Chemoresistance-associated alternative splicing signatures in serous ovarian cancer

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Abstract. Primary platinum-based chemoresistance occurs in ~30% of patients with serous ovarian cancer. Chemoresistance is the main cause of disease recurrence, and accurate predictors to identify these patients with chemoresistance are required. Alternative splicing (AS) is a post-transcriptional modification process that is altered in cancer. A possible association between AS and chemoresistance is unclear and needs to be studied comprehensively in ovarian cancer. In the present study, RNA-sequencing data and clinical information for 320 patients with ovarian serous cystadenocarcinoma (OV) were downloaded from The Cancer Genome Atlas (TCGA) database. Splicing events were determined using the TCGA SpliceSeq tool. Seven types of AS events were identified. Univariate and multivariate logistic analyses were performed, and predictive models for OV chemoresistance were established, as well as a splicing network. A total of 22,036 AS events were identified in 7,404 genes, with 915 AS events detected in 677 genes that were significantly associated with chemoresistance in patients with OV. A receiver operating characteristic (ROC) curve was constructed for resistance predictive models composed of the most significant AS events. The area under the ROC curve was 0.931, indicating strong and efficient prediction of chemoresistance. Additionally, the high-risk score was associated with shorter overall survival. The splicing correlation network suggested a potential role of splicing factors in chemoresistance. In summary, the present study created a powerful predictor for primary platinum-based chemoresistance in patients with OV, identified splicing networks that could be involved in potential mechanisms of chemoresistance and provided potential targets to overcome chemoresistance.

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Introduction

Ovarian cancer was the fifth-leading cause of cancer-associated mortality in females in 2015 in the United States, with 295,414 newly-diagnosed cases globally in 2018 and 184,799 cancer-associated deaths globally in 2018 (1,2). Statistical analyses indicate that 90% of ovarian cancer cases are epithelial, with serous carcinoma being the most common pathological type with a 5-year survival rate of 43% (3). Conventional treatment for epithelial ovarian cancer involves cytoreductive surgery followed by platinum- and taxane-based chemotherapy (4). However, development of resistance to chemotherapy eventually induces recurrence after treatment (5). An accurate and robust predictive marker of chemoresistance is urgently required to improve individualized treatment and enhance the prognosis and survival of patients with epithelial ovarian cancer. Previous studies have identified a number of chemoresistance-associated biomarkers, such as reactive stroma signature, markers of cancer stem cells and miRNAs (6-10), but they have not been used in clinical practice. Effective predictors of primary platinum-based chemotherapy resistance would provide novel strategies for treating patients with epithelial ovarian cancer.

Dysregulation of genomic expression serves a critical role in tumorigenesis and chemoresistance in epithelial ovarian cancer. Previous progress in developing genomics-based and precision-targeted therapies has provided novel strategies for treating patients with ovarian cancer (11). However, previous studies have only focused on gene expression levels rather than investigating how alternative splicing (AS) can affect transcript architecture (12,13).

AS is a post-transcriptional modification process that produces a variable mature mRNA transcript from a single gene by removing different intronic or exonic regions from the precursor mRNA and subsequently combining the spliced exons (14,15). AS generates mRNAs with different stabilities or coding potentials, enabling quantitative control of protein production and achieving distinct protein functions (16). AS serves crucial roles in specialized muscle functions (17), angiogenesis (18) and pathological processes, including hearing loss (19), Huntington's disease (20) and cancer (21). Emerging evidence suggests that AS is associated with tumorigenic processes, such as tumor proliferation, invasion, metastasis and apoptosis (22). Splicing factors perform splicing by binding

to pre-mRNAs, influencing exon selection and selecting the splicing site (23). Splicing factors are expressed differentially between normal and cancerous tissues (24,25). Therefore, identifying AS signature profiles and exploring splicing factors may reveal useful cancer biomarkers.

An analysis of AS in cancer has become possible with the advent of deep-sequencing techniques that allow the discovery of previously unknown prognostic and therapeutic biomarkers for patients with cancer. Prognostic predictors based on AS events have been identified in patients with various types of cancer, including ovarian cancer (26-28). However, to the best of our knowledge, no systematic analyses of chemoresistance-associated AS in ovarian cancer have been performed, even though these are urgently required due to the major role of chemoresistance in disease recurrence. In the present study, The Cancer Genome Atlas (TCGA) RNA-sequencing (RNA-seq) data was used to investigate whether AS events could serve as predictors of primary platinum-based chemotherapy resistance in serous ovarian carcinoma.

Materials and methods

Data acquisition. AS profiles were analyzed using the TCGA SpliceSeq tool version 1 provided by the MD Anderson Cancer Center (https://bioinformatics.mdanderson.org/TCGASpliceSeq/) (29). Seven types of AS events were quantified using the percent spliced-in (PSI) value: Exon skip (ES), alternate promoter (AP), alternate terminator (AT), alternative acceptor site (AA), alternate donor site (AD), retained intron (RI) and mutually exclusive exons (ME). The PSI values for the seven types of AS in ovarian serous cystadenoma (OV) were downloaded from TCGA SpliceSeq. AS events with a standard deviation >0.05 and a PSI value >75% were included. Clinical information for the TCGA-OV cohort was obtained from the TCGA database (https://portal.gdc.cancer.gov/projects/TCGA-OV) (30). Individuals who met the following criteria were included in the present study: i) Patients diagnosed with serous ovarian cancer; ii) patients who received platinum-based chemotherapy; and iii) patients with well-defined responses to chemotherapy. Patients without AS information were excluded from the present study. A total of 63 splicing factors and their information were obtained from SpliceAid 2 (31). Level three mRNA expression data of splicing factors were also acquired from the TCGA database.

