SLAMF7 predicts prognosis and correlates with immune infiltration in serous ovarian carcinoma

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Research Article

Keywords: ovarian carcinoma, SLAM family members, prognosis, immune infiltration

Posted Date: July 27th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2938493/v1

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Abstract

Background: Signaling lymphocytic activation molecule family members (SLAMFs) play a critical role in immune regulation of malignancies. However, the function of SLAMFs in ovarian cancer (OV) is rarely studied.

Methods: The expression analysis of SLAMFs was conducted based on the TCGA-OV and GEO databases. Immunohistochemistry (IHC) was further performed on tissue arrays (n = 98) to determine the expression of SLAMF7. Kaplan-Meier plotter and multivariate Cox regression model were used to evaluate the correlation of SLAMF7 expression with survival outcomes of patients. The molecular function of SLAMF7 in OV was further investigated using Gene Set Enrichment Analysis (GSEA).

Results: SLAMF7 mRNA expression were significantly upregulated in OV tumor tissue compared to normal tissue. IHC revealed that SLAMF7 expression was located in the interstitial parts of tumor tissue, and higher SLAMF7 expression was associated with favorable survival outcomes. GSEA demonstrated that SLAMF7 is involved immune-related pathways. Further analysis showed that SLAMF7 had a strong correlation with the T cell-specific biomarker (CD3) but not with the B cell (CD19, CD22, and CD23) and NK cell-specific biomarkers (CD85C, CD336, and CD337). Furthermore, IHC analysis confirmed that SLAMF7 was expressed in tumor-infiltrating T cells, and the IHC score of SLAMF7 was positively correlated with CD3 (r = 0.846, P < 0.001).

Conclusions: SLAMF7 is expressed in the interstitial components of clinical OV tissue, and higher SLAMF7 expression indicated a favorable prognosis for patients with OV. Additionally, SLAMF7 is involved in T-cell immune infiltration in OV.

1 Introduction

Ovarian cancer (OV) is the most lethal malignant gynecological tumor, with 70% of patients being diagnosed at advanced stages and experiencing poor 5-year survival rates (below 45%)[1]. Despite advancements in diagnosis and treatment, OV remains a major public health concern and a significant threat to women's lives[2]. To improve clinical outcomes for patients, it is crucial to enhance our understanding of the underlying pathophysiology of the disease and identify new biomarkers and potential therapeutic targets. The molecular characteristics of OV are highly complex and diverse. Several studies have highlighted the influence of tumor-infiltrating lymphocytes (TILs) on the clinical outcomes of patients with OV[3-6]. The signaling lymphocytic activation molecules (SLAM) family, including SLAMF1-9, are transmembrane receptors expressed on immune cells and involved in immune regulation, including T and B cell activation, natural killer cell-mediated cytotoxicity, and cytokine production[7-11].

Emerging evidence indicates that SLAMFs play multifunctional roles in tumorigenesis and progression[12-14]. For example, SLAMF1 has been found to promote hematologic tumor cell survival and proliferation[15], and induce methotrexate resistance in choriocarcinoma cells[16]. However, in colorectal cancer, SLAMF1 acts as an anti-tumor biomarker[17]. In multiple myeloma, SLAMF6 expression
is associated with an aggressive clinical phenotype, including higher tumor burden and rapid disease progression[18]. These findings suggest that SLAMFs could serve as new tumor markers, diagnostic tools, or potential therapeutic targets for controlling tumor progression. However, the role of SLAMFs in OV has received limited attention. In this study, we assessed the prognostic value of SLAMFs expression in OV using bioinformatic analysis combined with immunohistochemistry (IHC) analyses of clinical samples. Furthermore, we explored the underlying mechanisms associated with SLAMFs in OV.

2 Materials And Methods

2.1 Data collection and bioinformatics analysis

The expression features and prognostic value of SLAMFs in OV were analyzed using the GEPIA 2 web server (http://gepia2.cancer-pku.cn/). RNA sequencing data of GSE9891 (including 267 OV tissues and 18 borderline tumor tissues), GSE18520 (including 53 OV tissues and ten normal ovarian samples), and GSE32062 (including 270 OV tissues) were obtained from the NCBI-GEO database (https://www.ncbi.nlm.nih.gov/geo/) for the analysis of SLAMF7 mRNA expression between serous OV and normal ovarian tissues, as well as to validate the prognostic values of SLAMFs. Patients were categorized into high- and low-expression groups based on the optional cut-off value of SLAMFs.

