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ASCC1 Promotes Malignant Progression of Pancreatic Cancer by Regulating the NF- κ B Signaling Pathway

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Abstract

Background: Pancreatic cancer is a highly aggressive malignancy with an extremely low five-year survival rate, underscoring an urgent need to identify novel molecular targets. ASCC1, a key component of the ASC-1 complex, plays a critical role in transcriptional regulation and DNA repair. Recent studies have suggested that ASCC1 might be involved in tumorigenesis; however, its function in pancreatic cancer remains largely undefined.

Methods: In this study, we utilized the TCGA-PAAD dataset to screen for prognostically relevant genes using Cox regression and LASSO regression analyses, which identified ASCC1 as a candidate marker. The expression of ASCC1 in pancreatic cancer tissues was subsequently validated by immunohistochemistry and Western blot analyses. ASCC1 knockdown cell lines were established to assess cellular proliferation, migration, and invasion via CCK-8, Transwell, and wound healing assays, respectively. Changes in NF- κ B and EMT-related protein expression were evaluated by Western blot. In vivo, a subcutaneous xenograft model in nude mice was employed to validate the functional impact of ASCC1 knockdown. Additionally, GSEA and ssGSEA were applied to explore the involvement of related signaling pathways and immune characteristics.

Results: ASCC1 was significantly overexpressed in pancreatic cancer and correlated with poor clinical outcomes. Knockdown of ASCC1 markedly inhibited cell

proliferation, migration, and invasion, accompanied by reduced expression levels of phosphorylated p65 and phosphorylated I κ B α , and suppression of the EMT process. In the nude mouse xenograft model, ASCC1 knockdown significantly retarded tumor growth. Furthermore, GSEA revealed enrichment of the NF- κ B signaling pathway, while immune analyses indicated that high ASCC1 expression was associated with increased infiltration of various immune cell subsets and a positive correlation with immune checkpoint molecules.

Conclusions: The findings indicate that high ASCC1 expression in pancreatic cancer is associated with adverse prognosis and may promote tumor progression by activating the NF- κ B pathway, inducing EMT, and modulating the tumor immune microenvironment. ASCC1 may thus serve as a promising prognostic biomarker and therapeutic target for pancreatic cancer.

Keywords:

ASCC1; Pancreatic cancer; NF- κ B; EMT

Introduction

Pancreatic cancer is a highly aggressive malignancy with an exceptionally poor prognosis. According to the American Cancer Society and epidemiological studies, pancreatic cancer ranks among the top ten in annual incidence. Owing to the deep-seated anatomical location of the pancreas and the insidious nature of its early symptoms, approximately 80% of patients are diagnosed at advanced stages, thereby losing the optimal opportunity for surgical resection. Consequently, the five-year survival rate remains below 10%^[1]. Current treatment approaches mainly rely on surgical resection, chemotherapy, and radiotherapy; however, pancreatic cancer's strong resistance to chemo- and radiotherapy, coupled with its high degree of heterogeneity, often results in unsatisfactory therapeutic outcomes^[2]. Therefore, it is imperative to elucidate the molecular mechanisms underlying pancreatic cancer development and to identify novel prognostic biomarkers and therapeutic targets to improve treatment efficacy and patient survival.

In recent years, advancements in molecular biology and high-throughput sequencing technologies have made it possible to elucidate the underlying mechanisms of tumor development through analyses of gene expression profiles and signaling pathways^[3]. Among the myriad pathways regulating tumor cell proliferation, invasion, and metastasis, the NF- κ B signaling pathway has been recognized for its pivotal role in mediating inflammation, cell survival, and immune regulation, and it has been shown to play a crucial role in pancreatic cancer as well as other solid tumors^[4]. Moreover, epithelial-mesenchymal transition (EMT)—a process by which cells acquire enhanced migratory and invasive capabilities—is closely associated with the malignant progression of pancreatic cancer^[5]. Existing studies indicate that there is a complex crosstalk between the NF- κ B pathway and EMT, which together contribute to the

malignant remodeling of the tumor microenvironment and facilitate immune evasion, thereby further exacerbating tumor aggressiveness^[6].

ASCC1 (Activating Signal Cointegrator 1 Complex Subunit 1) encodes a pivotal component of the ASC-1 complex, which plays a crucial role in regulating gene transcription, DNA damage repair, and cellular signal transduction^[7]. The ASCC1 protein features an N-terminal KH-type RNA binding motif that is essential for the transcriptional activation mediated by the ASC-1 complex, particularly in activating AP-1 transcriptional activity. Moreover, through synergistic interactions with other transcription factors, such as NF- κ B and serum response factor (SRF), the ASC-1 complex integrates multiple signaling pathways to regulate downstream target gene expression^[8]. In addition, ASCC1, as a constituent of the ASCC complex, directly participates in DNA repair mechanisms, especially by maintaining genomic stability through homologous recombination (HDR) or single-strand annealing (SSA) pathways^[9]. Although the majority of research on ASCC1 has traditionally focused on neuromuscular disorders, such as spinal muscular atrophy and congenital bone fractures^[10], recent studies have begun to implicate ASCC1 in tumorigenesis. For example, genetic variations in ASCC1 have been significantly associated with an increased risk of Barrett's esophagus and esophageal adenocarcinoma^[11]. Furthermore, in head and neck paragangliomas, somatic mutations in the ASCC1 gene have been identified and are believed to drive tumor development^[12]. Therefore, these findings suggest that ASCC1 not only plays an essential role in maintaining normal cellular functions and genomic integrity but also that its aberrant expression or mutation may serve as a critical molecular marker for tumor initiation, progression, and prognosis.

In this study, we first performed a bioinformatics analysis of pancreatic cancer patient samples from TCGA, integrating univariate and multivariate Cox regression analyses to screen for prognosis-related genes, and systematically evaluated the expression and prognostic significance of ASCC1. Subsequently, the expression of ASCC1 in clinical samples was validated by Western blotting and immunohistochemistry, while its effects on cell proliferation, migration, and invasion were assessed in both in vitro and in vivo models. Furthermore, Gene Ontology (GO), KEGG pathway, and Gene Set Enrichment Analysis (GSEA) were employed to investigate the downstream signaling pathways regulated by ASCC1, with a particular focus on the NF- κ B pathway and epithelial–mesenchymal transition (EMT)-related processes. Additionally, single-sample Gene Set Enrichment Analysis (ssGSEA) was applied to evaluate the correlation between ASCC1 expression and the infiltration of various immune cell subsets, as well as the expression of immune checkpoint molecules in the pancreatic cancer immune microenvironment. Collectively, our findings reveal that ASCC1 is aberrantly overexpressed in pancreatic cancer and is associated with poor prognosis, suggesting that ASCC1 may serve as a potential therapeutic target. These results provide novel insights into the molecular mechanisms underlying pancreatic cancer progression and support the development of individualized treatment strategies.

Methods

1. Bioinformatics analysis

1.1 TCGA Database Data Download

Clinical information and genomic data for pancreatic cancer were obtained from the TCGA-PAAD database. The downloaded clinical data were subjected to preliminary screening and cleaning, where patients' basic information (including age, gender, tumor stage, treatment modalities, etc.) was processed, and missing values and outliers were removed to ensure data quality and consistency. Subsequently, expression levels for each gene were extracted from the samples based on gene IDs, and the data were normalized. Gene annotation files were then used to annotate each gene ID to guarantee accurate labeling of gene functions and associated information. Finally, the clinical and gene expression data were integrated to establish a comprehensive dataset that encompasses patients' clinical characteristics alongside their gene expression profiles.

1.2 Prognostic Gene Screening

Using the R package "survival," we conducted univariate Cox regression analysis on all genes within the TCGA-PAAD dataset to identify those significantly associated with overall patient survival ($P < 0.001$). Subsequently, we extracted a set of genes related to gastrointestinal tumors from the GeneCards database and intersected this set with the previously identified prognostic genes to further narrow down the candidate pool. The R package "glmnet" was then employed to perform LASSO regression for variable selection, with cross-validation used to determine the optimal regularization parameter λ , thereby yielding a gene signature with the greatest prognostic predictive value. The genes selected via LASSO regression were subsequently incorporated into a multivariate Cox regression model for further screening, ultimately identifying independent prognostic genes along with their hazard ratios, 95% confidence intervals, and P values. The performance of the prognostic model was evaluated using receiver operating characteristic (ROC) curves generated by the "timeROC" package, and the area under the curve (AUC) was calculated.

