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ORIGINAL RESEARCH

Integrative Multi-Omics Analysis of Identified SKA3 as a Candidate Oncogene Correlates with Poor Prognosis and Immune Infiltration in Lung Adenocarcinoma

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Background: Spindle and kinetochore-associated complex subunit 3 (SKA3) plays important roles in promoting the migration and the invasion of various human cancer cells. There are a few studies on SKA3 in lung adenocarcinoma (LUAD), but the in-depth analysis of the expression of SKA3 and the correlated possible immune mechanism of SKA3 in LUAD are not clear.

Methods: In our study, the expression and survival data of SKA3 were analyzed in LUAD using TIMER, Oncomine, UALCAN, cBioPortal, LinkedOmics, Human Protein Atlas, and Kaplan–Meier plotter. Then, quantitative PCR was used to verify the expression differences of SKA3 between LUAD tissues of mice and the normal tissues.

Results: We established that the expression of SKA3 in the LUAD group was remarkably higher than that in the normal group. Additionally, high SKA3 expression was linked to poorer survival in LUAD. Moreover, SKA3 expression had a remarkable negative correlation with the immune infiltration of B cells, macrophages, and CD4+ T cells. SKA3 was markedly negatively related to the immune type biomarkers of T cells and B cells in LUAD. The elevated expression of SKA3 with LUAD in enriched B cells, CD4+ T cells, CD8+ T cells, macrophages and Treg cells had worse prognosis, respectively. Functional network analysis showed that SKA3 regulated the mitotic cell cycle, mitosis, chromosome segregation and cell division via pathways.

Conclusion: In summary, our study suggested that SKA3 was highly expressed in LUAD and SKA3 might function as a prognostic biomarker in LUAD. Besides, SKA3 may be a candidate oncogene, which correlates with poor prognosis and immune infiltration in lung adenocarcinoma.

Keywords: SKA3, prognosis, immune infiltration, LUAD

Introduction

Lung cancer is a worldwide health issue because it is a major cause of cancer-related deaths.[1] Lung adenocarcinoma (LUAD) constitutes the most frequent histological type of lung cancer and can be divided into five types: acinar, squamous, solid, papillary and micropapillary.[1] Because of early metastasis, the prognosis of LUAD is generally poor, and average five-year survival rate is less than 20%.[2] Although there are many treatments for LUAD, such as chemotherapy, radiotherapy and targeted therapy, the tumor is still progressing rapidly and the mortality rate is high.[3] Therefore, finding suitable immune-related markers is very important for the prognosis of LUAD.

The Spindle and kinetochore-associated complex subunit (SKA) complex consists of three subunits, including SKA1, SKA2 and SKA3, which work together. The SKA complex is an important component of mitosis in human cells and

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establishes a stable mitochondrial–microtubule interaction with the Ndc80 network.[4,5] SKA3 regulates the robust dynamic dance of microtubule attachment and mitotic progression in the Ska complex.[6] Studies have shown that overexpression of SKA3 can promote the migration along with the invasion of many different human cancer, such as prostate cancer,[7] colorectal adenoma,[8] cervical cancer,[9] breast cancer,[10,11] hepatocellular carcinoma,[12,13] glioblastoma,[14] laryngeal squamous cell carcinoma[15] and renal cell carcinoma.[16] There are a few studies on SKA3, which is associated with lung cancer metastasis and poor prognosis in patients with LUAD.[17] However, the multi-omics analysis in LUAD of SKA3 remains to be elucidated, and the correlated possible immune mechanism of SKA3 is not clear.

Herein, we analyzed the expression of SKA3 in LUAD in the TIMER and Oncomine databases. Then, we employed the Kaplan–Meier plotter, as well as the PrognoScan web resources to study the prognostic value and clinical characteristics between SKA3 and LUAD. Besides, we employed TIMER to explore the relationship of SKA3 expression with the immune infiltration in LUAD. Our study suggested that SKA3 was highly expressed in LUAD and SKA3 might function as a prognostic biomarker in LUAD. Besides, SKA3 may be a candidate oncogene, which correlates with poor prognosis and immune infiltration in lung adenocarcinoma.

Methods

Microarray Data Collection

Gene Expression Omnibus (GEO) is a public database that can archive microarray and other forms of high-throughput omics data. The expression profiles of GSE13213 and GSE31210 are obtained in GEO database. Gene expression data of LUAD and normal lung tissues in HTSeq-FPKM and clinical information of LUAD samples were achieved from TCGA database.

Oncomine Analysis

Oncomine is an online cancer microarray database (www.oncomine.org).[18] SKA3 mRNA expression in LUAD was explored with the Oncomine. $P < 0.01$, fold change > 1 and gene rank top 10% were considered significant.