2.2 Patient samples and immunohistochemical analysis

The feature of SLAMF7 protein expression was analyzed for tissue arrays from 98 serous OV patients from the EH cohort diagnosed between 2010 and 2021. All patients were diagnosed based on pathological examination, and informed consent was obtained from all individual participants included in the study. The disease stage of each OV patient was determined according to the standards of the International Federation of Gynecology and Obstetrics (FIGO) 2018 for OV. The study was approved by the Ethics Committee of the Affiliated Shanghai East Hospital of Tongji University School of Medicine (approve number: 2022-204), and all experimental procedures were conformed to the principles of the World Medical Association Declaration of Helsinki.

Immunohistochemistry (IHC) was performed on paraffin-embedded tissue array slides. Briefly, paraffin slides were subjected to antigen retrieval using a 1:50 dilution of EnVision FLEX Target Retrieval Solution (Dako DM828, Agilent Technologies Inc. Santa Clara, CA, USA) at 95°C for 15 min, followed by 1 h of cooling to room temperature (20°C to 25°C). Slides were then washed twice with phosphate-buffered saline (PBS) and blocked with EnVision FLEX Peroxidase-Blocking Reagent (Dako SM801) for 10 min. After blocking, slides were washed twice with PBS and incubated overnight at room temperature in SLAMF7 primary antibody (ab230954) with 1:2000 dilution or CD3 primary antibody (MAB0740, MXB Biotechnologies) with 1:200 dilution in a humidified chamber. Post incubation, slides were washed twice with PBS and incubated with secondary EnVision FLEX/HRP Detection Reagent (Dako SM802) for 20 min. Visualization was performed using EnVision FLEX DAB+ Chromogen (Dako K827) in EnVision FLEX Substrate Buffer (Dako SM803). Finally, slides were counterstained in hemalum for 3 min, dehydrated, cleared, and coverslipped. Paraffin sections were visually evaluated by a Gynecological pathologist. The
visual inspection grading was classified as negative (score 0), weak (score 1), moderate (score 2), or strong (score 3), and the groups were defined as low SLAMF7 expression (score 0-1) and high SLAMF7 expression (score 2-3).

2.3 Correlation and enrichment analysis in OV

Gene Set Enrichment Analysis (GSEA) was performed to investigate the function of SLAMF7 in OV. The SLAMF7 expression data was stratified into low and high groups to annotate biological functions using 1000 permutations. The Reactome pathways were illustrated using a cluster profiler ($P<0.01$). Tumor Immune Estimation Resource (TIMER, https://cistrome.shinyapps.io/timer/) was utilized to assess the correlation between SLAMF7 expression and the infiltration levels of different immune cells, including tumor purity, B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells. To consolidate the analysis results, the correlation module in the TIMER platform was further employed to analyze the association between SLAMF7 and specific marker genes of immune cells.

2.4 Statistics

A Student t-test was used to compare the means, while the Mann-Whitney U test was used to evaluate the correlation between SLAMF expression and clinicopathological variables. The Log-rank test was used to assess the influence of SLAMF7 expression on overall survival (OS), defined as the time elapsed between treatment initiation and death from any cause. A multivariable Cox proportional hazards model was fitted using prespecified variables, including age, FIGO stage (I-II vs. III-IV), lymph node invasion, neoplasm location (unilateral vs. bilateral), and histological grade (G1-2 vs. G3-4). Spearman's rank correlation coefficient was calculated to evaluate the relationship between SLAMF7 expression and lymphocyte-specific biomarkers. All data analyses were performed using IBM SPSS Statistics 22.0 for Windows (IBM Corp, Inc., New York, USA) and GraphPad Prism 8.0.2 for Windows (Graph Pad Software, Inc., California, USA). The difference was considered significant according to the p-value: *: $P<0.05$ **: $P<0.01$, ***: $P<0.001$.

