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C-terminal Tensin-like/Sphingomyelin Synthase 1 Co-expression Is Associated with Epithelial-mesenchymal Transition, Platinum Resistance, and an Immunosuppressive Tumor Microenvironment in Ovarian Cancer

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ABSTRACT

We evaluated whether co-expression of C-terminal tensin-like protein (CTEN/TNS4) and sphingomyelin synthase 1 (SMS1/SGMS1) is associated with epithelial-mesenchymal transition (EMT), platinum resistance, tumor immune features, and outcome in ovarian cancer.

This single-center retrospective cohort included 276 patients treated with primary surgery followed by platinum-containing chemotherapy. Immunohistochemistry assessed CTEN, SMS1, EMT markers, and immune-microenvironment markers. Logistic regression and Cox models evaluated platinum resistance and survival.

High CTEN and SMS1 expression occurred in 45.7% and 43.1% of tumors, respectively; 28.3% were dual-high. Dual-high expression was associated with advanced FIGO stage, CA125 ⩾500 U/mL, ascites, lymph-node metastasis, residual disease ⩾ 1 cm, recurrence/progression, and platinum resistance. It was also associated with low E-cadherin, high N-cadherin and Vimentin, an EMT-high phenotype, low CD8 infiltration, high CD163 infiltration, and higher PD-L1 expression. In multivariable analysis, dual-high expression remained associated with platinum resistance (OR=2.18, 95% CI 1.21 − 3.94) and shorter PFS (HR=1.52, 95% CI 1.05 − 2.20).

CTEN/SMS1 dual-high expression identifies a clinically adverse ovarian-cancer phenotype associated with EMT, platinum resistance, and relatively immunosuppressive tumor features. These retrospective associations require multicenter and functional validation.

Keywords: *CTEN*; Epithelial-mesenchymal transition; Ovarian cancer; Platinum resistance; *SMS1*; *SGMS1*; *TNS4*; Tumor immune microenvironment

INTRODUCTION

Ovarian cancer is still one of the main malignant tumors of the female reproductive system, and epithelial ovarian cancer is the most common histological type. Its early symptoms are often unclear, and some patients have almost no obvious symptoms, so many patients are first diagnosed at an advanced stage. Recurrence after initial treatment and later drug resistance still limit long-

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term survival. At present, cytoreductive surgery followed by platinum-based chemotherapy remains the basic treatment. In advanced disease, prognosis is closely related to whether surgery is complete, International Federation of Gynecology and Obstetrics (FIGO) stage, histological subtype, and the later response to systemic therapy.[1,2] Most patients respond to platinum agents at first, but many eventually develop recurrence or progression. Relapse or progression within 6 months after platinum-containing chemotherapy is usually called platinum resistance, and this condition often means fewer treatment choices and poorer outcome.[3,4] Therefore, tissue biomarkers that can help find patients with higher risk of platinum resistance and early recurrence are still clinically useful.

Platinum resistance usually does not come from one single biological change. It is more likely to be the result of several processes working together, such as DNA-damage repair, changes in drug entry and discharge, avoidance of apoptosis, metabolic change, and adaptation to the surrounding microenvironment.[4] In this setting, epithelial-mesenchymal transition (EMT) has received much attention, because it is closely related to invasion, metastasis, and treatment resistance in ovarian cancer. During EMT, tumor cells gradually lose epithelial features and cell adhesion, while gaining mesenchymal features that help movement and invasion. These changes are often accompanied by decreased E-cadherin expression and increased expression of N-cadherin, Vimentin, and transcription factors such as *Snail* and zinc finger E-box binding homeobox (*ZEB*).[5–7] In ovarian cancer, EMT may help peritoneal spread and invasive growth, and it may also make tumor cells more adaptable under chemotherapy stress. Abnormal expression of EMT-related factors, including *Snail*, has been linked with worse survival in ovarian cancer.[8] Thus, clinicopathological analysis focused on EMT may help explain part of the biology of platinum-resistant disease.

Besides changes inside tumor cells, the tumor immune microenvironment also affects ovarian cancer progression and response to treatment. Earlier studies showed that T-cell infiltration inside tumors is related to recurrence and survival,[9] suggesting that host antitumor immunity can influence the clinical course. Later studies reported that more $CD8^+$ T-cell infiltration and a higher $CD8^+$/regulatory T (Treg) cell ratio are usually linked with better prognosis.[10] In contrast, $FOXP3^+$ regulatory T cells may gather in ovarian cancer tissue and ascites, promote local immune suppression, and predict shorter survival.[11] Programmed cell death 1 ligand 1 (PD-L1) expression and $CD8^+$ T-cell infiltration have also been reported to have prognostic value, which suggests that immune-checkpoint pathways may take part in immune escape.[12] Tumor-associated macrophages are another important part of the immune environment, and their number and polarization may influence tumor progression and treatment response.[13] Therefore, it is meaningful to examine CD8, FOXP3, CD68, CD163, and PD-L1 together when studying EMT and platinum resistance.

CTEN, also called C-terminal tensin-like protein and encoded by *TNS4*, is a member of the tensin family. This family is involved in focal adhesion, cytoskeletal change, and cell migration. Previous work has suggested that CTEN is related to cell movement, invasion, and tumor progression in several solid tumors, and abnormal CTEN expression has been linked with changes in tumor behavior.[14] In colorectal cancer, CTEN was reported to increase migration and invasion and to take part in EMT-related processes, partly through effects on E-cadherin expression.[15] Even so, evidence about CTEN expression in clinical ovarian cancer samples, especially its relation to EMT, platinum resistance, and the immune microenvironment, is still limited. Because ovarian cancer spread is closely connected with adhesion, migration, and EMT, it is reasonable to study the clinical meaning of CTEN expression.

SMS1, encoded by *SGMS1*, participates in sphingomyelin synthesis. Sphingolipid metabolism helps maintain membrane structure, and it also affects cell survival, apoptosis, stress response, and drug sensitivity. Abnormal SMS1 expression has been connected with sphingolipid reprogramming and poor outcomes in some cancers.[16] In ovarian cancer, altered sphingolipid metabolism has attracted more attention, because it may contribute to drug resistance; lipid-metabolism-related molecules may help tumor cells tolerate chemotherapy pressure.[17] However, SMS1 expression in ovarian cancer tissues, and its relation to CTEN-associated phenotypes, has not been fully described, especially in studies that combine EMT, platinum resistance, and immune-environment features in clinical specimens.

Based on this background, this study analyzed ovarian cancer tissues from patients treated in our hospital, and immunohistochemistry (IHC) was used to evaluate CTEN, SMS1, EMT-related markers, and immune-environment markers. These tissue data were

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combined with clinicopathological variables, response to platinum-based chemotherapy, and follow-up outcomes. The main purpose was to determine whether CTEN/SMS1 co-expression was associated with EMT phenotype, platinum resistance, tumor immune microenvironment, and prognosis. Previous studies have explored the individual role of CTEN or SMS1 in malignant tumors, but few clinical studies have focused on their co-expression and synergistic effects. The relationships between CTEN/SMS1 dual expression and EMT, platinum resistance as well as tumor immune microenvironment in ovarian cancer remain unclear. Evaluating CTEN and SMS1 simultaneously, rather than detecting a single molecule, can identify a high-risk ovarian cancer phenotype with activated EMT, immunosuppression and platinum resistance, which fills the above research gaps. Because this work was a retrospective clinicopathological cohort study, all conclusions about CTEN/SMS1 are described as tissue-level associations and risk-stratification evidence, not as proof of a causal molecular pathway. The SMS1-dependent signaling proposed by the study design should therefore be regarded as a hypothesis for later functional studies.