The Human Protein Atlas

The Human Protein Atlas (https://www.proteinatlas.org/)[19] had amounts of proteomics and transcriptomics data. In our study, we used The Human Protein Atlas database to explore the protein expression of the SKA3 in cancer tissues and normal lung tissues by immunohistochemistry.

Survival Analysis

We employed the PrognoScan web resource (http://dna00.bio.kyutech.ac.jp/PrognoScan/index.html)[20] along with the Kaplan–Meier plotter (http://kmplot.com/)[21] to analyze the prognosis of SKA3 expression in LUAD. Cox $p < 0.05$ in PrognoScan, logrank $p < 0.05$ in Kaplan–Meier plotter web resource were considered significant.

UALCAN Analysis

UALCAN is a comprehensive online tool for analyzing cancer OMICS data (http://ualcan.path.uab.edu/index.html).[22] We analyzed the promoter methylation profile of SKA3 in UALCAN web resource. We stratified LUAD based on types, stage, nodal metastasis, age, gender and status of smoking.

TIMER Analysis

The TIMER is a comprehensive database, which is able to analyze the immune invasion level of different tumors and the expression of gene in different tumors (https://cistrome.shinyapps.io/timer/).[23] We explored SKA3 expression in LUAD and the relation of SKA3 expression with immune infiltrates (B cells, dendritic cells, CD4+ T cells, macrophages, CD8+ T cells, as well as neutrophils) using the TIMER. Then, we used TIMER database to analyze the type biomarkers of CD8 + T cells, dendritic cells, T cells, macrophages, B cells, as well as neutrophils in LUAD.

C-bioPortal Analysis

We employed the c-BioPortal open resource (http://cbioportal.org)[24] to assess the SKA3 alterations in the TCGA-LUAD samples. The study parameters included mutation, CNVs, and mRNA expression.

LinkedOmics Analysis

We used the LinkedOmics (http://cbioportal.org)[25] to analyze SKA3 co-expression through Pearson's correlation coefficient, showing in volcano plots and heat maps. Then, we analyzed GO, KEGG in webgestalt; meanwhile, we examined target networks of miRNAs by miRDB. The rank criterion constituted FDR < 0.05, and 1000 simulations were performed.

GSEA Analysis

GSEA was performed to clarify the molecular mechanisms of the prognostic gene signature. GSEA was performed in GSEA v. 4.2.2 and was searched to determine the enriched biological processes, cellular components, molecular functions, KEGG pathway associated with survival of the high-risk group. FDR < 0.05 and |NES| > 1 were considered statistically significant.

Establishment of Lung Adenocarcinoma in Nude Mice

AdCre recombinant adenovirus was provided by microbix company. The 6-week-old mice were anesthetized by intraperitoneal injection of 45 mg/kg pentobarbital sodium, and then the co precipitate of AdCre: CaPi (AdCre: CaPi: 50ul AdCre with titer of 2.5 $\times 10^7$pfu added to 69ul MEM, followed by 6ul of 2.5 mol/L calcium chloride) was dripped into the nasal cavity of the mice.[26] About 125 μL was dripped into the mice twice and repeated every 5 days, twice for 42 days. The mice were killed at 42 days after induction, and their lung tissues were paraffin embedded, sectioned and stained with HE. The mice were euthanized according to the review principles stipulated in China's national standards for ethical review of animal welfare. Recommended procedures for CO2 euthanasia: The initial CO2 flow should be constant at 20% to 30% V/min and increase the CO2 flow rate after loss of consciousness.[27] At the same time, CO2 airflow should be maintained for at least 1 minute after clinical death to avoid reversal. At last, cervical dislocation was performed after CO2 euthanasia. Three 6-week-old mice were obtained from the Shanghai Jihui experimental animal breeding Co., Ltd (China) (n = 3 in lung adenocarcinoma group and paracancerous group).

Quantitative Real-Time PCR (RT-qPCR)

We used the Trizol (Invitrogen) to extract total RNA from mice tissues. We used M-MLV Reverse Transcriptase (Promega) reverse transcribed into cDNA. SYBR Premix Ex Taq (TaKaRa) was used in the amplification process on with ABI StepOnePlus real-time PCR system (Applied Biosystems). Actin was used as an internal control. We analyzed the qRT-PCR results with the $2^{-\Delta\Delta Ct}$ method. The primers were as follows: Forward: 5'-GTTAGCACAGACCAGTAGAA-3' and Reverse: 5'-GCCATTAGATAGTTCCAGAATC-3'. All qRT-PCR data were performed with at least 2 repeats.

