Am J Transl Res 2026;18(4):3250-3263
www.ajtr.org /ISSN:1943-8141/AJTR0167229

Original Article

SKA3 promotes cell growth and metastasis and is associated with poor prognosis in patients with esophageal cancer

Heng Yang[1,2*], Hua-Lin Xu[3*], Qiang Guo[2*], Jing-Hui Fan[4], Yao-Sen Zhang[1], Jia-Long Guo[2,5], Heng-Lun Liang[1]

*[1]Department of Thoracic Surgery, The Affiliated Panyu Center Hospital, Guangzhou Medical University, Guangzhou, Guangdong, China; [2]Department of Cardiothoracic Surgery, Taihe Hospital, Hubei University of Medicine, Shiyan, Hubei, China; [3]Department of Research, Guangzhou Red Cross Hospital of Jinan University, Guangzhou, Guangdong, China; [4]Department of Research, The Affiliated Panyu Center Hospital, Guangzhou Medical University, Guangzhou, Guangdong, China; [5]Department of Thoracic Surgery, The Central Hospital of Wuhan, Wuhan, Hubei, China. *Co-first authors.*

Received July 4, 2025; Accepted February 25, 2026; Epub April 15, 2026; Published April 30, 2026

Abstract: Objectives: Overexpression of Spindle and Kinetochore Associated complex subunit 3 (SKA3) has been strongly implicated in tumor prognosis, proliferation, and metastasis in various cancers. However, its role in esophageal cancer (ESCA) remained unexplored. This study aims to investigate the function of SKA3 in ESCA. Methods: The expression and prognostic value of SKA3 in ESCA were analyzed through bioinformatics, followed by experimental validation using clinical tissue samples from our hospital. Functional assays were performed to elucidate the role of SKA3 in promoting ESCA cell proliferation and migration. Additionally, the relationship between SKA3 expression and the tumor immune microenvironment was examined. Results: SKA3 was significantly overexpressed in ESCA, with its elevated expression strongly correlating with key clinical features such as Barrett's esophagus, deeper tumor infiltration, lymph node metastasis, advanced pathologic stage, and poor prognosis. Genes co-expressed with SKA3 were enriched in processes related to cell division, DNA replication, the cell cycle, and the p53 signaling pathway. Knockdown of SKA3 significantly reduced ESCA cell proliferation and migration. Moreover, SKA3 overexpression was associated with higher stromal, immune, and ESTIMATE scores. Conclusion: SKA3 contributes to poor prognosis and modulates the immune microenvironment in ESCA. Inhibition of SKA3 expression effectively suppressed tumor growth and metastasis.

Keywords: SKA3, esophageal cancer, bioinformatics, prognosis

Introduction

Esophageal cancer (ESCA) presents a significant global health challenge, ranking as the eighth most commonly diagnosed cancer and the sixth leading cause of cancer-related mortality worldwide [1-3]. The burden of ESCA is particularly severe in China, where it is the third most prevalent cancer and the fourth leading cause of cancer-related death [4, 5], accounting for over half of the global incidence and mortality. A key challenge in managing ESCA is its frequent absence of early symptoms, exacerbated by its high prevalence in economically underdeveloped and remote areas. As a result, diagnosis often occurs at advanced stages, marked by local invasion and lymph node metastasis, with a five-year survival rate of less than 25% [6]. Despite advancements in treatments such as surgery, radiotherapy, chemotherapy, and immunotherapy, patient outcomes remain poor due to high rates of recurrence and metastasis. Therefore, identifying novel biomarkers and therapeutic targets is critical for improving early diagnosis and treatment strategies for ESCA.

The Spindle and Kinetochore Associated Complex subunit-3 (SKA3) gene encodes a protein essential for cell division and mitosis. It is a key component of the protein complexes involved in spindle formation and chromosome separation during mitosis [7]. SKA3 is a multifunctional protein involved in critical processes such as

https://doi.org/10.62347/KYDP3214

cell cycle regulation and proliferation. Overexpression of SKA3 has been reported in various malignancies, including breast, lung, and liver cancers, where it contributes to tumorigenesis and progression [7-18]. For example, Zhang et al. reported that SKA3 promotes breast cancer cell proliferation and invasion by activating the Ak strain Transforming Protein Kinase B (AKT)/WNT/Beta-catenin (β-catenin) signaling pathway [8], while Ruan et al. demonstrated that SKA3 knockdown inhibits tumor growth by downregulating Polo-like kinase 1 (PLK-1) expression [9]. However, the functional roles and underlying mechanisms of SKA3 in ESCA remain unexplored. This study thus combines bioinformatic analysis with *in vitro* experiments to examine systematically the oncogenic roles, molecular mechanisms, and clinical relevance of SKA3 in ESCA, aiming to identify new therapeutic targets for the disease.

Materials and methods

The Cancer Genome Atlas (TCGA) databases

The Cancer Genome Atlas (TCGA) (https://www.cancer.gov/) is a landmark cancer genomics program established by the National Cancer Institute (NCI) and the National Human Genome Research Institute (NHGRI) in the United States. Transcriptome data from the TCGA database, including both cancerous and normal tissues of patients with ESCA, were obtained from the Xiantao Academic database. This dataset comprised 11 normal and 163 cancerous tissue samples. Clinical data for patients with ESCA were also collected.

Clinical patients with ESCA

Paraffin blocks of cancerous tissues and adjacent normal tissues were obtained from 77 patients with ESCA treated between January 2015 and December 2020, for whom complete clinical information was available. These patients had not undergone preoperative radiation therapy, chemotherapy, or immunotherapy. Additionally, cancerous and matched normal tissues were collected from patients undergoing surgery at Taihe Hospital between April and June 2021, with post-surgical pathologic confirmation for Reverse Transcription Polymerase Chain Reaction (RT-PCR) and western blotting. This cohort included 10 patients, both male and female, who had not received preoperative radiation therapy, chemotherapy, or immunotherapy. Paraffin tissue samples were randomly selected from the specimen database, along with normal and cancer tissues obtained during surgery. All patients provided informed consent, and the study was approved by the Ethics Committee of Taihe Hospital (No. 2021KS028) in Shiyan City.

Immunohistochemistry (IHC)

Tissue sections were processed by baking at 60°C for 2 hours, followed by dewaxing, hydration, antigen retrieval, and blocking. Sections were then incubated overnight at 4°C with 1:100 SKA3 antibodies (BS-7848R, Bioss, China). Afterward, they were incubated with a 1:300 horseradish peroxidase (HRP)-conjugated secondary antibody (A0208, Beyotime Biotechnology, China) for 1 hour at room temperature, followed by diaminobenzidine (DAB) and hematoxylin staining. The sections were dehydrated, coverslipped, and prepared for interpretation. A random double-blind approach was applied for slide interpretation by at least two senior physicians with an Associate Chief Physician title or higher. If their assessments matched, the consensus result was adopted; If not, a third physician of similar seniority was consulted to reach a final decision. IHC staining results were evaluated based on both the percentage of positive cells and the intensity of staining. The scoring system was as follows: 1) 0-5% positive cells, score = 0; 2) 5-25%, score = 1; 3) 26-50%, score = 2; 4) 51-75%, score = 3; 5) > 75%, score = 4. Staining intensity was classified as: 1) No staining, score = 0; 2) Light yellow, score = 1; 3) Yellow, score = 2; 4) Brown-yellow, score = 3. The final staining index was calculated as the product of the staining range and intensity, with each patient group receiving the corresponding index score for both esophageal squamous cell carcinoma (ESCC) tissue and the associated normal esophageal mucosal epithelium.

Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Cancer tissues from patients with ESCA and their corresponding adjacent normal esophageal tissues were homogenized using a grinder, and an appropriate amount of Trizol (R401-

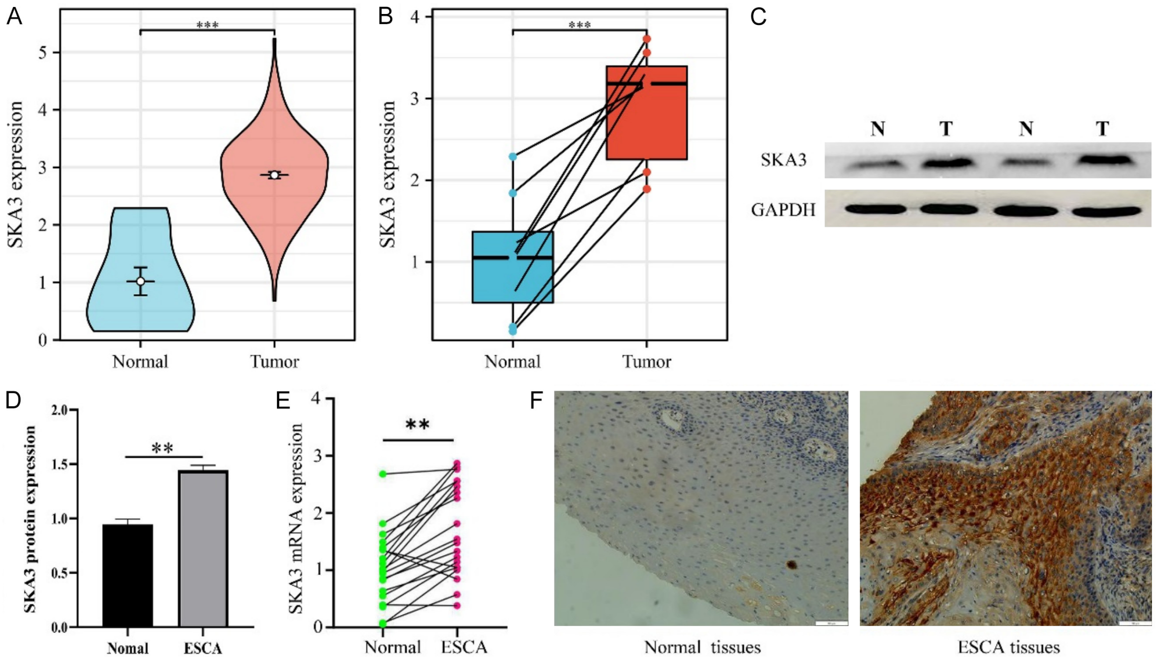

Figure 1. SKA3 expression is significantly increased in ESCA. A, B. SKA3 expression in ESCA from the TCGA database; C-F. SKA3 expression in ESCA from our hospital, assessed using RT-PCR, western blotting, and IHC. Note: ESCA, esophageal cancer; IHC, immunohistochemistry; N, normal tissues; T, ESCA tissues; RT-PCR, Reverse Transcription Polymerase Chain Reaction; TCGA, The Cancer Genome Atlas; **, P < 0.01; ***, P < 0.001.

01, Vazyme Biotech, China) was added to the ECA109 cells and ESCA tissues to facilitate cell release and preserve RNA integrity. Total RNA was reverse-transcribed into cDNA according to the manufacturer's instructions using the HiScript 1st Strand cDNA Synthesis Kit (R211-01, Vazyme Biotech, China). Quantitative PCR was performed with a SYBR Green PCR Kit (Q221-01, Vazyme Biotech, China) on the ABI 7500 real-time PCR system. The thermocycling conditions were as follows: 95°C for 30 seconds, followed by 40 cycles of 95°C for 10 seconds and 60°C for 30 seconds, with an annealing step at 60°C for 1 minute and a final extension at 95°C for 15 seconds. The relative expression of SKA3 was calculated using the $2^{-\Delta\Delta CT}$ method. The following primers were used for qPCR: SKA3 forward, TGAGCGGTACATCGTATCCCA; SKA3 reverse, GGGGTTACAATTACGGGCTCT; β-actin forward, 5'-GTCAGGTCATCACTATCGGCAAT-3', β-actin reverse, 5'-AGAGGTCTTTACGGATGTCAACGT-3'.

Western blotting

For protein expression analysis, cancerous and adjacent normal esophageal tissues were minced, and RIPA protein lysis solution was added to the ECA109 cells and ESCA tissues. After sufficient dissolution, Phenylmethylsulfonyl fluoride (PMSF) and Protease Inhibitor (PI) were incorporated, mixed thoroughly, and stored on ice. The protein lysis solution was prepared according to standard protocols and subjected to quantitative analysis and denaturation using the Bicinchoninic Acid (BCA) method. Sodium Dodecyl Sulfate - PolyAcrylamide Gel Electrophoresis (SDS-PAGE) was used to separate the proteins, followed by gel cutting based on the required molecular weight and transfer to a Polyvinylidene Difluoride (PVDF) membrane. The membrane was incubated at room temperature for 2 hours with 5% skim milk powder after electrotransfer. It was then incubated overnight at 4°C with primary antibodies against SKA3 (1:500, bs-7848R, Bioss, China) and Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) (1:2000, 10494-1-AP, Proteintech, China). Afterward, it was incubated with HRP-conjugated secondary antibody (1:10,000, A0208, Beyotime Biotechnology, China) for 2 hours at room temperature. Protein development was performed, and the grayscale value of the target band was analyzed using Image Lab software.

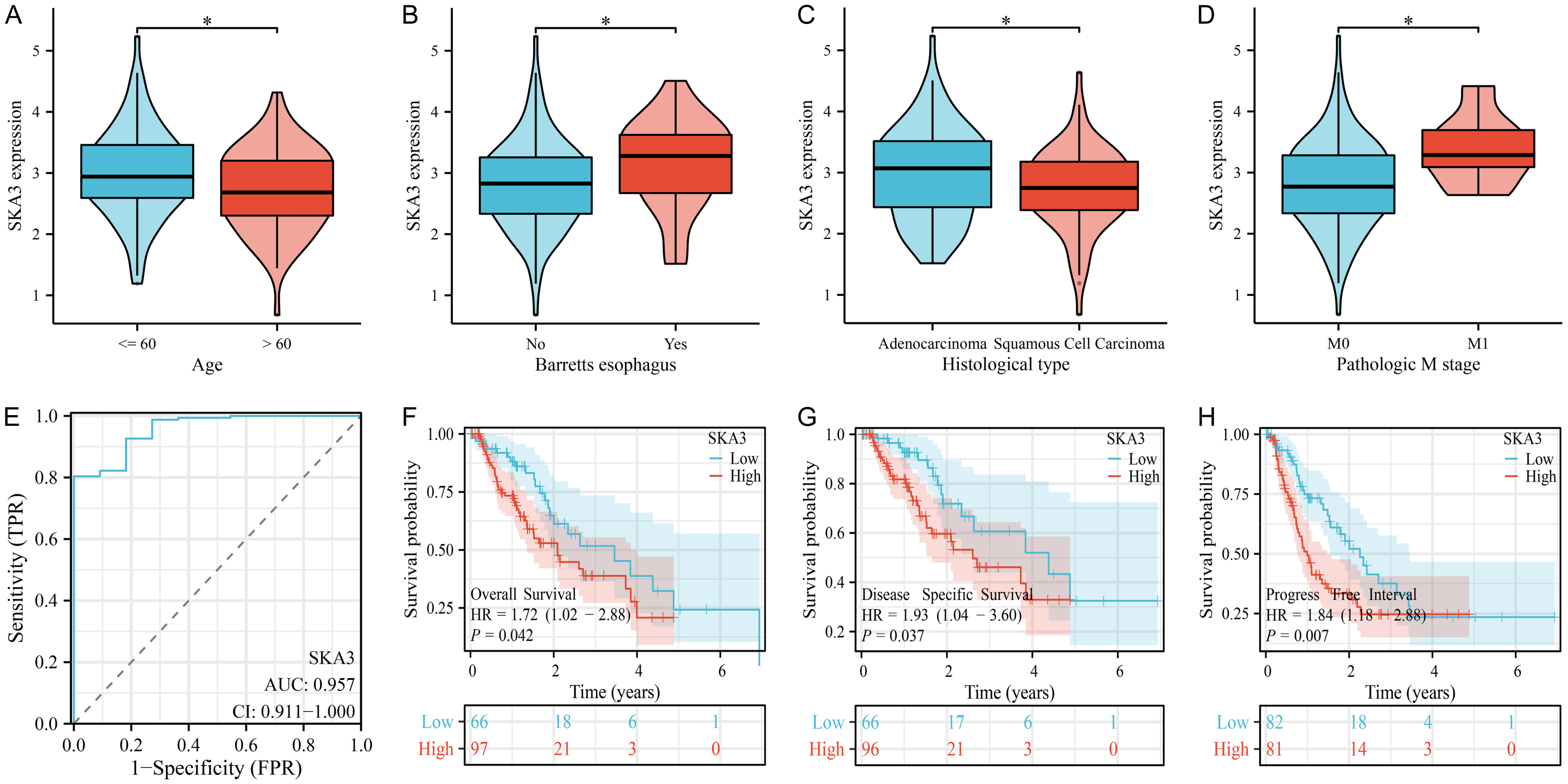

Figure 2. SKA3 expression correlates with clinical features and poor prognosis in ESCA from the TCGA database. A. Age; B. Barrett's esophagus; C. Histologic type; D. M stage; E. Diagnostic value assessed by ROC curve; F. OS; G. DSS; H. PFI. Note: PFI, progression-free interval; ESCA, esophageal cancer; ROC, receiver operating characteristic; DSS, disease-specific survival; TCGA, The Cancer Genome Atlas; OS, overall survival; *, $P < 0.05$.