1.3 Differential Gene Screening and Volcano Plot Generation

Samples were divided into high and low ASCC1 expression groups based on the median value. Differential expression analysis was performed using the R package "limma" to identify significantly differentially expressed genes (criteria: $|\log_2FC| > 1$ and $P < 0.05$). The results were visualized by generating a volcano plot using the "ggplot2" package.

1.4 Functional Enrichment Analysis

GSEA software (version 4.2) was used to perform enrichment analysis on predefined gene sets, enabling the identification of significantly enriched signaling pathways. The analysis evaluated the normalized enrichment score (NES) and statistical significance was assessed with thresholds set at $P < 0.05$ and $FDR < 0.25$. Additionally, the ClusterProfiler package was employed to conduct Gene Ontology (GO) and KEGG pathway enrichment analyses on the differentially expressed genes. This approach allowed us to identify terms significantly enriched in biological processes, cellular components, molecular functions, and metabolic pathways.

1.5 Immunoinfiltration and Immune Checkpoint Correlation Analysis

The ssGSEA algorithm was utilized to assess the infiltration levels of various immune cell types in each sample based on predefined immune cell-specific gene sets. Subsequently, the correlation between ASCC1 expression levels and the infiltration levels of different immune cell populations was determined. Additionally, clinical and gene expression data from the TCGA database were integrated, and Spearman correlation analysis was performed to evaluate the relationship between ASCC1 expression and the expression levels of multiple immune checkpoint molecules.

2. Immunohistochemistry

Five pathological specimens from pancreatic cancer patients were obtained from the Affiliated Hospital of Yangzhou University, and both tumor tissues and adjacent non-tumor tissues were sectioned. The study protocol was approved by the Ethics Committee of the Yangzhou University Affiliated Hospital (Approval No. YXYLL-2025-036). Tissue samples were first subjected to slide baking and drying, followed by deparaffinization with xylene and rehydration through a graded ethanol series. Antigen retrieval was then performed, after which the sections were incubated with a primary antibody against ASCC1 at 4 °C for 14–16 hours. The following day, slides were washed three times for 2 minutes each with tris-buffered saline containing Tween (TBST) to remove unbound primary antibody, and then incubated with the secondary antibody at room temperature for 30 minutes. After a further series of three TBST washes (2 minutes each), the sections were developed with DAB for 5 minutes and counterstained with hematoxylin. Finally, the slides were dehydrated through a graded ethanol series, cleared, and mounted with neutral gum. Microscopic evaluation was performed to assess both the area and intensity of positive staining. Scoring for the extent of positive staining was as follows: 0 for 0–5%, 1 for 6–30%, 2 for 31–70%, and 3 for greater than 71%. A total score of ≤ 7 was considered negative expression, whereas a score > 7 was defined as positive expression.

3. Western Blot Analysis

Antibodies for ASCC1 (12301-1-AP), GAPDH (60004-1-Ig), E-cadherin (20874-1-AP), N-cadherin (22018-1-AP), SNAIL (13099-1-AP), p65 (10745-1-AP), and I κ B- α (10268-1-AP) were purchased from Proteintech, whereas antibodies for

phosphorylated p65 (#3033) and phosphorylated I κ B- α (#2859) were obtained from Cell Signaling Technology. Samples from each experimental group were collected and lysed in an equal volume of RIPA lysis buffer. Protein concentrations were determined using a BCA assay, and sample loading volumes were adjusted accordingly based on the measured concentrations. Following electrophoresis, proteins were transferred onto a polyvinylidene fluoride (PVDF) membrane, which was subsequently blocked with 5% nonfat milk for 1 hour at room temperature. The membranes were then incubated with the primary antibodies overnight at 4 °C. After three washes with TBST (5 minutes each), the membranes were incubated with appropriate secondary antibodies at room temperature for 90 minutes. Finally, enhanced chemiluminescence substrate was applied, and protein bands were visualized using a gel imaging system.

4. Quantitative Real-Time PCR (qPCR)

Total RNA was extracted from cells using TRIzol reagent, and first-strand cDNA was synthesized via reverse transcription. Quantitative real-time PCR was then performed using SYBR Green chemistry to measure ASCC1 mRNA expression levels. Relative expression levels were calculated using the $\Delta\Delta C_t$ method with GAPDH as the internal control. The primer sequences were as follows (5'-3'):

ASCC1:Forward,AAGCTTGGTACCATGTACCCATACGATGTTCCAGATTACGC
TGCTTTACCTCGTCTC;Reverse,ACTAGTGGATCCTCAGGAGAAGTCAAT.
GAPDH:Forward,CGGAGTCAACGGATTTGGTCGTAT;
Reverse,AGCCTTCTCCATGGTGGTGAAGAC'.

5. Cell Culture and Construction of ASCC1 Knockdown Cell Lines

Pancreatic cancer cell lines Capan1 and Capan2 were purchased from Pricella. Capan1 cells were maintained in IMDM medium supplemented with 20% fetal bovine serum, while Capan2 cells were cultured in McCoy's 5A medium. Both cell lines were incubated in a humidified incubator at 37 °C with 5% CO₂. Cells in the logarithmic growth phase were seeded into 6-well plates, with the volume of lentiviral working solution determined according to the desired multiplicity of infection (MOI). Subsequently, ASCC1-targeting knockdown plasmids and corresponding negative control plasmids were transfected into the cells using Polybrene as a transfection enhancer. After 24 hours of infection, the virus-containing medium was discarded, and 48 hours post-infection, puromycin selection was applied to eliminate uninfected cells.

6. CCK-8 Cell Proliferation Assay

For the cell proliferation assay, 3,000 cells per well from both the ASCC1 knockdown group and the negative control group were seeded into 96-well plates. At 24, 48, and 72 hours post-seeding, 10 μ l of CCK-8 reagent was added to each well, and the plates

were incubated in the dark at 37 °C for 3 hours. Absorbance was then measured at 450 nm using a microplate reader to evaluate cell proliferation at the specified time points.

7. Transwell Migration Assay

2×10^4 cells were resuspended in 200 μ l of serum-free medium and gently added to the upper chamber of a Transwell insert. The lower chamber was pre-filled with 600 μ l of medium containing 10% fetal bovine serum as a chemoattractant. The Transwell plates were then incubated at 37 °C in a 5% CO₂ humidified incubator for 48 hours. After incubation, the inserts were removed and the migrated cells on the lower surface of the membrane were fixed with paraformaldehyde and subsequently stained with crystal violet. Finally, the migrated cells were imaged under a microscope to assess the migration capacity.

8. Wound Healing Assay

Cells were seeded in 6-well plates and allowed to reach complete confluence. A straight-line wound was then created in the cell monolayer using a 1,000 μ l pipette tip. After gently washing with phosphate-buffered saline (PBS) to remove detached cellular debris, fresh medium was added, and the cells were further incubated under standard conditions. The wound areas were captured at 0 and 48 hours, and the rate of wound closure was compared between groups to assess cell migration capabilities.

9. Animal Experiment

Four-week-old female nude mice were purchased from the Experimental Animal Center of Yangzhou University (Animal Experiment Ethics Approval No. YXYLL-2025-017). DLD1 cells were injected subcutaneously into the axillary region of the forelimbs at a concentration of 2.5×10^4 cells/ μ l. Tumor growth was monitored in both the negative control and ASCC1 knockdown groups, with tumor volumes measured every 3 days using the formula: tumor volume (mm³) = $0.5 \times (\text{length} \times \text{width}^2)$. When the maximum tumor volume reached approximately 1500 mm³, the mice were euthanized, and tumor tissues were collected for subsequent analyses.