Statistical Analyses

Data were analyzed in GraphPad Prism 8 software. Student's *t*-test was used to analyze measurement data. Cox regression multivariate analysis was used for survival analysis. $P < 0.05$ was considered to be statistically significant.

Results

The Expression Levels of SKA3 in LUAD

Our study was divided into three parts, including expression, signature and mechanism analysis of SKA3. The details were shown in the workflow (Figure 1A). To determine SKA3 expression in diverse kinds of cancer, we analyzed the SKA3 expression by TIMER and Oncomine database. We established that SKA3 expression was elevated in most cancers in contrast with the non-cancer tissues, such as lung, breast, colorectal, leukemia, ovarian, prostate cancers (Figure 1B and C). Furthermore, the Oncomine data demonstrated that the mRNA expression of SKA3 in LUAD tissues

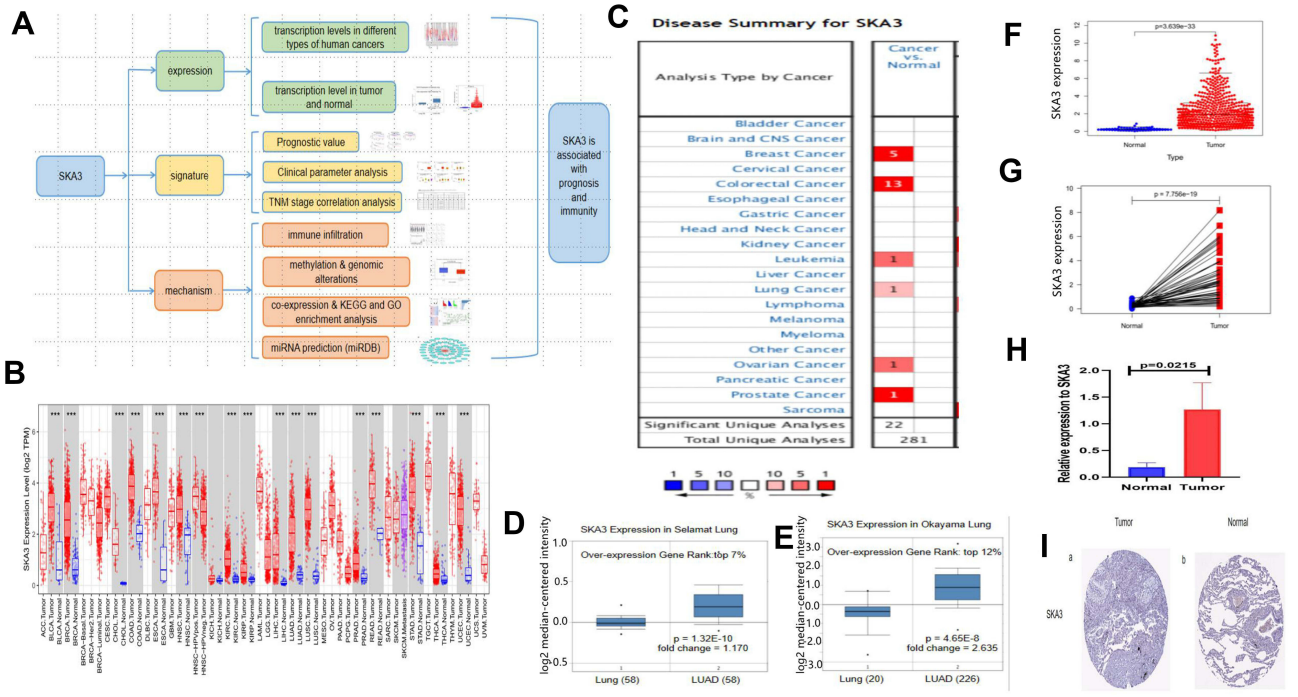

Figure 1 SKA3 expression levels in diverse kinds of human cancers and transcription level in LUAD. (**A**) Workflow of this study. (**B**) SKA3 expression levels in diverse kinds of tumors in TCGA web resource (TIMER). (**C**) Increased or decreased SKA3 in diverse tumors vs healthy tissues in Oncomine web resource. (**D** and **E**) Levels of SKA3 mRNA remarkably higher in LUAD than in healthy tissue. Box plot indicating SKA3 mRNA levels in Selamat lung, Okayama lung by Oncomine. (**F**) The expression levels of SKA3 in tumor and non-cancer tissues of LUAD patients in TCGA database. (**G**) Comparison of SKA3 expression pre-disease and post-disease in the same sample. (**H**) Histogram of the difference between SKA3 expression levels in lung adenocarcinoma tissues of mice and normal lung tissues. (**I**) Immunohistochemical (IHC) staining of SKA3 expression in lung adenocarcinoma samples and normal tissues in the Human Protein Atlas. a and b represent the expression of SKA3 in tumors and normal tissues, respectively. ***, $p < 0.001$.