3 Results

3.1 SLAMF7 mRNA expression level and prognostic value in OV

We investigated the mRNA expression levels of SLAM family members in 424 cases of OV samples from the TCGA and 88 cases of normal ovarian tissues from the GTEX database. The results revealed significantly higher expression of SLAMF2 and SLAMF7 (Figure 1A, $P<0.05$) in tumor tissue compared to normal tissue, while the other members showed no statistically significant difference (Supplementary Figure 1A). The Log-rank test revealed that lower mRNA expression of SLAMF2 was significantly correlated with shorter overall survival (OS) (Figure 1B, $P=0.016$), and lower mRNA expression of SLAMF7 was significantly correlated with shorter disease-free survival (DFS) and OS (Figure 1C, $P=0.05$). No statistically significant differences were observed for the other members (Supplementary Figures 2 and 3). We further analyzed the mRNA expression level of SLAMF7 in relation to other clinicopathological
features. Patients with early-stage I or II cancer had significantly higher SLAMF7 mRNA expression in the tumor than those with advanced-stage (FIGO III or IV) cancer (Figure 1D). However, there was no correlation between SLAMF7 expression and tumor grade. Further analysis of the GEO database (GSE9891) confirmed higher expression of SLAMF7 in tumor tissue (Figure 1E), and high SLAMF7 expression (GSE18521, and GSE32062) indicated a favorable OS of OV patients (Figure 1F). A multivariable Cox proportional hazards model was performed, adjusting for prespecified variables, including age, FIGO stage (I-II vs. III-IV), lymph node invasion, neoplasm location (Unilateral vs. Bilateral), histological grade (G1-2 vs. G3-4), tumor residual (No vs. Yes), and primary therapy outcome (PR&CR vs. RD&SD). The results indicated that high SLAMF7 expression was an independent prognostic marker for DFS ($HR = 0.691$, 95% CI $0.527-0.906$, $P = 0.007$) and OS ($HR = 0.616$, 95% CI $0.449-0.845$, $P = 0.003$) in OV (Supplementary Tables 1 and 2). These findings demonstrate that SLAMF7 is a potentially favorable prognostic biomarker involved in the progression of OV.

### 3.2 Evaluation of SLAMF7 protein in OV samples

IHC analysis of tissue array slides showed a positive signal for SLAMF7 in the interstitial components of the tumor tissue. Among the analyzed samples, 68 patients showed negative to weak staining (low expression), while 32 patients showed moderate to strong staining (high expression) in tumor tissues (Figures 2A and 2B). Table 1 provides the basic features of patients. Further analysis demonstrated that patients with high SLAMF7 expression had higher rates of lymph node invasion compared to those with low SLAMF7 expression (Table 2). The median follow-up time after surgery was 53.0 months (3-86 months), and during the follow-up period, 48 patients died. The Log-rank test showed that patients with low SLAMF7 expression had a shorter median OS (47.0 months, 95% CI 27.6-66.1 months) compared to patients with high SLAMF7 expression (not reached) (Figure 2C). Cox regression analysis adjusted for related clinical factors indicated that high SLAMF7 expression was an independent protective factor for patients’ OS ($HR = 0.421$, $P = 0.031$, Table 3). These findings suggest that SLAMF7 is expressed in the interstitial components of OV tissue and that higher SLAMF7 expression is associated with a better patient prognosis.

### 3.3 The relationship between SLAMF7 and immune cell infiltration in OV

Studies have shown that SLAMF7 can directly regulate immune cells and the immune environment[19], so we speculate that the SLAMF7 could modulate tumor progression through immune-related pathways in OV. We discovered the genes that differed between low and high expression groups of SLAMF7 mRNA in OV. GSEA analysis revealed that the SLAMF7 is involved in the T cytotoxic pathway, Fceri-mediated Nf Kb activation, and immunoregulatory interactions between a lymphoid and a non-lymphoid cell (Figure 3A). Furthermore, immune infiltration analysis indicated that SLAMF7 mRNA expression was positively associated with infiltrating T cells, Th1 cells, B cells, T Reg, DC, NK CD56dim cell, cytotoxic cells, macrophages, iDC, T helper cells, Tem, neutrophils, aDC, CD8 T cells, and TFH (Figure 3B, $P<0.05$). These results suggested that SLAMF7 was significantly associated with immune infiltration in OV. To
investigate the potential relationship between SLAMF7 and interstitial lymphocytes, we further analyzed the correlation between SLAMF7 and specific lymphocyte biomarkers.

The results revealed a strong correlation between SLAMF7 and T cell markers, including CD3D \((P<0.05, r = 0.66)\), CD3E \((P<0.05, r = 0.66)\), and CD3G \((P<0.05, r = 0.68)\), as shown in Figure 3C. However, a weak correlation was observed between SLAMF7 and marker genes of B cells and NK cells (Figures 3D and 3E). To further investigate the relation between SLAMF7 and T cells, an IHC analysis of T cell-specific marker CD3 was performed for a tissue array of OV. The results indicated that the expression location of CD3 was identical to SLAMF7 (Figure 4A), and a significantly positive correlation existed between CD3 and SLAMF7 expression (Figure 4B). In summary, these findings suggested that SLAMF7 modulates T-cell immune infiltration and affects the prognosis of OV.