Statistical analysis. Univariate logistic regression analyses were performed to assess the predictive value of AS events for primary platinum-based chemotherapy resistance. Subsequently, the top 30 most significant AS events from the univariate analyses were included in multivariate logistic regression analyses to build prediction models for each type of AS event individually and for all types of AS events combined. The Akaike information criterion was applied to select the most appropriate risk model (32). The prediction accuracy of the risk models was evaluated by receiver operating characteristic (ROC) analysis. Patients were classified into high- and low-risk groups, with the median score as the cut-off value. Kaplan-Meier analysis and a log-rank test were performed to estimate the difference in overall survival (OS) time between the high- and low-risk groups.

Table I. Demographic and clinical characteristics of ovarian serous cystadenocarcinoma cases in The Cancer Genome Atlas datasets involved in developing alternative splicing signatures to predict primary platinum-based chemoresistance.

Characteristics	Resistance cases, n	Sensitive cases, n	P-value
Sample number	95	225	
Age, years			0.734
<60	56	128	
≥60	39	97	
Stage			0.027
FIGO I/II	1	16	
FIGO III/IV	94	209	
Grade			0.788
Low	12	26	
High	81	194	
Unknown	2	5	
Debulking status			<0.001
Optimal	53	164	
Suboptimal	36	36	
Unknown	6	25	

FIGO, International Federation of Gynecology and Obstetrics.

Resistance-associated splicing factor genes were identified using univariate logistic regression analysis. Pearson's correlation test was used to determine whether expression of the splicing factor genes was significantly associated with the PSI values of resistance-associated AS events. The regulatory network map was built based on the significantly correlated splicing factors and AS events.

All analyses were performed using R (version 3.5.2; www.r-project.org). P<0.05 was considered to indicate a statistically significant difference, unless otherwise specified. Differences in clinicopathologic parameters between chemosensitive and chemoresistant groups, including age, grade, FIGO (International Federation of Gynecology and Obstetrics) stage and debulking status (33), were tested by unpaired t-test or the χ^2 test.

Procedures. R was used to perform the univariate and multivariate logistic analyses and build chemoresistance prediction models. UpSet plots were generated using UpSetR (version 1.4.0; https://cran.r-project.org/web/packages/UpSetR/index.html). The pROC package (version 1.13.0; https://cran.r-project.org/src/contrib/Archive/pROC/) was used to create ROC curves and to calculate the area under the curve (AUC). The Functional Annotation Result Summary tool version 6.8 (https://david.ncifcrf.gov/summary.jsp) from the Database for Annotation, Visualization, and Integrated Discovery (version 6.8) was used for Gene Ontology (GO) (http://geneontology.org) analysis of the corresponding genes (34). The gene interaction network and correlation network were visualized using Cytoscape (version 3.7.1; https://cytoscape.org).
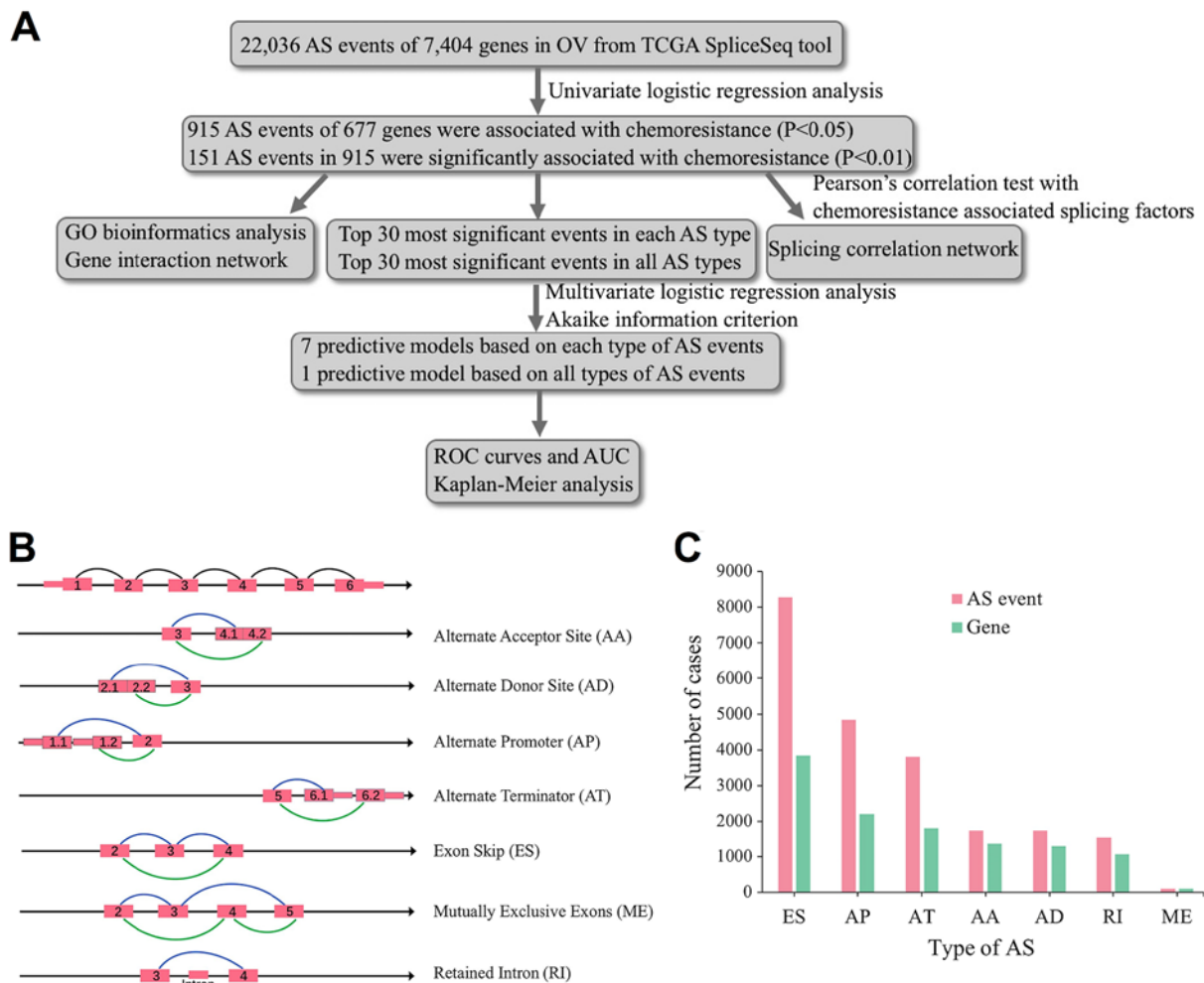
Figure 1. Overview of the seven types of AS. (A) Flowchart of the present study. (B) Illustrations of the seven types of AS events, including AA, AD, AP, AT, ES, ME and RI. (C) Number of AS events and involved genes from 320 patients with OV. AS, alternative splicing; OV, ovarian serous cystadenocarcinoma; AA, alternate acceptor site; AD, alternate donor site; AP, alternate promoter; AT, alternate terminator; ES, exon skip; ME, mutually exclusive exons; RI, retained intron; TCGA, The Cancer Genome Atlas; GO, Gene Ontology; ROC, receiver operating characteristic; AUC, area under the curve.