10. Statistical Analysis

Statistical analyses were conducted using GraphPad Prism (version 8.0) and SPSS software (version 22.0). First, the data were subjected to tests for normality and homogeneity of variance. Depending on the outcome of these tests, appropriate statistical methods were chosen. For comparisons between two groups, if the data followed a normal distribution, a two-tailed independent samples t-test was employed; otherwise, a non-parametric test such as the Mann–Whitney U test was used. For comparisons involving multiple groups, one-way ANOVA was applied. Survival data were analyzed using Kaplan–Meier survival curves, with the log-rank test used to compare differences between groups. Correlation analyses were performed using

Spearman's rank correlation coefficient. A P-value of < 0.05 was considered statistically significant in all tests.

Results

Screening of Prognostic Genes in Pancreatic Cancer

To identify key genes associated with the prognosis of pancreatic cancer patients, we first performed univariate Cox regression analysis on the TCGA-PAAD dataset and selected genes that significantly influenced patient survival. Subsequently, we intersected these prognosis-related genes with a set of gastrointestinal tumor-related genes obtained from the GeneCards database, resulting in a total of 76 candidate genes (Figure 1A). To further narrow down the candidate pool, LASSO regression analysis was performed, and the optimal model was determined based on the selected λ value (Figures 1B–C). On this basis, the 28 genes identified from the LASSO regression were subjected to multivariate Cox regression analysis, ultimately yielding 12 independent prognostic genes, including MET, WNT7A, CA12, EFNB2, PTCH2, GMNN, SRD5A1, RPN1, RNF216, MYO5B, CAPN10, and ASCC1 (Figure 1D).

Among these 12 genes, MET, PTCH2, RPN1, RNF216, and CAPN10 acted as protective factors, whereas WNT7A, CA12, EFNB2, GMNN, SRD5A1, MYO5B, and ASCC1 were identified as risk factors. Correlation analysis further revealed close interactions among these genes (Figure 1E). Moreover, a heat map of gene expression based on clinical variables demonstrated distinct expression patterns of these 12 prognostic genes in pancreatic cancer patients (Figure 1F). Notably, the mechanistic role of ASCC1 in pancreatic cancer has not been previously reported, and its potential biological functions remain unclear. Therefore, we selected ASCC1 as the focus of our study to further explore its role in pancreatic cancer progression. The protein interaction network of ASCC1, as shown in Figure 1G, provides preliminary insights into its possible molecular functions.

Pan-Cancer Analysis of ASCC1

We systematically evaluated ASCC1 expression across 33 cancer types using data from the TCGA database. Our analysis revealed that ASCC1 is differentially expressed in 24 tumor types (Figure 2A), with a predominance of high expression observed in most tumors, suggesting an important role in tumorigenesis. Furthermore, prognostic analyses indicated that in 10 cancer types, ASCC1 expression levels were significantly correlated with patient survival, with high ASCC1 expression generally associated with poorer outcomes (Figure 2B). Collectively, these findings imply that ASCC1 not only exhibits aberrant expression across multiple tumors but also may serve as a potential prognostic biomarker with valuable clinical applications.

Expression of ASCC1 in Pancreatic Cancer and Its Impact on Prognosis

Differential expression analysis of non-paired samples revealed that ASCC1 mRNA levels were significantly elevated in pancreatic tumor tissues compared to adjacent normal tissues (Figure 3A). Based on the median expression level of ASCC1, pancreatic cancer patients were stratified into high-expression ($n = 90$) and low-expression ($n = 89$) groups. Kaplan–Meier survival analysis demonstrated that patients with high ASCC1 expression had significantly shorter overall survival compared to those with low expression ($P < 0.001$; Figure 3B), with median survival times of 518 days and 1059 days for the high- and low-expression groups, respectively. A prognostic nomogram constructed based on clinical features further confirmed the prognostic value of ASCC1 (Figure 3D). Moreover, receiver operating characteristic (ROC) curve analysis showed an area under the curve (AUC) of 0.874 for the 5-year survival prediction in pancreatic cancer patients (Figure 3E), indicating that ASCC1 has strong prognostic predictive capability. The calibration curve further validated the accuracy of the prognostic model in predicting patient outcomes (Figure 3F).

Expression of ASCC1 in Clinical Specimens of Pancreatic Cancer Patients

Pathological specimens from five pancreatic cancer patients who underwent radical resection were collected. Western blot analysis was used to assess the protein expression levels of ASCC1 in tumor tissues compared to the corresponding adjacent normal tissues (Figure 4A). The results revealed that ASCC1 protein expression was significantly higher in pancreatic tumor tissues than in adjacent normal tissues (Figure 4B). To further confirm this observation, immunohistochemical analysis was performed on the pancreatic tumor sections; the results were consistent with the Western blot findings (Figure 4C). Quantitative evaluation indicated that the staining score for ASCC1 in pancreatic tumor tissues was 9 (scores >7 being defined as positive), whereas the adjacent normal tissues scored only 1 (scores ≤ 7 being defined as negative), thereby further confirming the elevated expression of ASCC1 in pancreatic cancer.

Effect of ASCC1 Expression on the Biological Behavior of Pancreatic Cancer Cells

To investigate the role of ASCC1 in pancreatic cancer cell behavior, we established ASCC1 knockdown cell lines. First, the knockdown efficiency was confirmed by qPCR and Western blot analysis (Figures 5A–B), which demonstrated that all three designed plasmids could stably reduce ASCC1 expression. The CCK-8 assay revealed that the absorbance values at 72 hours in the ASCC1 knockdown group were significantly lower than those in the control group ($P < 0.001$; Figure 5C), indicating that ASCC1 silencing suppressed the proliferative capacity of pancreatic cancer cells. Subsequently, Western blot analysis of EMT-related proteins showed that knockdown of ASCC1 significantly upregulated the epithelial marker E-cadherin, while the mesenchymal marker N-cadherin and the transcription factor SNAIL were markedly downregulated (Figure 5D), suggesting that ASCC1 may promote cell invasiveness.

through the regulation of the EMT process. Furthermore, the Transwell migration assay revealed that the number of migrated cells in both Capan1 and Capan2 ASCC1 knockdown groups was significantly lower than that in the control group (Figure 5E). Concurrently, the wound healing assay demonstrated a reduced cell migration rate in the ASCC1 knockdown group compared to the control group (Figure 5F). Collectively, these findings indicate that ASCC1 knockdown significantly inhibits the proliferation, migration, and invasion of pancreatic cancer cells in vitro.

ASCC1 knockdown suppresses tumor growth in nude mice in vivo

In the in vivo experiment, Capan1 cells with stable ASCC1 knockdown and control cells were subcutaneously injected into nude mice (Figure 6A-B). Tumor volume was measured every 3 days after tumor formation, and growth curves were plotted (Figure 6C). Upon sacrificing the mice at the experimental endpoint, the excised tumor tissues were weighed (Figure 6D). Results demonstrated that the ASCC1-knockdown group exhibited significantly smaller tumor volumes compared to the control group ($P < 0.0001$), with markedly reduced tumor weights ($P < 0.001$). These findings collectively demonstrate that ASCC1 knockdown significantly suppresses the proliferative capacity of pancreatic cancer cells in vivo.

ASCC1 Promotes Pancreatic Cancer Progression Through the NF- κ B Signaling Pathway

Based on the median expression level of ASCC1, samples were divided into high and low expression groups, and differentially expressed genes (DEGs) between the two groups were visualized using a volcano plot (Figure 7A). GO and KEGG pathway enrichment analyses of these DEGs revealed significant enrichment in various molecular functions, biological processes, and cellular components, particularly in pathways such as pancreatic secretion, neuroactive ligand–receptor interaction, signaling receptor activator activity, receptor–ligand activity, and potassium ion transmembrane transport (Figure 7B). In addition, GSEA demonstrated significant enrichment of the NF- κ B signaling pathway and EMT-related pathways in the ASCC1 high-expression group (Figure 7C).

