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Identification of SLAMF7 as an immune-related biomarker associated with the tumor microenvironment in colon adenocarcinoma using multi-omics and single-cell analyses

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Abstract

Objective To investigate the expression profiles, clinical implications, and tumor immunoregulatory roles of signaling lymphocyte activation molecule family member 7 (SLAMF7) in colon adenocarcinoma (COAD) using multi-omics data combined with clinical sample validation, thereby

providing theoretical evidence for the identification of immune biomarkers for COAD.

Methods Transcriptomic, clinicopathological, and mutation datasets for COAD were retrieved from the TCGA and GEO databases. Eighteen pairs of colon cancer tissues and matched adjacent non-tumorous tissues were collected from our hospital, and quantitative real-time PCR (qRT-PCR) was performed to detect SLAMF7 mRNA expression. Bioinformatic analyses were conducted to evaluate the associations between SLAMF7 expression and clinicopathological features, as well as patient prognosis. Receiver operating characteristic (ROC) curves and a prognostic nomogram were constructed. Differentially expressed gene (DEG) screening, Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Gene Set Enrichment Analysis (GSEA) were performed. The cBioPortal and MEXPRESS platforms were used to analyze gene mutation and DNA methylation profiles. The GSCA platform was applied to assess immune cell infiltration and drug sensitivity, and the TISCH database was utilized to identify the cellular origin of SLAMF7 at the single-cell level.

Results SLAMF7 was significantly downregulated in COAD tumor tissues, a finding validated by multiple GEO datasets and clinical qRT-PCR results. Low SLAMF7 expression correlated with advanced TNM stage and distant metastasis. Patients with high SLAMF7

expression exhibited longer overall survival (OS) and disease-free interval (DFI); however, SLAMF7 was not an independent prognostic factor for OS in COAD patients. The nomogram demonstrated favorable performance in predicting 1-year and 3-year survival probabilities. Samples with high SLAMF7 expression were enriched in multiple anti-tumor immune pathways. SLAMF7 expression was positively correlated with the infiltration abundance of cytotoxic T cells and natural killer (NK) cells and was co-expressed with multiple immune checkpoint genes. Single-cell analyses demonstrated that SLAMF7 was predominantly expressed in tumor-infiltrating CD8+ T cells and NK cells, whereas malignant tumor cells barely expressed SLAMF7. SLAMF7 exhibited a low mutation frequency in COAD, and DNA methylation was not the primary mechanism underlying its downregulation. Furthermore, SLAMF7 expression was significantly associated with sensitivity to multiple chemotherapeutic and targeted agents.

Conclusions SLAMF7 is downregulated in colon adenocarcinoma and reflects the magnitude of anti-tumor immune infiltration in tumor tissues, serving as a potential biomarker for evaluating the immune microenvironment status of COAD.

Keywords colon adenocarcinoma; SLAMF7; immune-cell infiltration; tumor immune microenvironment; prognosis analysis; single-cell

transcriptomics

1.Introduction

Colon adenocarcinoma (COAD) is one of the most prevalent malignant tumors of the digestive system worldwide, characterized by high incidence and mortality rates that pose a significant threat to public health [1-3]. Surgical resection remains the mainstay of clinical treatment, supplemented by chemotherapy and targeted therapy. Although immune checkpoint inhibitor therapy has achieved encouraging outcomes for advanced COAD, clinical benefits are largely confined to patients with microsatellite instability-high (MSI-H) tumors. Most patients exhibit limited immunotherapeutic responses and heterogeneous prognoses. The tumor immune microenvironment (TIME) exerts pivotal effects on tumor initiation, invasion, metastasis, and therapeutic response[4-6]. Consequently, the identification of reliable immune-related biomarkers is critical for patient risk stratification and the design of individualized immunotherapeutic regimens. Accumulating evidence has demonstrated that immune-related genes not only modulate tumor progression but also correlate closely with immune infiltration status[7]. For instance, the infiltration levels of regulatory T cells, macrophages, dendritic cells, and CD8+ T cells are significantly associated with survival outcomes in COAD patients[8]. Furthermore, the molecular features of the immune

microenvironment reflect tumor biological heterogeneity and provide a theoretical foundation for the development of targeted and immunotherapeutic strategies[9].

Signaling lymphocyte activation molecule family member 7 (SLAMF7) is an immune receptor primarily expressed on NK cells and CD8+ T cells, participating in immune cell activation, adhesion, and cytotoxic killing [10-13]. SLAMF7 is a well-established therapeutic target for multiple myeloma; elotuzumab, an anti-SLAMF7 monoclonal antibody, has received FDA approval for this hematologic malignancy. Increasing evidence indicates that dysregulated SLAMF7 expression contributes to immune evasion and disease progression in solid tumors. In kidney renal clear cell carcinoma (KIRC), high SLAMF7 expression is associated with unfavorable survival outcomes [14].In breast cancer, elevated SLAMF7 expression correlates with clinical prognosis [15, 16]. In ovarian cancer, SLAMF7 upregulation enhances cancer cell survival, migration, invasion, and epithelial-mesenchymal transition [17, 18]. Studies in breast and ovarian cancer further link SLAMF7 to immune suppression, T cell exhaustion, and immunotherapy responsiveness, implying its potential value in solid tumor immunomodulation. Although a previous study indicated that SLAMF7 expression correlates with clinical outcomes in COAD patients receiving cytokine-induced killer cell-based immunotherapy, the biological functions and clinical significance of

SLAMF7 as an immunoregulatory molecule in COAD remain to be fully elucidated [19, 20].

Public multi-omics databases enable the efficient high-throughput screening of candidate tumor biomarkers. In this study, we integrated pan-cancer transcriptomic, clinicopathological, mutation, methylation, and single-cell sequencing datasets, combined with qRT-PCR validation using clinical tissue specimens. We systematically analyzed SLAMF7 expression in COAD and explored its relationships with clinicopathological parameters, patient prognosis, immune cell infiltration, and drug sensitivity. Additionally, single-cell datasets were utilized to pinpoint the cellular source of SLAMF7 expression. Our findings advance the mechanistic understanding of COAD molecular pathology and tumor immunity, providing supporting evidence for follow-up basic and translational investigations targeting SLAMF7.

2 Materials and methods

2.1 Data acquisition and processing

Gene expression and clinical datasets comprising 480 COAD samples and 41 paired adjacent non-tumorous samples were downloaded from the TCGA database. FPKM values were converted to transcripts per million (TPM) for downstream analyses. Two additional COAD datasets, GSE44076 and GSE113513, were obtained from the GEO database for

the external validation of SLAMF7 mRNA expression.

2.2 Clinical Sample Collection

Eighteen pairs of primary colon adenocarcinoma tissues and matched adjacent non-tumorous tissues were collected at Guangxi Medical University Cancer Hospital between April 2026 and June 2026 for mRNA expression validation.

2.3 Quantitative Reverse-Transcription PCR

Total RNA was isolated from clinical specimens and reverse-transcribed into complementary DNA (cDNA). Quantitative real-time PCR (qRT-PCR) was performed to amplify target transcripts. Relative gene expression was quantified using the $2^{-\Delta\Delta Ct}$ method. Each experiment was performed in biological triplicate with five technical replicates per sample. The primer sequences for SLAMF7 were as follows: forward, 5′-ACCCTCATCTATATCCTTTGGCA-3′; reverse, 5′-CACCAACGGAACCGACCAG-3′.

2.4 Mutation, Methylation, and Drug Sensitivity Analyses

The cBioPortal database was employed to evaluate SLAMF7 mutation frequency, variant loci, and mutation types in COAD. The MEXPRESS platform was used to visualize correlations between SLAMF7 methylation status and gene expression. The Gene Set Cancer Analysis (GSCA) database was utilized to analyze associations of SLAMF7 expression with immune cell infiltration abundance and drug sensitivity.

The Tumor Immune Single-Cell Hub (TISCH) database was used for the single-cell level visualization of SLAMF7 expression across distinct cell subpopulations.

2.5 Statistical Analyses

All statistical computations and data visualization were performed using R software (version 4.2.1). The Wilcoxon rank-sum test was applied for differential expression comparisons of unpaired samples. The paired t-test was adopted for paired sample comparisons where normality assumptions were satisfied. Spearman correlation tests were used for correlation analyses. Differences in immune cell infiltration between SLAMF7 high- and low-expression subgroups were assessed using the Wilcoxon rank-sum test. Receiver operating characteristic (ROC) curves were generated using the pROC package. Survival curves were plotted via the Kaplan-Meier method, and survival differences were evaluated using the log-rank test. The Kruskal-Wallis test, Mann-Whitney U test, and chi-square test were appropriately applied for univariate analyses. $P < 0.05$ was defined as the threshold for statistical significance.Gene expression and clinical datasets comprising 480 COAD samples and 41 paired adjacent non-tumorous samples were downloaded from the TCGA database. FPKM values were converted to transcripts per million (TPM) for downstream analyses. Two additional COAD datasets, GSE44076 and GSE113513, were obtained from the GEO database for external

validation of SLAMF7 mRNA expression.

3.Results

3.1 Pan-cancer and COAD specific differential expression of SLAMF7

Pan-cancer transcriptomic analyses from TCGA revealed significant differences in SLAMF7 expression between tumor and corresponding normal tissues across multiple cancer types (Figure 1A). SLAMF7 was markedly upregulated in lung adenocarcinoma (LUAD), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), and head and neck squamous cell carcinoma (HNSC) ($P < 0.001$). In contrast, significant downregulation was observed in thyroid carcinoma (THCA), colon adenocarcinoma (COAD), and rectal adenocarcinoma (READ) ($P < 0.05$).