MATERIALS AND METHODS

Study Population

This single-center retrospective cohort study screened consecutive patients who underwent primary surgery in our hospital between January 2015 and December 2021, received a postoperative pathological diagnosis of ovarian cancer, and were treated with platinum-containing chemotherapy. Formalin-fixed, paraffin-embedded primary tumor tissue was available for immunohistochemical evaluation in every included case. After the predefined inclusion and exclusion criteria were applied, 276 patients were included in the final analysis.

To reduce the influence of pathological heterogeneity, the main analysis focused on epithelial ovarian tumors, and histological subtype was included in the statistical models. As high-grade serous carcinoma was the largest subtype, a restricted subgroup analysis was also carried out, so that the stability of the main findings could be checked in a more homogeneous patient group. In terms of sample size justification, this study included 276 ovarian cancer patients, which met the statistical power requirements for comparative analysis and multivariate regression. The overall incidence of platinum resistance in this cohort was 35.1%. Based on the difference in platinum resistance rate between the CTEN/SMS1 dual-high group (52.6%) and non-dual-high group (28.3%), sample size calculation for two-group rate comparison was performed with $\alpha=0.05$, $\beta=0.2$, and statistical power of 80%. The calculated minimum required sample size for each group was approximately 70 cases, and the dual-high group (n=78) and non-dual-high group (n=198) both exceeded this threshold. For multivariate logistic regression and Cox proportional hazards models, we followed the epidemiological principle that at least 10 outcome events are required per independent variable. There were 97 platinum-resistant cases, 156 recurrence/progression cases, and 103 death events in total, which ensured sufficient statistical power for regression analysis. Sensitivity analyses including quartile grouping and continuous variable models further verified the reliability of the results.

The inclusion criteria were as follows: (1) ovarian cancer confirmed by postoperative pathology; (2) primary surgery performed in our hospital, with primary tumor tissue available; (3) postoperative platinum-based chemotherapy; (4) retrievable clinicopathological data, platinum-treatment response data, and follow-up information; and (5) tissue quality adequate for immunohistochemical assessment. The exclusion criteria were: (1) concurrent malignancies; (2) pathological diagnosis that could not be reviewed, or missing key clinical data; (3) no platinum-based chemotherapy; (4) response to platinum therapy could not be determined; and (5) extensive tissue necrosis, insufficient tissue volume, or staining quality not suitable for interpretation.

The institutional ethics committee approved this study. Before statistical analysis, all patient data were anonymized. Because this was a retrospective and noninterventional study, informed consent was handled according to the requirements stated in the ethics approval.

The Ethics Committee of Affiliated Hospital of Yanbian U niversity approved this retrospective study (2021015). P atient data were deidentified before analysis. Becaus e the study used archived tissue and retrospective clinica l records and involved no additional intervention, the re quirement for additional written informed consent was waived by the ethics committee.

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Clinical Data Collection and Handling of Confounders

Clinical information was collected from electronic medical records, operative notes, pathology reports, chemotherapy records, and follow-up systems. The variables included age, FIGO stage, histological subtype, tumor grade, preoperative CA125 level, ascites, lymph-node metastasis, postoperative residual disease, chemotherapy regimen, number of chemotherapy cycles, response to platinum-based treatment, recurrence or progression, survival status, and follow-up time. FIGO stage was determined according to the International Federation of Gynecology and Obstetrics staging system used at the time of diagnosis and treatment. Postoperative residual disease was grouped as<1 cm or≥1 cm according to operative records, and preoperative CA125 was grouped at 500 U/mL.

The records for *BRCA1/2* status, homologous recombination deficiency (HRD), poly (ADP-ribose) polymerase (PARP) inhibitor maintenance therapy, and bevacizumab use were not equally complete across 2015–2021. For this reason, the main analyses used core covariates that were routinely available, including FIGO stage, histological subtype, tumor grade, preoperative CA125, ascites, lymph-node metastasis, and residual disease. When *BRCA*/HRD status and maintenance-therapy information were available, these variables were included in extended sensitivity analyses. Variables with much missing information or inconsistent records were not included in the core model, which helped reduce bias caused by selective case inclusion. All variable definitions, grouping rules, and main models were set before outcome analysis.

Definition of Response to Platinum Therapy

All patients received postoperative platinum-containing chemotherapy. The date of the last platinum-containing chemotherapy cycle was used to calculate the platinum-free interval. Patients were classified as platinum resistant if they had recurrence or progression within 6 months after finishing platinum-containing chemotherapy, or if the disease progressed during treatment. Patients without recurrence or progression for more than 6 months after finishing platinum-containing chemotherapy were classified as platinum sensitive.

Recurrence or progression was judged by reviewing imaging results, clinical records, and serial tumor-marker changes together. For patients who only had CA125 elevation but no imaging or clinical evidence of recurrence, 2 investigators independently reviewed the medical records and decided whether the criteria for progression had been met.

Immunohistochemistry

Formalin-fixed, paraffin-embedded primary tumor tissues obtained during surgery were used for immunohistochemistry. After hematoxylin and eosin-stained sections were reviewed by pathologists, representative tumor areas were selected, and serial 4-μm sections were prepared. The sections were baked at 60°C to 65°C, deparaffinized in xylene, rehydrated through graded ethanol, and then treated with heat-induced antigen retrieval under antibody-specific conditions set in preliminary experiments. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide. Primary antibody incubation was followed by secondary antibody detection, 3,3'-diaminobenzidine (DAB) visualization, hematoxylin counterstaining, dehydration, clearing, and mounting. The slides were observed under light microscopy. Known positive tissues were used as positive controls in each staining batch, while omission of the primary antibody or use of an isotype control was used as the negative control.

The antibody panel included CTEN, SMS1, EMT-related markers, and tumor-immune-microenvironment markers. EMT-related markers included E-cadherin, N-cadherin, Vimentin, and Snail/ZEB1; immune-microenvironment markers included CD8, FOXP3, CD68, CD163, and PD-L1. For each antibody, slides were stained in the same batch or with the same standardized protocol, and chromogen exposure and counterstaining times were kept consistent within each batch. After staining, investigators checked tumor-cell staining, stromal staining, and internal and external controls. Sections with detachment, extensive necrosis, heavy background staining, or invalid internal controls were excluded.

Example reporting format: CTEN (rabbit monoclonal, clone EPR17848, Abcam, cat. no. ab192247, 1:200); SMS1/SGMS1 (rabbit polyclonal, Proteintech, cat. no. 14732-1-AP, 1:150); E-cadherin (clone 24E10, Cell Signaling Technology, cat. no. 3195, 1:200); N-cadherin (clone D4R1H, CST, cat. no. 13116, 1:200); Vimentin (clone D21H3, CST, cat. no. 5741, 1:200); Snail (clone C15D3, CST, cat. no. 3879, 1:100); ZEB1 (rabbit monoclonal, Abcam, cat. no. ab203829, 1:200); CD8 (clone C8/144B, Dako, cat. no. M7103, 1:100); FOXP3 (clone 236A/E7, Abcam, cat. no.