To experimentally validate the activation status of the NF- κ B pathway, we performed Western blot analysis of key pathway components. The results showed that knockdown of ASCC1 in Capan1 and Capan2 cells led to a marked decrease in the levels of phosphorylated p65 and phosphorylated I κ B- α , while the total levels of p65 and I κ B- α remained largely unchanged (Figure 7D). These findings suggest that ASCC1 may promote pancreatic cancer progression by enhancing the activation of the NF- κ B signaling pathway.

ASCC1 and the Tumor Immune Microenvironment

To further explore the relationship between ASCC1 and the immune microenvironment in pancreatic cancer, we employed the single-sample Gene Set Enrichment Analysis (ssGSEA) algorithm to evaluate the infiltration levels of immune cell subsets across samples. The results revealed that ASCC1 expression was significantly positively correlated with the infiltration of various immune cell types, including T helper cells, central memory T cells, Th2 cells, Th1 cells, activated dendritic cells, effector memory T cells, dendritic cells, total T cells, macrophages, NK CD56dim cells, mast cells, B cells, regulatory T cells, immature dendritic cells, eosinophils, cytotoxic cells, neutrophils, and follicular helper T cells. In contrast, negative correlations were observed between ASCC1 expression and the infiltration of plasmacytoid dendritic cells and Th17 cells (Figure 8A).

Further analysis of immune checkpoint molecules demonstrated that ASCC1 expression was positively correlated with the expression of several key immune checkpoint genes, including CD274, PDCD1, LAG3, CTLA4, HAVCR2, TIGIT, and CD276 (Figure 8B). These findings suggest that ASCC1 may contribute to the formation of an immunosuppressive tumor microenvironment by coordinating immune cell recruitment and upregulating immune checkpoint molecules in pancreatic cancer.

Discussion

In this study, we systematically investigated the expression pattern and prognostic significance of ASCC1 in pancreatic cancer by integrating bioinformatics analysis based on the TCGA-PAAD dataset, validation using clinical tissue samples, and functional assays both in vitro and in vivo. Our major findings are as follows: (1) ASCC1 is significantly upregulated in pancreatic cancer tissues at both the mRNA and protein levels compared with adjacent normal tissues; (2) patients with high ASCC1 expression exhibited significantly shorter overall survival, and multivariate Cox regression analysis confirmed ASCC1 as an independent prognostic factor with a high hazard ratio; (3) functional assays demonstrated that ASCC1 knockdown markedly inhibited pancreatic cancer cell proliferation, migration, and invasion, and suppressed tumor growth in a subcutaneous xenograft model in nude mice; (4) enrichment analyses, including GO, KEGG, and GSEA, indicated that ASCC1 may promote malignant progression by activating the NF- κ B signaling pathway and inducing EMT; (5) immune infiltration analyses revealed that high ASCC1 expression was positively correlated with various immune cell types and immune checkpoint molecules, suggesting that ASCC1 may contribute to tumor immune evasion by reshaping the immunosuppressive tumor microenvironment. Taken together, our results reveal that ASCC1 plays a crucial role in promoting pancreatic cancer progression and may serve as a novel prognostic biomarker and therapeutic target. The overexpression of ASCC1 in tumor tissues and its association with poor prognosis highlight its potential clinical value in risk stratification and outcome prediction. Mechanistically, ASCC1 appears to facilitate tumor growth and metastasis via NF- κ B pathway activation and EMT induction, consistent with the known roles of

these pathways in tumorigenesis. Additionally, its correlation with immune cell infiltration and checkpoint expression further implies that ASCC1 may be involved in the regulation of tumor immunity, offering new insights into immune-based therapeutic strategies.

Pancreatic cancer is a highly lethal malignancy characterized by a low diagnosis rate and extreme aggressiveness, which present significant challenges to clinical management. According to statistical data, the five-year survival rate for pancreatic cancer is less than 10%, with the majority of patients being diagnosed at advanced stages. In this context, the discovery of novel prognostic biomarkers is crucial for risk stratification and the development of individualized treatment strategies^[13]. Our results demonstrate that the expression of ASCC1 is significantly elevated in pancreatic cancer tissues compared to adjacent normal tissues, and patients in the high-expression group exhibit a dramatically reduced median survival time of only 518 days versus 1059 days in the low-expression group. Furthermore, ROC curve analysis revealed that ASCC1 has a high predictive accuracy for 5-year survival, with an AUC of 0.874, thereby supporting its potential as a prognostic indicator. These findings are in line with current trends in prognostic biomarker research, which, despite advances in high-throughput sequencing and big data analysis, still have not identified a sufficient number of robust molecular markers for clinical prognostication in pancreatic cancer^[14]. Our data suggest that ASCC1 not only independently predicts overall survival in pancreatic cancer patients but its high expression may also reflect the activation of key oncogenic pathways, thereby providing novel insights for early intervention in this devastating disease.

The NF- κ B signaling pathway is recognized as a critical regulator of cell proliferation, anti-apoptosis, and inflammatory responses in a variety of solid tumors, with its aberrant activation being closely linked to cancer invasion, metastasis, and therapeutic resistance^[15-17]. Our Western blot results demonstrate that in pancreatic cancer cells with ASCC1 knockdown, the levels of phosphorylated p65 and phosphorylated I κ B α are significantly reduced, while the total levels of p65 and I κ B α remain largely unaltered. This observation suggests that ASCC1 predominantly modulates the activation state of the NF- κ B signaling pathway. The pivotal role of NF- κ B signaling in pancreatic cancer has been well documented^[18, 19]. Our data further extend this paradigm by linking high ASCC1 expression to sustained NF- κ B pathway activation, which in turn may enhance cellular resistance to apoptosis, and drive cell proliferation—processes that collectively facilitate tumor progression^[20]. In addition to NF- κ B activation, EMT is a fundamental mechanism by which tumor cells acquire enhanced migratory and invasive capacities^[21]. In our experiments, ASCC1 knockdown resulted in a pronounced upregulation of the epithelial marker E-cadherin, alongside a marked downregulation of the mesenchymal marker N-cadherin and the transcription factor SNAIL. These findings indicate that ASCC1 may promote pancreatic cancer cell invasion by facilitating EMT. Previous studies have highlighted a complex interplay between the NF- κ B pathway and EMT, wherein NF- κ B not only directly regulates the expression of genes involved in EMT but also upregulates

transcription factors such as SNAIL to further drive the EMT process^[22]. Consequently, our results suggest that ASCC1, by sustaining NF- κ B activation, may contribute to the induction of EMT, thereby providing a molecular basis for the enhanced invasiveness and metastatic potential observed in pancreatic cancer cells.

In recent years, the tumor immune microenvironment has increasingly been recognized as a critical determinant of both tumor progression and therapeutic response^[23]. Our ssGSEA analysis revealed that high ASCC1 expression is positively correlated with the infiltration of various immune cell types, including T helper cells, memory T cells, natural killer cells, macrophages, and regulatory T cells. In contrast, ASCC1 expression exhibited a negative correlation with the infiltration of certain anti-tumor immune cells, such as plasmacytoid dendritic cells and Th17 cells. These observations indicate that elevated ASCC1 levels may not only exert a direct effect on tumor cell behavior but also modulate immune cell recruitment and function, ultimately shaping an immunosuppressive microenvironment that facilitates tumor immune evasion. Furthermore, additional analysis of immune checkpoint molecules demonstrated that ASCC1 expression is positively correlated with the expression of key immune checkpoints, including PD-L1, PD-1, and CTLA-4. Although immune checkpoint inhibitors have demonstrated promising efficacy in several solid tumors, pancreatic cancer remains notably resistant due to its unique immunological landscape^[24-26]. Our data suggest that ASCC1 may contribute to tumor immune escape by upregulating immune checkpoint molecules, leading to impaired function of immune effector cells. In the context of pancreatic cancer, the aberrant overexpression of ASCC1, together with its role in modulating the NF- κ B pathway, may not only promote tumor cell proliferation but also reinforce an immunosuppressive TME through the enhancement of immune checkpoint expression.