Results

Comprehensive analysis of AS events in the OV data. The overall process of the present study is described in Fig. 1A. Integrated AS event signatures for 320 patients with OV were curated from the TCGA database (Table I). Seven types of AS events were identified, as shown in Fig. 1B. A total of 22,036 AS events were detected in 7,404 genes, suggesting that one gene might have had more than one AS event. The following numbers of AS events were detected for each type: 8,280 ES events in 3,835 genes; 1,535 RI events in 1,073 genes; 4,841 AP events in 2,196 genes; 3,806 AT events in 1,801 genes; 1,735 AD events in 1,291 genes; 1,741 AA events in 1,357 genes; and 98 ME events in 96 genes (Fig. 1C). The most common type of AS events was ES, followed by AP and AT events.

Chemoresistance-associated AS events in the OV data. The univariate logistic regression analyses of OV data from the TCGA database identified 915 AS events associated with chemotherapy resistance in patients with OV (P<0.05; Table SI). Among these, 151 AS events were significantly associated with chemotherapy resistance (P<0.01; Table SII), 407

AS events were risk factors for chemotherapy resistance [odds ratio (OR)>1], and 508 were protective factors for chemotherapy resistance (OR<1). The distribution of 677 genes involved in 915 AS events was visualized in the UpSet plot (Fig. 2A). A total of 640 genes had only one type of AS event associated with chemoresistance, whereas 37 genes had more than one type of AS event associated with them. For example, ES, AA and AD events in GPR56 were all significantly associated with chemoresistance (Table SI).

GO bioinformatics analysis was performed on 677 genes with AS events. A total of 13 biological processes and 6 molecular functions were identified in the GO analysis (P<0.01; Fig. 2B). These genes were found to be significantly associated with 'protein binding' and 'negative regulation of transcription from RNA polymerase II promoter'. The gene interaction network analysis for these 677 genes revealed a hub that included RHOA, POLR2G, RPS9, DYNLL1 and RPL13A (the top 5 genes with higher degree of connectivity) (Fig. 2C).

Chemoresistance predictors for patients with OV. The top 30 most significant events for each AS type (except for ME, which had only 6 events) and for all types of AS events were

Table II. General characteristics of chemoresistance predictors for ovarian cancer.