Table 1. Relationship between SKA3 overexpression and clinical features in ESCA from our hospital, using IHC scores

Characteristic	N	Expression of SKA3	*P* value
Gender			
Female	59	8.44±2.373	0.800
Male	18	8.28±2.421	
Age			0.09
< 60	42	8.81±2.233	
≥ 60	35	7.91±2.466	
T stage			0.017
T1	16	6.81±2.281	
T2	24	8.75±2.048	
T3	34	8.76±2.400	
T4	3	10.00±2.369	
N stage			
No	30	9.17±2.119	0.023
Yes	47	7.91±2.412	
Pathologic stage			0.011
Stage I-II	46	7.85±2.336	
Stage III-IV	31	9.23±2.156	
Overall survival			0.04
Alive	23	6.78±2.567	
Dead	48	9.19±2.070	

Note: ESCA, esophageal cancer; IHC, immunohistochemistry.

SKA3 expression levels in ESCA

This study assessed SKA3 expression levels in cancer and adjacent normal tissues from both TCGA ESCA datasets and patients from our hospital, using RT-PCR and western blotting to measure SKA3 protein and mRNA. Additionally, SKA3 protein expression levels in cancer and adjacent normal tissues from patients with ESCA were determined through IHC.

Relationship between clinical characteristics, diagnostic value, and prognosis of SKA3 in patients with ESCA

To investigate the relationship between SKA3 expression and clinicopathologic characteristics, SKA3 expression data were integrated with clinical features from both the TCGA database and our hospital's ESCA cohort. SKA3 expression was categorized, and the association between SKA3 levels and clinical features was analyzed. Receiver operating characteristic (ROC) analysis was performed to evaluate the diagnostic significance of SKA3 expression in cancer and adjacent normal tissues of patients with ESCA. Survival analysis was conducted to assess the effect of high and low SKA3 expression on the prognosis of patients with ESCA, based on the optimal *P*-value.

SKA3-related prognostic nomogram

The TNM Staging System, a widely recognized prognostic indicator for cancer individuals [19], was used to examine the correlation between TNM stage and SKA3 expression levels, alongside 1-year, 3-year, and 5-year survival rates in patients with ESCA using nomograms and risk models. The survival analysis revealed a significant decline in prognosis for high-risk patients with ESCA.

Functions involved in SKA3 co-expressed genes

Co-expression analysis was performed to explore the functions and regulatory networks of genes involved in biological processes. ESCA data from the TCGA database were utilized to identify genes co-expressed with SKA3, applying a minimum correlation coefficient of 0.4. This approach aimed to elucidate the role of SKA3 in cell division and mitosis, as well as its associations with other genes, providing valuable insight into the underlying mechanisms. Gene Ontology (GO) annotations were then applied to investigate the functional roles of the co-expressed SKA3 genes [20].

Mechanisms and protein networks involved in SKA3 co-expressed genes

Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, a method commonly used to explore signaling pathways involving single or multiple genes, was employed to examine the signaling mechanisms of SKA3 co-expressed genes [20]. The STRING database was used to construct protein-protein interaction (PPI) networks among the SKA3 co-expressed genes [21]. Co-expressed genes of SKA3 were imported into the STRING database, and the PPI network was visualized with a binding score threshold of 0.9.

Inhibition of SKA3 expression in ESCA cells

Human ESCA ECA109 cells (Wuhan Procell Biotechnology, CL-0077) were cultured in DMEM supplemented with 10% fetal bovine serum

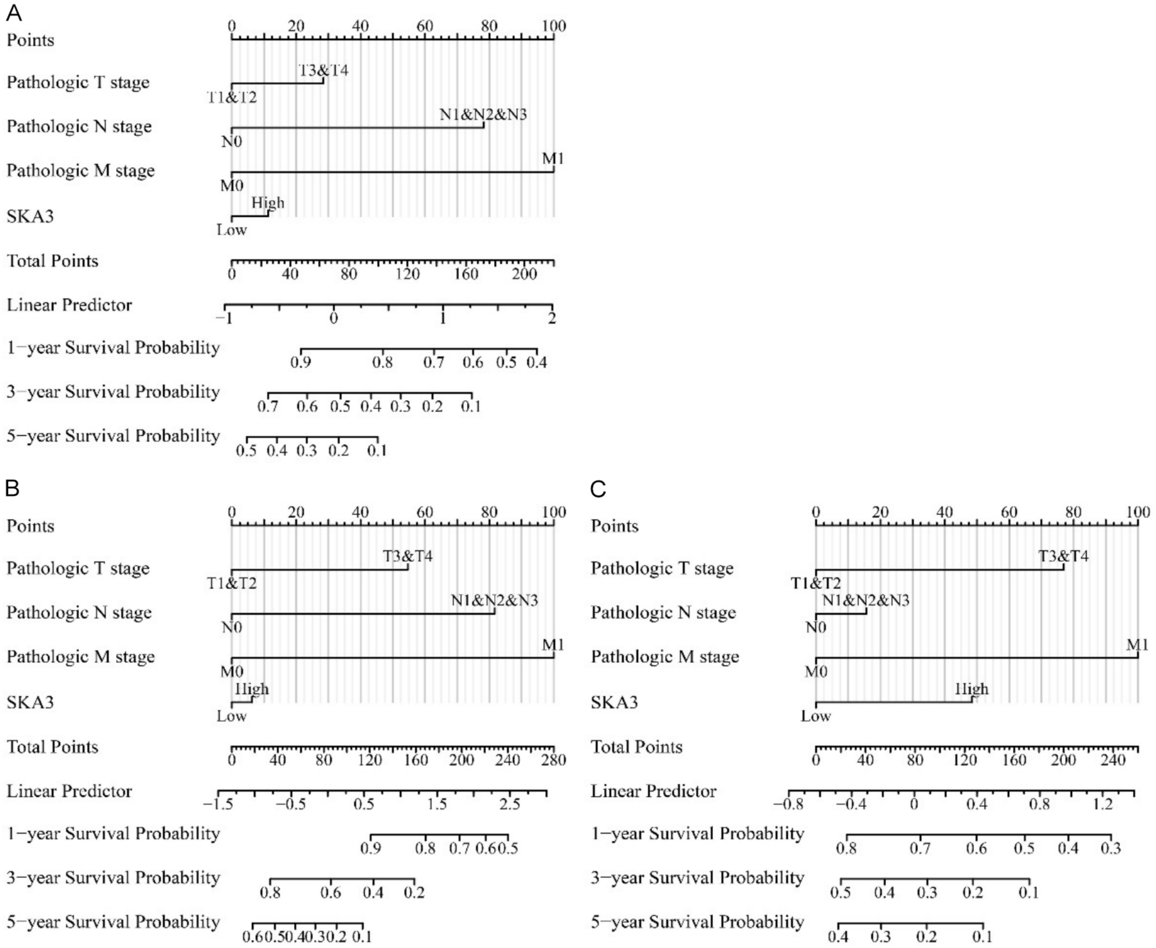

Figure 3. Prognostic nomograms for OS, DSS, and PFI in patients with ESCA. A. OS; B. DSS; C. PFI. Note: PFI, progression-free interval; ESCA, esophageal cancer; DSS, disease-specific survival; OS, overall survival.

and 1% penicillin-streptomycin. Interfering RNAs targeting SKA3 were designed and constructed using Gemera. The siRNA sequences included Si-SKA3-1 (5'-GCGACUUGAGAAUUGAUAUCCTT-3', 5'-GAUAAUCUUCA AAGUCGCT-3'), Si-SKA3-2 (5'-GUUCAGACUCUAAAGGAUGTT-3', 5'-CAUCCUUAGAGU GAACTT-3'), and nonspecific siRNA (si-NC, 5'-UUCUCCGAACGUGUCACGUTT-3', 5'-ACGUGACACGUUCGGAGAATT-3') as the control. According to the manufacturer's instructions, siRNA was transfected into ECA109 cells using the lipo3000 transfection reagent. After 24 hours of incubation, SKA3 expression was assessed by RT-PCR and western blotting.