Despite the multi-level experimental evidence presented in this study revealing the pivotal role of ASCC1 in pancreatic cancer, several limitations remain. First, the majority of the data were derived from the TCGA database and a limited number of clinical specimens, resulting in a relatively small sample size; hence, future studies involving larger, multi-center cohorts are warranted for further validation. Second, the detailed molecular mechanisms by which ASCC1 regulates the NF- κ B signaling pathway, EMT progression, and the tumor immune microenvironment require more in-depth functional investigations. Lastly, given the pronounced heterogeneity of pancreatic cancer, ASCC1 may exert differential effects across various molecular subtypes or clinical stages—a factor that necessitates further focused exploration in future research.

Abbreviations

ASCC1: Activating Signal Cointegrator 1 Complex Subunit 1, NF- κ B: Nuclear Factor kappa-light-chain-enhancer of activated B cells, EMT: Epithelial-Mesenchymal Transition, TCGA: The Cancer Genome Atlas, GSEA: Gene Set Enrichment Analysis, ssGSEA: single-sample Gene Set Enrichment Analysis, CCK-8: Cell Counting Kit-8,

qPCR: quantitative real-time Polymerase Chain Reaction, GO: Gene Ontology, KEGG: Kyoto Encyclopedia of Genes and Genomes, ROC: Receiver Operating Characteristic, AUC: Area Under the Curve, DEGs: Differentially Expressed Genes

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Availability of data and materials

All data in this study are available in (<https://portal.gdc.cancer.gov/>), which are public functional genomics data repositories.

Author contributions

YQC: Data curation, Validation, Writing – original draft, Writing – review & editing, Conceptualization, Funding acquisition, Visualization. RWF: Resources, Validation, Writing – review & editing. CHL, FW, FCW: Data curation, Resources, Writing – review & editing. NZW: Formal Analysis, Methodology, Writing – review & editing. JTC, HZ: Project administration, Resources, Supervision, Writing – review & editing. YQC, YC: Data curation, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

Ethics approval and consent to participate

Before commencing the investigation, informed consent was obtained from all patients. This investigation was approved by the Ethics Committee of the Yangzhou University Affiliated Hospital (reference number: YXYLL-2025-036)

Consent for publication

Not applicable.

Competing interests

The authors have declared that they have no competing interests.

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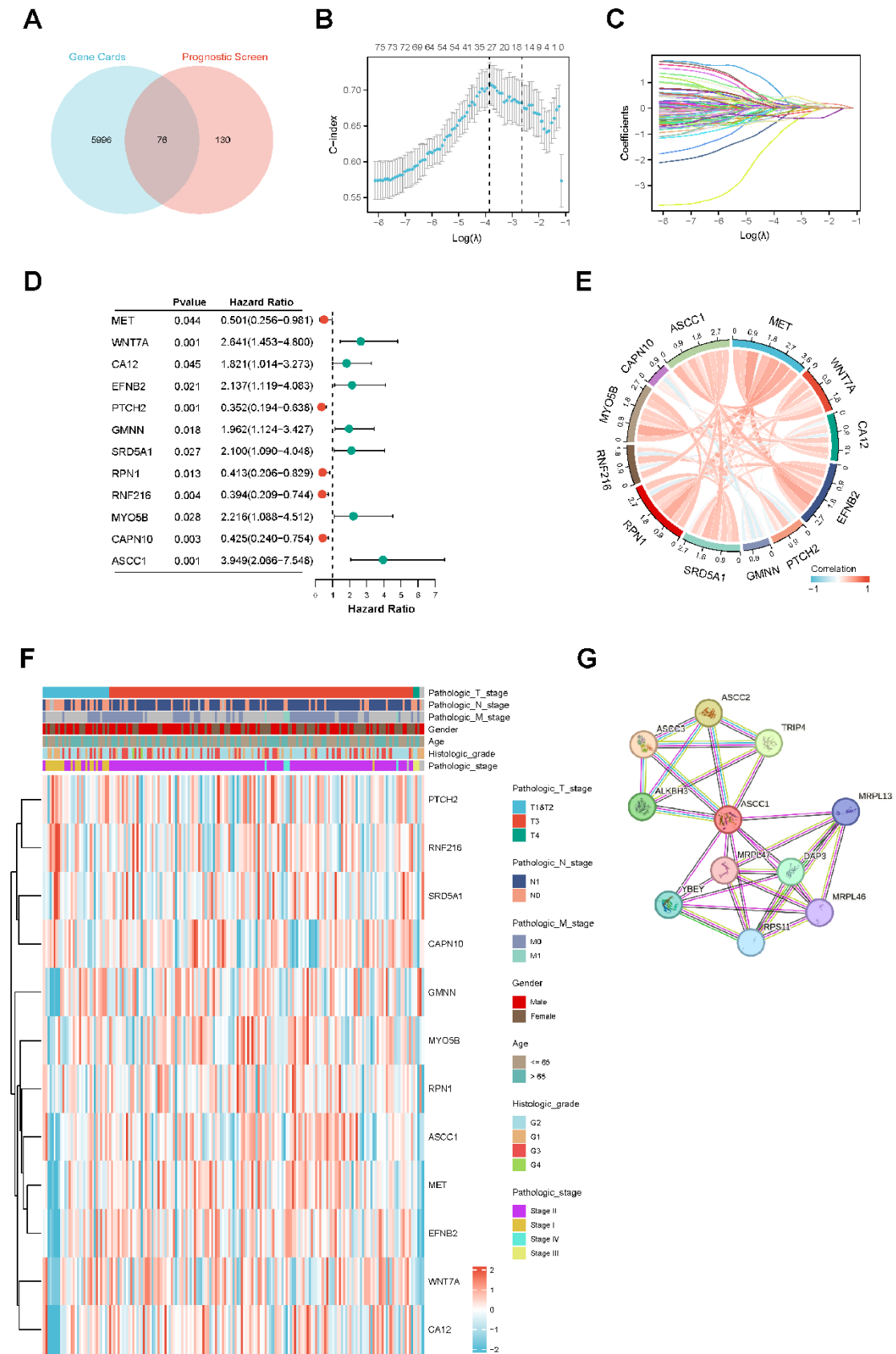


Figure 1: Screening of Prognostic Genes in Pancreatic Cancer

(A) Venn diagram showing the intersection between prognostic genes identified via univariate Cox regression analysis and gastrointestinal tumor-related genes from GeneCards. (B–C) LASSO regression analysis and corresponding cross-validation curves. (D) Forest plot derived from multivariate Cox regression analysis. (E) Correlation analysis plot among the prognostic genes. (F) Heatmap of prognostic genes stratified by clinical variables.