Discussion

SLAM family receptors include nine members: SLAMF1-SLAMF9, which play pivotal roles in immune activation[20] and suppression[21]. Recent evidence has disclosed that SLAM is also involved in tumor pathophysiology, even as new tumor biomarkers[17], diagnostic tools, or powerful therapeutic targets[22]. Moreover, the roles of SLAM are implicated and diverse. Take SLAMF1, for example, in lymphoma[15] and choriocarcinoma[16], it can promote tumor survival and proliferation; in colorectal tumor, SLAMF1 act as an anti-tumor biomarker[17]. The present study finds that SLAMF7 is a protective factor in OV. Further, IHC showed that SLAMF7 expression was located in interstitial components of tumor tissue.

SLAMF7 has both cancer-promoting and tumor-suppressing effects on human cancers[23]. The tumor-promoting role of SLAMF7 is supported by evidence that in multiple myeloma, lymphoma, and clear cell renal cell[24], SLAFM7 is associated with tumor invasion and metastasis. However, high SLAMF7 expression in lymph node metastatic positive breast cancer was a strongly favorable prognosticator[25]. A related study showed that knockdown of SLAMF7 mRNA repressed malignancy and cisplatin resistance of OV cells[26]. Another study revealed that SLAMF7 is related to the recurrence of OV[27]. However, the function experiment was conducted only at the OV cell level, and there was no comparative research in clinical ovarian tissue. In this article, we used IHC analysis and public datasets to further explore the expression and prognostic value of SLAMFs in OV. Our findings indicate that SLAMF7 exhibits significantly high expression in TCGA- OV samples and is associated with the FIGO stage and lymphatic invasion of the tumor. The survival analysis revealed that highly expressed SLAMF7 was associated with longer OS and DFS in patients with OV. Cox regression adjusted for related clinical factors showed that high SLAMF7 expression was an independent protective factor for patients’ OS. IHC analysis of 98 tissue array slides showed positive SLAMF7 in the interstitial components of the tumor tissue, and higher SLAMF7 expression indicated a better prognosis of patients.

Immune infiltration is closely related to the malignancy of OV[28]. Tumor-infiltrating lymphocytes (TILs) have long been known to exist in OV[29]. The accumulation of TILs in OV indicates increased survival, while immunosuppressive regulatory T-cells (Tregs) are linked to poor clinical outcomes[5]. Bolster tumor-
reactive TILs may inhibit tumor progression effectively[30]. Based on the previous findings, we hypotheses that the SLAMF7 may regulate tumor progression through interstitial cells in tumor immune microenvironments, and the GSEA showed that SLAMF7 was related to multi-immune pathways and lymphocytes. Further analysis indicated that SLAMF7 was significantly correlated with T cell markers but not with B cell or NK cell markers. We further verified the relation between SLAMF7 and T cells in clinical samples, indicating that SLAMF7 may regulate the progression of OV by regulating T-cell infiltration. Thus, immune infiltration data analysis is consistent with the findings of clinical samples.

There are certain limitations to this study. Firstly, OV comprises various pathological types, but we only analyzed the prognostic value of SLAMF7 in serous OV. Further investigations are required to assess the prognostic value of SLAMF7 in other pathological types. Secondly, due to the small sample size of this study, we were only able to find a positive relationship between SLAMF7 expression and T cells in OV. However, the definite mechanism needs further exploration. In summary, our study revealed that SLAMF7 is expressed in interstitial T cells of OV tissue. Higher SLAMF7 expression is a protective prognostic marker for patients with OV, and the potential mechanism may be associated with T-cell infiltration and tumor immune microenvironment regulation.

Declarations

Funding Statement: This study was funded by the National Natural Science Foundation of China (grant number 82260587), the Natural Science Foundation of Jiangxi Province (grant number 20202ACB206006) and The Top-level Clinical Discipline Project of Shanghai Pudong (grant number PWYgf2021-07).

Conflict of Interest: The authors declared that they have no conflicts of interest to this work.

Author Contributions: Conceptualization, Y.D and J.C.; Methodology, L.Z., Y.X. and J.C.; Validation, Y.D; Formal analysis, L.Z. and Y.D.; Writing—original draft, Y.D. and C.D.; Writing—review and editing, Y.D.; Visualization, Y.D.; Supervision, J.C.; Funding acquisition, J.C. All authors have read and agreed to the published version of the manuscript.