Alternative splicing	Formula	OR (95% CI) (High vs. low)
AA	25.108023-4.442929*'TSACC-8246-AA'+3.315118* 'FAM111A-16027-AA'-5.747130*'SERPINA1-29121-AA'-7.858303* 'TNK1-38931-AA'-10.248344*'USHBP1-48249-AA'-11.439197* 'DNAAF3-52039-AA'-11.843241*'POLM-79455-AA'	7.14 (3.65-14.85)
AD	-12.309890-6.338744*'RNF220-2559-AD'-9.644038*'GBP3-3711-AD' +4.0052598*'CLEC16A-34006-AD'+9.383993*'GPR56-36585-AD' +14.723866*'TADA2A-40522-AD'+3.932024*'YIF1B-49610-AD' +7.967860*'YBEY-60918-AD'+5.746687*'ZSCAN25-80706-AD'	9.55 (5.18-18.71)
AP	8.898032-4.064766*'ANGEL2-9775-AP'-3.745283* 'KIAA0391-27213-AP'+2.045282*'PPP1R13L-50435-AP'-9.158386* 'FAM110A-58466-AP'-5.969009*'RBM47-69086-AP'-6.474537* 'MYO10-71601-AP'-5.998058*'NUDT1-78608-AP'+2.415810* 'MID1-88461-AP'	6.68 (4.06-11.41)
AT	1.333149+1.932604*'FPGT-TNNI3K-3457-AT'+6.408309* 'ADAMTSL4-7486-AT'-6.490989*'TMEM180-12952-AT'+3.711496* 'CSTF3-14883-AT'-5.673945*'KLC1-29468-AT'+4.2497568* 'JUP-40930-AT'-4.365262*'ZNF544-52425-AT'-5.382822* 'RAPH1-57077-AT'+1.901203*'KRBOX1-64325-AT'-3.998685* 'ST3GAL6-65794-AT'	11.95 (6.78-22.31)
ES	8.884081+12.456874*'BTAF1-12524-ES'+3.040003*'SPAG9-42494-ES' +6.529384*'GAA-44021-ES'-6.241932*'RBM6-64950-ES'-5.268264* 'SLC10A7-70775-ES'+6.110066*'TRAPPC13-72245-ES'-6.730836* 'PNISR-77056-ES'-4.439848*'PEX2-84241-ES'-5.337279* 'EEF1D-98099-ES'-12.321102*'COL1A2-1412008-ES'	22.05 (10.31-54.59)
ME	-2.554826+2.383275*'ATE1-91855-ME'+2.300138*'GOLT1B-92984-ME' +5.824629*'RAB28-265743-ME'	2.13 (1.36-3.35)
RI	-14.038586+3.701273*'POLR2G-16420-RI'+11.008279*'GLG1-37565-RI' -5.099955*'C17orf58-43119-RI'+3.088192*'CDKN2D-47553-RI'-4.112494* 'UNC50-54643-RI'-10.031071*'ID1-58896-RI'-7.797206*'HOPX-69372-RI' -1.959239*'TTC23L-71732-RI'+4.305603*'SOD2-78304-RI'+3.626741* 'PILRB-80936-RI'+2.983951*'VPS28-85606-RI'	10.23 (5.52-20.39)
All	14.333930-8.697005*'SERPINA1-29121-AA'+5.713988*'SMIM7-48190-AD'- 9.355225*'TRAPPC6B-27360-ES'+9.130642*'GAA-44021-ES'-2.458800* 'PDE4D-72144-AP'-5.978179*'RBM6-64950-ES'-4.543145* 'SLC10A7-70775-ES'+11.331974*'TRAPPC13-72245-ES'-5.850540* 'FAM49B-85160-ES'-6.577849*'EEF1D-98099-ES'+5.275202* 'CLEC16A-34006-AD'-11.452096*'COL1A2-1412008-ES'-8.721743* 'ZNF544-52425-AT'	64.88 (22.55-284.86)

OR, odds ratio; AA, alternate acceptor site; AD, alternate donor site; AP, alternate promoter; AT, alternate terminator; ES, exon skip; ME, mutually exclusive exons; RI, retained intron.

selected as candidates to identify the independent predictive model for chemoresistance in OV (Table SIII). Multivariate logistic regression analysis was performed for the 30 candidate events for each AS type and for all AS types combined, and the Akaike information criterion was used to select the most appropriate risk model (32). The predictive models are presented in Table II. The median score was used as the cut-off value, the patients were divided into high- and low-risk groups, and the OR for each model was calculated. ROC curves were generated and the AUCs were determined to evaluate the effectiveness of the chemoresistance predictive models. The seven predictors that were built using the seven types of AS events displayed considerable power in distinguishing the chemotherapy response of patients with OV. The model based on ES events was the most effective predictor among the models based on each type of AS event, with an AUC of 0.894 (Fig. 3).
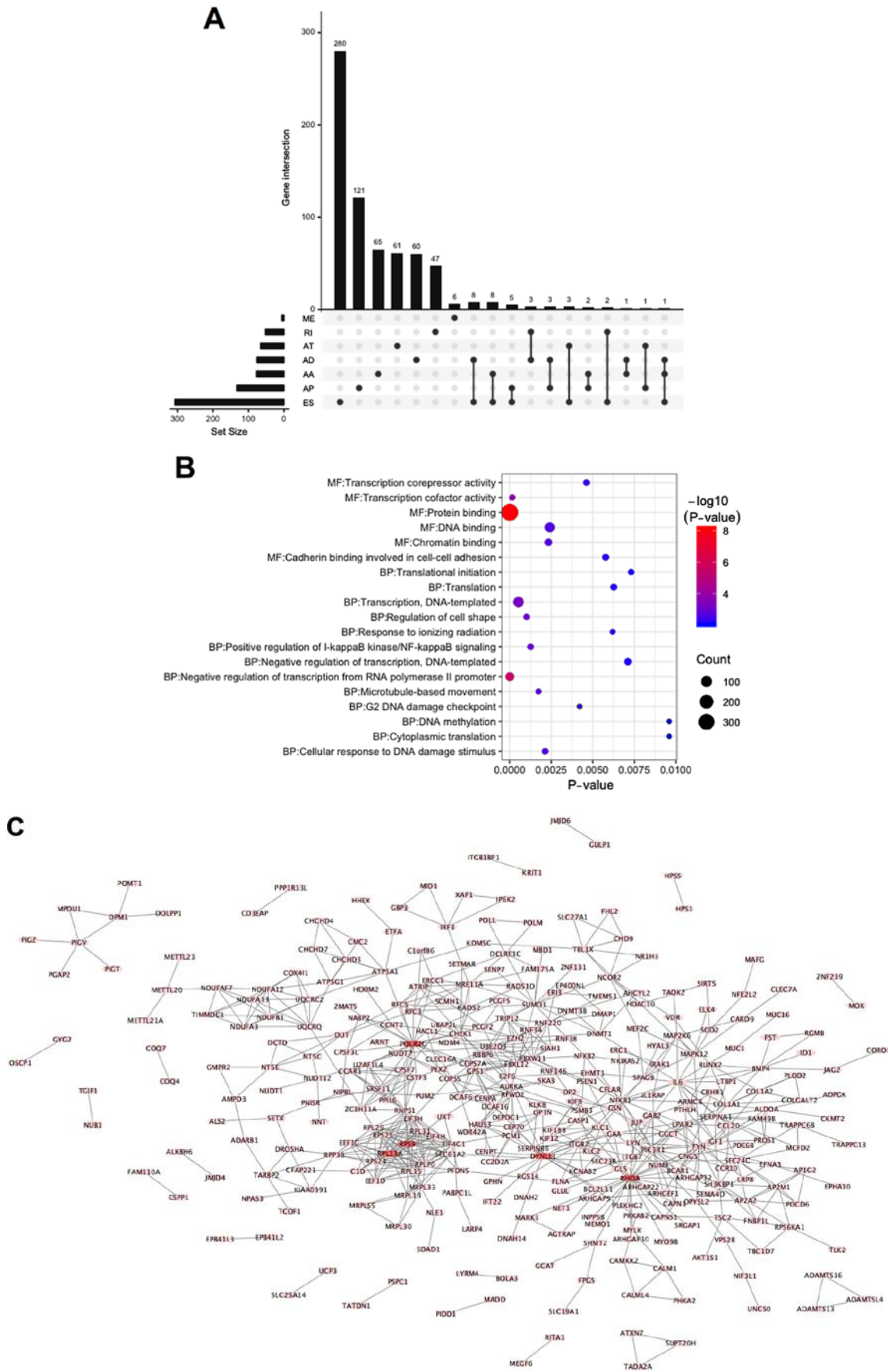
Figure 2. UpSet plot, GO analysis and gene network of chemoresistance-associated AS in OV. (A) UpSet plot of interactions between the seven types of chemo-resistance-associated AS events in OV. (B) GO analysis of chemoresistance-associated AS events in OV. (C) Gene network of chemoresistance-associated AS in OV generated by Cytoscape. AS, alternative splicing; OV, ovarian serous cystadenocarcinoma; AA, alternate acceptor site; AD, alternate donor site; AP, alternate promoter; AT, alternate terminator; ES, exon skip; ME, mutually exclusive exons; RI, retained intron; GO, Gene Ontology; BP, biological process; MF, molecular function.