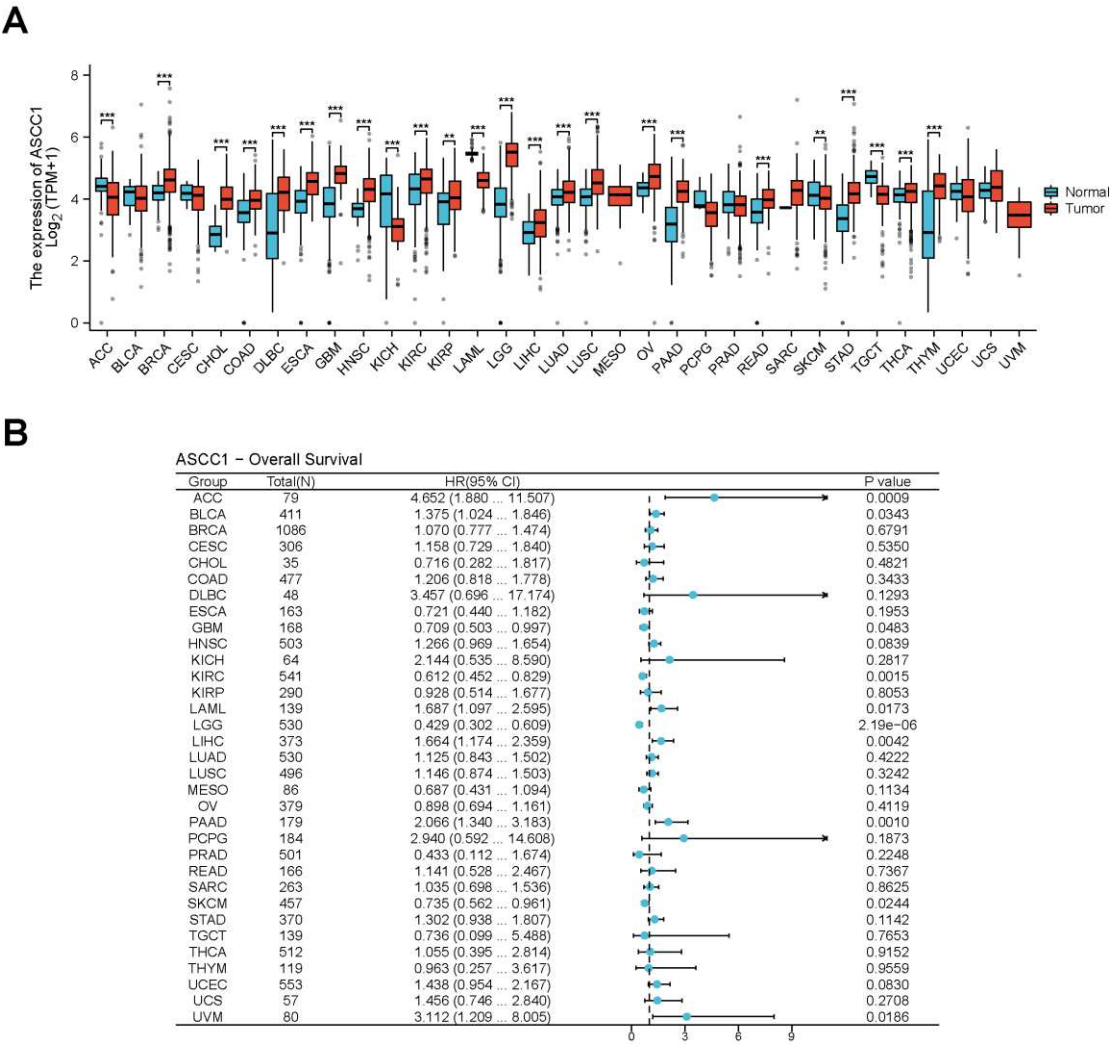


Figure 2: Pan-Cancer Analysis of ASCC1

(A) Boxplot of differential ASCC1 expression in a pan-cancer analysis. (B) Forest plot of survival analysis for ASCC1 in a pan-cancer analysis.

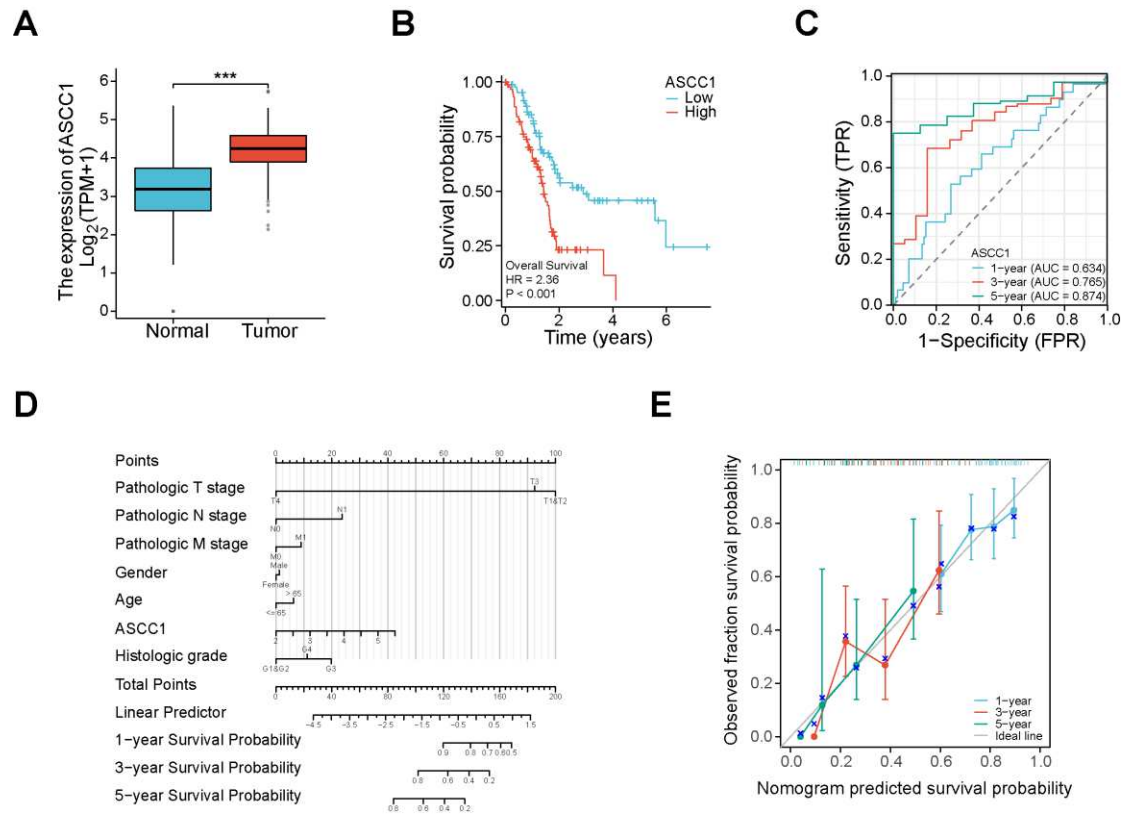


Figure 3: Expression of ASCC1 in Pancreatic Cancer and Its Impact on Prognosis

(A) ASCC1 mRNA expression levels in pancreatic tumor tissues and adjacent normal tissues. (B) Survival curves for pancreatic cancer patients stratified by high and low ASCC1 expression. (C) ROC curves from the nomogram predicting 1-year, 3-year, and 5-year prognosis in pancreatic cancer patients. (D) Nomogram constructed based on clinical parameters. (F) Calibration curve for the nomogram predicting 1-year, 3-year, and 5-year prognosis in pancreatic cancer patients.