To characterize SLAMF7 expression specifically in COAD, multi-cohort validations were performed. TCGA-COAD paired sample analysis demonstrated substantial SLAMF7 downregulation in tumor tissues relative to matched adjacent tissues ($P < 0.001$, Figure 1B). Unpaired sample analysis further confirmed this robust differential expression ($P < 0.001$, Figure 1C). Two independent GEO datasets corroborated these findings: SLAMF7 mRNA levels were significantly lower in tumor tissues in GSE44076 ($P < 0.01$, Figure 1D) and GSE113513 ($P < 0.05$,

Figure 1E). Consistent with public database results, qRT-PCR assays using our institutional clinical samples confirmed that SLAMF7 mRNA expression was significantly decreased in colon adenocarcinoma tissues compared with paired adjacent non-tumorous tissues ($P < 0.05$, Figure 1F).Consistent with public database results, q-PCR assays using our institutional clinical samples confirmed that SLAMF7 mRNA expression was significantly decreased in colon adenocarcinoma tissues compared with paired adjacent non-tumorous tissues ($P < 0.05$, Figure 1F).

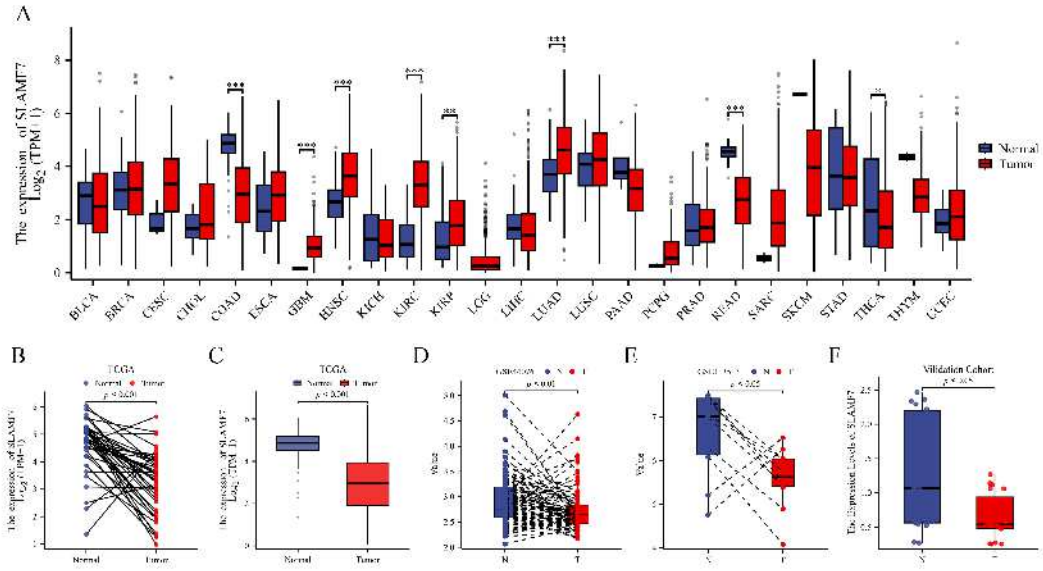


Figure 1 mRNA expression profiles of SLAMF7 across pan-cancer and colon adenocarcinoma

A: Expression distribution of SLAMF7 between tumor and normal tissues in the TCGA pan-cancer cohort; B: SLAMF7 expression in paired TCGA-COAD samples; C: SLAMF7 expression in unpaired TCGA-COAD samples; D: SLAMF7 expression in the GSE44076 dataset; E: SLAMF7 expression in the GSE113513 dataset; F: qPCR validation cohort using clinical samples from this study. N, normal tissue; T, tumor tissue. Asterisks indicate statistical significance.

3.2 Correlations between SLAMF7 expression and clinicopathological features of colon adenocarcinoma

TCGA-COAD patients were stratified into SLAMF7 high-expression and low-expression subgroups according to the median SLAMF7 expression value. Baseline clinicopathological variables were compared between the two groups (Table 1). No significant inter-group differences were observed for sex, age, T stage, carcinoembryonic antigen (CEA) concentration, perineural invasion, or lymphovascular invasion ($P > 0.05$), indicating well-balanced baseline characteristics. However, key parameters reflecting tumor metastasis and disease stage differed significantly. The proportion of patients with N1 disease was higher in the SLAMF7 low-expression subgroup (13.4% vs. 9.2%), whereas the proportion of M0 stage patients was lower (39.5% vs. 44.6%). Regarding overall TNM staging, the low-expression subgroup contained more stage IV patients (9.2% vs. 4.9%) and fewer stage II patients (17.6% vs. 22.5%). All aforementioned differences reached statistical significance ($P < 0.05$).

Stratified analyses demonstrated that SLAMF7 expression correlated closely with COAD disease progression (Figure 2). Patients with M0 stage disease exhibited significantly higher SLAMF7 expression than those with distant metastasis ($P < 0.001$, Figure 2C). Within TNM staging, SLAMF7 expression was significantly higher in stage II tumors compared with stage IV tumors ($P < 0.001$, Figure 2D). No significant correlations were detected between SLAMF7 expression and CEA level, perineural invasion, or lymphatic invasion. When stratified by survival

status, surviving patients displayed significantly higher SLAMF7 expression than deceased patients ($P < 0.05$, Figure 2H). Collectively, low SLAMF7 expression correlated with advanced TNM stage, distant metastasis, and unfavorable clinical outcomes, suggesting that reduced SLAMF7 may serve as a marker of aggressive biological behavior in COAD.

Table 1 Baseline clinicopathological characteristics of patients with colon adenocarcinoma

characteristics	Low expression of SLAMF7	High expression of SLAMF7	pvalue
n	239	239	
Gender, n (%)			0.71
Female	111 (23.2%)	115 (24.1%)	
Male	128 (26.8%)	124 (25.9%)	
Age, n (%)			1.00
<= 65	97 (20.3%)	97 (20.3%)	
> 65	142 (29.7%)	142 (29.7%)	
Pathologic T stage, n (%)			0.98
T1	5 (1%)	6 (1.3%)	
T2	41 (8.6%)	42 (8.8%)	
T3	161 (33.8%)	162 (34%)	
T4	31 (6.5%)	29 (6.1%)	
Pathologic N stage, n (%)			0.05
N0	130 (27.2%)	154 (32.2%)	
N1	64 (13.4%)	44 (9.2%)	
N2	45 (9.4%)	41 (8.6%)	
Pathologic M stage, n (%)			0.01
M0	164 (39.5%)	185 (44.6%)	
M1	43 (10.4%)	23 (5.5%)	
Pathologic stage, n (%)			0.02
Stage I	38 (8.1%)	43 (9.2%)	
Stage II	82 (17.6%)	105 (22.5%)	
Stage III	71 (15.2%)	62 (13.3%)	
Stage IV	43 (9.2%)	23 (4.9%)	
CEA level, n (%)			0.85
<= 5	93 (30.7%)	103 (34%)	
> 5	52 (17.2%)	55 (18.2%)	
Perineural invasion, n (%)			0.11
No	77 (42.5%)	58 (32%)	
Yes	20 (11%)	26 (14.4%)	
Lymphatic invasion, n (%)			0.76
No	129 (29.7%)	137 (31.6%)	
Yes	84 (19.4%)	84 (19.4%)	

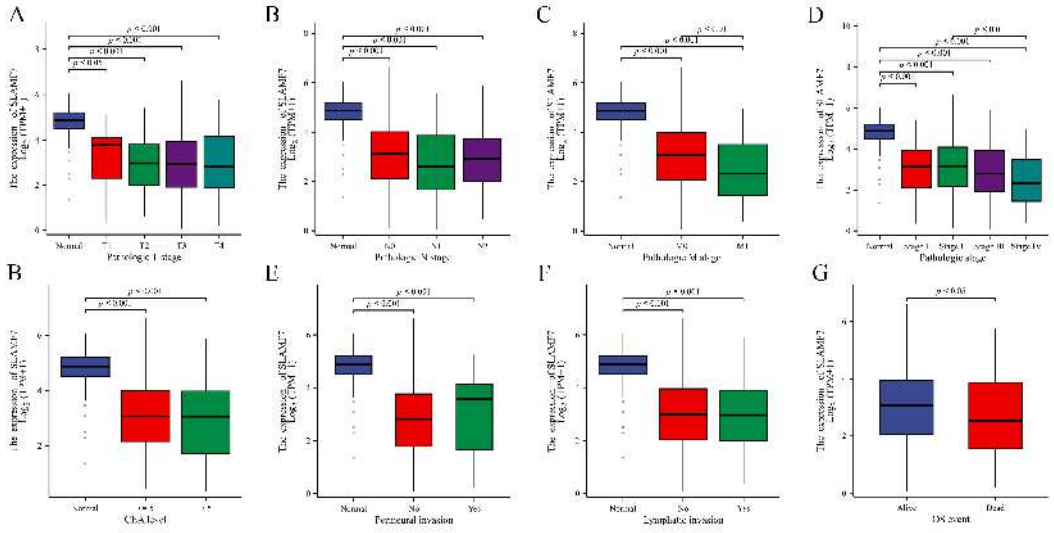


Figure 2 Association between SLAMF7 expression and clinicopathological features in colon adenocarcinoma

A: SLAMF7 expression across pathological T stages; B: SLAMF7 expression across pathological N stages; C: SLAMF7 expression across pathological M stages; D: SLAMF7 expression across overall pathological stages; E: SLAMF7 expression stratified by serum CEA levels; F: SLAMF7 expression in patients with or without vascular invasion; G: SLAMF7 expression in patients with or without lymphatic invasion; H: SLAMF7 expression stratified by overall survival outcome.

