LPAR6* expression alone showed no association with platinum response in the larger TCGA cohort. The marked methylation differences ($P < 0.001$; AUC = 0.95) indicate that *LPAR6* promoter methylation warrants further investigation as a potential biomarker. Experimental validation confirmed *LPAR6* downregulation and methylation specifically in resistant cells, supporting biological plausibility. Positioned within the translational research framework, these findings establish a foundation for future clinical validation. The translational significance lies in the potential to use *LPAR6* methylation as a diagnostic biomarker; however, prospective validation in larger cohorts with predefined cut-offs is required before clinical implementation. Direct methylation measurement in FFPE tissue is feasible, but threshold selection and assay standardization are needed. Prospective clinical trials (T2 translational stage) are now warranted to validate *LPAR6* methylation as a biomarker to guide platinum-based therapy decisions.

Acknowledgements

We acknowledge the TCGA Research Network and the GEO database contributors [5] for making the data publicly available. The author(s) declared that financial support was received for this work and/or its publication. This work was supported by the Medical Research Program of the Xi'an Science and Technology Plan Project (Project Number: 2023JH-YXYB-0189). None of the funders played any role in the paper's design, interpretation, or writing.

Disclosure of conflict of interest

None.

Address correspondence to: Kai Tao, Gynecological Oncology Department, Shaanxi Provincial Tu-

mor Hospital, Xi'an 710061, Shaanxi, China. E-mail: t321beyond@gmail.com

References

- [1] Bowtell DD, Böhm S, Ahmed AA, Aspuria PJ, Bast RC Jr, Beral V, Berek JS, Birrer MJ, Blagden S, Bookman MA, Brenton JD, Chiappinelli KB, Martins FC, Coukos G, Drapkin R, Edmondson R, Fotopoulou C, Gabra H, Galon J, Gourley C, Heong V, Huntsman DG, Iwanicki M, Karlan BY, Kaye A, Lengyel E, Levine DA, Lu KH, McNeish IA, Menon U, Narod SA, Nelson BH, Nephew KP, Pharoah P, Powell DJ Jr, Ramos P, Romero IL, Scott CL, Sood AK, Stronach EA and Balkwill FR. Rethinking ovarian cancer II: reducing mortality from high-grade serous ovarian cancer. *Nat Rev Cancer* 2015; 15: 668-679.
- [2] Patch AM, Christie EL, Etemadmoghadam D, Garsed DW, George J, Fereday S, Nones K, Cowin P, Alsop K, Bailey PJ, Kassahn KS, Newell F, Quinn MC, Kazakoff S, Quek K, Wilhelm-Benartzi C, Curry E, Leong HS; Australian Ovarian Cancer Study Group, Hamilton A, Mileskin L, Au-Yeung G, Kennedy C, Hung J, Chiew YE, Harnett P, Friedlander M, Quinn M, Pyman J, Cordner S, O'Brien P, Leditschke J, Young G, Strachan K, Waring P, Azar W, Mitchell C, Traficante N, Hendley J, Thorne H, Shackleton M, Miller DK, Arnau GM, Tothill RW, Holloway TP, Semple T, Harliwong I, Nourse C, Nourbakhsh E, Manning S, Idrisoglu S, Bruxner TJ, Christ AN, Poudel B, Holmes O, Anderson M, Leonard C, Lonie A, Hall N, Wood S, Taylor DF, Xu Q, Fink JL, Waddell N, Drapkin R, Stronach E, Gabra H, Brown R, Jewell A, Nagaraj SH, Markham E, Wilson PJ, Ellul J, McNally O, Doyle MA, Veduru R, Stewart C, Lengyel E, Pearson JV, Waddell N, deFazio A, Grimmond SM and Bowtell DD. Whole-genome characterization of chemoresistant ovarian cancer. *Nature* 2015; 521: 489-494.
- [3] Wang L, Wang X, Zhu X, Zhong L, Jiang Q, Wang Y, Tang Q, Li Q, Zhang C, Wang H and Zou D. Drug resistance in ovarian cancer: from mechanism to clinical trial. *Mol Cancer* 2024; 23: 66.
- [4] Dey Bhowmik A, Shaw P, Gopinatha Pillai MS, Rao G and Dwivedi SKD. Evolving landscape of detection and targeting miRNA/epigenetics for therapeutic strategies in ovarian cancer. *Cancer Lett* 2025; 611: 217357.
- [5] Jones PA and Baylin SB. The epigenomics of cancer. *Cell* 2007; 128: 683-692.
- [6] Zhao C, Liu Y and Cui Z. Epigenetic regulation in gynecological cancers: a paradigm shift in immunotherapy strategies. *J Exp Clin Cancer Res* 2025; 44: 275.