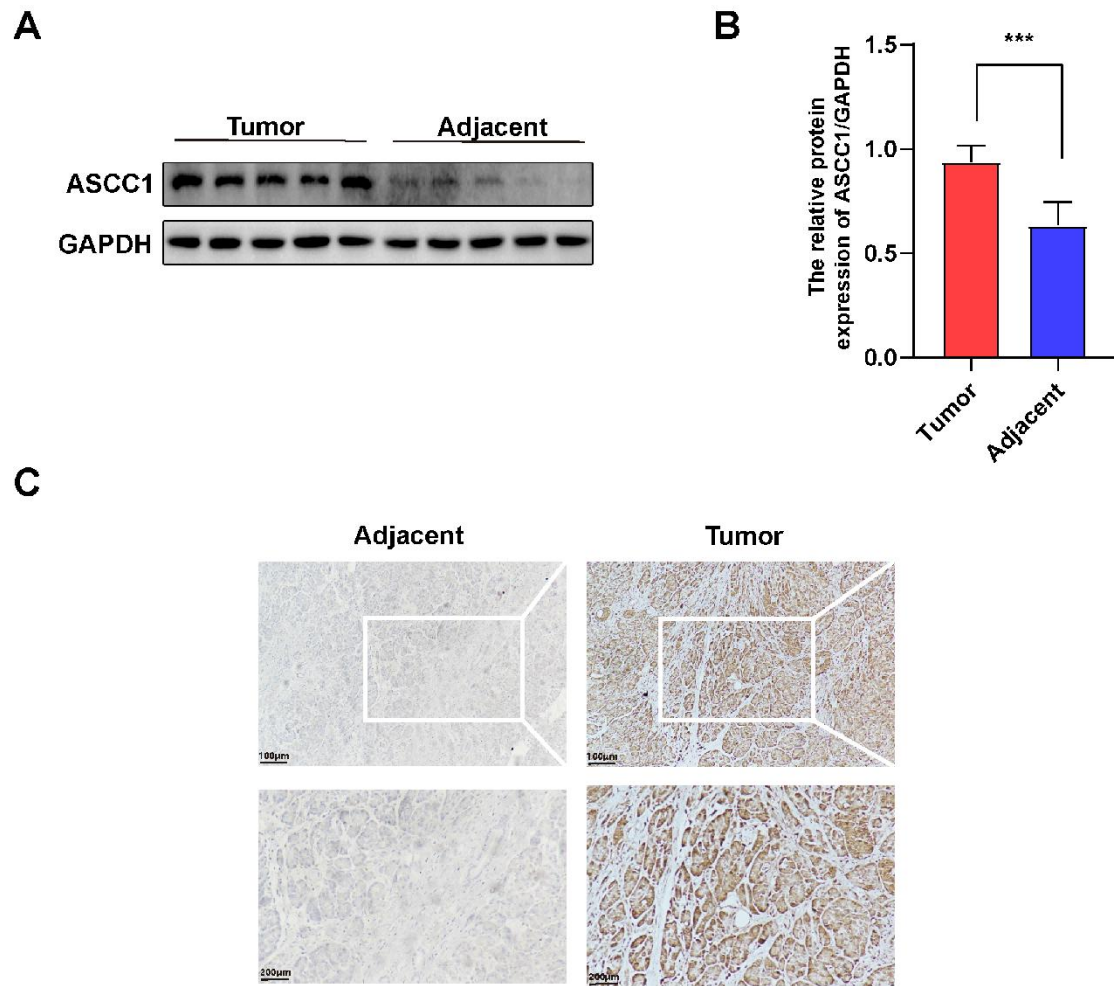


Figure 4: Expression of ASCC1 in Clinical Specimens of Pancreatic Cancer Patients

(A) Western blot analysis showing ASCC1 protein expression levels in pancreatic tumor tissues and adjacent normal tissues. (B) Bar graph representing the quantitative analysis of Western blot grayscale values. (C) Immunohistochemical detection of ASCC1 protein expression in pancreatic tumor tissues and adjacent normal tissues.

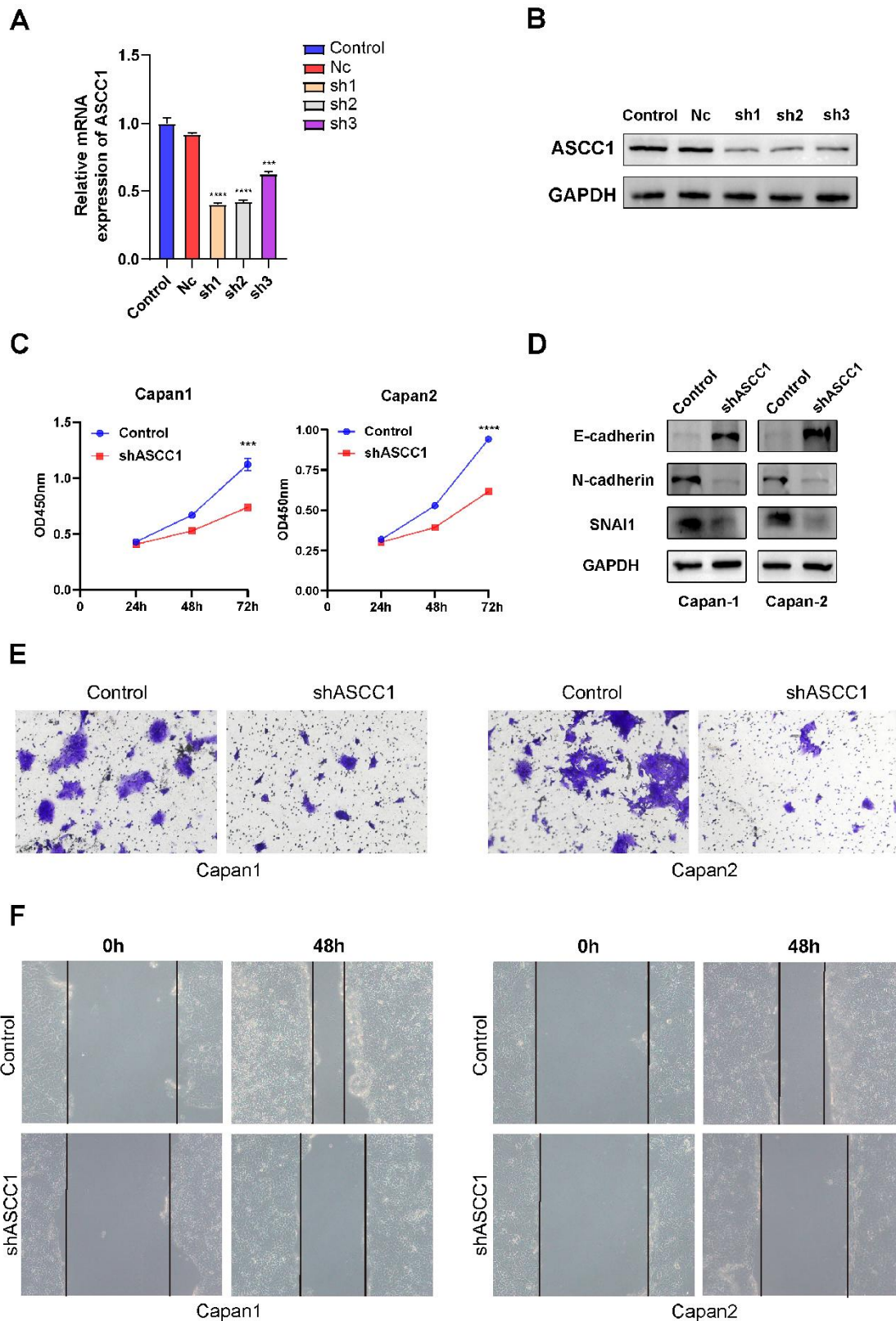


Figure 5: Effect of ASCC1 Expression on the Biological Behavior of Pancreatic Cancer Cells

(A–B) qPCR and Western blot analyses assessing the efficiency of ASCC1 knockdown. (C) CCK-8 assay evaluating the proliferative capacity of pancreatic cancer cells. (D) Western blot analysis of EMT-related protein expression levels. (E) Transwell invasion assay assessing the invasive ability of

pancreatic cancer cells. (F) Wound healing assay evaluating the migratory capacity of pancreatic cancer cells.