3.3 Prognostic value of SLAMF7 and construction of a prognostic prediction model

ROC curve analysis yielded an area under the curve (AUC) value of 0.859 (95% CI: 0.797–0.921) for SLAMF7 in discriminating COAD from normal tissues, indicating favorable diagnostic performance (Figure 3A). Risk distribution visualization illustrated that patients in the high-risk subgroup (SLAMF7 low expression) had higher mortality and shorter survival duration (Figure 3B).

Kaplan-Meier survival analysis showed that high SLAMF7 expression was associated with superior clinical outcomes. Patients with high SLAMF7 expression demonstrated significantly prolonged OS (HR =

0.65, P = 0.034, Figure 3C) and DFI (HR = 0.68, P = 0.033, Figure 3E). Although disease-specific survival (DSS) showed a trend toward better outcomes for the high-expression group, the difference was not statistically significant (HR = 0.63, P = 0.077, Figure 3D).

Subgroup analyses revealed that high SLAMF7 expression predicted improved OS among patients with T3–T4 advanced stage tumors (HR = 0.61, P = 0.020) and among stage III–IV patients (HR = 0.59, P = 0.043). By contrast, no significant survival difference was observed within the N1–N2 lymph node metastasis subgroup (HR = 0.71, P = 0.175). Cox proportional hazards regression analyses were performed in 477 patients with colon adenocarcinoma to evaluate prognostic factors for OS. Univariate analysis demonstrated that age > 65 years, T3/T4 stage, N1/N2 stage, and distant metastasis (M1) represented significant risk factors for OS ($P < 0.05$). Conversely, high SLAMF7 expression acted as a protective factor against death (HR = 0.648, 95% CI: 0.433–0.969, P = 0.034).

Variables showing statistical significance in the univariate model were further incorporated into the multivariate Cox regression model. Multivariate analysis identified age > 65 years, T3/T4 stage, N2 stage, and distant metastasis (M1) as independent adverse prognostic factors for OS ($P < 0.05$) (Table 2) . After adjustment for these clinical confounders, the association between SLAMF7 expression and OS lost statistical

significance (HR = 0.656, 95% CI: 0.418–1.030, P = 0.067).

A nomogram integrating SLAMF7 expression, age, T stage, and lymphovascular invasion was constructed to quantitatively predict 1-year, 3-year, and 5-year OS probabilities (Figure 3H). Calibration curve analysis demonstrated good concordance between nomogram-predicted survival probabilities and observed clinical outcomes (Figure 3I), supporting its predictive accuracy and potential clinical applicability. In summary, SLAMF7 represents a promising diagnostic biomarker for COAD; its low expression correlates with poor prognosis without constituting an independent prognostic variable. The nomogram incorporating SLAMF7 enables effective survival risk stratification for COAD patients.

Table 2 Univariate and multivariate Cox regression analyses of prognostic factors for overall survival in patients with colon adenocarcinoma

Characteristics	Total(N)	HR(95% CI) Univariate analysis	P value	HR(95% CI) Multivariate analysis	P value
Gender	477				
Female	226	Reference			
Male	251	1.101 (0.746 - 1.625)	0.627		
Age	477				
<= 65	194	Reference		Reference	
> 65	283	1.610 (1.052 - 2.463)	0.028	1.895 (1.166 - 3.079)	0.010
Pathologic T stage	476				
T1&T2	94	Reference		Reference	
T3	322	2.576 (1.183 - 5.612)	0.017	3.391 (1.036 - 11.100)	0.044
T4	60	7.021 (2.993 - 16.473)	< 0.001	7.014 (1.964 - 25.056)	0.003
Pathologic N stage	477				
N0	283	Reference		Reference	
N1	108	1.681 (1.019 - 2.771)	0.042	1.046 (0.565 - 1.937)	0.886
N2	86	4.051 (2.593 - 6.329)	< 0.001	2.315 (1.317 - 4.071)	0.004
Pathologic M stage	414				
M0	348	Reference		Reference	
M1	66	4.193 (2.683 - 6.554)	< 0.001	2.402 (1.398 - 4.126)	0.002
SLAMF7	477				
Low	239	Reference		Reference	
High	238	0.648 (0.433 - 0.969)	0.034	0.656 (0.418 - 1.030)	0.067

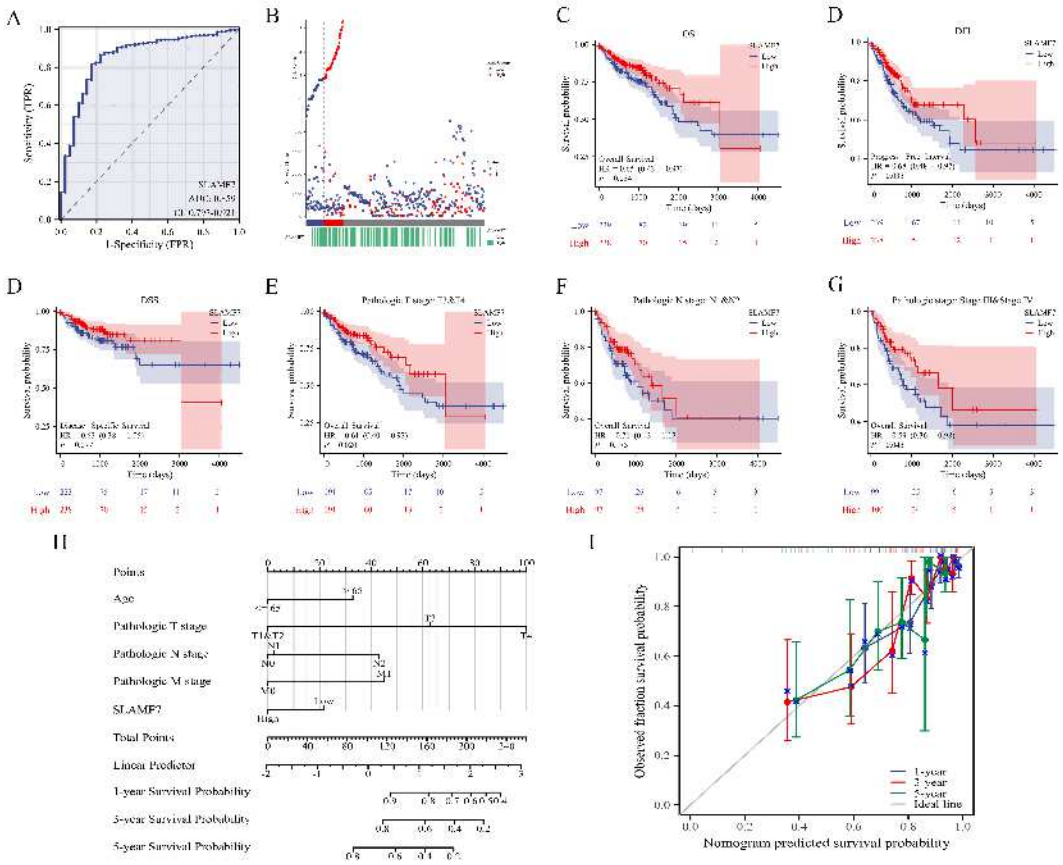


Figure 3 Diagnostic and prognostic analysis of SLAMF7 and construction of a prognostic nomogram for patients with colon adenocarcinoma

A: ROC curve for diagnostic performance of SLAMF7 expression in COAD; B: Heatmap showing SLAMF7 expression distribution in COAD samples; C: Kaplan–Meier survival curve for overall survival (OS); D: Survival curve for disease-free survival (DFS); E: Survival curve for disease-specific survival (DSS); F: OS survival analysis in the T3–T4 subgroup; G: OS survival analysis in the N1–N2 subgroup; H: Prognostic nomogram for COAD patients; I: Calibration curve of the nomogram to assess the consistency between model-predicted values and observed outcomes.

3.4 Differentially expressed gene profiling and functional enrichment analyses

To dissect potential molecular mechanisms mediated by SLAMF7 in COAD, DEG analysis was performed comparing SLAMF7 high- and low-expression subgroups. Applying thresholds of |log2FC| > 2 and adjusted P < 0.05, 4,222 DEGs were identified, of which 4,084 genes

were upregulated and 138 genes were downregulated (Figure 4A). Co-expression analysis indicated that SLAMF7 was positively correlated with immune activation-associated genes, including CD48, IRF4, MPEG1, and IKZF1, and negatively correlated with several stromal and proliferation-related genes (Figure 4B).

A protein-protein interaction (PPI) network built via the STRING database revealed robust interactions between SLAMF7 and immune-relevant molecules CD48, SH2D1B, and CD244 (Figure 4C). MCODE algorithm mining identified a core functional module comprising immune-related genes: CD2, CD3D, CD3G, CD48, CD53, FCGR3A, KLRK1, LCK, SH2D1A, and TYROBP, suggesting the coordinated participation of these genes in COAD immunoregulation (Figure 4D).

GO and KEGG enrichment analyses demonstrated that DEGs derived from the SLAMF7 high-expression subgroup were predominantly enriched in biological processes such as the regulation of immune responses, leukocyte-mediated immunity, cell adhesion, and NK cell-mediated cytotoxicity. Cellular component terms centered on cell membrane compartments and secreted vesicles. Molecular function enrichment included immune receptor activity and cytokine receptor activity. Key KEGG pathways encompassed cytokine-cytokine receptor interaction, chemokine signaling pathways, and NK cell-mediated

cytotoxicity (Figure 4E). These results imply that SLAMF7 modulates the COAD immune microenvironment through cytokine signaling, cell adhesion events, and NK cell-dependent cytotoxicity.