was remarkably higher in contrast with that in healthy tissues ($P < 0.01$). The fold changes were 1.170 (Selamat lung) and 2.635 (Okayama lung), while the overexpression genes ranked top 7% (Selamat lung) and top 12% (Okayama lung) (Figure 1D and E). Interestingly, data from TCGA still demonstrated that the expression of SKA3 in the LUAD group was remarkably higher in contrast with that in the normal tissues (Figure 1F and G). In addition, qRT-PCR assays revealed that SKA3 was highly expressed in LUAD mice tissues at the mRNA level, but was poorly expressed in adjacent normal LUAD mice tissues. The relative mRNA expression level of SKA3 in cancer tissues was higher than in adjacent normal tissues (Figure 1H, P=0.0215), which was consistent with our bioinformatics analysis. We also compared SKA3 expression with immunohistochemical (IHC) staining in LUAD samples and normal tissues in the Human Protein Atlas. The results showed that there was no significant difference in protein levels, which was worthy of further experimental verification (Figure 1I).

Prognostic Significance of SKA3 in LUAD

Based on the differential expression of SKA3 in LUAD, we further investigated the relationship of the expression of SKA3 with the prognosis. By analyzing the PrognoScan, GSE13213 (225 samples) and GSE31210 (204 samples) presented high SKA3 expression was linked to poor prognosis (PFS HR = 0.42, Cox P = 0.000191; OS HR = 1.24, Cox P = 0.000602; RFS HR = 1.16, Cox P = 0.000006) (Figure 2A–C). To further evaluate and verify the prognostic capacity of SKA3 in LUAD, Kaplan–Meier plotter was used, and the results demonstrated that the high SKA3 expression group was linked to poor LUAD patients prognosis (OS HR = 1.85, P = 2.8e-05; RFS HR = 1.88, P = 0.018) (Figure 2D and E). The results were consistent with these findings of GEO data, which verify the reliability of the results.