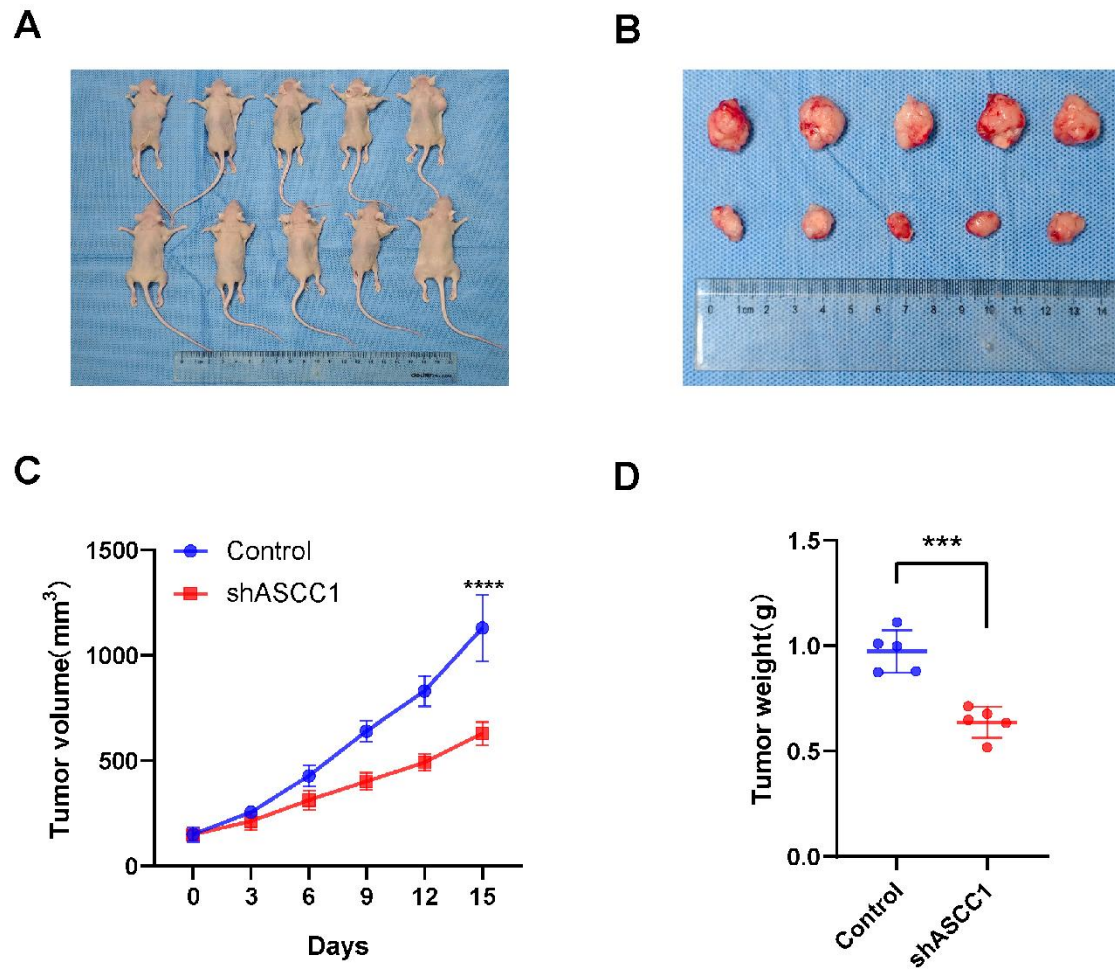


Figure 6: ASCC1 knockdown suppresses tumor growth in nude mice in vivo

(A–B) Representative images of tumors from the control group and the ASCC1 knockdown group in the nude mouse subcutaneous xenograft model. (C) Tumor volume growth curve over time. (D) Bar graph comparing tumor weights.

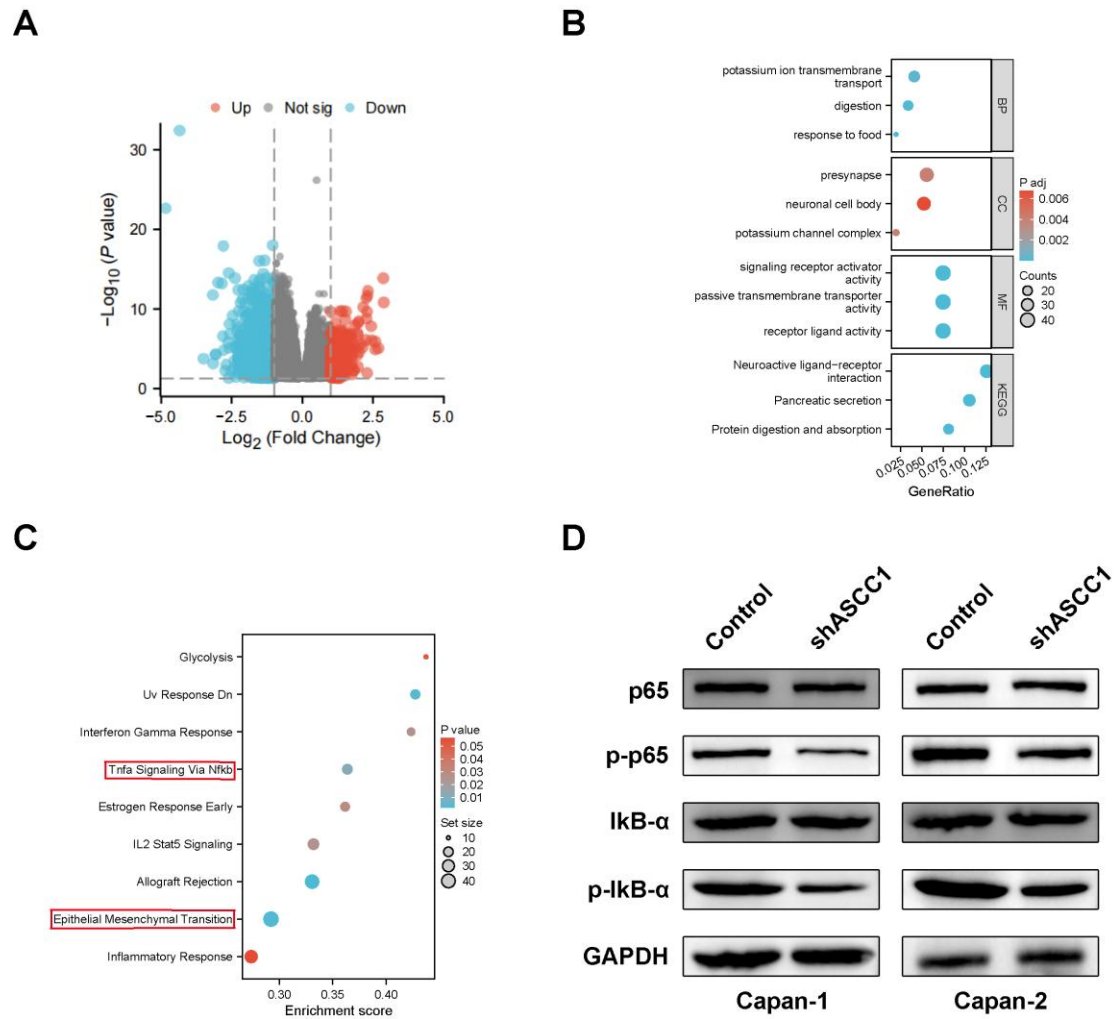


Figure 7: ASCC1 Promotes Pancreatic Cancer Progression Through the NF- κ B Signaling Pathway

(A) Volcano plot of differentially expressed genes. (B) GO and KEGG pathway enrichment analysis chart. (C) GSEA enrichment analysis plot. (D) Western blot analysis showing changes in key proteins within the NF- κ B signaling pathway.

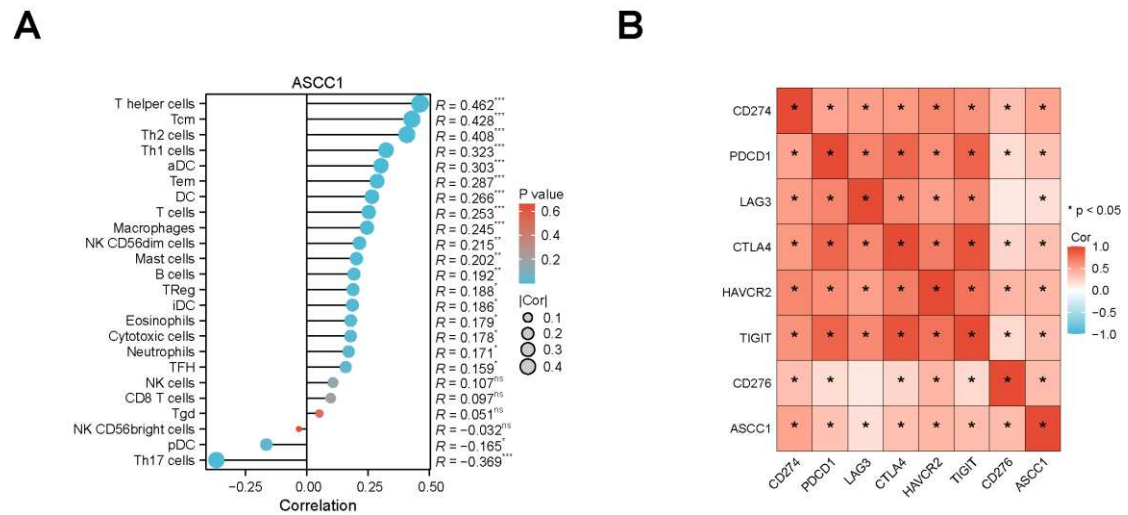


Figure 8: ASCC1 and the Tumor Immune Microenvironment

(A) Immunoinfiltration analysis plot. (B) Heatmap of immune checkpoint correlations.