Table III. Information for AS event candidates involved in the model based on all types of AS events.

Gene	OR (95% CI)	P-value	Type	Exon
SMIM7	41.5291 (8.0671-228.7526)	0.00024	AD	7.2
COL1A2	0.0043 (0.0003-0.0549)	0.00061	ES	23:24:25:26:27:28:29:30:31:32:33:34:35:36:37:45:46:47
GAA	130.2634 (13.7266-1,594.0712)	0.00075	ES	2.2
EEF1D	0.0247 (0.0035-0.1586)	0.00133	ES	6
ZNF544	0.0508 (0.0107-0.2331)	0.00142	AT	10.2
SLC10A7	0.0916 (0.0257-0.3090)	0.00151	ES	13
FAM49B	0.0373 (0.0064-0.2078)	0.00186	ES	5
RBM6	0.0705 (0.0163-0.2871)	0.00233	ES	4:05:06
TRAPPC6B	0.0109 (0.0009-0.1213)	0.00235	ES	4
CLEC16A	10.9329 (3.0549-40.9032)	0.00237	AD	11.2
PDE4D	0.3015 (0.1554-0.5813)	0.00274	AP	1
TRAPPC13	25.9213 (4.4025-167.8280)	0.00322	ES	9
SERPINA1	0.03190 (0.0043-0.2147)	0.00357	AA	2.4

OR, odds ratio; AA, alternate acceptor site; AD, alternate donor site; AP, alternate promoter; AT, alternate terminator; ES, exon skip; AS, alternative splicing.
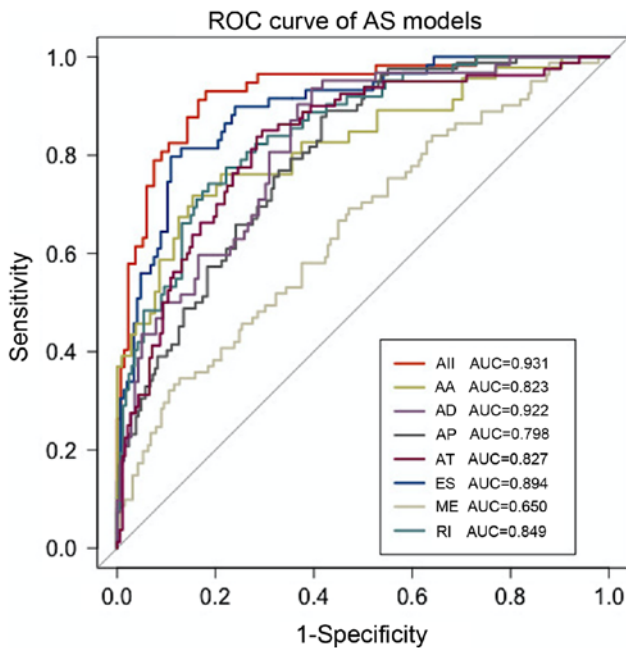

Figure 3. ROC curves with AUCs of chemoresistance predictors built by one type or all seven types of AS events in ovarian serous cystadenocarcinoma. AS, alternative splicing; ROC, receiver operating characteristic; AUC, area under the curve; AA, alternate acceptor site; AD, alternate donor site; AP, alternate promoter; AT, alternate terminator; ES, exon skip; ME, mutually exclusive exons; RI, retained intron.