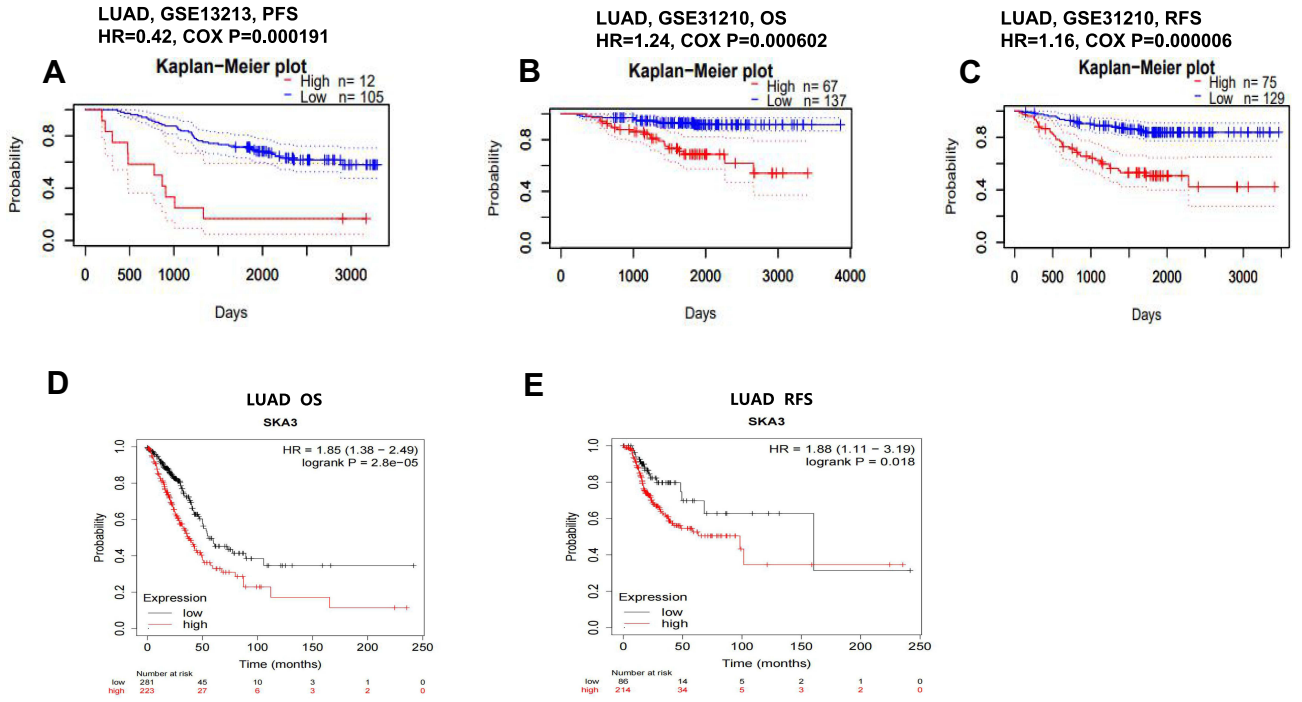

Figure 2 Kaplan–Meier survival curves comparing the high and low expression of SKA3 in LUAD. (**A**–**C**) Survival curves of PFS, OS and RFS in two LUAD cancer cohorts (PrognoScan) [GSE13213 (n = 125) and GSE31210 (n = 204)]. (**D** and **E**) OS and FP survival curves of lung cancer (Kaplan–Meier Plotter). (n = 1114, n = 596).

SKA3 Transcription in Subgroups of LUAD Patients

To further analyze the subgroups of multiple clinical characteristics of SKA3, the expression level of SKA3 in TCGA-LUAD samples was displayed by using the UALCAN web resource. The level of expression of SKA3 in LUAD was higher relative to the non-cancer tissues (Figure 3A, $P < 0.001$). High expression of SKA3 in LUAD was associated with gender, age, race, smoking and stages (Figure 3B–F). Then, we examined the association of SKA3 expression with the clinical features of OS with 316 LUAD patients by univariate analysis and multivariate analysis. The over-expression of SKA3 was linked to worse OS both univariate analysis and multivariate analysis (Table 1, $P < 0.01$). These results indicated that SKA3 maybe a prognostic factor independent of LUAD.

SKA3 Expression is Linked to the Immune Infiltration and Prognostic Value in LUAD

Previous studies have documented that immune infiltration is linked to the prognosis of lung cancer.[28,29] Therefore, we used TIMER web resource to analyze whether SKA3 was linked to the immune invasion level in LUAD. Our findings demonstrated that SKA3 expression had marked negatively relationships with the infiltration levels of B cells (r = −0.222, P = 8.57e-07), CD4+ T cells (r = −0.131, P = 3.83e-03) and macrophages (r = −0.092, P = 4.31e-02) in LUAD (Figure 4A). Interestingly, SKA3 copy number variations (CNV) also had remarkable associations with the invading levels of B cells, CD4+ T cells and macrophages in Figure 4B.

To explore the relationship of the expression of SKA3 with the immune cell markers, we used TIMER database to analyze the type biomarkers of CD8 + T cells, neutrophils, T cells, dendritic cells, B cells, as well as macrophages. The results suggested that SKA3 in LUAD is positively related to CD8A and CD8B in CD8 + T cells. SKA3 in LUAD was negatively related to CD3E and CD2 in T cells. SKA3 in LUAD is negatively related to CD19 and CD79A in B cells. SKA3 in LUAD is positively related to NOS2 and PTGS2 in macrophages (Table 2). The results indicated that SKA3 expressions in LUAD were related to immune infiltration.

We have confirmed that the elevated expression of SKA3 was linked to poor prognosis, and we have also known that SKA3 was associated with immune infiltration. Therefore, we speculate that the SKA3 expression in LUAD affects the prognosis may be because of immune infiltration. Then, we employed the Kaplan Meier plotter to do a prognosis