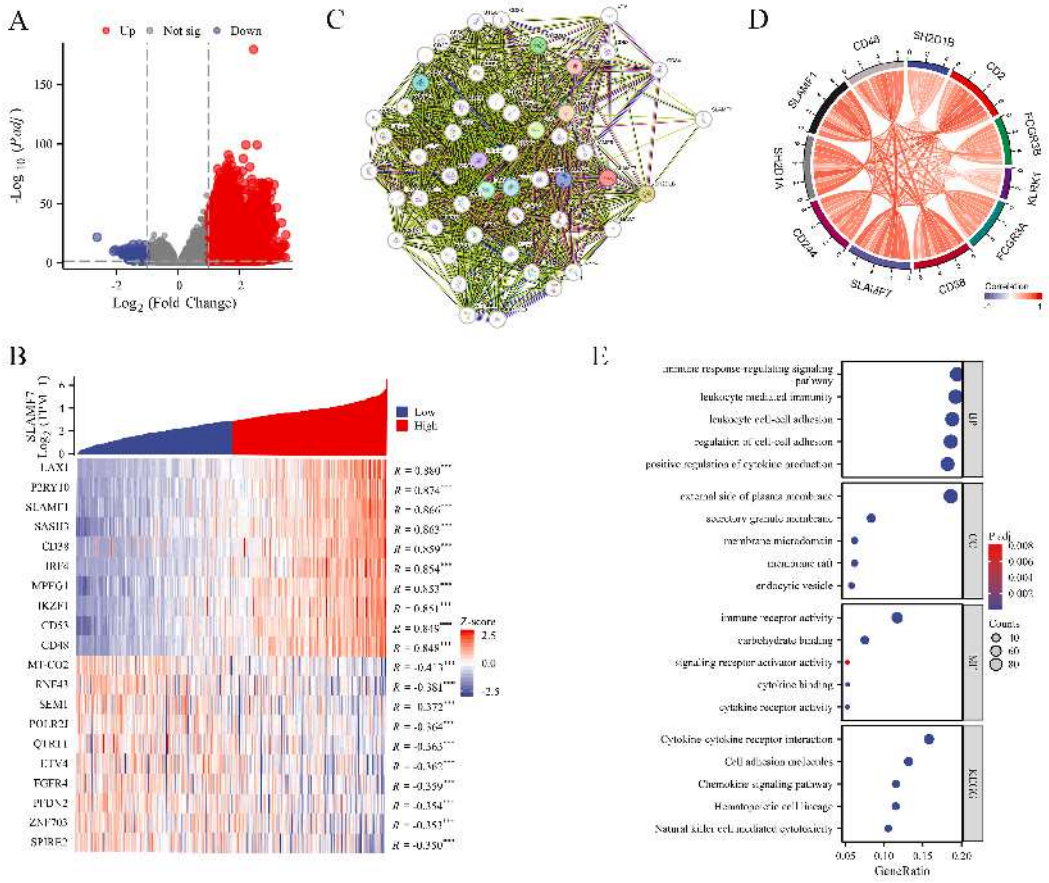


Figure 4 Differential genes, co-expression, PPI network and functional enrichment analysis between SLAMF7 high- and low-expression groups

A: Volcano plot of differentially expressed genes between SLAMF7 high- and low-expression groups. Red dots indicate upregulated genes, blue dots indicate downregulated genes, and grey dots represent genes with no significant difference; B: Co-expression heatmap of SLAMF7 and immune-related genes, with corresponding correlation coefficients annotated; C: Protein-protein interaction (PPI) network of differentially expressed genes; D: Circle plot showing core interacting genes of SLAMF7; E: Bubble plot for GO and KEGG functional enrichment analysis. BP, biological process; CC, cellular component; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes pathway.

3.5 Gene set enrichment analysis (GSEA)

GSEA was performed to further characterize biological pathways

associated with SLAMF7 status. Multiple anti-tumor immune-related gene sets were significantly enriched in the SLAMF7 high-expression subgroup (FDR < 0.001). Enriched immune-checkpoint-related signatures included the PD-1 signaling pathway (NES = 2.222, Figure 5A) and the CTLA4-signaling pathway (NES = 2.149, Figure 5B). Cytokine and chemokine-associated pathways comprised cytokine-cytokine receptor interaction (NES = 2.011, Figure 5C), chemokine receptor chemokine binding (NES = 2.187, Figure 5D), JAK-STAT signaling (NES = 1.747, Figure 5E), and chemokine-signaling pathways (NES = 1.891, Figure 5G). Immune-cell interaction and cytotoxic function signatures included lymphocyte-non-lymphocyte immunoregulatory interactions (NES = 2.321, Figure 5F) and NK cell-mediated cytotoxicity (NES = 1.921, Figure 5H). Collectively, GSEA results support that high SLAMF7 expression correlates with robust anti-tumor immune activation in COAD; SLAMF7 may exert tumor-suppressive effects by modulating PD-1/CTLA4 immune-checkpoint signaling, augmenting cytokine signal transduction, and potentiating NK cell cytotoxicity.

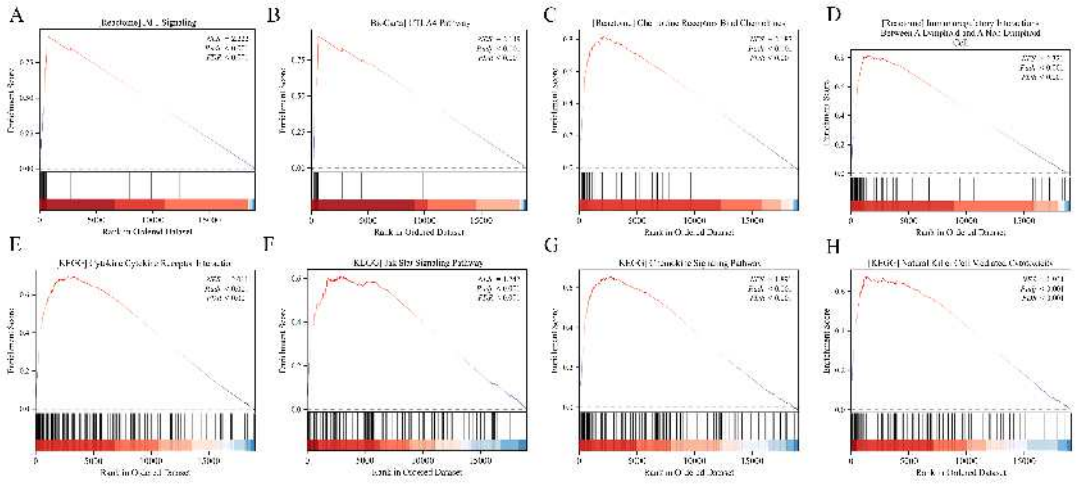


Figure 5 Results of GSEA enrichment analysis

A: PD-1 signaling pathway; B: CTLA4 pathway; C: Chemokine receptors bind chemokines pathway; D: T lymphocyte–non-T lymphocyte interactions at the immunological synapse pathway; E: Cytokine–cytokine receptor interaction pathway; F: JAK-STAT signaling pathway; G: Chemokine signaling pathway; H: Natural killer cell-mediated cytotoxicity pathway. NES, normalized enrichment score; FDR, false discovery rate. Red blocks indicate high-expression enrichment of the gene set, while blue blocks indicate low-expression enrichment.

3.6 SLAMF7 genetic-alteration landscape and methylation features

Within the TCGA-COAD cohort, the overall genetic-alteration frequency for SLAMF7 was 7 %. Major alteration types included mRNA overexpression, gene amplification, and missense mutation; mRNA up regulation constituted the most prevalent event (Figure 6A). The S139N missense mutation mapped to the Ig-3 functional domain of SLAMF7 protein (Figure 6B). Comparison between SLAMF7 altered and non altered groups revealed distinct distributions for tumor mutational burden (TMB), fraction of genome altered values, and total mutation counts (Figure 6C-E). mRNA-expression distributions varied across missense

mutation, truncating mutation, wild type, and mutation unknown subgroups (Figure 6F).

Oncoprint visualization of high frequency driver gene mutations demonstrated that APC, TTN, and KRAS were frequently mutated in both SLAMF7 high and low expression subgroups. In the high expression subgroup, APC exhibited the highest mutation rate at 72.6 %, followed by TTN (52.3 %) and KRAS(42.7 %). Statistically significant inter-subgroup differences in mutation frequencies existed for APC, KRAS, SYNE1, FAT4, and RYR2 ($P < 0.05$), with variant classes including missense, nonsense, frameshift insertion/deletion, and splice site mutations. These observations indicate divergent genomic mutation profiles between SLAMF7 high and low expression COAD subsets and suggest that SLAMF7 expression status may correlate with distinct tumor mutational backgrounds.

MEXPRESS platform based methylation analysis showed that SLAMF7 CpG site methylation levels correlated with colorectal polyp history, tumor stage, body mass index (BMI), and OS. Several CpG site methylation events displayed weak positive correlations with SLAMF7 mRNA expression, whereas copy number variations showed no obvious association with methylation status. This finding indicates that DNA methylation is not the predominant mechanism driving SLAMF7 down regulation in COAD (Figure 6H).