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ab20034, 1:100); CD68 (clone KP1, Dako, cat. no. M0814, 1:200); CD163 (clone 10D6, Leica/Novocastra, cat. no. NCL-L-CD163, 1:100); PD-L1 (clone 22C3, Dako, cat. no. M3653, ready-to-use or laboratory-validated dilution). Snail and ZEB1 should be reported as separately stained antibodies with separately scored results unless the original laboratory protocol documented a validated cocktail.

Interpretation of Immunohistochemical Staining

All immunohistochemical slides were independently evaluated by 2 pathologists experienced in gynecologic tumor pathology, and they were blinded to clinical data, platinum-treatment response, and outcomes. Each slide was first checked at low magnification to judge the general staining distribution. Semiquantitative scoring was then performed in representative tumor regions or immune-cell hot spots. If the 2 pathologists assigned different high/low expression categories, or if the difference in H-score or positive-cell count was beyond the prespecified range, the final score was decided after joint review. Interobserver agreement was evaluated by the intraclass correlation coefficient (ICC) or kappa statistic and is shown in Table 1.

Table 1. Scoring thresholds and interobserver agreement for immunohistochemical markers

Marker	Scoring method	Cut-off value	Agreement index	Purpose
CTEN	H-score	112	0.83	High/low expression grouping
SMS1	H-score	105	0.81	High/low expression grouping
E-cadherin	H-score	92	0.79	Low-expression definition
N-cadherin	H-score	78	0.80	High-expression definition
Vimentin	H-score	86	0.82	High-expression definition
Snail/ZEB1	H-score	72	0.76	High-expression definition
PD-L1	H-score	58	0.74	High-expression definition
CD8	Positive cells/HPF	32	0.85	Low-infiltration definition
FOXP3	Positive cells/HPF	18	0.78	High-infiltration definition
CD68	Positive cells/HPF	45	0.80	High-infiltration definition
CD163	Positive cells/HPF	34	0.82	High-infiltration definition

CD8: cluster of differentiation 8; CD68: cluster of differentiation 68; CD163: cluster of differentiation 163; CTEN: C-terminal tensin-like protein; FOXP3: forkhead box P3; HPF: high-power field; PD-L1: programmed cell death 1 ligand 1; SMS1: sphingomyelin synthase 1; ZEB1: zinc finger E-box binding homeobox 1.

CTEN, SMS1, E-cadherin, N-cadherin, Vimentin, Snail/ZEB1, and PD-L1 were evaluated semiquantitatively with the H-score method. The percentages of positive cells at staining intensities of 0, 1, 2, and 3 were recorded, and the H-score was calculated as Σ (Pi×i), where Pi means the percentage of positive cells at the corresponding intensity and i means staining intensity. Final H-scores ranged from 0 to 300. CD8, FOXP3, CD68, and CD163 were evaluated by counting positive immune cells. For each case, 5 representative high-power fields were selected while avoiding necrosis, hemorrhage, and obvious tissue folding, and the mean number of positive cells was calculated.

CTEN, SMS1, and EMT-related tumor-cell markers were scored mainly according to cytoplasmic, membranous, or nuclear staining patterns in tumor cells. Immune-cell markers were counted as positive infiltrating cells within tumor nests and stroma. PD-L1 was assessed by combining staining in tumor cells and tumor-associated immune cells, and cases were divided into high or low expression according to the cut-off values set before statistical analysis. All cut-off values were fixed before the main outcome analysis. Table 1 shows the scoring method, cut-off value, and interobserver concordance for each marker. All cut-off values were predefined based on cohort median H-scores or median positive cell counts prior to statistical analysis. Median dichotomization was selected due to good repeatability and consistency with routine pathological binary grouping. To offset potential information loss caused by categorization, we supplemented analyses with continuous H-scores and quartile grouping. PD-L1 and all other markers were

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evaluated using a unified in-house H-score system instead of commercial companion diagnostic criteria such as tumor proportion score (TPS) or combined positive score (CPS). These median-based thresholds are only used for academic grouping and intergroup comparison; their clinical diagnostic value needs further verification in larger cohorts.

CTEN/SMS1 Co-expression Groups

Patients were divided into 4 groups according to CTEN and SMS1 expression status: CTEN-low/SMS1-low, CTEN-high/SMS1-low, CTEN-low/SMS1-high, and CTEN-high/SMS1-high. Patients with both high CTEN and high SMS1 expression were defined as the CTEN/SMS1 dual-high group; all other patients were placed in the non-dual-high group.

Definitions of EMT Phenotype and Immunosuppression-related Phenotype

The EMT-high phenotype was defined as low E-cadherin expression together with high expression of at least 1 mesenchymal marker among N-cadherin, Vimentin, and Snail/ZEB1. Cases that did not meet these rules were classified as non-EMT-high. Because EMT is continuous and not completely uniform, this definition was used only to describe a combined tissue-level phenotype; it was not treated as proof of a functional EMT mechanism. The definition of EMT-high phenotype adopted in this study is the classic and widely recognized standard for EMT evaluation in ovarian cancer clinicopathological research. We selected E-cadherin (epithelial marker) as the negative indicator and N-cadherin, Vimentin, Snail/ZEB1 (mesenchymal markers) as positive indicators, which can effectively distinguish different EMT status. Since EMT is a continuous dynamic process, alternative marker combinations or definition criteria may lead to different results. We unified this predefined definition to ensure the consistency and comparability of grouping in the whole cohort.

The immunosuppression-related phenotype was defined as low CD8 infiltration together with high CD163 infiltration and/or high PD-L1 expression. FOXP3 and CD68 were used as additional immune-environment indicators in correlation analyses, but they were not required for defining this combined phenotype. To avoid saying more than the data can support, this indicator is called an immunosuppression-related phenotype.

Follow-up and Outcomes

Patients were followed by outpatient review records, hospitalization records, imaging examinations, telephone follow-up, and medical-record system queries. Follow-up ended in May 2026. The main outcomes were platinum resistance, progression-free survival (PFS), and overall survival (OS). PFS was the time from surgery to first recurrence, disease progression, death, or last follow-up. OS was the time from surgery to death or last follow-up. Patients who did not have the corresponding event by the end of follow-up were censored.

Statistical Analysis

Categorical variables are shown as numbers and percentages, and they were compared with the chi-square test or Fisher exact test. Continuous variables are shown as mean±standard deviation or median with interquartile range according to their distribution, and they were compared with the t test, Mann-Whitney U test, or suitable nonparametric tests. The relationship between CTEN and SMS1 expression was evaluated with Spearman correlation analysis. Missing data were systematically checked before modeling. The missing rate of core variables (clinicopathological features, IHC scores, platinum response and survival outcomes) was less than 2%. Variables with high missing rates, including BRCA1/2 status, HRD status, PARP-inhibitor maintenance therapy and bevacizumab application, were excluded from the core models and only used in extended sensitivity analyses. Complete-case analysis was strictly adopted for all main analyses, and no outcome-driven data imputation was performed. Baseline comparison confirmed that cases with missing core data had no significant differences in age, FIGO stage and histological subtype compared with complete cases, indicating that the bias caused by complete-case analysis was minimal and acceptable.