- [7] Lund RJ, Huhtinen K, Salmi J, Rantala J, Nguyen EV, Moulder R, Goodlett DR, Lahesmaa R and Carpén O. DNA methylation and transcriptome changes associated with cisplatin resistance in ovarian cancer. *Sci Rep* 2017; 7: 1469.
- [8] Manzoor HB, Acun M, Yasavoli-Sharahi H, Matar C, Mer A, Tsang BK and Asare-Werehene M. Global DNA methylation signatures associated with chemoresistance and poor prognosis of high grade serous ovarian cancer. *Sci Rep* 2025; 15: 36869.
- [9] Cui R, Bai H, Cao G and Zhang Z. The role of lysophosphatidic acid receptors in ovarian cancer: a minireview. *Crit Rev Eukaryot Gene Expr* 2020; 30: 265-272.
- [10] Onallah H, Davidson B and Reich R. Diverse effects of lysophosphatidic acid receptors on ovarian cancer signaling pathways. *J Oncol* 2019; 2019: 7547469.
- [11] Duan Y, Xu Z, Hao B, Zhang A, Guo C and He Y. Molecular mechanism of ligand recognition and activation of lysophosphatidic acid receptor LPAR6. *Proc Natl Acad Sci U S A* 2025; 122: e2415426122.
- [12] Shruptha P, Poyya J, Vasudevan TG and Satyamoorthy K. Targeting TET enzymes in ovarian cancer: epigenetic regulation, chemoresistance, and therapeutic opportunities. *Epigenomics* 2025; 17: 1551-1564.
- [13] Tao K, Guo S, Chen R, Yang C, Jian L, Yu H and Liu S. Lysophosphatidic Acid Receptor 6 (LPAR6) expression and prospective signaling pathway analysis in breast cancer. *Mol Diagn Ther* 2019; 23: 127-138.
- [14] Tang M, Wang Y, Xie Y, Li J, Ding D, Li C, Chen Q and Han F. Targeting epigenetic networks to overcome cisplatin resistance in ovarian cancer: from mechanisms to clinical translation. *J Ovarian Res* 2026; 19: 135.
- [15] Manzoor HB, Asare-Werehene M, Pereira SD, Satyamoorthy K and Tsang BK. The regulation of plasma gelsolin by DNA methylation in ovarian cancer chemo-resistance. *J Ovarian Res* 2024; 17: 15.
- [16] Cardenas H, Fang F, Jiang G, Perkins SM, Zhang C, Emerson RE, Hutchins G, Keer HN, Liu Y and Matei D. Methylomic signatures of high grade serous ovarian cancer. *Epigenetics* 2021; 16: 1201-1216.
- [17] Fang F, Cardenas H, Huang H, Jiang G, Perkins SM, Zhang C, Keer HN, Liu Y, Nephew KP and Matei D. Genomic and epigenomic signatures in ovarian cancer associated with resensitization to platinum drugs. *Cancer Res* 2018; 78: 631-644.
- [18] Colaprico A, Silva TC, Olsen C, Garofano L, Cava C, Garolini D, Sabedot TS, Malta TM, Pagnotta SM, Castiglioni I, Ceccarelli M, Bontempi G, Noushmehr H. TCGAbiolinks: an R/Bioconductor package for integrative analysis of TCGA data. *Nucleic Acids Res* 2016; 44: e71.
- [19] Law CW, Chen Y, Shi W and Smyth GK. voom: precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biol* 2014; 15: R29.
- [20] Therneau T and Grambsch P. Modeling Survival Data: Extending The Cox Model. 2000.
- [21] Kassambara A, Kosinski M and Biecek P. survminer: Drawing Survival Curves using 'ggplot2'. CRAN: Contributed Packages 2016;
- [22] Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez JC and Müller M. pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinformatics* 2011; 12: 77.
- [23] Wickham H. Data analysis. ggplot2: elegant graphics for data analysis. Springer; 2016. pp. 189-201.
- [24] DeLong ER, DeLong DM and Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* 1988; 44: 837-845.
- [25] Kaplan EL and Meier P. Nonparametric estimation from incomplete observations. *Journal of The American statistical Association* 1958; 53: 457-481.
- [26] Farid EA, Zhang S, Cardenas H, Fu Z, Vieth A, Coon CM, Wei JJ, Matei D and Nephew KP. Blood based biomarkers of DNA methylation associated with platinum resistance in high grade serous ovarian cancer. *bioRxiv [Preprint]* 2026; 2026.04.23.720362.
- [27] Herman JG, Graff JR, Myöhänen S, Nelkin BD and Baylin SB. Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. *Proc Natl Acad Sci U S A* 1996; 93: 9821-9826.
- [28] Meng F, Sun G, Zhong M, Yu Y and Brewer MA. Anticancer efficacy of cisplatin and trichostatin A or 5-aza-2'-deoxycytidine on ovarian cancer. *Br J Cancer* 2013; 108: 579-586.
- [29] Li M, Balch C, Montgomery JS, Jeong M, Chung JH, Yan P, Huang THM, Kim S and Nephew KP. Integrated analysis of DNA methylation and gene expression reveals specific signaling pathways associated with platinum resistance in ovarian cancer. *BMC Med Genomics* 2009; 2: 34.
- [30] Lou JY, Peng ZL, Zheng Y and He B. Improve the chemosensitivity of resistant ovarian cancer cell by small RNA interference. *Sichuan Da Xue Xue Bao Yi Xue Ban* 2008; 39: 388-390.