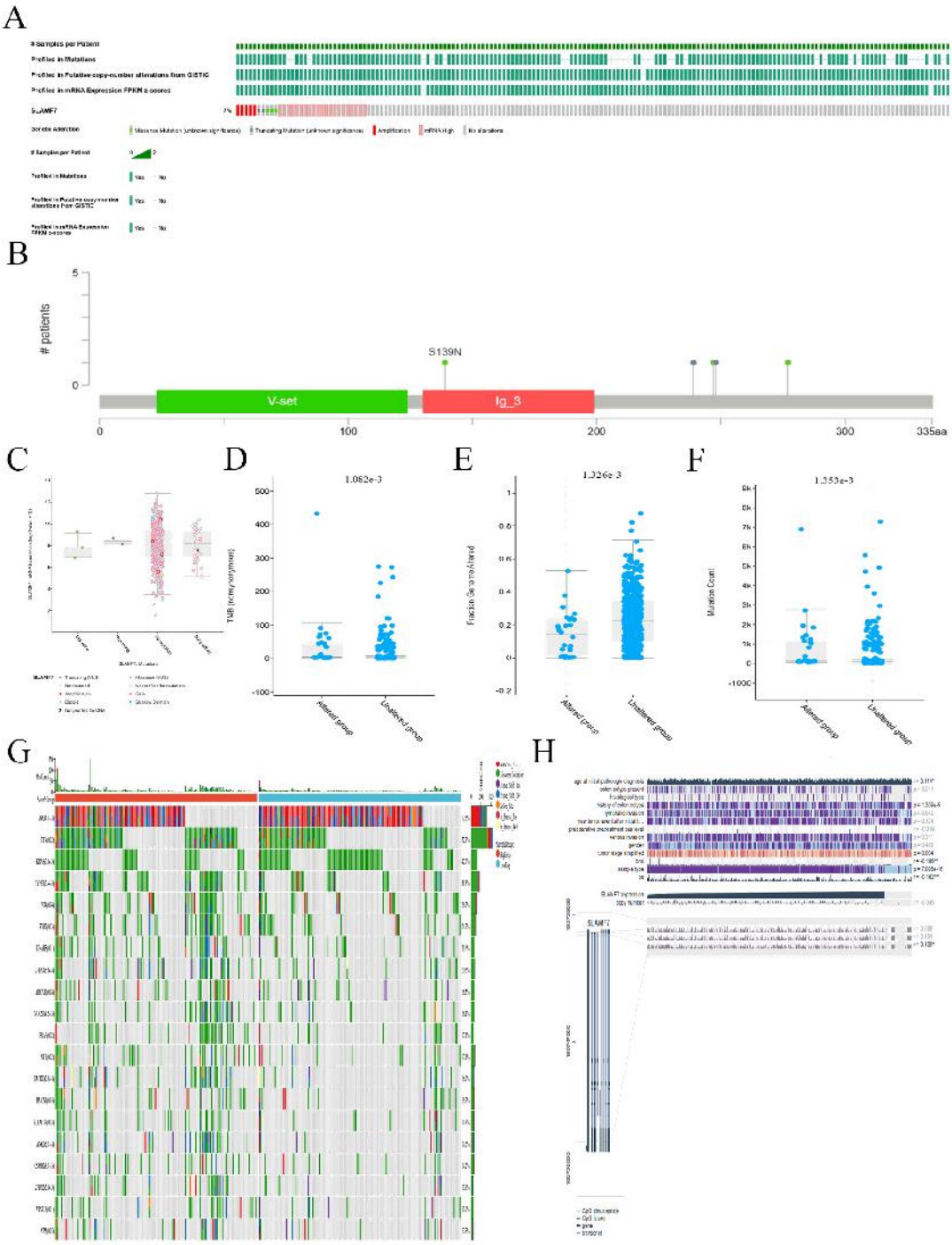


Figure 6 Genomic alteration and methylation analysis of SLAMF7 in COAD

A: Overview of global SLAMF7 gene alterations; B: Distribution of mutation sites across the SLAMF7 protein domain, with the representative missense mutation S139N annotated; C: Comparison of tumor mutational burden (TMB) between the altered and non-altered groups; D: Comparison of the proportion of genomic changes between the altered and non-altered groups; E: Comparison of total mutation counts between the altered and non-altered groups; F: Comparison of SLAMF7 mRNA expression levels between wild-type and altered samples; G: Oncoprint plot showing co-mutations in SLAMF7-altered samples, with different colors representing distinct alteration types; H: Correlation analysis among SLAMF7 methylation status, gene expression and

clinicopathological parameters in COAD using the MEXPRESS platform.

3.7 Association between SLAMF7 expression and the tumor immune microenvironment

Given the central importance of the immune microenvironment in COAD oncogenesis, immune escape, and prognosis, we systematically analyzed relationships between SLAMF7 expression, immune cell infiltration abundance, immune gene set scores, and immune marker gene expression. Pan-cancer correlation heatmaps indicated broad associations between SLAMF7 expression and anti tumor immune cell infiltration across multiple malignancies (Figure 7A). In the COAD cohort, the SLAMF7 high expression subgroup exhibited greater infiltration abundances of effector T cells, NK cells, and dendritic cells alongside elevated enrichment scores for immune activation related gene sets (Figure 7B-C). Correlation quantification demonstrated that SLAMF7 expression was positively associated with infiltration levels of cytotoxic T cells, B cells, dendritic cells, macrophages, and NK cells. The strongest correlation occurred with cytotoxic T cells ($R = 0.785$, $P < 0.001$); significant correlations with certain suppressive immune cell populations were also observed, implying SLAMF7 participates in complex immune regulatory networks in COAD (Figure 7D).

Radar plot visualization showed positive correlations between SLAMF7 and most chemokines as well as MHC-I/MHC-II antigen presentation

related genes (HLA family genes), suggesting potential involvement in tumor antigen presentation (Figure 7E). Meanwhile, SLAMF7 exhibited strong positive co-expression with multiple immune inhibitory checkpoint genes including PDCD1, CTLA4, LAG3, TIGIT, and HAVCR2(Figure 7F). Collectively, SLAMF7 expression closely reflects immune cell infiltration magnitude within COAD tumors and likely participates in anti-tumor immune responses by modulating immune cell infiltration and immune related gene programs.

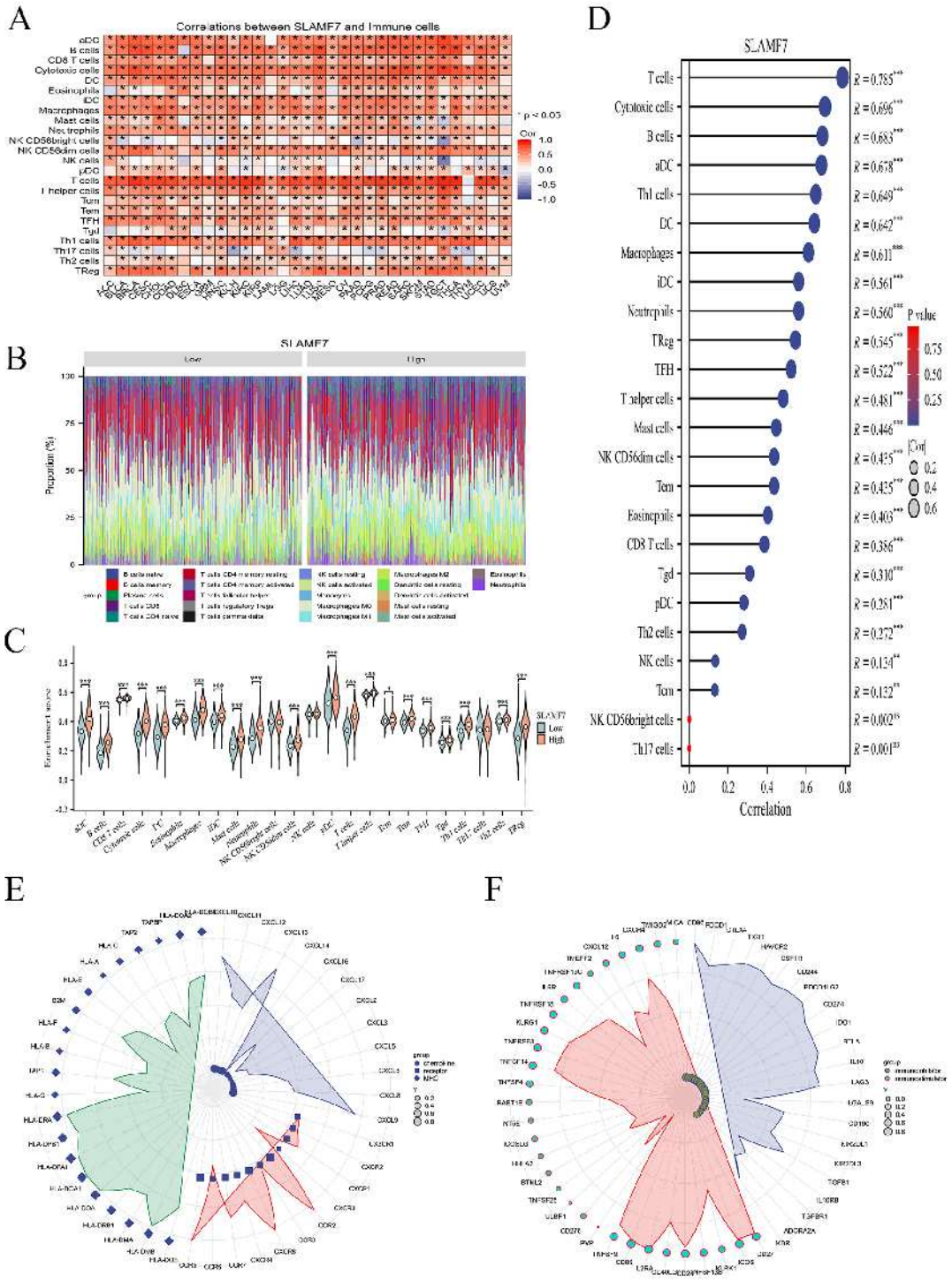


Figure 7 Correlation analysis between SLAMF7 expression and the tumor immune microenvironment in COAD

A: Heatmap showing correlations between SLAMF7 expression and immune cell infiltration levels across pan-cancer cohorts; B: Distribution of immune cell infiltration abundance in SLAMF7 high- and low-expression groups; C: Comparison of enrichment scores for immune-related gene sets between SLAMF7 high- and low-expression groups; D: Correlation between SLAMF7 expression and immune cell infiltration levels, with correlation coefficients (*R*) and *P* values annotated; E: Radar plot illustrating correlations of SLAMF7 with

chemokines and major histocompatibility complex (MHC) molecules; F: Radar plot showing correlations between SLAMF7 and the expression of immune marker genes.