Cell Counting Kit-8 (CCK-8)

After washing ECA109 cells with phosphate-buffered saline (PBS), trypsin digestion was terminated by adding culture medium, followed by centrifugation and cell counting. The ECA109 cells were seeded at a density of 1 × 10^4 cells/mL in a 96-well plate and incubated overnight under optimal conditions. After 24 hours, 10 μL of CCK-8 reagent was added to both the si-NC and SKA3 inhibition groups, and absorbance was measured at 450 nm using an iMark microplate reader.

5-Ethynyl-2'-deoxyuridine (EDU)

After counting ECA109 cells, they were seeded in a 24-well plate and cultured until reaching approximately 90% confluence. EDU solution was then added, and the cells were incubated for 24 hours. Following incubation, the cells were washed with PBS, fixed with 4% paraformaldehyde for 30 minutes, and stained with DAPI for 15 minutes. After removing excess fluorescent stain with PBS, the cells were examined under a fluorescence microscope.

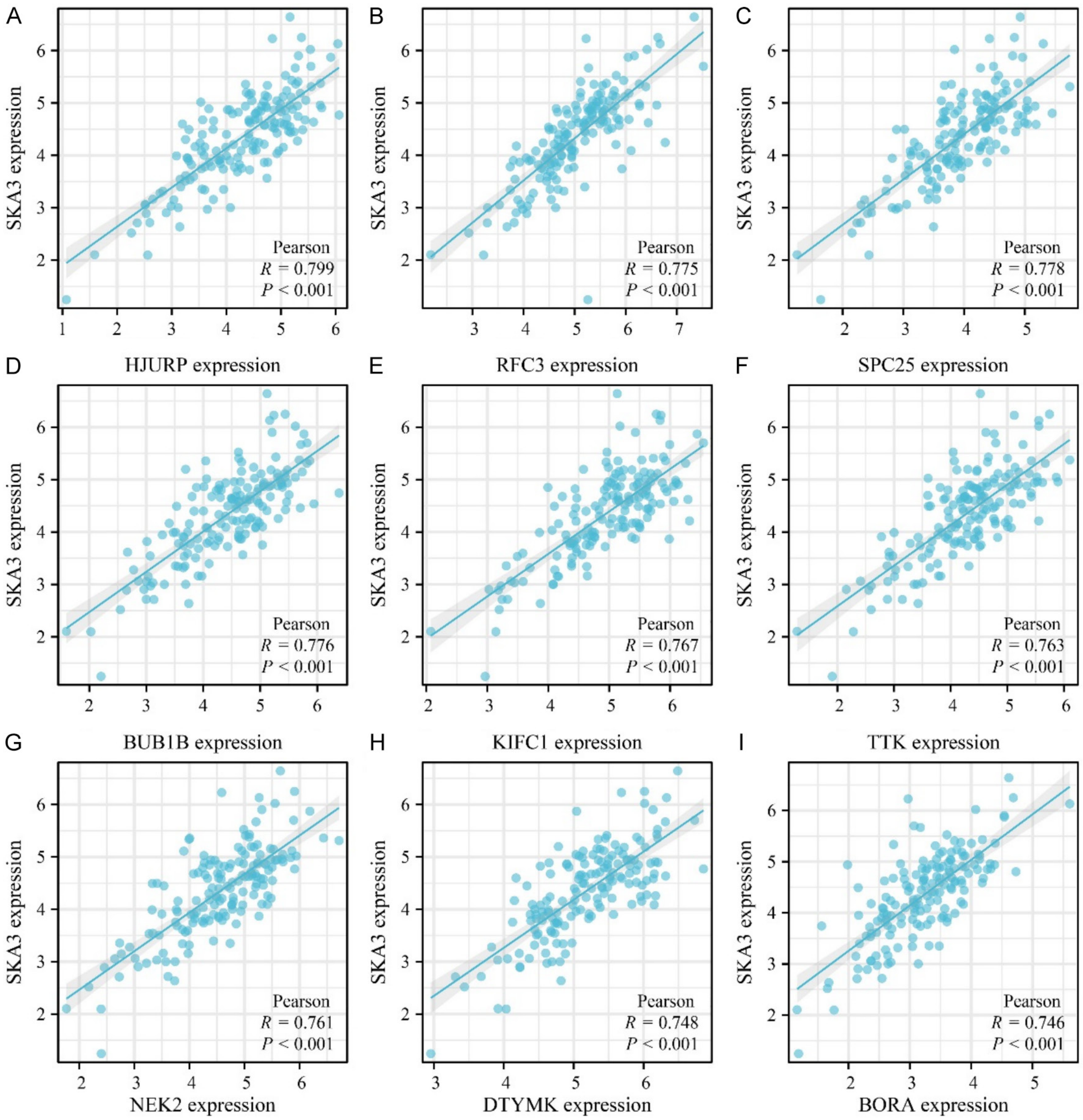

Figure 4. SKA3 co-expressed genes. A. HJURP; B. RFC3; C. SPC25; D. BUB1B; E. KIFC1; F. TTK; G. NEK2; H. DTYMK; I. BORA.

Wound healing

ECA109 cells were seeded in a 6-well plate with 2 mL of culture medium per well and incubated in a 5% CO_2 incubator at room temperature. Upon reaching 90% confluence, scratches were made in the wells using a 200 μL pipette tip, ensuring the scratches were as perpendicular as possible to the horizontal axis of the well. The cells were then washed with PBS and imaged immediately, with a second set of images taken after 24 hours to assess cell migration.

Relationship between SKA3 expression and immune microenvironment

To calculate immune scores and assess the relative expression levels of immune cells in ESCA tissues from the TCGA database, the Estimation of STromal and Immune cells in MAlignant Tumors using Expression data (ESTIMATE) and Single-Sample Gene Set Enrichment Analysis (ssGSEA) methods were employed. SKA3 expression data were integrated with immune scores and immune cell data

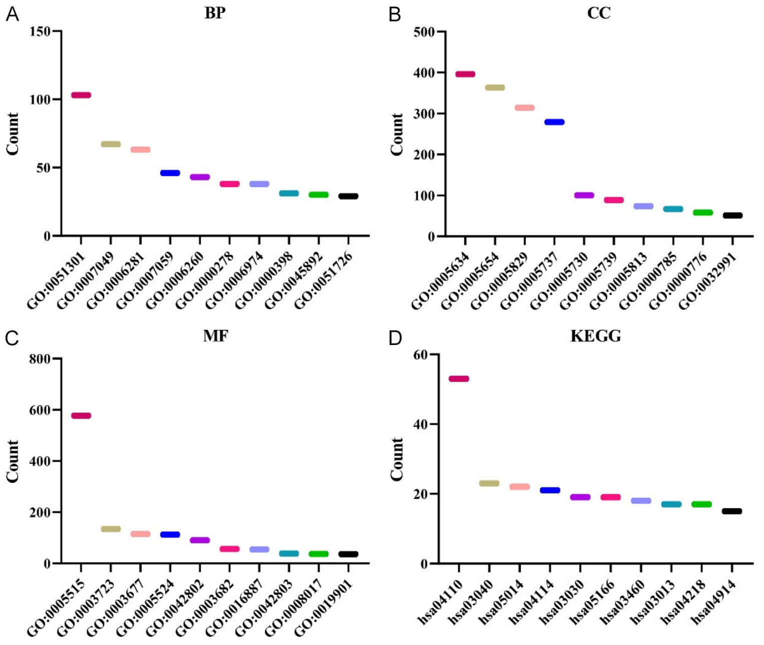

Figure 5. Functions and mechanisms of SKA3 co-expressed genes. A. Biological processes (BP); B. Cellular components (CC); C. Molecular functions (MF); D. KEGG pathway analysis. Note: KEGG, Kyoto Encyclopedia of Genes and Genomes.

using Perl. Correlation analysis was performed to explore the relationship between SKA3 expression, immune scores, and immune cell levels.