Factors related to platinum resistance were analyzed by logistic regression, and odds ratios (ORs) with 95% confidence intervals (CIs) were reported. Multivariable models mainly included variables with clear clinical meaning and routine availability during diagnosis and treatment, such as age, FIGO stage, histological subtype, tumor grade, preoperative CA125, ascites, lymph-node metastasis, residual disease, EMT-high phenotype, immunosuppression-related phenotype, and CTEN/SMS1 dual-high expression. To reduce collinearity, CTEN high expression, SMS1 high

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expression, and CTEN/SMS1 dual-high expression were not entered into the same core model at the same time. Model stability was checked by sensitivity analyses and collinearity diagnostics.

Survival analyses were performed with the Kaplan-Meier method and log-rank test. Cox proportional hazards models were used to identify factors associated with PFS and OS, and hazard ratios (HRs) with 95% CIs were reported. The proportional hazards assumption was examined with Schoenfeld residuals or time-interaction terms, and collinearity was evaluated with the variance inflation factor (VIF) for all multivariate models. All variables included in the regression models had VIF values less than 3, and key variables such as CTEN/SMS1 dual-high expression had VIF values below 2, demonstrating no obvious multicollinearity in all models. To further avoid collinearity, CTEN high expression, SMS1 high expression and CTEN/SMS1 dual-high expression were not incorporated into the same core model simultaneously. To reduce the possibility of false-positive results from multiple comparisons, the Benjamini-Hochberg method was also used to control the false discovery rate (FDR). The added value of predictive models was evaluated by comparing changes in AUC or C-index within the same analysis set, and these added-value results were considered exploratory. All statistical tests were two-sided, and $p<.05$ was regarded as statistically significant.

RESULTS

Baseline Clinicopathological Characteristics

In total, 276 patients with pathologically confirmed ovarian cancer who received platinum-based chemotherapy were included. The median age was 57 years; 169 patients (61.2%) were younger than 60 years, and 107 patients (38.8%) were 60 years or older. FIGO stage I–II disease was found in 76 patients (27.5%), while 200 patients (72.5%) had FIGO stage III–IV disease. High-grade serous carcinoma was the most common histological subtype, with 190 cases (68.8%). Postoperative residual disease was <1 cm in 161 patients (58.3%) and ≥1 cm in 115 patients (41.7%). According to the response to platinum-based therapy, 179 patients were platinum sensitive (64.9%), and 97 patients were platinum resistant (35.1%). The median follow-up time was 52.0 months. During follow-up, 156 patients had recurrence or disease progression, and 103 patients died. In the whole cohort, the median PFS was 23.4 months, and the median OS was 61.7 months (Table 2).

Relationship Between CTEN and SMS1 Expression

With the median immunohistochemical H-scores used as cut-off values, high CTEN expression was found in 126 patients (45.7%), and low CTEN expression was found in 150 patients (54.3%). High SMS1 expression was found in 119 patients (43.1%), and low SMS1 expression was found in 157 patients (56.9%). Among patients with high CTEN expression, 61.9% also had high SMS1 expression, which was clearly higher than the 27.3% seen in patients with low CTEN expression. Spearman correlation analysis showed a positive relationship between CTEN and SMS1 expression ($r=0.36$, $p<.001$, Table 3).

Association of CTEN/SMS1 Dual-high Expression with Clinicopathological Features

In the full cohort, 78 patients (28.3%) had CTEN/SMS1 dual-high expression, while 198 patients (71.7%) did not. Compared with the non-dual-high group, the CTEN/SMS1 dual-high group had higher proportions of FIGO stage III–IV disease, CA125≥500 U/mL, ascites, lymph-node metastasis, postoperative residual disease≥1 cm, recurrence/progression, and platinum resistance. Age, histological subtype, and tumor grade did not show statistically significant differences (Table 4).

Association of CTEN/SMS1 Dual-High Expression with the EMT Phenotype

Low E-cadherin expression, high N-cadherin expression, and high Vimentin expression were more frequent in the CTEN/SMS1 dual-high group than in the non-dual-high group. High Snail/ZEB1 expression also appeared more common in the dual-high group, although the result did not reach statistical significance. In the combined EMT phenotype analysis, the EMT-high phenotype was present in 55.1% of the CTEN/SMS1 dual-high group, compared with 32.8% of the non-dual-high group ($p<.001$, Table 5).

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Table 2. Baseline clinicopathological features of 276 patients with ovarian cancer

Clinicopathological variable	No. (%)
Total number of patients	276
Age, median, years	57
Age <60 years	169 (61.2)
Age ≥60 years	107 (38.8)
FIGO stage I–II	76 (27.5)
FIGO stage III–IV	200 (72.5)
High-grade serous carcinoma	190 (68.8)
Endometrioid carcinoma	37 (13.4)
Clear-cell carcinoma	28 (10.1)
Mucinous carcinoma	12 (4.3)
Other subtypes	9 (3.3)
Low/intermediate-grade tumor	54 (19.6)
High-grade tumor	222 (80.4)
CA125 <500 U/mL	117 (42.4)
CA125 ≥500 U/mL	159 (57.6)
No ascites	101 (36.6)
Ascites present	175 (63.4)
No lymph-node metastasis	130 (47.1)
Lymph-node metastasis present	146 (52.9)
Residual disease <1 cm	161 (58.3)
Residual disease ≥1 cm	115 (41.7)
Platinum sensitive	179 (64.9)
Platinum resistant	97 (35.1)
Recurrence/progression	156 (56.5)
Death	103 (37.3)
Median follow-up, months	52.0
Median PFS, months	23.4
Median OS, months	61.7

CA125: cancer antigen 125; FIGO: International Federation of Gynecology and Obstetrics; OS: overall survival; PFS: progression-free survival; U/mL: units per milliliter.

Table 3. Relationship between CTEN and SMS1 expression

CTEN expression status	SMS1 low expression	SMS1 high expression	*p*
CTEN low expression, n=150	109 (72.7)	41 (27.3)	<.001
CTEN high expression, n=126	48 (38.1)	78 (61.9)	
Spearman correlation: $r = 0.36$, $p<.001$			

CTEN: C-terminal tensin-like protein; r: correlation coefficient; SMS1: sphingomyelin synthase 1.

Association of CTEN/SMS1 Dual-High Expression with the Tumor Immune Microenvironment

Low CD8 infiltration was more common in the CTEN/SMS1 dual-high group than in the non-dual-high group (59.0% vs 39.4%, p=.003). High CD163 infiltration and high PD-L1 expression were also more frequent in the dual-high group. FOXP3 and CD68 showed upward trends in the dual-high group, but the

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differences were not statistically significant. According to the combined definition, the immunosuppression-related phenotype was present in 43.6% of the CTEN/SMS1 dual-high group and 31.3% of the non-dual-high group (Table 6).

Association of CTEN/SMS1 Dual-High Expression with Platinum Resistance

High CTEN expression, high SMS1 expression, and CTEN/SMS1 dual-high expression were all more frequent in the platinum-resistant group than in the platinum-sensitive group. Multivariable logistic regression showed that residual disease ≥1 cm, EMT-high phenotype, and CTEN/SMS1 dual-high expression remained independently associated with platinum resistance. FIGO stage and the immunosuppression-related phenotype were no longer statistically significant in the multivariable model, which suggests that their associations may partly overlap with tumor burden, EMT phenotype, and molecular co-expression status (Table 7).

Histological Adjustment, Subgroup Analysis, and Sensitivity Analysis

After adjustment for histological subtype, the direction of the association between CTEN/SMS1 dual-high expression and platinum resistance was still consistent. In the subgroup limited to patients with high-grade serous carcinoma, the associations of CTEN/SMS1 dual-high expression with platinum resistance and shorter PFS were also generally unchanged. When continuous H-scores, quartile-based grouping, and FDR correction were used, the main results remained mostly stable. However, borderline findings, such as high PD-L1 expression, high FOXP3 infiltration, and interaction analyses, should still be interpreted as exploratory (Table 8).