3.8 Correlations of SLAMF7 with immune-cell infiltration and drug-treatment sensitivity

The GSCA platform was utilized to validate SLAMF7 immune microenvironment associations and further explore its links to chemotherapeutic and targeted drug sensitivity in COAD. SLAMF7 expression was significantly positively correlated with infiltration abundance for the majority of immune cell types; the strongest correlations were observed for T cells ($R = 0.785$), cytotoxic cells ($R = 0.696$), and B cells ($R = 0.683$) (Figure 8A). These results independently corroborated that high SLAMF7 expression corresponds to enhanced anti-tumor immune cell infiltration in COAD.

Analyses using GDSC and CTRP drug response databases revealed significant correlations between SLAMF7 expression and IC50 values for numerous chemotherapeutic and targeted agents. Within the GDSC dataset, high SLAMF7 expression positively correlated with IC50 values for 17-AAG, cytarabine, and docetaxel, indicating potential drug resistance. Conversely, high SLAMF7 expression negatively correlated with IC50 values for CAL-101, PIK-93, and UNC0638, suggesting improved sensitivity toward these compounds (Figure 8B). CTRP database analyses yielded concordant trends: high SLAMF7 expression

predicted enhanced sensitivity to trametinib, alvocidib, and dinaciclib, yet implied resistance to axitinib, AZD4547, and foretinib (Figure 8C). These data highlight SLAMF7 as a candidate biomarker for predicting therapeutic responses in COAD.

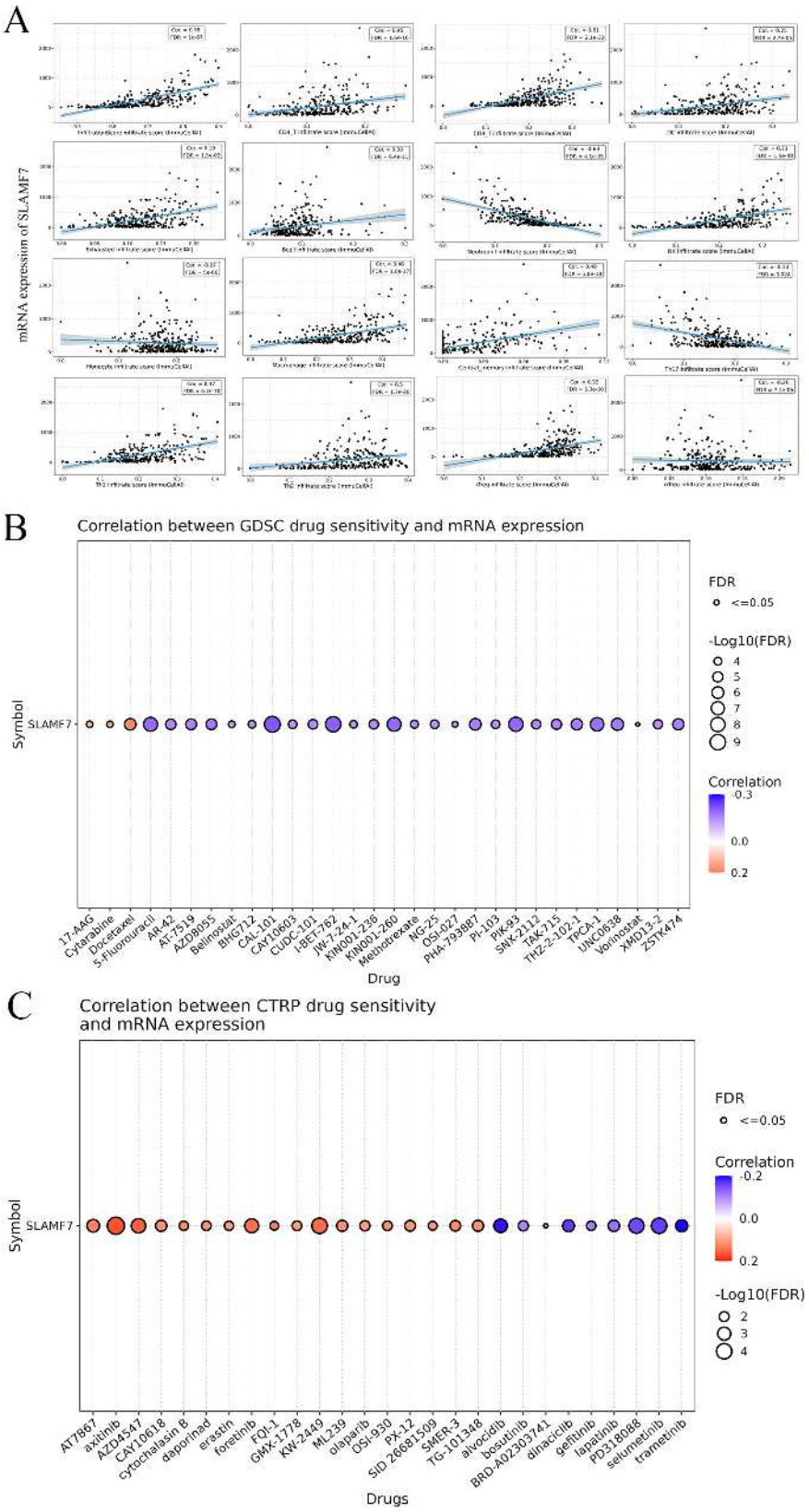


Figure 8 Analysis of the associations of SLAMF7 expression with immune infiltration and drug sensitivity in COAD based on the GSCA platform

A: Scatter plot showing the correlation between SLAMF7 expression and immune cell infiltration

levels; B: Bubble plot of correlations between SLAMF7 expression and drug sensitivity from the GDSC database. Bubble color represents the correlation coefficient, and bubble size denotes $-\log_{10}$(FDR); C: Bubble plot of correlations between SLAMF7 expression and drug sensitivity from the CTRP database.

3.9 Single cell transcriptomic dissection of SLAMF7 cellular expression sources in COAD

To pinpoint the cellular origin and distribution of SLAMF7 within the COAD immune microenvironment, two single-cell RNA sequencing datasets (Smart-seq2 and 10× Genomics) from the GSE146771 dataset hosted on the TISCH platform were analyzed. UMAP clustering of Smart-seq2 data partitioned tumor microenvironment cells into B cells, $CD4^+$ T cells, $CD8^+$ T cells, dendritic cells, macrophages, NK cells, malignant epithelial cells, and stromal cell subsets. SLAMF7 signal was predominantly enriched in immune cell clusters and barely detectable in malignant tumor cells (Figure 9A). Violin plot quantification demonstrated that SLAMF7 was most highly expressed in $CD8^+$ T cells and NK cells (Figure 9B).

Analyses of the 10× sequencing dataset reproduced these observations: cell clustering patterns and SLAMF7 expression distribution closely recapitulated the Smart seq2 findings (Figure 9C). SLAMF7 was again predominantly detected in $CD8^+$T cells and NK cells, whereas malignant cells showed negligible expression (Figure 9D). Results from both single cell platforms confirm that SLAMF7 in COAD is primarily produced by

tumor infiltrating immune cells (predominantly $CD8^+$ T cells and NK cells), rather than malignant tumor cells per se. This single cell evidence mechanistically explains the observed associations between SLAMF7 and immune cell infiltration phenotypes in colon adenocarcinoma.

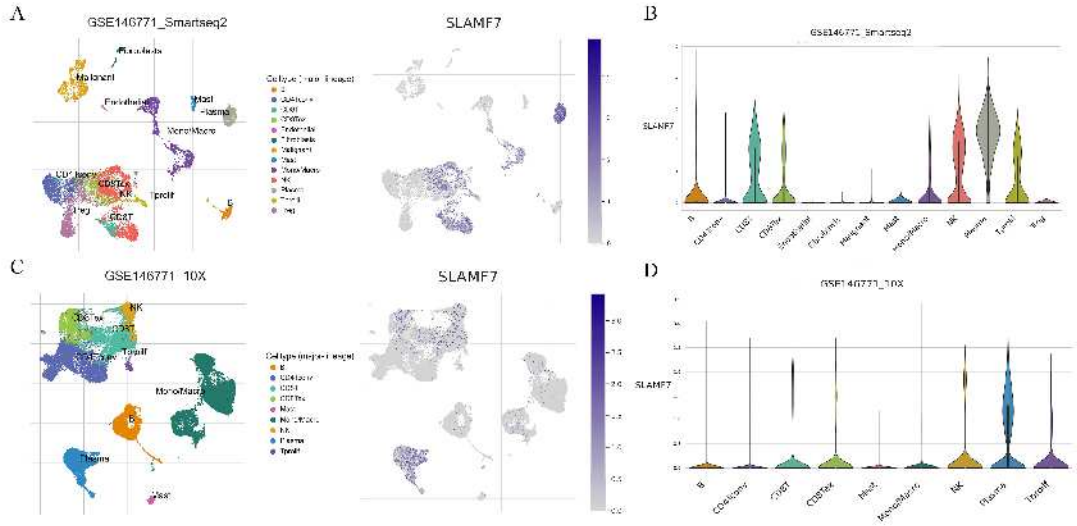


Figure 9 Analysis of SLAMF7 expression characteristics at the single-cell level in COAD from the GSE146771 dataset using the TISCH platform

A: UMAP plot of cell clusters and projected UMAP visualization of SLAMF7 gene expression from Smarter-seq2 sequencing; B: Violin plot showing SLAMF7 expression across cell subpopulations from Smarter-seq2 sequencing; C: UMAP plot of cell clusters and projected UMAP visualization of SLAMF7 gene expression from 10× Genomics sequencing; D: Violin plot showing SLAMF7 expression across cell subpopulations from 10× Genomics sequencing. The results revealed that SLAMF7 was predominantly expressed in cytotoxic immune cells such as $CD8^+$ T cells and NK cells, with low expression in malignant tumor cells.