Disclosure: The study was approved by the ethical committee of the Shanghai East Hospital

Data availability: Data available on request from the corresponding author

References


Tables

Table 1. The basic characteristics of patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number(n=98)</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>56.2 ± 10.4</td>
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<td>FIGO stage</td>
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<tr>
<td>-</td>
<td>68 (69.4)</td>
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<tr>
<td>-</td>
<td>30 (30.6)</td>
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<tr>
<td>Lymph node invasion</td>
<td>34 (34.7)</td>
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<td>Neoplasm location</td>
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<tr>
<td>Unilateral</td>
<td>67 (68.4)</td>
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<tr>
<td>Bilateral</td>
<td>31 (31.6)</td>
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<tr>
<td>Histological grade</td>
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<tr>
<td>G1-2</td>
<td>29 (29.6)</td>
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<tr>
<td>G3-4</td>
<td>69 (70.4)</td>
</tr>
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Table 2. The differences of clinical features between patients with lower SLAMF7 expression and higher SLAMF7 expression in tumor tissue
<table>
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<th>variable</th>
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<th>Higher SLAMF7 expression (n=31)</th>
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<tr>
<td>Age</td>
<td>57.1 ± 9.9</td>
<td>54.1 ± 11.3</td>
<td>0.69</td>
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<tr>
<td>FIGO stage</td>
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<td></td>
<td></td>
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<tr>
<td>-</td>
<td>49 73.1</td>
<td>19 61.3</td>
<td>0.24</td>
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<tr>
<td>-</td>
<td>18 26.9</td>
<td>12 38.7</td>
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<tr>
<td>Lymph node invasion (N1)</td>
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<td>Unilateral</td>
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<td>21 (67.7)</td>
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<td>10 (32.3)</td>
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<td>Histological grade</td>
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<td>G1-2</td>
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<td>G3-4</td>
<td>46 (68.7)</td>
<td>23 (74.2)</td>
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Table 3. Multivariable cox proportional HRs for tissue SLAMF7 expression level on progression overall survival of patients

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<th>Variable</th>
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<td>Age (continuous)</td>
<td>1.014 (0.983-1.047)</td>
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<td>FIGO stage (- vs -)</td>
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<td>1.455 (0.705-3.003)</td>
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<td>Neoplasm location (Unilateral tumor vs Bilateral tumor)</td>
<td>0.871 (0.434-1.750)</td>
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<td>Histological grade (G1-2 vs G3-4)</td>
<td>0.968 (0.498-1.882)</td>
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<td>SLAMF7 expression (lower expression vs higher expression)</td>
<td>0.421 (0.192-0.925)</td>
<td>0.031</td>
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Figures
Figure 1

The expression features of SLAMF members in the public databases. **A.** The comparison of SLAMF2 and SLAMF7 expression between serous ovarian cancer (OV) and normal ovarian tissue. **B.** The influence of SLAMF2 expression on patients’ overall survival (OS). **C.** The influence of SLAMF2 expression on the disease-free survival (DFS) and OS of patients. **D.** The differences in expression of SLAMF7 between different clinical stages (stage I-II vs. stage III-IV), lymph node metastasis (N0 vs. N1), and histologic
grade (G1-2 vs. G3-4). E. Gene differential expression between serous OV and normal ovarian tissue in the GEO database (GSE9891). F. OS outcomes of patients with low SLAMF7 and high SLAMF7 in the GEO databases (GSE18521 and GSE32062).

Figure 2
The expression features of SLAMF7 in clinical serous ovarian cancer (OV) samples. A. The expression distribution of SLAMF7 in clinical samples. B. Features of different SLAMF7 expression levels in clinical samples. C. Overall survival of patients with low and high SLAMF7 expression.

Figure 3
The relation between SLAMF7 expression and immune infiltration in ovarian cancer (OV) in the TCGA-OV database. A. Gene enrichment analysis of SLAMF7 and related pathways. B. The correlation between SLAMF7 expression and immune cell infiltration in OV. C. The correlation between SLAMF7 expression and T cell-specific biomarkers. D. The correlation between SLAMF7 expression and B cell-specific biomarkers. E. The correlation between SLAMF7 expression and NK cell-specific biomarkers.
The correlation of SLAMF7 and CD3 expression in clinical serous ovarian cancer samples. **A.** The immunohistochemistry (IHC) analysis of SLAMF7 and CD3 in clinical samples. **B.** The correlation between the expression levels of SLAMF7 and CD3.

**Supplementary Files**

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- Supplementaryfigure2.tif
- Supplementaryfigure3.tif