Table 4. Relationship between CTEN/SMS1 dual-high expression and clinicopathological characteristics

Clinicopathological variable	Non-dual-high group, n=198	CTEN/SMS1 dual-high group, n=78	*p*
Age ≥60 years	71 (35.9)	36 (46.2)	.11
FIGO stage III–IV	133 (67.2)	67 (85.9)	.002
High-grade serous carcinoma	132 (66.7)	58 (74.4)	.21
High-grade tumor	154 (77.8)	68 (87.2)	.08
CA125 ≥500 U/mL	105 (53.0)	54 (69.2)	.01
Ascites present	116 (58.6)	59 (75.6)	.008
Lymph-node metastasis present	96 (48.5)	50 (64.1)	.02
Residual disease ≥1 cm	70 (35.4)	45 (57.7)	.001
Recurrence/progression	102 (51.5)	54 (69.2)	.008
Platinum resistance	56 (28.3)	41 (52.6)	<.001

CA125: cancer antigen 125; CTEN: C-terminal tensin-like protein; FIGO: International Federation of Gynecology and Obstetrics; SMS1: sphingomyelin synthase 1; U/mL: units per milliliter.

Table 5. Relationship between CTEN/SMS1 dual-high expression and EMT markers

EMT marker	Non-dual-high group, n=198	CTEN/SMS1 dual-high group, n=78	*p*
Low E-cadherin expression	80 (40.4)	48 (61.5)	.002
High N-cadherin expression	75 (37.9)	44 (56.4)	.005
High Vimentin expression	84 (42.4)	49 (62.8)	.002
High *Snail*/*ZEB1* expression	69 (34.8)	37 (47.4)	.05
EMT-high phenotype	65 (32.8)	43 (55.1)	<.001

CTEN: C-terminal tensin-like protein; EMT: epithelial-mesenchymal transition; SMS1: sphingomyelin synthase 1; ZEB1: zinc finger E-box binding homeobox 1.

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Table 6. Relationship between CTEN/SMS1 dual-high expression and tumor immune microenvironment markers

Immune-microenvironment marker	Non-dual-high group, n=198	CTEN/SMS1 dual-high group, n=78	*p*
Low CD8 infiltration	78 (39.4)	46 (59.0)	.003
High FOXP3 infiltration	69 (34.8)	36 (46.2)	.08
High CD68 infiltration	80 (40.4)	41 (52.6)	.07
High CD163 infiltration	76 (38.4)	42 (53.8)	.02
High PD-L1 expression	69 (34.8)	38 (48.7)	.03
Immunosuppression-related phenotype	62 (31.3)	34 (43.6)	.04

CD8: cluster of differentiation 8; CD68: cluster of differentiation 68; CD163: cluster of differentiation 163; CTEN: C-terminal tensin-like protein; FOXP3: forkhead box P3; PD-L1: programmed cell death 1 ligand 1; SMS1: sphingomyelin synthase 1.

Table 7. Factors related to platinum resistance and logistic regression analysis

Variable	Platinum-sensitive, n=179	Platinum-resistant, n=97	*p*	Multivariable OR	95% CI	*p*
FIGO stage III–IV	121 (67.6)	79 (81.4)	.01	1.71	0.89–3.29	.11
Residual disease ≥1 cm	61 (34.1)	54 (55.7)	.001	1.91	1.09–3.35	.02
CA125 ≥500 U/mL	94 (52.5)	65 (67.0)	.02	1.31	0.74–2.33	.36
High CTEN expression	70 (39.1)	56 (57.7)	.004	—	—	—
High SMS1 expression	66 (36.9)	53 (54.6)	.005	—	—	—
CTEN/SMS1 dual-high expression	37 (20.7)	41 (42.3)	<.001	2.18	1.21–3.94	.01
EMT-high phenotype	57 (31.8)	51 (52.6)	.001	2.14	1.22–3.74	.008
Immunosuppression-related phenotype	50 (27.9)	46 (47.4)	.001	1.65	0.91–2.98	.10

To avoid collinearity, high CTEN expression, high SMS1 expression, and CTEN/SMS1 dual-high expression were not entered together in the multivariable model. CA125: cancer antigen 125; CI: confidence interval; CTEN: C-terminal tensin-like protein; EMT: epithelial-mesenchymal transition; FIGO: International Federation of Gynecology and Obstetrics; OR: odds ratio; SMS1: sphingomyelin synthase 1; U/mL: units per milliliter.

Table 8. Subgroup and sensitivity analyses for CTEN/SMS1 dual-high expression

Model or population	Outcome	Effect size	95% CI	*p*
Overall cohort, adjusted for clinical variables	Platinum resistance	2.18	1.21–3.94	.01
Adjusted additionally for histological subtype	Platinum resistance	2.05	1.10–3.82	.02
High-grade serous carcinoma subgroup	Platinum resistance	2.26	1.08–4.71	.03
Continuous H-score model	Platinum resistance	1.38 per 50 points	1.08–1.77	.01
Main model after FDR correction	Platinum resistance	2.18	1.21–3.94	.03
Overall cohort, adjusted for clinical variables	PFS	1.52	1.05–2.20	.03
High-grade serous carcinoma subgroup	PFS	1.47	1.00–2.16	.05

CI: confidence interval; CTEN: C-terminal tensin-like protein; FDR: false discovery rate; PFS: progression-free survival; SMS1: sphingomyelin synthase 1.

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CTEN-associated Phenotypes Stratified by SMS1 Expression

Among patients with low SMS1 expression, high CTEN expression showed no significant statistical association with the EMT-high phenotype, platinum resistance, or the immunosuppression-related phenotype. Among patients with high SMS1 expression, the statistical association between CTEN and platinum resistance was more prominent, and the EMT-high phenotype also showed an increasing trend (Table 9). Exploratory interaction analysis revealed a potential statistical interaction between CTEN and SMS1 for platinum resistance. This finding only presents a clinical stratification pattern and raises a hypothesis for subsequent mechanistic exploration, rather than proving an SMS1-dependent molecular mechanism (Table 10).

Association of CTEN/SMS1 Dual-high Expression with Patient Prognosis

Follow-up analysis showed that median PFS was 17.6 months in the CTEN/SMS1 dual-high group and 28.4 months in the non-dual-high group. Median OS was also shorter in the dual-high group than in the non-dual-high group (50.2 vs 67.8 months). Multivariable Cox regression showed that FIGO stage III–IV, residual disease ≥1 cm, platinum resistance, and CTEN/SMS1 dual-high expression were associated with shorter PFS. For OS, platinum resistance remained an independent poor prognostic factor, while CTEN/SMS1 dual-high expression did not reach statistical significance (Table 11).

Incremental Value of Predictive Models

For predicting platinum resistance, the model that included only clinical variables had an AUC of 0.71. After adding the EMT-high phenotype and the immunosuppression-related phenotype, the AUC increased to 0.75. When CTEN/SMS1 dual-high expression was further added, the AUC increased to 0.78. For PFS prediction, the C-index of the clinical model was 0.66, and it increased to 0.69 after CTEN/SMS1 dual-high expression was added. These findings suggest that CTEN/SMS1 co-expression may add some information, although the improvement was not large and still needs external validation (Tables 12 and 13).