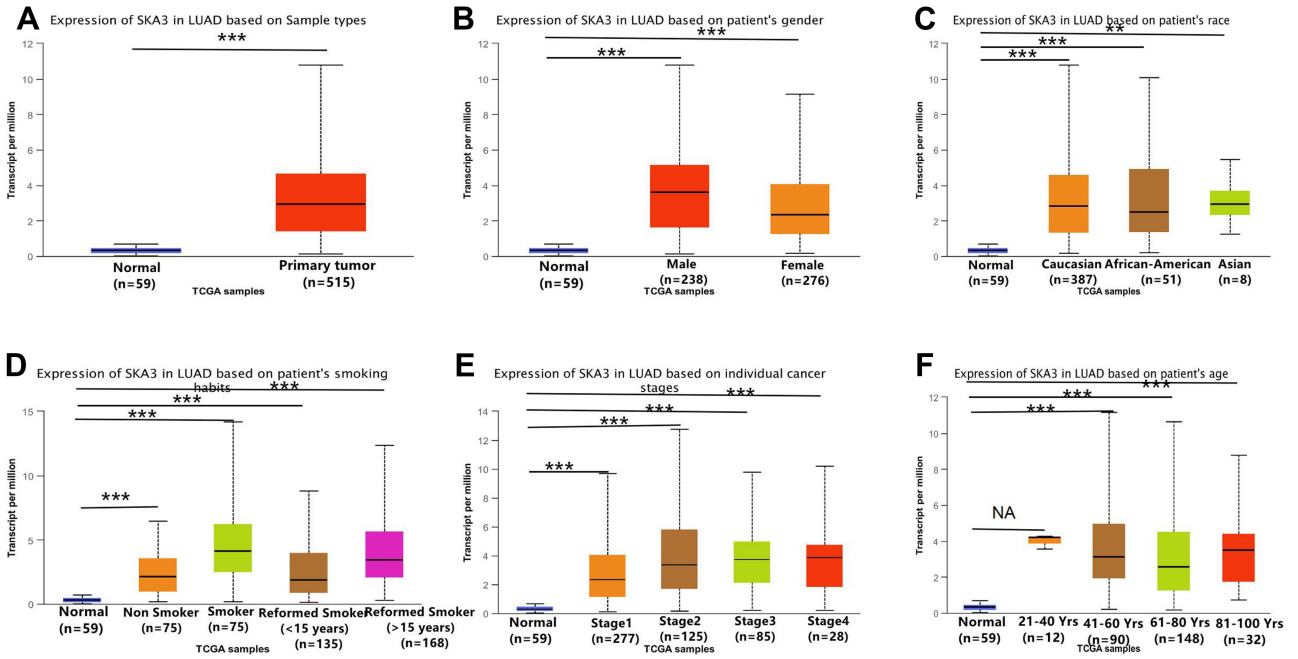

Figure 3 SKA3 transcription in subgroups of LUAD patients, stratified on the basis of types, gender, age, race, smoking and stages (UALCAN). (**A**) Boxplot shows expression of SKA3 in LUAD and normal patients. (**B**) Boxplot shows expression of SKA3 in normal people of either gender or male or female LUAD patients. (**C**) Boxplot shows relative expression of SKA3 in normal people of any ethnicity or Caucasian, African-American or Asian ethnicity in LUAD. (**D**) Boxplot shows relative expression of SKA3 in normal people of either smoker or non-smoker, smoker, reformed smoker (<15 years) or reformed smoker (>15 years). (**E**) Boxplot shows relative expression of SKA3 in normal people or in LUAD patients in stages 1, 2, 3 or 4. (**F**) Boxplot shows relative expression of SKA3 in healthy individuals of any age or in LUAD patients aged 21–40, 41–60, 61–80, or 81–100 years. The edges of the box are the 25th and 75th percentiles. The significance of difference in SKA3 expression between groups by the *t*-test. **, $p < 0.01$; ***, $p < 0.001$.

investigation based on the expression of SKA3 in LUAD correlations with immune cells subgroup. These data demonstrated that the high expression of SKA3 with LUAD in abundant B cells (HR = 1.76), CD4+ T cells (HR = 2.18), CD8+ T cells (HR = 1.99), macrophages (HR = 1.90) and Treg cells (HR = 1.92) had worse prognosis, respectively ($P < 0.05$) (Figure 4C, E, G, I and K). Nevertheless, there is no remarkable relationship between the high SKA3 and the prognosis of LUAD in the enriched Th1 cells (Figure 4M, HR = 1.36, $P = 0.22$). The high expressions of SKA3 of LUAD had significant relationship in decreased B cells (HR = 2.18), decreased CD4+ T cells (HR = 2.75), decreased

Table 1 Univariate Analysis and Multivariate Analysis of the Correlation of SKA3 Expression with OS Among Lung Adenocarcinoma from TCGA

Parameter	Univariate Analysis				Multivariate Analysis			
	HR	HR.95L	HR.95H	p value	HR	HR.95L	HR.95H	p value
Age	1.00	0.98	1.02	0.84	1.01	0.99	1.03	0.18
Gender	1.04	0.72	1.49	0.85	0.94	0.65	1.37	0.76
Stage	1.65	1.40	1.95	**2.58E-09**	1.92	1.20	3.07	**0.01**
T	1.63	1.32	2.02	**8.60E-06**	1.20	0.94	1.53	0.13
M	1.76	0.96	3.20	0.07	0.42	0.13	1.43	0.17
N	1.79	1.46	2.20	**2.41E-08**	0.98	0.66	1.46	0.92
SKA3	1.11	1.05	1.18	**5.07E-04**	1.44	1.15	1.81	**1.68E-03**

Note: Bold values indicate p<0.05.
Abbreviations: HR, hazard ratio; CI, confidence interval.