4.Discussion

Colon adenocarcinoma represents a multi-gene-driven malignancy that progresses through multi-step oncogenic events. Dysregulation of the tumor immune microenvironment constitutes a core mechanism driving tumor proliferation, invasion, metastasis, and therapeutic resistance [21-23]. Identification of immune-related biomarkers can facilitate

improved risk stratification and advance individualized immunotherapy for patients with colon adenocarcinoma.

SLAMF7 is an immunoregulatory molecule that modulates immune cell activation and cytotoxic function, and it is closely implicated in the initiation and progression of diverse human cancers [12]. High SLAMF7 expression has been linked to tumor invasion and metastasis in multiple myeloma, lymphoma, and KIRC [24-26]. Findings regarding ovarian cancer remain controversial: some studies have reported significant SLAMF7 up-regulation associated with unfavorable patient prognosis, whereas other investigations have drawn opposite conclusions, whereby elevated SLAMF7 predicts favorable clinical outcomes [17]. In high-risk neuroblastoma, increased SLAMF7 levels are similarly correlated with better patient prognosis[27].Previous studies have documented down-regulated SLAMF7 expression in primary or metastatic colorectal cancer tissues relative to normal colonic tissues[11]. Moreover, high SLAMF7 expression serves as a potential biomarker for favorable therapeutic responses among colorectal cancer patients receiving cytokine induced killer cell based immunotherapy[20]. Nevertheless, most existing evidence is derived from cellular or animal models, and validations in large-scale clinical cohorts are still lacking[19].

In this multi-omics study integrating public-database resources and institutional clinical specimens, we confirmed consistent SLAMF7 down

regulation in COAD tissues across TCGA, multiple GEO datasets, and our q-PCR clinical cohort. Low SLAMF7 expression correlated with advanced TNM stage and distant metastasis. Patients with high SLAMF7 expression displayed superior OS and DFI, and this prognostic benefit persisted within T3-T4 and stage III-IV subgroups. However, multivariate Cox regression demonstrated that SLAMF7 was not an independent prognostic factor for OS in COAD patients. This may reflect complex multi-factorial transcriptional regulation of SLAMF7, as well as cohort heterogeneity and limited sample size that could obscure true biological relationships. Accordingly, multi-center large patient cohorts are required to validate whether SLAMF7 can serve as an independent prognostic biomarker. The nomogram incorporating SLAMF7 alongside established clinical risk factors achieved favorable survival prediction performance and may support clinical risk stratification.

Our genomic variant and methylation analyses revealed a low overall mutation rate for SLAMF7 in COAD. Distinct mutation landscapes existed between SLAMF7 high and low expression subgroups. DNA methylation changes only weakly correlated with SLAMF7 expression, implying that promoter methylation is not the major cause of SLAMF7 down regulation in COAD. Other regulatory layers, including transcription factor control and non coding RNA mediated epigenetic repression, likely contribute and merit experimental follow up.

Significant differences in the mutation frequencies of canonical COAD driver genes such as APC and KRAS between SLAMF7 subgroups suggest potential crosstalk between tumor driver mutation backgrounds and SLAMF7 transcriptional status, which warrants further mechanistic inquiry.

The infiltration of immune cells within the tumor immune microenvironment is closely associated with the malignancy of colon adenocarcinoma. Enhancing the anti-tumor activity of tumor-reactive tumor-infiltrating lymphocytes (TILs) can effectively suppress tumor progression[28-30]. Gene Set Enrichment Analysis (GSEA) indicated that SLAMF7 is closely linked to multiple immune signaling pathways and lymphocyte-associated functions. Further correlation analysis revealed that SLAMF7 expression is significantly positively correlated with the infiltration levels of T cells, B cells, and NK cells, as well as the expression of their signature marker genes. This suggests that SLAMF7 may influence colon adenocarcinoma progression by modulating TIL infiltration. Notably, significant enrichment of regulatory T cell (Treg)-related features was also observed in the SLAMF7 high-expression subgroup. Integrating previous findings from hepatocellular carcinoma studies, we hypothesize that SLAMF7 may mediate the polarization of immunosuppressive macrophages, thereby remodeling the tumor microenvironment and ultimately contributing to

resistance against immune checkpoint blockade therapy[31, 32]. Accumulating evidence supports the pivotal role of SLAMF7 in regulating immune cell polarization, immune response activation, and anti-tumor effects[12]. Synthesizing these results, we propose that SLAMF7 remodels the tumor immune microenvironment in colon adenocarcinoma by modulating the functions of various immune cells, including TILs and Tregs, consequently altering the overall anti-tumor immune status. Due to these dual biological effects, SLAMF7 represents a sophisticated yet promising therapeutic target for solid tumor immunotherapy, holding potential for the development of bispecific antibodies and combination regimens with immune checkpoint inhibitors.

Drug sensitivity analyses based on the GSCA platform uncovered significant associations between SLAMF7 expression and responses to diverse chemotherapeutic and targeted agents. Given its cellular expression pattern, SLAMF7 associated drug response phenotypes are likely mediated by remodeling of the immune microenvironment and altered immune cell function rather than direct intrinsic drug sensitivity changes in tumor cells. These bioinformatic observations offer preliminary clues for designing chemo-immunotherapy combination regimens for COAD but require validation using cellular models, animal experiments, and prospective clinical cohorts.

This study has several notable limitations. First, validation is restricted to

mRNA level measurements from clinical specimens; protein level immunohistochemical confirmation of SLAMF7 expression in COAD tissues remains absent. Second, all public database analyses are retrospective and subject to selection bias; our nomogram model was validated only internally and lacks external cohort verification. Third, this work generates mechanistic hypotheses purely derived from in silico multi-omics analyses. Functional cellular and in vivo animal experiments are needed to delineate the precise molecular mechanisms whereby SLAMF7 modulates $CD8^+$ T cell and NK cell driven anti tumor immunity in colon adenocarcinoma.

5.Conclusion

This multi-omics study demonstrates that SLAMF7 is down regulated in colon adenocarcinoma and correlates with immune functional pathways and immune cell infiltration profiles. Although SLAMF7 cannot act as an independent prognostic biomarker, it is associated with humoral immunity, complement activation, and enrichment of diverse immune cell subsets including regulatory T cells. These results expand our understanding of COAD immunobiology and provide new theoretical foundations for future translational research targeting SLAMF7.

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Accordance statement

All data and results in this manuscript are true and reliable, without any fabrication, falsification or plagiarism, and all authors have made substantial contributions to the study in line with the authorship criteria.

Author contributions

All authors contributed to the study conception and design.All authors read and approved the final manuscript.Lin-Yao Cheng: writing-original draft preparation. Guang Liu: writing-review, and editing. Yi-Bang Lao: data analyses and interpretation.Jing-Mao Lao: writing-review, and editing. Wei Deng: supervision. All authors participated in writing or revising the manuscript.Xiao-Bo Wei: conceived and designed this project.

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Data availability

The raw data supporting the conclusions of this article were obtained from the following public databases:

TCGA-COAD dataset:

https://portal.gdc.cancer.gov/projects/TCGA-COAD

GEO datasets:

GSE44076(https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE44

076),

GSE113513(https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE113513)

cBioPortal COAD cohort:https://www.cbioportal.org

MEXPRESS:https://mexpress.ugent.be

GSCA: https://guolab.wchscu.cn/GSCA/#/expression

TISCH: http://tisch.comp-genomics.org

All data generated or analysed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate

All COAD and paired adjacent normal tissue specimens used in this retrospective work were collected and stored under the ethical approval issued by the Science and Technology Ethics Committee of Guangxi Medical University Cancer Hospital(Approval No. KY2025650).

The study was conducted in accordance with the Declaration of Helsinki. All methods were carried out in accordance with relevant guidelines and regulations.Informed consent was obtained from all subjects and/or their legal guardian(s).