Table 9. CTEN-associated EMT, platinum-resistance, and immunosuppression-related phenotypes grouped by SMS1 expression

SMS1 status	CTEN status	No.	EMT-high phenotype	Platinum resistance	Immunosuppression-related phenotype
SMS1 low expression	CTEN low expression	109	34 (31.2)	28 (25.7)	32 (29.4)
SMS1 low expression	CTEN high expression	48	16 (33.3)	15 (31.3)	15 (31.3)
p	.80	.47	.81		
SMS1 high expression	CTEN low expression	41	15 (36.6)	13 (31.7)	15 (36.6)
SMS1 high expression	CTEN high expression	78	43 (55.1)	41 (52.6)	34 (43.6)
p	.06	.03	.47		

[a]CTEN: C-terminal tensin-like protein; EMT: epithelial-mesenchymal transition; SMS1: sphingomyelin synthase 1.

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Table 10. Exploratory interaction analysis

Interaction term	OR	95% CI	*p*
CTEN × SMS1 for EMT-high phenotype	1.54	0.94–2.74	.09
CTEN × SMS1 for platinum resistance	1.76	1.03–3.42	.04
CTEN × SMS1 for immunosuppression-related phenotype	1.28	0.78–2.21	.23

Interaction analyses were exploratory and were used only to suggest that CTEN-associated phenotypes may vary across SMS1 expression backgrounds. They do not independently prove an SMS1-dependent molecular mechanism. CI: confidence interval; CTEN: C-terminal tensin-like protein; EMT: epithelial-mesenchymal transition; OR: odds ratio; SMS1: sphingomyelin synthase 1.

Table 11. Relationship between CTEN/SMS1 dual-high expression and patient survival outcomes

Survival outcome	Non-dual-high group, n=198	CTEN/SMS1 dual-high group, n=78	*p*
Median PFS, months	28.4	17.6	<.001
1-year PFS rate	74.2%	60.3%	.02
3-year PFS rate	41.4%	24.4%	.008
Median OS, months	67.8	50.2	.006
3-year OS rate	72.7%	61.5%	.07
5-year OS rate	52.2%	38.1%	.04

CTEN: C-terminal tensin-like protein; OS: overall survival; PFS: progression-free survival; SMS1: sphingomyelin synthase 1.

Table 12. Multivariable Cox regression analysis of PFS and OS

Variable	PFS HR	95% CI	*p*	OS HR	95% CI	*p*
FIGO stage III–IV	1.54	1.03–2.30	.04	1.62	0.98–2.68	.06
Residual disease ≥1 cm	1.72	1.19–2.48	.004	1.41	0.90–2.22	.14
Platinum resistance	2.14	1.50–3.05	<.001	1.91	1.23–2.96	.004
EMT-high phenotype	1.36	0.96–1.94	.09	1.19	0.76–1.86	.44
Immunosuppression-related phenotype	1.24	0.86–1.80	.25	1.28	0.82–2.01	.28
CTEN/SMS1 dual-high expression	1.52	1.05–2.20	.03	1.39	0.88–2.21	.16

CI: confidence interval; CTEN: C-terminal tensin-like protein; EMT: epithelial-mesenchymal transition; HR: hazard ratio; OS: overall survival; PFS: progression-free survival; SMS1: sphingomyelin synthase 1.

Table 13. Added value of predictive models

Outcome	Model	AUC or C-index	Increment
Platinum resistance	Clinical-variable model	0.71	—
Platinum resistance	Clinical variables + EMT/immune phenotypes	0.75	+0.04
Platinum resistance	Clinical variables + EMT/immune phenotypes + CTEN/SMS1	0.78	+0.03
PFS	Clinical-variable model	0.66	—
PFS	Clinical variables + CTEN/SMS1	0.69	+0.03

AUC: area under the curve; C-index: concordance index; CTEN: C-terminal tensin-like protein; EMT: epithelial-mesenchymal transition; PFS: progression-free survival; SMS1: sphingomyelin synthase 1.

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DISCUSSION

Using clinical data and tumor-tissue specimens from 276 patients with ovarian cancer, this study evaluated the relationship between CTEN/SMS1 expression and EMT phenotype, platinum resistance, tumor immune microenvironment, and prognosis. CTEN expression was positively related to SMS1 expression, and CTEN/SMS1 dual-high expression was associated with several unfavorable clinicopathological features, including advanced FIGO stage, increased CA125, ascites, lymph-node metastasis, postoperative residual disease, recurrence/progression, and platinum resistance. Further analyses showed that dual-high tumors were more likely to have an EMT-high phenotype and immune-environment features suggesting relative immunosuppression, such as low CD8 infiltration, high CD163 infiltration, and higher PD-L1 expression. Survival analysis showed shorter PFS and OS in the dual-high group. In multivariable Cox regression, the association with PFS remained clear, while the independent association with OS did not reach statistical significance. Overall, these findings suggest that CTEN/SMS1 co-expression may be related to ovarian cancer progression, platinum resistance, and higher recurrence risk; however, the evidence is still clinical association and should not be taken as proof of a causal molecular mechanism.

Treatment of ovarian cancer still mainly depends on surgery combined with platinum-containing chemotherapy, but recurrence and platinum resistance remain major problems for long-term disease control.[1–4] Platinum resistance is affected by several factors together, including DNA-damage repair, drug transport, avoidance of apoptosis, higher cellular plasticity, metabolic change, and changes in the tumor microenvironment.[4] In this cohort, 35.1% of patients were platinum resistant, which broadly reflects the clinical situation in which recurrent or resistant disease often develops after initial treatment, especially in advanced ovarian cancer. Platinum resistance was much more common in patients with CTEN/SMS1 dual-high tumors than in those without dual-high expression. After adjustment for FIGO stage, residual disease, EMT-high phenotype, and immunosuppression-related phenotype, CTEN/SMS1 dual-high expression was still associated with platinum resistance. This result suggests that CTEN/SMS1 co-expression may identify a phenotype more inclined to treatment resistance. Even so, platinum resistance is also affected by tumor stage, completeness of cytoreduction, tumor burden, molecular background, and later treatment. Therefore, CTEN/SMS1 dual-high expression should be understood as a risk-related marker, not as evidence that CTEN/SMS1 dual-high expression is the only or decisive cause of platinum resistance.

EMT is an important biological process in ovarian cancer invasion, spread, and treatment resistance. During EMT, tumor cells lose epithelial characteristics and cell-cell adhesion, and they gain stronger migration, invasion, and anti-apoptotic ability. Typical molecular changes include lower E-cadherin expression and higher expression of N-cadherin, Vimentin, Snail, and ZEB.[5–7] In this study, low E-cadherin expression was more common in the CTEN/SMS1 dual-high group, and high N-cadherin and Vimentin expression were also more common. The combined EMT-high phenotype was significantly enriched in the dual-high group. These data suggest that CTEN/SMS1 dual-high tumors are more likely to show an EMT-activated tissue phenotype. Snail/ZEB1 showed an increasing trend in the dual-high group but did not reach the usual level of statistical significance. This result does not invalidate the EMT analysis; it more likely reflects the heterogeneity of EMT in clinical samples. EMT is not a simple yes-or-no state, but a changing process that includes partial EMT, mixed epithelial-mesenchymal states, and reversible phenotype changes. For this reason, individual EMT markers do not have to change in exactly the same way in clinical cohorts.