Statistical analysis

The Wilcoxon rank-sum test was used to determine the expression level of SKA3 in TCGA ESCA tissues, while a t-test was applied to evaluate its effect on cell growth and metastasis. ROC analysis was performed to assess the diagnostic value of SKA3 in ESCA, with the area under the curve (AUC) as the evaluation criterion. Survival analysis, nomograms, and risk models were used to explore the relationship between SKA3 expression and survival time in patients with ESCA. Additionally, correlation analysis was conducted to identify co-expressed genes of SKA3 and their association with the immune microenvironment. A *P*-value of < 0.05 was considered significant.

Results

SKA3 overexpressed in ESCA

Analysis of the TCGA database revealed a significant increase in SKA3 expression in both unpaired (**Figure 1A**) and paired ESCA tissues (**Figure 1B**). Western blotting and RT-PCR results from patients at our hospital also showed a marked increase in SKA3 mRNA expression in ESCA tissues (**Figure 1C-E**), with IHC analysis confirming elevated SKA3 protein expression in these tissues (**Figure 1F**).

Overexpression of SKA3 is associated with age, tumor subtypes, distant metastasis, T stage, lymph node metastasis, Barrett's esophagus, and radiotherapy in patients with ESCA

In the TCGA database, SKA3 overexpression was significantly associated with clinical factors such as age, tumor subtype, distant metastasis, and Barrett's esophagus in patients with ESCA (**Figure 2A-D**). In our hospital's cohort, grouping patients by SKA3 expression revealed significant associations with T stage, lymph node metastasis, and distant metastasis (**Table 1**).

Overexpression of SKA3 is associated with diagnosis and poor prognosis in patients with ESCA

The AUC for SKA3 expression in both cancer and adjacent tissues of patients with ESCA was 0.957, based on TCGA data (**Figure 2E**). Survival analysis indicated that SKA3 overexpression was associated with overall survival (OS), disease-specific survival (DSS), and disease progression (**Figure 2F-H**). Similarly, in our hospital's ESCA cohort, SKA3 overexpression correlated with OS (**Table 1**).

SKA3-related nomogram is related to the survival time of patients with ESCA

The TNM stage serves as a key prognostic indicator for cancer individuals [19]. SKA3 expression was significantly correlated with the prognosis of patients with ESCA. A nomogram was constructed to illustrate the relationship between T stage, N stage, M stage, and SKA3

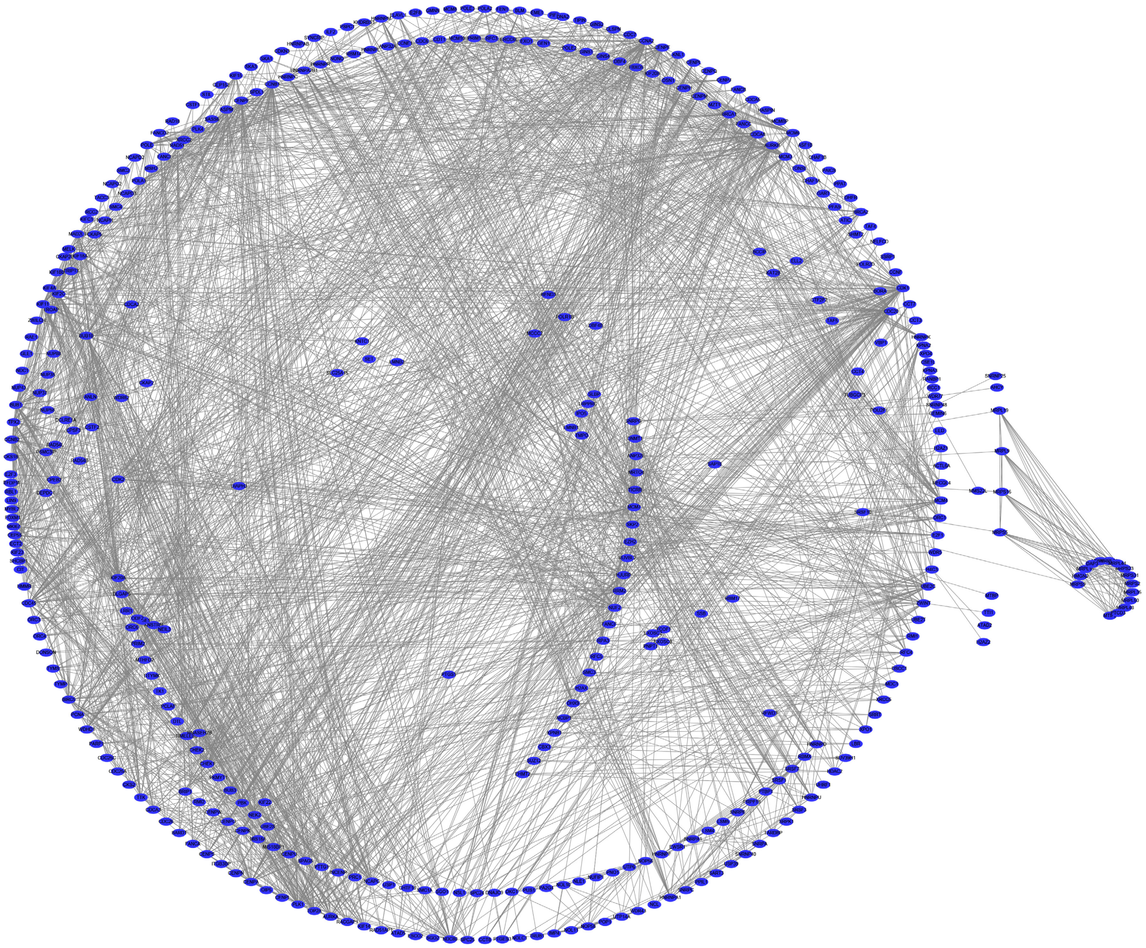

Figure 6. PPI network of SKA3 co-expressed genes. Note: PPI, protein-protein interaction.

expression with OS, DSS, and progression-free interval (PFI) at 1, 3, and 5 years for patients with ESCA (**Figure 3**).

Functions, mechanisms, and PPI networks involved in SKA3 co-expressed genes

A total of 708 genes were identified as co-expressed with SKA3, the majority of which exhibited positive co-expression. The top 9 co-expressed genes, based on correlation coefficients, were visualized (**Figure 4**). These genes are involved in critical biological functions such as cell division, DNA replication, DNA repair, cell cycle regulation, mRNA splicing, and kinetochore localization (**Figure 5A-C**). They also contribute to various mechanisms, including cell cycle regulation, DNA replication, oocyte meiosis, homologous recombination, nuclear-cytoplasmic transport, base excision repair, mismatch repair, cellular aging, the p53 signaling pathway, and antioxidant activity (**Figure 5D**). The PPI network of SKA3 co-expressed genes is shown in **Figure 6**.

Inhibiting SKA3 expression can inhibit ESCA cell proliferation and metastasis

RT-PCR and immunoblotting confirmed the successful establishment of a cell model with suppressed SKA3 expression (**Figure 7A**, **7B**). CCK-8 and EDU assays demonstrated that inhibition of SKA3 expression significantly suppressed ESCA cell proliferation, with statistically significant differences (**Figure 7C-E**). Additionally, wound healing assays revealed that inhibiting SKA3 expression notably reduced ESCA cell migration, with significant differences (**Figure 8**).