The model based on all types of AS events exhibited the best efficiency with an AUC of 0.931. The information for AS event candidates involved in this model is presented in Table III. This model was utilized in univariate and multivariate logistic analyses of chemotherapy resistance together with common clinical characteristics. A high-risk score was an independent risk factor for chemoresistance (Table IV).

To verify the prognostic value of these predictive models, Kaplan-Meier analysis and log-rank tests were performed for each model. The results indicated that the patients in the high-risk groups in risk models based on AP, ES, RI and all types of AS events had shorter survival time compared with patients in the low-risk groups (Fig. 4). In the risk model based on all types of AS events, the median OS time for the high- and low-risk groups were 1,341 and 1,875 days, respectively (Fig. 4H).

Potential correlation network of AS splicing factors. AS is regulated primarily by splicing factors. Therefore, it is crucial to determine whether key splicing factors regulate chemoresistance-associated AS events in OV. Univariate logistic analyses revealed that the mRNA expression levels of five splicing factors were associated with chemoresistance. Information of these splicing factors was obtained from SpliceAid2 and was shown in Table V. Subsequently, correlation analyses of the expression levels of the five splicing factors and the PSI values of 151 AS events were performed (P<0.01 in univariate analyses). A splicing correlation network was generated from the significant correlations (P<0.05; Fig. 5A) between 70 chemoresistance-associated AS events, including 38 protective and 32 adverse AS events, and the 5 splicing factors. Most of the protective AS events were positively correlated with the expression of splicing factors, such as AP PSI value of SH3YL1 with expression of PTBP1, AD PSI value of RPL15 with expression of YBX1, AP PSI value of CLUL1 with expression of SYNCRIP. Most of the adverse AS events were negatively correlated with the expression of splicing factors, such as AT PSI value of UBAP2L with expression of TRA2B, ES PSI value of RPS24 with expression of SYNCRIP, ES PSI value of RHOA with expression of ELAVL4. Representative correlations between AS events and splicing factors are shown in the dot plots (Fig. 5B-G).

Table IV. Univariate and multivariate logistic regression analyses for chemoresistance in The Cancer Genome Atlas datasets.

Characteristics	Univariate			Multivariate		
	OR	95% CI	P-value	OR	95% CI	P-value
Age, years						
<60	1 (reference)			1 (reference)		
≥60	0.90	0.52-1.53	0.741	1.72	0.77-3.91	0.270
Stage						
FIGO I/II	1 (reference)			1 (reference)		
FIGO III/IV	4.10	0.94-41.14	0.186	inf	0-inf	0.989
Grade						
Low	1 (reference)			1 (reference)		
High	0.99	0.43-2.42	0.978	0.37	0.06-1.61	0.290
Debulking status						
Optimal	1 (reference)			1 (reference)		
Suboptimal	2.53	1.38-4.63	0.012	5.13	2.01-15.21	0.007
Risk score						
Low	1 (reference)			1 (reference)		
High	64.88	22.55-284.86	<0.001	192.07	41.20-2087.85	<0.001

FIGO, International Federation of Gynecology and Obstetrics; OR, odds ratio; inf, infinity.

Discussion

Previous studies have focused on the function of single AS events associated with ovarian cancer. Elevated expression of gluta-thione-specific γ-glutamylcyclotransferase splicing variants has been related to poor outcomes in ovarian cancer (35). Researchers have also found that an increased level of the mesenchymal spliced variant CD44s and reduced expression of the epithelial variant CD44v promotes epithelial-mesenchymal transition and invasion of ovarian cancer cells (36). A splice variant of the tetraspanin KAI1 mitigates its tumor-suppressive function, inducing cell migration and resulting in poor prognosis (37). Chemotherapy sensitivity is the main factor influencing survival in serous ovarian cancer (38). However, to the best of our knowledge, only a few studies have investigated the potential role of AS events in chemotherapy resistance of ovarian cancer (39,40). AS events of the multidrug resistance-associated protein 1 gene in ovarian tumors have been reported to confer resistance to doxorubicin therapy (39). Overexpression of the VIII-deficient excision repair cross-complementing group 1 (ERCC1) exon is able to enhance cisplatin sensitivity in ovarian cancer cell lines by reducing the protein expression levels of ERCC1 (40). The present study demonstrated that the ES event of the ERCC1 gene was a protective factor for chemotherapy resistance, with an OR of 0.069 and a 95% CI of 0.008-0.638 (Table SI), indicating that these results are consistent with the aforementioned study. Hence, these studies demonstrated the potential role of AS in chemotherapy resistance of OV, and further systematic studies of AS signatures in OV may help to identify potential biomarkers and targets for chemoresistance.

The present study systemically analyzed the role of AS signatures in chemotherapy resistance using data from 320 patients with OV from the TCGA database, and then built powerful resistance predictors. A total of 22,036 AS events were detected in 7,404 genes. Approximately 38% of the AS events were ES, and the risk model based on ES events exhibited high efficiency. ES events can be validated by PCR. Thus, future research should investigate associations between ES events and chemotherapy resistance in more detail. The predictive model based on all types of AS had the best efficiency, with the AUC of the ROC curve reaching 0.931. This was much higher than the AUC for models based on a single type of AS and was more efficient than previous predictors based on single mRNA expression (AUC, 0.8056) (41), the lncRNA signature (AUC, 0.83) (42) or the clinical serum CA125/ascites leptin (AUC, 0.846) (43). These combined results suggest that this model could provide accurate predictions of chemotherapy resistance in patients with OV.