CTEN/TNS4 is a member of the tensin family and is involved in focal adhesion, cytoskeletal regulation, and cell migration. Previous studies have shown that CTEN contributes to movement, invasion, and EMT-related processes in several solid tumors.[14,15] Lu et al[18] reported that CTEN promotes EMT, migration, and invasion in non-small-cell lung cancer cells, supporting a connection between CTEN and stronger tumor-cell movement. Asiri et al[19] also showed that CTEN may promote EMT-related changes in colorectal cancer through stabilization of Src. These studies provide a biological background for the association seen here between CTEN/SMS1 dual-high expression and the EMT-high phenotype. Although this study did not include functional experiments, the association of high CTEN expression with EMT phenotype, ascites, lymph-

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node metastasis, and increased residual disease is understandable when considering the role of CTEN in focal adhesion and cell movement. Still, this study cannot prove that CTEN directly causes EMT, and it also cannot show that CTEN must regulate EMT through SMS1. The data mainly support a tissue-level association between CTEN/SMS1 dual-high expression and an EMT-activated phenotype. Combined with the existing biological literature, we further explained the potential biological basis of the interaction between CTEN and SMS1. CTEN regulates cell adhesion, cytoskeleton remodeling and cell migration, while SMS1 dominates sphingomyelin synthesis and sphingolipid metabolism, which maintains cell membrane structure and modulates apoptosis and drug sensitivity. Functionally, CTEN-induced changes in cell adhesion and cytoskeleton can alter the microenvironment of cell membrane, thereby affecting the distribution and function of sphingolipids synthesized by SMS1. In turn, sphingolipid reprogramming mediated by SMS1 can change cell membrane fluidity and intracellular signal transduction, and amplify the pro-EMT and pro-metastatic effects of CTEN. The combined dysregulation of CTEN and SMS1 may synergistically promote platinum resistance in ovarian cancer cells. It is emphasized that this is only a hypothetical mechanistic explanation based on published studies.

SMS1/SGMS1 is involved in sphingomyelin synthesis and sphingolipid metabolism. Sphingolipids are important structural components of cell membranes, and they also affect apoptosis, stress response, survival signaling, and drug response.[16,17] In cancer biology, sphingolipid metabolites such as ceramide, sphingomyelin, and sphingosine-1-phosphate are considered to influence the balance between cell death and survival. Bataller et al[22] summarized evidence that abnormal sphingolipid metabolism is related to drug resistance in several cancers, and that it may change treatment sensitivity by affecting membrane properties, apoptotic thresholds, and signal transduction. In ovarian cancer, abnormal sphingolipid metabolism has also been connected with treatment resistance.[17] This study found a positive relationship between CTEN and SMS1 expression, and patients with CTEN/SMS1 dual-high expression had a higher rate of platinum resistance. These findings suggest that a CTEN-related invasive phenotype may be linked with SMS1-associated lipid-metabolic or membrane-signaling states. However, this study did not measure sphingolipid metabolites, SMS1 enzyme activity, or downstream metabolic pathways. The exact metabolites and signaling routes that may connect SMS1 with platinum resistance remain unclear. Future studies using lipidomics, cellular perturbation experiments, and drug-sensitivity assays will be needed to describe the biological relationship between CTEN/SMS1 and platinum resistance. To further verify the interaction between CTEN and SMS1 and the underlying mechanism, a series of functional experiments could be performed in future studies, including CTEN/SMS1 knockdown, overexpression and rescue experiments in ovarian cancer cell lines to observe changes of EMT phenotype and platinum sensitivity; ovarian cancer organoid models can be used to simulate tumor microenvironment; lipidomics analysis can also be applied to detect alterations of sphingolipid metabolites. These functional validations are necessary to clarify the biological interaction of CTEN and SMS1.

One important part of this study was the analysis stratified by SMS1 expression. In tumors with low SMS1 expression, high CTEN expression was not statistically associated with the EMT-high phenotype, platinum resistance, or the immunosuppression-related phenotype. In tumors with high SMS1 expression, however, high CTEN expression was associated with a clearly higher rate of platinum resistance, and the EMT-high phenotype also showed an upward trend. Exploratory interaction analysis further suggested a possible statistical interaction between CTEN and SMS1 for platinum resistance. These results are consistent with the CTEN/SMS1 co-expression hypothesis, but the evidence is limited to stratified and interaction-based clinical analyses, rather than knockdown, overexpression, or rescue experiments. The most cautious explanation is that the CTEN-associated platinum-resistant phenotype is more obvious in a high-SMS1 background, appearing as an SMS1-related, or SMS1-dependent-like, clinical feature. The current data do not prove that CTEN must act through SMS1 to have biological effects. This point is important, because conclusions from a retrospective clinical study should not be extended too far.

The tumor immune microenvironment plays an important role in ovarian cancer progression and treatment response. Previous studies showed that T-cell infiltration inside tumors is associated with recurrence and survival,[9] and that higher $CD8^+$ T-cell infiltration or

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a higher $CD8^+$/Treg ratio usually indicates a better antitumor immune state.[10] In contrast, enrichment of $FOXP3^+$ regulatory T cells may promote local immune suppression and is related to poorer prognosis.[11] The meaning of PD-L1 expression in ovarian cancer is complicated. PD-L1 may suggest tumor immune escape, but it may also be an adaptive response to existing intratumoral immunity. Webb et al[20] reported that PD-L1 expression in high-grade serous ovarian cancer is associated with tumor-infiltrating T cells, and its prognostic meaning should be understood within the wider immune context. Therefore, PD-L1 should not be treated as a single adverse marker alone; it is better evaluated together with CD8, regulatory T cells, macrophage infiltration, and the spatial arrangement of tumor tissue.

In this cohort, CTEN/SMS1 dual-high tumors more often showed low CD8 infiltration, together with increased CD163 infiltration and PD-L1 expression, suggesting a relatively immunosuppressive microenvironment. CD163 is often used as a marker related to M2-like macrophage infiltration. Hensler et al[21] proposed that M2-like tumor-associated macrophages can drive clinically meaningful immunosuppression in metastatic ovarian cancer. In the present study, FOXP3 and CD68 only showed upward trends in the dual-high group and did not reach statistical significance, suggesting that CTEN/SMS1 dual-high expression may not be accompanied by synchronized changes in all immune-cell subsets. Instead, the immune profile seemed to be mainly marked by reduced CD8 infiltration, increased CD163 infiltration, and higher PD-L1 expression. This pattern also shows the heterogeneity of the ovarian cancer immune microenvironment, and no single immune marker can characterize the entire microenvironment. Immunohistochemical assessment of CD8, FOXP3, CD68, CD163, and PD-L1 is suitable for research based on clinical specimens, but it cannot fully define immune-cell function, spatial distribution, or cell-cell interaction. Validation with multiplex immunofluorescence, spatial transcriptomics, or flow cytometry is therefore still needed.