SKA3 expression is associated with ESCA immune microenvironment

Correlation analysis showed that SKA3 overexpression was significantly correlated with stro-

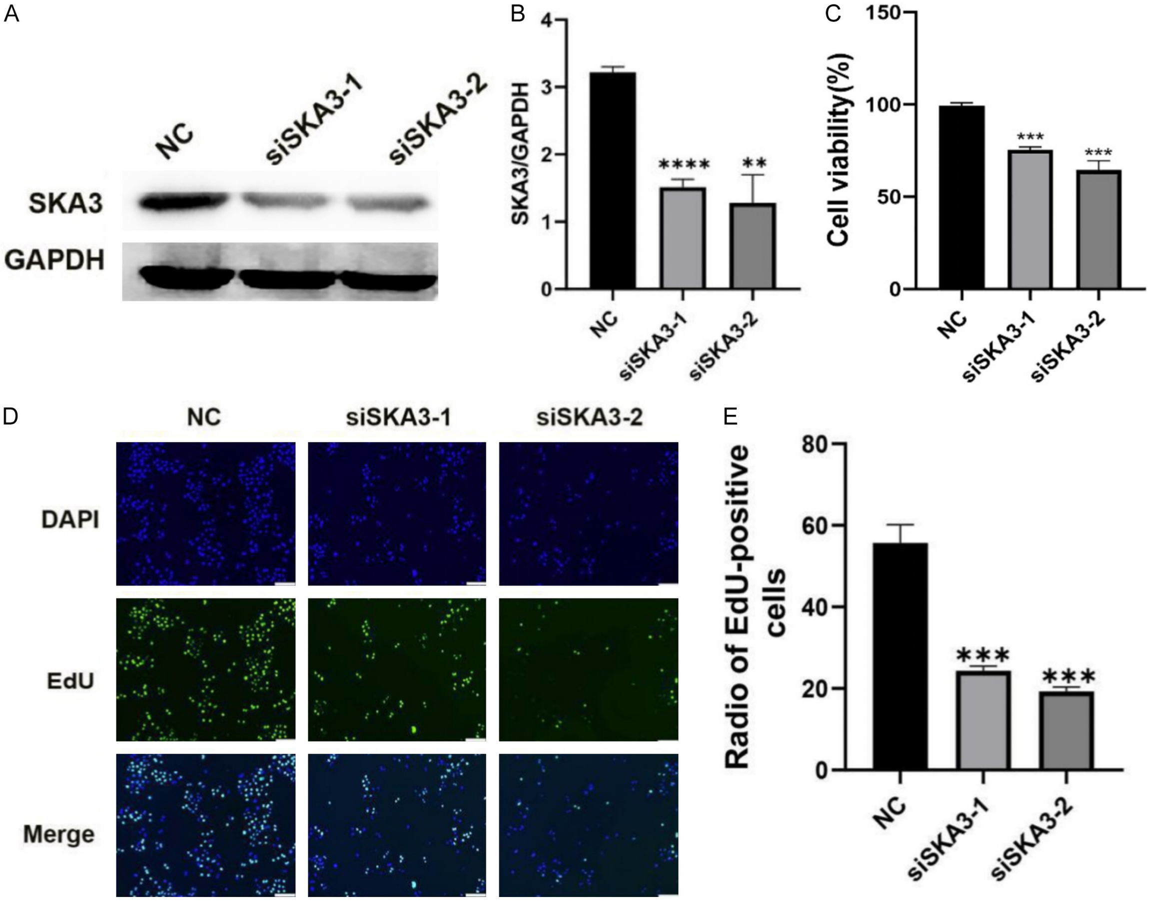

Figure 7. Inhibition of SKA3 expression inhibits ESCA cell growth. A, B. Cell models established using western blotting and RT-PCR; C. Cell viability measured by CCK-8 assay; D, E. Cell growth assessed by EDU assay. Note: ESCA, esophageal cancer; RT-PCR, Reverse Transcription Polymerase Chain Reaction; CCK-8, Cell Counting Kit-8; EDU, 5-ethynyl-2'-deoxyuridine; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001.

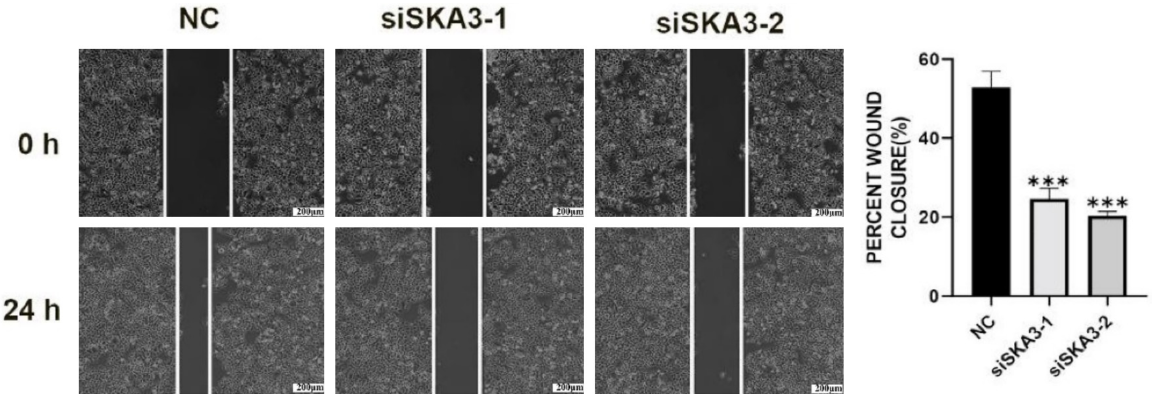

Figure 8. Inhibition of SKA3 expression inhibits ESCA cell migration, as measured by wound healing assay. Note: ESCA, esophageal cancer; ***, P < 0.001.

mal score (r = 0.321), immune score (r = 0.378), and ESTIMATE score (r = 0.381) (**Figure 9A-C**). Stromal, immune, and ESTIMATE scores exhibited significant statistical differences between high- and low-SKA3 expression groups (**Figure 9D**). Moreover, SKA3 overexpression was strongly correlated with immune cell infiltration, including ESCA Th2 cells (r = 0.436), mast cells

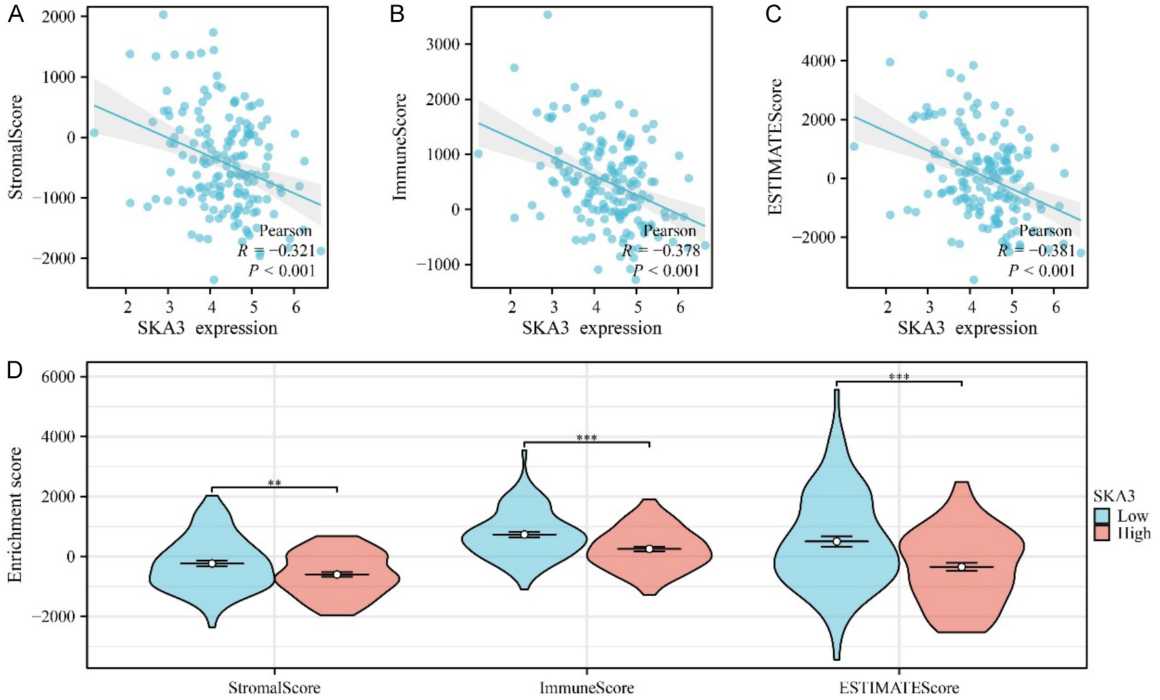

Figure 9. Overexpression of SKA3 correlates with stromal score, immune score, and ESTIMATE score in ESCA. A-C. Correlation analysis of SKA3 with stromal score, immune score, and ESTIMATE score; D. Expression analysis of SKA3 in relation to stromal score, immune score, and ESTIMATE score. Note: ESCA, esophageal cancer; **, P < 0.01; ***, P < 0.001.