Additionally, the present study investigated the potential role of splicing factors in chemotherapy resistance. Five splicing factors were associated with chemotherapy resistance, and their possible targets were identified. These results suggested that splicing factors were involved in chemotherapy resistance in patients with serous ovarian cancer. Further work is required to determine whether regulation of these specific splicing factors could increase the sensitivity to chemotherapy and prevent disease recurrence.

The present study presented some limitations. The present study was based on RNA-seq data from the TCGA database. Validation using other databases or larger cohorts is required in future studies. Numerous splicing events and splicing factors that may be associated with the biological behavior of OV were identified and should be further evaluated in future experimental studies.

Table V. Information for splicing factors in the correlation network from SpliceAid 2.

Splicing factors	Gene names	Descriptions	Expression in normal ovary tissue	Expression in ovarian cancer
hnRNP I (PTB)	PTBP1	Polypyrimidine tract binding protein 1. In the context of CALCA gene, PTB enhances exon 4 inclusion (PMID:9858533). nPTB functionally compensates for PTB and is upregulated when PTB is removed (PMID:17679092).	P(3)_M(4)	H(1)_M(4)_L(5)
HTra2beta1	TRA2B	Splicing factor arginine/serine-rich 10.	P(3)_L(4)	H(1)_M(4)_L(5)
HuD	ELAVL4	Embryonic lethal abnormal vision Drosophila-like 4 (Hu antigen D).	A(4)	A(1)_L(4)_A(5)
YB-1	YBX1	Y box binding protein 1.	P(3)_M(4)	H(1)_M(4)_M(5)
hnRNP Q	SYNCRIP	Synaptotagmin binding cytoplasmic RNA interacting protein.	P(3)_M(4)	M(1)_M(4)_L(5)

(1) Human Protein Atlas; (2) Human Protein Reference Database; (3) Human Proteinpedia; (4) Human Transcriptome Map; (5) Cancer Genome Anatomy Project. A, absent; L, low; M, medium; H, high; P, present.
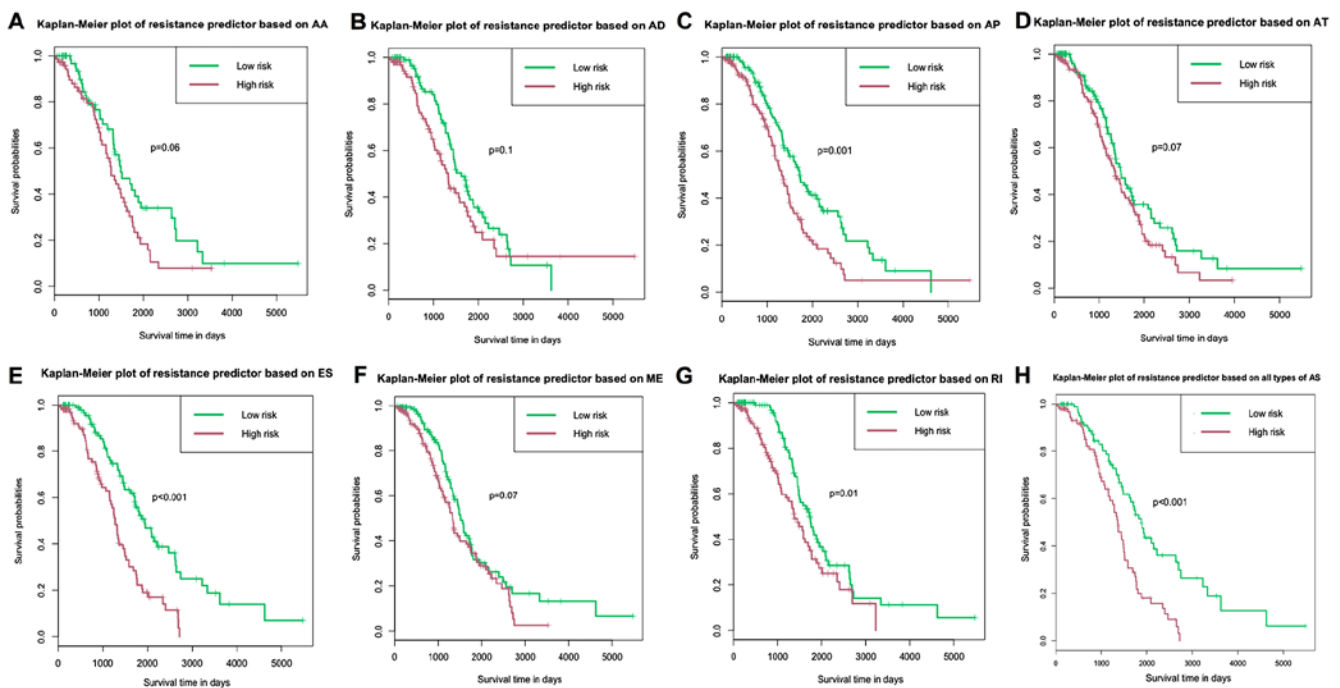

Figure 4. Kaplan-Meier plots of chemoresistance predictors for patients with OV. (A) Kaplan-Meier curves of chemoresistance predictors built with AA events for patients with OV. (B) Kaplan-Meier curves of chemoresistance predictors built with AD events for patients with OV. (C) Kaplan-Meier curves of chemoresistance predictors built with AP events for patients with OV. (D) Kaplan-Meier curves of chemoresistance predictors built with AT events for patients with OV. (E) Kaplan-Meier curves of chemoresistance predictors built with ES events for patients with OV. (F) Kaplan-Meier curves of chemoresistance predictors built with ME events for patients with OV. (G) Kaplan-Meier curves of chemoresistance predictors built with RI events for patients with OV. (H) Kaplan-Meier curves of the chemoresistance predictor built with all types of AS events for patients with OV. AS, alternative splicing; OV, ovarian serous cystadenocarcinoma; AA, alternate acceptor site; AD, alternate donor site; AP, alternate promoter; AT, alternate terminator; ES, exon skip; ME, mutually exclusive exons; RI, retained intron.