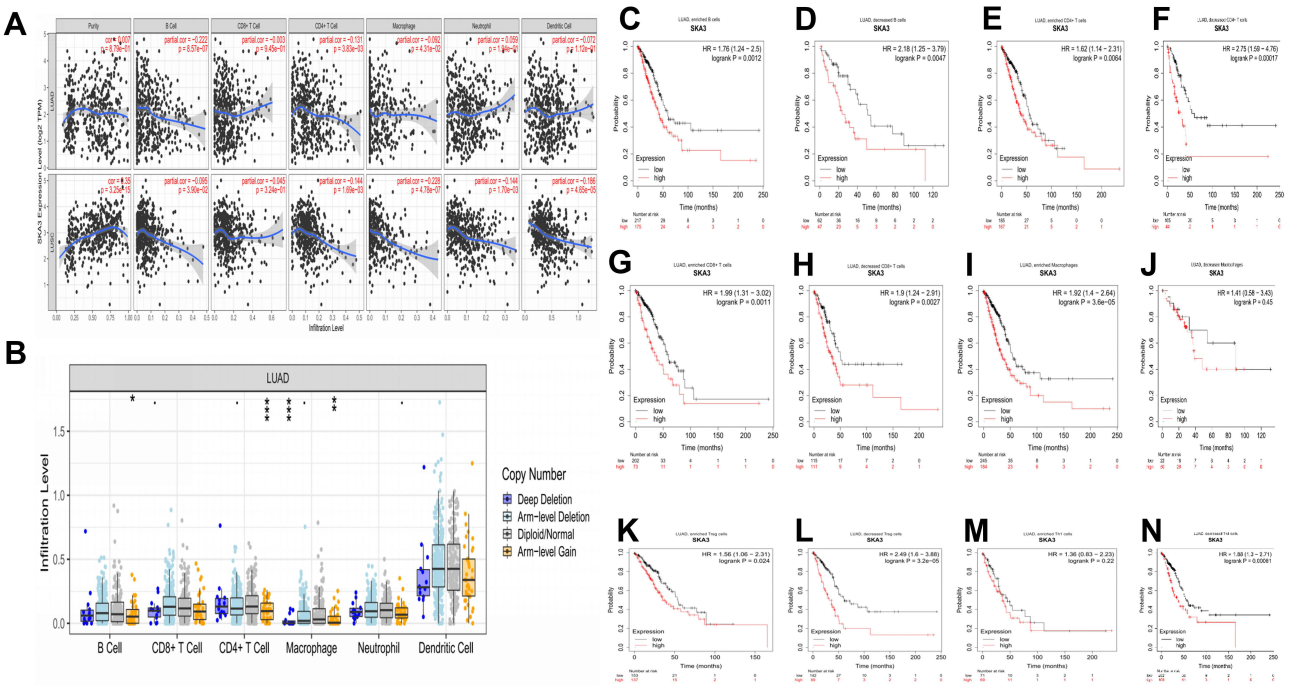

Figure 4 The expression of SKA3 in LUAD was related to immune infiltration and Prognostic value. (**A**) SKA3 expression has remarkable negatively correlations with the infiltration levels of B cells, CD4+ T cells and macrophages in LUAD. (**B**) SKA3 CNV influences the infiltration level of B cells, CD4+ T cells and macrophages in LUAD. P-value Significant Codes: *** < 0.001, ** for p < 0.01, * for p < 0.05. (**C**–**N**) Kaplan Meier analysis of prognostic correlation with different immune cells subgroup in LUAD.

CD8+ T cells (HR = 1.90), decreased Treg cells (HR = 2.49) and decreased Th1 cells (HR = 1.88), respectively (P < 0.05) (Figure 4D, F, H, J, L and N).

The data demonstrated that high SKA3 expression in LUAD may influence the prognosis through immune infiltration.

Promoter Methylation and Genomic Alterations Level of SKA3 in LUAD

In order to find other possible mechanism of SKA3, we analyzed the promoter methylation and genomic alterations of SKA3 in LUAD by UALCAN database. The methylation levels of SKA3 in LUAD were lower than normal tissue (Figure 5A, P = 5.91E-02). In addition, we stratified LUAD based on types, stage, nodal metastasis, age, gender and smoking status. These results showed that SKA3 methylation levels of the stage (normal relative to stage 3, normal relative to stage 4), nodal metastasis (normal in contrast with N2), age (normal compared with 40–60 years, normal relative to 61–80 years), gender (normal vs male) and smoking status (normal vs non-smoker, normal vs smoker) were lower than normal in LUAD (Figure 5B–F, P < 0.05). Nonetheless, the methylation levels of SKA3 had no remarkable associations in other different subgroups of LUAD.