Clinical trial number

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests

References

1. Shek, D.; Akhuba, L.; Carlino, M. S.; Nagrial, A.; Moujaber, T.; Read, S. A.; Gao, B.; Ahlenstiel, G., Immune-Checkpoint Inhibitors for Metastatic Colorectal Cancer: A Systematic Review of Clinical Outcomes. *Cancers (Basel)* **2021,** 13, (17).
2. Kanani, A.; Veen, T.; Soreide, K., Neoadjuvant immunotherapy in primary and metastatic colorectal cancer. *Br J Surg* **2021,** 108, (12), 1417–1425.
3. Andre, T.; Cohen, R.; Salem, M. E., Immune Checkpoint Blockade Therapy in Patients With Colorectal Cancer Harboring Microsatellite Instability/Mismatch Repair Deficiency in 2022. *Am Soc Clin Oncol Educ Book* **2022,** 42, 1–9.
4. Rajbhandary, S.; Dhakal, H.; Shrestha, S., Tumor immune microenvironment (TIME) to enhance antitumor immunity. *Eur J Med Res* **2023,** 28, (1), 169.
5. Fang, F.; Zhang, T.; Li, Q.; Chen, X.; Jiang, F.; Shen, X., The tumor immune-microenvironment in gastric cancer. *Tumori* **2022,** 108, (6), 541–551.
6. Lv, B.; Wang, Y.; Ma, D.; Cheng, W.; Liu, J.; Yong, T.; Chen, H.; Wang, C., Immunotherapy: Reshape the Tumor Immune Microenvironment. *Front Immunol* **2022,** 13, 844142.
7. Wang, G.; Chen, B.; Su, Y.; Qu, N.; Zhou, D.; Zhou, W., CEP55 as a Promising Immune Intervention Marker to Regulate Tumor Progression: A Pan-Cancer Analysis with Experimental Verification. *Cells* **2023,** 12, (20).
8. Gu, X.; Dai, X.; Huang, Y.; Zhang, Y.; Dong, L.; Gao, C.; Wang, F., Differential roles of highly expressed PFKFB4 in colon adenocarcinoma patients. *Sci Rep* **2023,** 13, (1), 16284.
9. Jin, Z.; Zhou, Q.; Cheng, J. N.; Jia, Q.; Zhu, B., Heterogeneity of the tumor immune microenvironment and clinical interventions. *Front Med* **2023,** 17, (4), 617–648.
10. Cheng, L.; Wu, H.; Chen, Z.; Hao, H.; Zheng, X., Gut microbiome at the crossroad of genetic variants and behavior disorders. *Gut Microbes* **2023,** 15, (1), 2201156.
11. Buller, C. W.; Mathew, P. A.; Mathew, S. O., Roles of NK Cell Receptors 2B4 (CD244), CS1 (CD319), and LLT1 (CLEC2D) in Cancer. *Cancers (Basel)* **2020,** 12, (7).
12. Altalbawy, F. M. A.; Babamuradova, Z.; Baldaniya, L.; Singh, A.; Singh, K. U.; Ballal, S.; Sabarivani, A.; Sead, F. F.; Alam, R.; Alshahrani, M. Y., The multifaceted role of CS1 (SLAMF7) in immunoregulation: Implications for cancer therapy and autoimmune disorders. *Exp Cell Res* **2025,** 447, (1), 114516.
13. Grosicki, S.; Bednarczyk, M.; Barchnicka, A.; Grosicka, O., Elotuzumab in the treatment of relapsed and refractory multiple myeloma. *Future Oncol* **2021,** 17, (13), 1581–1591.
14. O'Connell, P.; Hyslop, S.; Blake, M. K.; Godbehere, S.; Amalfitano, A.; Aldhamen, Y. A., SLAMF7 Signaling Reprograms T Cells toward Exhaustion in the Tumor Microenvironment. *J Immunol* **2021,** 206, (1), 193–205.

15. Assidi, M., Strong prognostic value of SLAMF7 protein expression in patients with lymph node-positive breast cancer. *Oncol Lett* **2022,** 24, (6), 433.
16. Blaye, C.; Darbo, E.; Debled, M.; Brouste, V.; Velasco, V.; Pinard, C.; Larmonier, N.; Pellegrin, I.; Tarricone, A.; Arnedos, M.; Commeny, J.; Bonnefoi, H.; Larmonier, C.; MacGrogan, G., An immunological signature to predict outcome in patients with triple-negative breast cancer with residual disease after neoadjuvant chemotherapy. *ESMO Open* **2022,** 7, (4), 100502.
17. Deng, Y.; Zhang, L.; Dai, C.; Xu, Y.; Gan, Q.; Cheng, J., SLAMF7 predicts prognosis and correlates with immune infiltration in serous ovarian carcinoma. *J Gynecol Oncol* **2024,** 35, (6), e79.
18. Zou, W.; Fang, X.; Qian, Z.; Nie, L., The lysosome-associated SLAMF7 inhibits the development of ovarian cancer by promoting lysosomal damage. *Mol Cell Endocrinol* **2025,** 606, 112586.
19. Torok, R.; Farkas, A.; Guttman, A.; Jarvas, G., Evaluation of Possible Processing Time Effects on the Global N-Glycosylation Profile of Human Blood Samples. *Curr Mol Med* **2020,** 20, (10), 840–846.
20. Li, X.; Zhou, H.; Huang, W.; Wang, X.; Meng, M.; Hou, Z.; Liao, L.; Tang, W.; Xie, Y.; Wang, R.; Yu, H.; Wang, L.; Zhu, H.; Wang, W.; Tan, J.; Li, R., Retrospective analysis of the efficacy of adjuvant cytokine-induced killer cell immunotherapy combined with chemotherapy in colorectal cancer patients after surgery. *Clin Transl Immunology* **2022,** 11, (1), e1368.
21. Lin, A.; Zhang, J.; Luo, P., Crosstalk Between the MSI Status and Tumor Microenvironment in Colorectal Cancer. *Front Immunol* **2020,** 11, 2039.
22. Zhou, X.; Lin, J.; Shao, Y.; Zheng, H.; Yang, Y.; Li, S.; Fan, X.; Hong, H.; Mao, Z.; Xue, P.; Zhang, S.; Sun, J., Targeting PLCG2 Suppresses Tumor Progression, Orchestrates the Tumor Immune Microenvironment and Potentiates Immune Checkpoint Blockade Therapy for Colorectal Cancer. *Int J Biol Sci* **2024,** 20, (14), 5548–5575.
23. Qiu, M.; Lan, C.; Lin, M.; Ma, H., Key immune cells in the tumor immune microenvironment of colorectal cancer: Roles and research advances (Review). *Oncol Rep* **2026,** 55, (3).
24. Hu, W. J.; Liu, M.; Bao, Y. N.; Su, B. W.; Sui, X.; Cui, X. H.; Wang, G. Y.; Wen, L.; Wen, R. K.; Wang, W. H.; Yang, H. X.; Luan, Z. L., Pregnane X receptor (PXR) as a prognostic marker and therapeutic target in kidney renal clear cell carcinoma (KIRC) via AMPK pathway regulation. *Sci Rep* **2025,** 15, (1), 35808.
25. Huang, S.; Hu, J.; Hu, M.; Hou, Y.; Zhang, B.; Liu, J.; Liu, X.; Chen, Z.; Wang, L., Cooperation between SIX1 and DHX9 transcriptionally regulates integrin-focal adhesion signaling mediated metastasis and sunitinib resistance in KIRC. *Oncogene* **2024,** 43, (39), 2951–2969.
26. Xu, A.; Zhang, J.; Zuo, L.; Yan, H.; Chen, L.; Zhao, F.; Fan, F.; Xu, J.; Zhang, B.; Zhang, Y.; Yin, X.; Cheng, Q.; Gao, S.; Deng, J.; Mei, H.; Huang, Z.; Sun, C.; Hu, Y., FTO promotes multiple myeloma progression by posttranscriptional activation of HSF1 in an m(6)A-YTHDF2-dependent manner. *Mol Ther* **2022,** 30, (3), 1104–1118.
27. Tang, X. X.; Shimada, H.; Ikegaki, N., Macrophage-mediated anti-tumor immunity against high-risk neuroblastoma. *Genes Immun* **2022,** 23, (3-4), 129–140.
28. Amador, C. D. M.; Conover, R. E.; Brown, M. C.; Lyniv, L. S.; Noldner, P. K.; Zhou, Y.; Gao, A. R.; McCutcheon, S. R.; Antonia, S. J.; Gersbach, C. A., RELB Reprograms Exhausted Tumor-Infiltrating Lymphocytes for Improved Adoptive Cell Therapy. *bioRxiv* **2025**.
29. Aydin, A. M.; Bunch, B. L.; Beatty, M.; Hajiran, A.; Dhillon, J.; Sarnaik, A. A.; Pilon-Thomas, S.; Poch, M. A., The Factors Affecting Expansion of Reactive Tumor Infiltrating Lymphocytes (TIL)

From Bladder Cancer and Potential Therapeutic Applications. *Front Immunol* **2021,** 12, 628063.

30. Aydin, A. M.; Hall, M.; Bunch, B. L.; Branthoover, H.; Sannasardo, Z.; Mackay, A.; Beatty, M.; Sarnaik, A. A.; Mullinax, J. E.; Spiess, P. E.; Pilon-Thomas, S., Expansion of tumor-infiltrating lymphocytes (TIL) from penile cancer patients. *Int Immunopharmacol* **2021,** 94, 107481.
31. Bruneau, A.; Hammerich, L., Slamming hepatocellular carcinoma: targeting immunosuppressive macrophages via SLAMF7 reprograms the tumor microenvironment. *Transl Cancer Res* **2024,** 13, (12), 6995–7001.
32. Zhu, M.; Wu, Y.; Zhu, T.; Chen, J.; Chen, Z.; Ding, H.; Tan, S.; He, J.; Zeng, Q.; Huang, X., Multifunctional Bispecific Nanovesicles Targeting SLAMF7 Trigger Potent Antitumor Immunity. *Cancer Immunol Res* **2024,** 12, (8), 1007–1021.