In summary, the present study determined that AS events provided valuable predictors for chemotherapy resistance. The model used provided efficient risk stratification for predicting chemotherapy resistance in patients with OV. A splicing correlation network was generated to explore the potential relationship between splicing factors and AS. A number of valuable targets were identified for future validation. The present study elucidated the role of AS events in primary platinum-based chemoresistance in patients with serous ovarian cancer and provided potential targets to overcome chemoresistance.
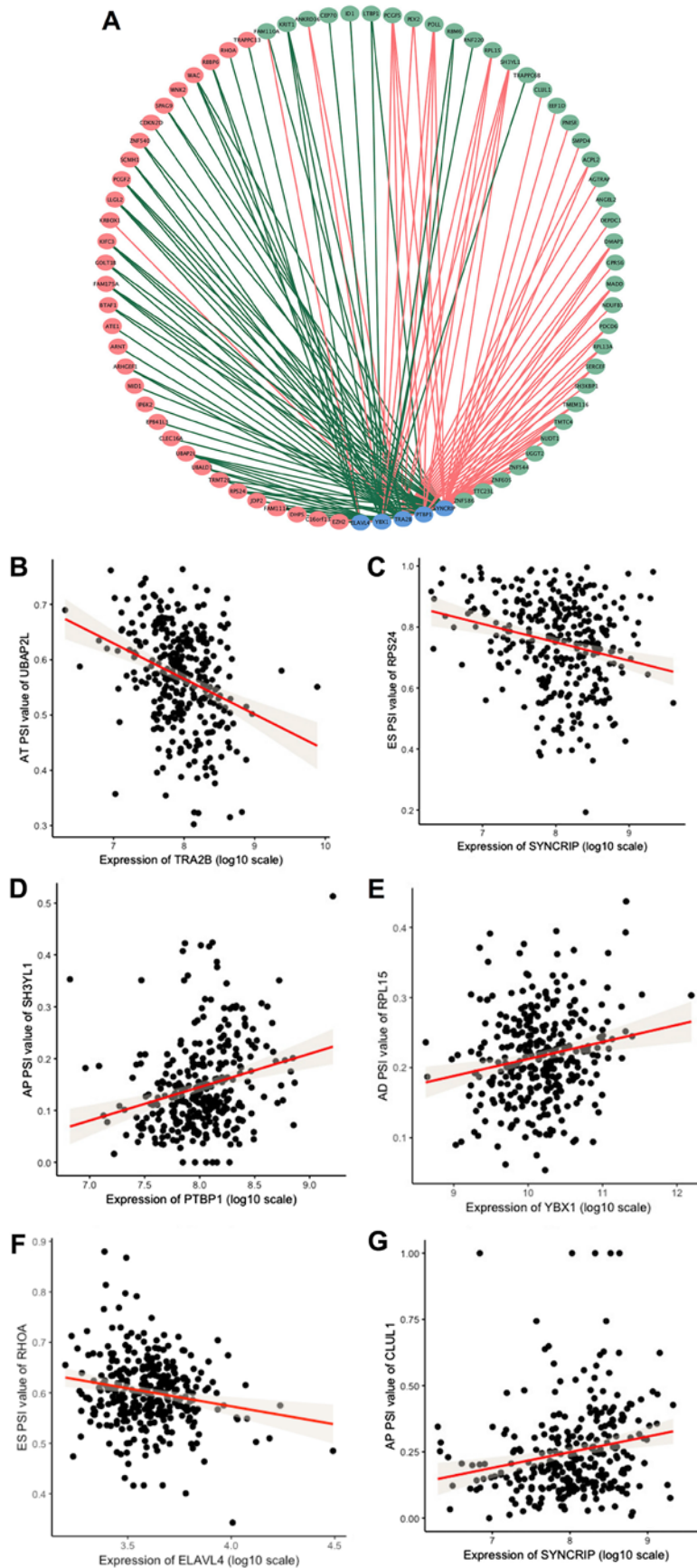
Figure 5. Splicing correlation network in OV. (A) Expression of five survival-associated splicing factors (blue circles) was positively (red lines) or negatively (green lines) correlated with the PSI values of 38 favorable (green circles) or 32 adverse prognosis AS events (red circles). (B) AT PSI value of UBAP2L was negatively correlated with expression of TRA2B. (C) ES PSI value of RPS24 was negatively correlated with expression of SYNCRIP. (D) AP PSI value of SH3YL1 was positively correlated with expression of PTBP1. (E) AD PSI value of RPL15 was positively correlated with expression of YBX1. (F) ES PSI value of RHOA was negatively correlated with expression of ELAVL4. (G) AP PSI value of CLUL1 was positively correlated with expression of SYNCRIP. $P<0.05$. AS, alternative splicing; OV, ovarian serous cystadenocarcinoma; PSI, percent spliced-in.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

TS and QY designed the study. TS performed the statistical analyses and wrote the manuscript. QY revised and edited the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

In the original article of the datasets, the trials were approved by the local institutional review boards of all participating centers, and informed consent was obtained from all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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