(r = -0.405), cytotoxic cells (r = -0.384), plasmacytoid dendritic cells (pDCs) (r = -0.383), immature dendritic cells (iDCs) (r = -0.373), and CD8$^+$ T cells (r = -0.331) (**Figure 10**).

Discussion

Extensive research highlights the critical role of genetic factors in the development of esophageal cancer (ESCA). Specific genetic alterations or mutations can increase the risk of developing ESCA and influence its progression [22-24]. For example, Secreted Phosphoprotein 1 (SPP1) expression is significantly upregulated in ESCA cells, where it promotes DNA damage repair and tumor cell survival. The SPP1/Janus Kinase 2 (JAK2)/Signal Transducer and Activator of Transcription 3 (STAT3) signaling axis plays a pivotal role in ESCA progression and radiation resistance, potentially enhancing treatment efficacy and patient survival [22]. Recently, SKA3 has garnered attention for its critical biological role in the progression of various cancers, including breast, lung, and liver cancers [8-18]. In liver cancer, SKA3 is notably overexpressed, and its upregulation correlates with clinicopathologic features and survival outcomes in patients with ESCA. Inhibition of SKA3 expression significantly reduces E2 Promoter-Binding Factor (E2F) transcription factor activity, suppresses cell proliferation, induces cell cycle arrest, and impedes tumorigenesis both *in vitro* and *in vivo*. This also results in a reduction in cyclin D1 levels and an increase in Cyclin-Dependent Kinase Inhibitor 1A (CDKN-1A) protein expression [16], suggesting that SKA3 inhibition may slow cancer progression.

The role of SKA3 in ESCA remains underexplored. This study used multi-omics data to construct a more integrated model that elucidates the functional roles of SKA3 overexpression. This approach clarified the distinct prognostic and therapeutic relevance of SKA3 in ESCA compared to its role in other malignancies. In clinical ESCA samples, significant increases in SKA3 mRNA and protein levels were observed, with SKA3 overexpression linked to age, tumor subtypes, distant metastasis, Barrett's esophagus, T stage, lymph node metastasis, and radiation therapy. The AUC for SKA3 was found to be 0.957, indicating its excellent diagnostic value for ESCA. SKA3 overexpression also correlates with OS, DSS, and disease

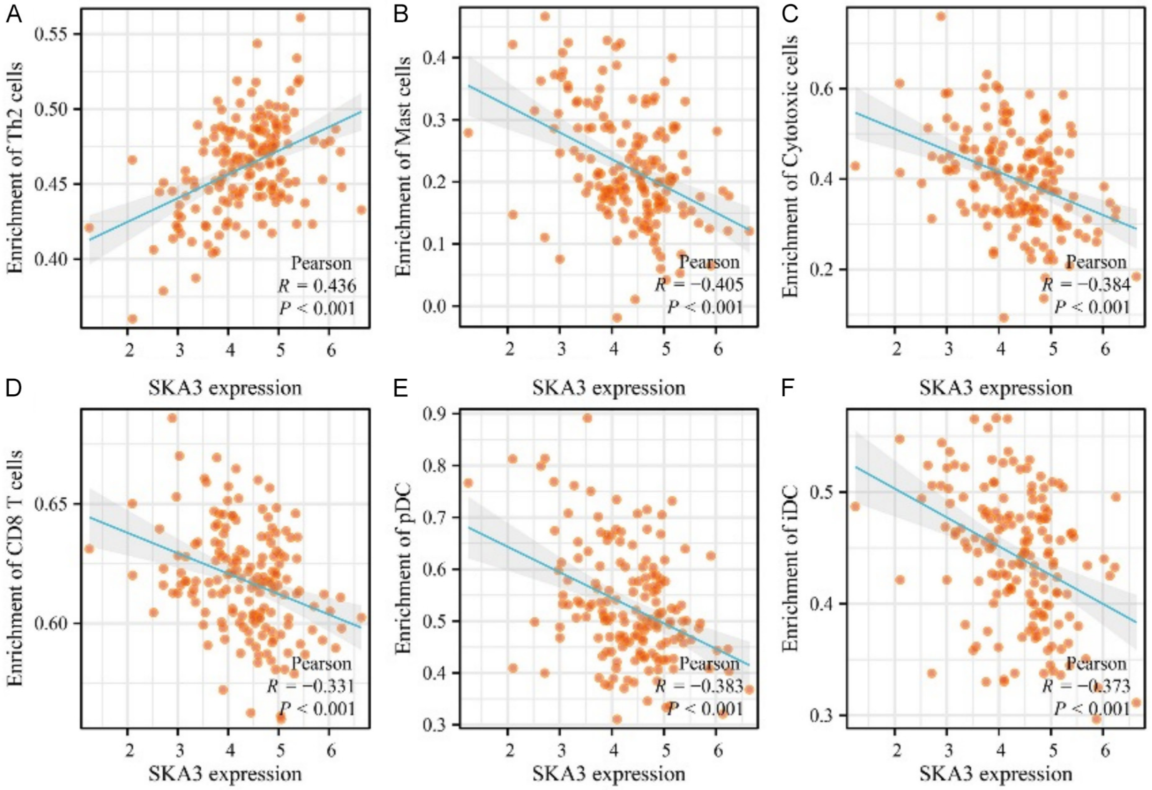

Figure 10. SKA3 overexpression is associated with immune cell infiltration levels. A. Th2 cells; B. Mast cells; C. Cytotoxic cells; D. $CD8^+$ T cells; E. pDC; F. iDC.

progression in patients with ESCA. Inhibiting SKA3 expression effectively disrupts the growth and migration of ESCA cells. These findings, along with other studies, support the role of SKA3 as an oncogene involved in cancer progression, suggesting its potential as a valuable biomarker for ESCA.

SKA3 overexpression is associated with critical biological functions, including cell division, chromosome separation, DNA replication, DNA repair, cell cycle regulation, and mitosis, as indicated by bioinformatic analysis. Inhibiting SKA3 expression significantly reduces ESCA cell proliferation and migration, highlighting its pivotal role in ESCA progression. KEGG pathway analysis further indicated that SKA3 is involved in key cellular processes, such as the cell cycle, DNA replication, and the p53 signaling pathway, all of which are strongly linked to ESCA progression [25-29]. For instance, the overexpression of Ubiquitin-Specific Peptidase 8 (USP8) in ESCC induces cell cycle arrest *via* the p53/p21 signaling axis and promotes apoptosis by modulating p53 target proteins, effectively inhibiting the growth of ESCC cells [27]. Additionally, a study by Hou et al. reported that SKA3 promotes hepatocellular carcinoma (HCC) progression by elevating the phosphorylation levels of CDK2 and p53 [28]. However, the specific relationship between SKA3 and the cell cycle, DNA replication, and p53 signaling pathway remains unexplored and warrants further investigation.

Genomic analysis, tissue sample assays, and cellular experiments have confirmed that SKA3, like other oncogenes, plays a pivotal role in carcinogenesis. The bioinformatic analysis in this study was based on a robust, reliable database, large sample sizes, and accurate results, which were further validated through *in vitro* tissue and cell experiments. However, this study has several limitations. The precise mechanisms by which SKA3 contributes to ESCA progression remain to be fully elucidated. Additionally, the role of SKA3 in regulating the immune microenvironment-potentially through pathways such as p53 signaling-requires further investigation. Overall, our findings position

SKA3 as a key driver of ESCA aggressiveness, linking its overexpression to adverse clinicopathologic features, poor prognosis, and an immunosuppressive microenvironment. The significant suppression of tumor growth and migration following SKA3 inhibition highlights its potential as a novel therapeutic target for halting ESCA progression and metastasis. In conclusion, SKA3 overexpression is strongly associated with an unfavorable prognosis and alterations in the tumor immune microenvironment in patients with ESCA.