The links among CTEN/SMS1 dual-high expression, EMT, platinum resistance, and an immunosuppressive microenvironment probably do not represent completely separate events. More likely, they may together describe a clinical phenotype with stronger tumor-cell plasticity and better adaptation to the surrounding microenvironment. EMT can reduce cell-cell adhesion, promote migration and invasion, and may influence chemotherapy sensitivity by increasing survival ability, changing cell-cycle states, or improving stress tolerance.[5–7] EMT may also connect with immune escape through changes in antigen presentation, immune-checkpoint molecule expression, and recruitment of immunosuppressive cell populations. Although this study cannot prove a causal relationship between EMT and immune-environment remodeling, patients with CTEN/SMS1 dual-high expression showed an EMT-high phenotype, more platinum resistance, and a relatively immunosuppressive immune profile at the same time. These findings suggest that the features may together reflect a more aggressive and treatment-resistant tumor state. This interpretation is still an integrated inference based on clinical association, and it cannot replace mechanistic experimental evidence. Accumulated previous studies have demonstrated that activated EMT in ovarian cancer can induce tumor immune remodeling and immune escape, including reduced antigen presentation, recruitment of M2-like macrophages and upregulation of PD-L1. Our results are consistent with these reports: the CTEN/SMS1 dual-high group presented concurrent high EMT phenotype and immunosuppressive microenvironment. Differently from previous studies focusing on single EMT marker or single immune molecule, this study firstly identified CTEN/SMS1 co-expression as a novel molecular phenotype linking EMT, platinum resistance and tumor immunosuppression in ovarian cancer.

For survival, the CTEN/SMS1 dual-high group had shorter median PFS and OS than the non-dual-high group. Multivariable Cox regression further showed that CTEN/SMS1 dual-high expression was independently associated with PFS, while its independent predictive value for OS did not reach statistical significance. This pattern is clinically reasonable. PFS more directly reflects the risk of recurrence or progression after initial treatment, so it may be more sensitive to residual disease, platinum resistance, EMT phenotype, and basic tumor biology. OS, in contrast, is affected by treatment after recurrence, maintenance therapy, targeted therapy, performance status, comorbidities, and follow-up time. In retrospective clinical cohorts, molecular or phenotypic markers may therefore show stronger prognostic value for PFS than for OS. The findings suggest that CTEN/SMS1 dual-high expression may be more useful for indicating disease-progression risk than for independently predicting overall survival.

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The incremental improvement in AUC and C-index was modest in this cohort, demonstrating that this molecular phenotype can only act as a supplementary indicator. Although it carries independent prognostic information beyond conventional clinicopathological factors, its clinical value is limited when used alone, and it cannot be developed as a standalone commercial diagnostic marker at this stage. Patients with CTEN/SMS1 dual-high expression tend to present activated EMT, platinum resistance and an immunosuppressive tumor immune microenvironment, so this signature helps identify patients at high risk of tumor recurrence and progression. If validated in large multicenter cohorts, CTEN/SMS1 can be applied as an auxiliary biomarker to assess the risks of recurrence and platinum resistance. Notably, this retrospective study cannot support CTEN or SMS1 as therapeutic targets, and we do not recommend any targeted therapy directed at these two molecules for now. In essence, our work identifies a clinically meaningful molecular phenotype of ovarian cancer, rather than a target available for direct clinical intervention. Further multicenter validation and development of combined biomarker panels are required to advance its clinical translation.

This study has several inherent limitations that need to be addressed. First, it is a single-center retrospective study, which may introduce selection bias associated with patient enrollment, treatment patterns and follow-up completeness. All participants underwent primary surgery and platinum-based chemotherapy at our hospital, so the results cannot be generalized to patients from other medical institutions or those receiving different treatment regimens. We did not conduct calibration analysis and decision-curve analysis to evaluate the practical utility of the prediction model due to data constraints, and the absence of an independent external validation cohort also weakens the generalizability of our conclusions. Multicenter prospective validation is therefore necessary in future work. Second, ovarian cancer is characterized by significant histological and molecular heterogeneity. High-grade serous carcinoma was the predominant subtype in our cohort, and subgroup analysis confirmed the stability of the associations between CTEN/SMS1 dual-high expression, platinum resistance and poor progression-free survival in this population. However, the sample sizes of other histological subtypes, including endometrioid carcinoma, clear-cell carcinoma and mucinous carcinoma, were insufficient. Given the distinct molecular features and platinum chemosensitivity among different subtypes, stratified analysis for rare subtypes could not be performed in the present study. Third, key confounding factors were not fully integrated into core analyses. BRCA mutation and homologous recombination deficiency (HRD) are critical factors influencing platinum response in ovarian cancer. Due to incomplete historical data between 2015 and 2021, information on BRCA/HRD status, maintenance therapy and post-recurrence treatment was missing, which may affect the correlation analysis of CTEN/SMS1 and platinum resistance. Related data should be comprehensively collected in subsequent studies for stratified exploration. Fourth, all biomarkers were detected via immunohistochemistry. Despite its wide use in clinicopathological research, this method is susceptible to variations in antibodies, staining protocols and scoring rules, which may compromise result reproducibility. We adopted median values as cut-offs to divide protein expression into high and low groups; this approach facilitates statistical analysis but may not be suitable for clinical diagnostic standards. In addition, dichotomizing continuous H-score data will result in information loss. We have adopted continuous variable models and quartile grouping as supplementary analyses to reduce this limitation. Fifth, this clinical cohort study lacks in vitro and in vivo functional experiments. For this reason, we cannot confirm that CTEN modulates epithelial-mesenchymal transition, platinum resistance or immune microenvironment remodeling through SMS1-dependent signaling. All findings in this study merely reflect clinicopathological associations, rather than definite causal relationships. Finally, our assessment of the tumor immune microenvironment has obvious deficiencies. We only detected five immune markers, which cannot fully depict the complete immune landscape, as natural killer cells, dendritic cells and various cytokines were not included. Standard immunohistochemistry cannot reflect the activation state of immune cells or intercellular interactions. Moreover, immune cells display prominent spatial heterogeneity in tumor tissues, and evaluating only representative microscopic fields may lead to sampling bias. Advanced technologies such as multiple immunofluorescence and spatial transcriptomics will be applied in future research to overcome these drawbacks. These limitations mean that the results should be interpreted cautiously, and prospective cohorts and mechanistic validation are needed.

In conclusion, this single-center retrospective cohort

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study demonstrated significant clinicopathological associations between CTEN/SMS1 dual-high expression and activated EMT phenotype, immunosuppressive tumor immune microenvironment, platinum resistance and shorter PFS in ovarian cancer. The association between CTEN and platinum resistance was more prominent in patients with high SMS1 expression, suggesting a potential cooperative relationship between the two molecules at the clinical level. It should be emphasized that all findings in this study are observational correlations, and no causal relationship or direct molecular mechanism was verified. CTEN/SMS1 dual-high expression has potential value as an auxiliary prognostic biomarker for ovarian cancer risk stratification. This study lacked external multicenter validation. Multicenter prospective cohort validation is absolutely essential before the clinical application of CTEN/SMS1-based risk stratification system. Further multicenter clinical validation, functional experiments and omics research are required to clarify the biological function of CTEN/SMS1 and facilitate its clinical translation.

STATEMENT OF ETHICS

The Institutional Review Board (IRB) / Ethics Committee of Affiliated Hospital of Yanbian University approved this study (2021015).

This study was retrospective and noninterventional; all patient data were deidentified and anonymized before analysis

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Not applicable.

DATA AVAILABILITY

The deidentified data supporting the findings of this study is available from the corresponding author upon reasonable request, subject to institutional ethics and data-protection requirements.

AI ASSISTANCE DISCLOSURE

No artificial intelligence (AI) tools were used in the preparation, writing, editing, or analysis of this manuscript.

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