Acknowledgements

This work was supported by the Panyu District Science and Technology Plan Project (2024-Z04-036).

Written informed consent was obtained from all participants.

Disclosure of conflict of interest

None.

Address correspondence to: Heng-Lun Liang, Department of Thoracic Surgery, The Affiliated Panyu Center Hospital, Guangzhou Medical University, Guangzhou, Guangdong, China. E-mail: Liangalun@126.com; Jia-Long Guo, Department of Cardiothoracic Surgery, Taihe Hospital, Hubei University of Medicine, Shiyan, Hubei, China. E-mail: GJL9988@126.com

References

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394-424.

[2] Short MW, Burgers KG and Fry VT. Esophageal cancer. Am Fam Physician 2017; 95: 22-28.

[3] Huang FL and Yu SJ. Esophageal cancer: risk factors, genetic association, and treatment. Asian J Surg 2018; 41: 210-215.

[4] Malhotra GK, Yanala U, Ravipati A, Follet M, Vijayakumar M and Are C. Global trends in esophageal cancer. J Surg Oncol 2017; 115: 564-579.

[5] Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ and He J. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-32.

[6] Orazbayev BA, Musulmanbekov K and Bukenov A. Analysis of treatment results of the thoracic part of oesophageal cancer. Open Access Maced J Med Sci 2019; 7: 82-87.

[7] Gaitanos TN, Santamaria A, Jeyaprakash AA, Wang B, Conti E and Nigg EA. Stable kinetochore-microtubule interactions depend on the Ska complex and its new component Ska3/C13Orf3. EMBO J 2009; 28: 1442-52.

[8] Zhang J, Liu Y, Pu S, He J and Zhou C. Spindle and kinetochore-associated complex subunit 3 accelerates breast cancer cell proliferation and invasion through the regulation of Akt/Wnt/β-catenin signaling. Breast Cancer Res Treat 2021; 186: 247-258.

[9] Ruan LW, Li PP and Jin LP. SKA3 promotes cell growth in breast cancer by inhibiting PLK-1 protein degradation. Technol Cancer Res Treat 2020; 19: 1533033820947488.

[10] Zhang C, Zhao S, Tan Y, Pan S, An W, Chen Q, Wang X and Xu H. The SKA3-DUSP2 axis promotes gastric cancer tumorigenesis and epithelial-mesenchymal transition by activating the MAPK/ERK pathway. Front Pharmacol 2022; 13: 777612.

[11] Wang C, Liu S, Zhang X, Wang Y, Guan P, Bu F, Wang H, Wang D, Fan Y, Hou S and Qiu Z. SKA3 is a prognostic biomarker and associated with immune infiltration in bladder cancer. Hereditas 2022; 159: 20.

[12] Chen C, Guo Q, Song Y, Xu G and Liu L. SKA1/2/3 serves as a biomarker for poor prognosis in human lung adenocarcinoma. Transl Lung Cancer Res 2020; 9: 218-231.

[13] Hu R, Wang MQ, Niu WB, Wang YJ, Liu YY, Liu LY, Wang M, Zhong J, You HY, Wu XH, Deng N, Lu L and Wei LB. SKA3 promotes cell proliferation and migration in cervical cancer by activating the PI3K/Akt signaling pathway. Cancer Cell Int 2018; 18: 183.

[14] Pang H, Zhou Y, Wang J, Wu H, Cui C and Xiao Z. SKA3 overexpression predicts poor outcomes in skin cutaneous melanoma patients. Transl Oncol 2022; 15: 101253.

[15] Hu DD, Chen HL, Lou LM, Zhang H and Yang GL. SKA3 promotes lung adenocarcinoma metastasis through the EGFR-PI3K-Akt axis. Biosci Rep 2020; 40: BSR20194335.

[16] Tang J, Liu J, Li J, Liang Z, Zeng K, Li H, Zhao Z, Zhou L and Jiang N. Upregulation of SKA3 enhances cell proliferation and correlates with poor prognosis in hepatocellular carcinoma. Oncol Rep 2021; 45: 48.

[17] Li C, Yang J, Lei S and Wang W. SKA3 promotes glioblastoma proliferation and invasion by enhancing the activation of Wnt/β-catenin signaling via modulation of the Akt/GSK-3β axis. Brain Res 2021; 1765: 147500.

[18] Feng D, Zhang F, Liu L, Xiong Q, Xu H, Wei W, Liu Z and Yang L. SKA3 serves as a biomarker for poor prognosis in kidney renal papillary cell carcinoma. Int J Gen Med 2021; 14: 8591-8602.

[19] Guo Q, Peng Y, Yang H and Guo J. Prognostic nomogram for postoperative patients with gastroesophageal junction cancer of no distant metastasis. Front Oncol 2021; 11: 643261.

[20] Xiang QM, Jiang N, Liu YF, Wang YB, Mu DA, Liu R, Sun LY, Zhang W, Guo Q and Li K. Overexpression of SH2D1A promotes cancer progression and is associated with immune cell infiltration in hepatocellular carcinoma via bioinformatics and in vitro study. BMC Cancer 2023; 23: 1005.

[21] Guo Q, Ke XX, Liu Z, Gao WL, Fang SX, Chen C, Song YX, Han H, Lu HL and Xu G. Evaluation of the prognostic value of STEAP1 in lung adenocarcinoma and insights into its potential molecular pathways via bioinformatic analysis. Front Genet 2020; 11: 242.

[22] Wang M, Sun X, Xin H, Wen Z and Cheng Y. SPP1 promotes radiation resistance through JAK2/STAT3 pathway in esophageal carcinoma. Cancer Med 2022; 11: 4526-4543.

[23] Guo Q, Liu XL, Jiang N, Zhang WJ, Guo SW, Yang H, Ji YM, Zhou J, Guo JL, Zhang J and Liu HS. Decreased APOC1 expression inhibited cancer progression and was associated with better prognosis and immune microenvironment in esophageal cancer. Am J Cancer Res 2022; 12: 4904-4929.

[24] Liu HS, Guo Q, Yang H, Zeng M, Xu LQ, Zhang QX, Liu H, Guo JL and Zhang J. SPDL1 overexpression is associated with the 18F-FDG PET/CT metabolic parameters, prognosis, and progression of esophageal cancer. Front Genet 2022; 13: 798020.

[25] Peake JD, Noguchi C, Lin B, Theriault A, O'Connor M, Sheth S, Tanaka K, Nakagawa H and Noguchi E. FANCD2 limits acetaldehyde-induced genomic instability during DNA replication in esophageal keratinocytes. Mol Oncol 2021; 15: 3109-3124.

[26] Lin J, Yang T, Peng Z, Xiao H, Jiang N, Zhang L, Ca D, Wu P and Pan Q. SLC1A5 silencing inhibits esophageal cancer growth via cell cycle arrest and apoptosis. Cell Physiol Biochem 2018; 48: 397.

[27] Sha B, Sun Y, Zhao S, Li M, Huang W, Li Z, Shi J, Han X, Li P, Hu T and Chen P. USP8 inhibitor-induced DNA damage activates cell cycle arrest, apoptosis, and autophagy in esophageal squamous cell carcinoma. Cell Biol Toxicol 2023; 39: 2011-2032.

[28] Hou Y, Wang Z, Huang S, Sun C, Zhao J, Shi J, Li Z, Wang Z, He X, Tam NL and Wu L. SKA3 Promotes tumor growth by regulating CDK2/P53 phosphorylation in hepatocellular carcinoma. Cell Death Dis 2019; 10: 929.

[29] Zhang W, Yan W, Qian N, Han Q, Zhang W and Dai G. Paired box 5 increases the chemosensitivity of esophageal squamous cell cancer cells by promoting p53 signaling activity. Chin Med J (Engl) 2022; 135: 606-618.