In order to further explore the mechanism of SKA3, we investigated the frequency and types of SKA3 alterations according to the TCGA-LUAD database by the cBioPortal. SKA3 was altered about 1.9% in LUAD patients (Supplementary Figure 1A). Regardless of smoking history, the proportion of people in the altered group of SKA3 was less than that in the non-altered group of SKA3 (Supplementary Figure 1B). Mutation frequency of SKA3 in the altered group was higher in contrast with the non-altered group at any mutation point (Supplementary Figure 1C). CNA and mutation frequency level with different datasets were shown in Supplementary Figure 1D. The results suggested that promoter methylation and genomic alterations may have little effect on SKA3.

Enrichment Analysis of SKA3 Networks in LUAD

To analyze SKA3 biological meaning in LUAD, we examine SKA3 co-expression mode in LUAD cohort from the TCGA by Function module of LinkedOmics. In Figure 6A, 4844 genes (dark red dots) exhibited remarkable positive associations with SKA3, while 6698 genes (dark green dots) showed remarkable negative relationships (false discovery

Table 2 Correlation Analysis Between SKA3 and Immune Cell Type Markers in TIMER Database

Description	Gene Markers	COAD			
		Tumor		Normal	
		COR	P	COR	P
CD8+ T cell	CD8A	0.098	0.03	0.23	0.086
	CD8B	0.12	0.0096	0.24	0.063
T cell	CD3E	−0.093	0.041	0.3	0.0022
	CD2	−0.086	0.06	0.25	0.053
B cell	CD19	−0.12	0.0091	0.23	0.086
	CD79A	−0.15	0.00087	0.24	0.062
Macrophage	INOS (NOS2)	0.091	0.047	−0.014	0.92
	IRF5	0.022	0.63	0.19	0.14
	COX2 (PTGS2)	0.087	0.57	−0.071	0.59
Dendritic cell	HLA-DQB1	−0.27	1.9E-09	0.053	0.69
	HLA-DRA	−0.32	2.8E-13	0.19	0.15
	HLA-DPA1	−0.32	1.3E-12	0.018	0.89
	BDCA-1 (CD1C)	−0.5	2.6E-31	0.34	0.0076
Neutrophils	CD66b (CEACAM8)	0.39	4.1E-18	−0.056	0.68
	CD11b (ITGAM)	−0.15	0.001	0.19	0.16
	CCR7	−0.23	2.3E-07	0.45	3E-04

Abbreviations: LUAD, lung adenocarcinoma; COR, R value of Spearman correlation.

rate [FDR] < 0.01). The top 50 positive and negative genes related to SKA3 were shown in the heatmap (Figure 6B and C). Significant Gene Ontology (GO) analysis of the top 15 positive co-expression genes showed that they were related to mitotic cell cycle, mitosis, chromosome segregation and cell division (webgestalt) (Figure 6D). KEGG pathway analysis showed enrichment in the cell cycle, progesterone mediated oocyte maturation, as well as oocyte meiosis cascades (Figure 6E). Of interest, KEGG and GO of GSEA also showed that SKA3 was associated with the cell cycle, pathways in cancer, p53 signaling pathway and meiotic cell cycle in LUAD (Supplementary Figure 2). From the result of Figure 6B, we selected top three positive co-expression genes. SKA3 expression was positively linked to the expression of CCNA2 (Pearson correlation = 0.91, $P < 0.01$), HJURP (Pearson correlation = 0.90, $P < 0.01$) and CDCA5 (Pearson correlation = 0.90, $P < 0.01$) (Supplementary Figure 3A–C). To further explore the targets of SKA3 in LUAD, we predicted the miRNA-target of SKA3 by miRDB. The result showed that it had 54 miRNA-target of SKA3 and which was visualized in Supplementary Figure 3D. The results may provide a potential regulatory network of SKA3 in LUAD.

Discussion

Although the treatment of lung cancer has improved, it is still one of the most malignant types of cancer, with a poor 5-year survival rate.[30] To help with treatment, we need to identify new biomarkers and study their molecular mechanisms. In recent years, with the rise of immunotherapy, it is gradually considered as a promising strategy for the treatment of cancer.[31] At present, the research on immunotherapy strategy of LUAD mainly focuses on radiotherapy, chemotherapy, immune checkpoint inhibitor, cancer vaccine, combination with other immunotherapeutic drugs.[32] However, only

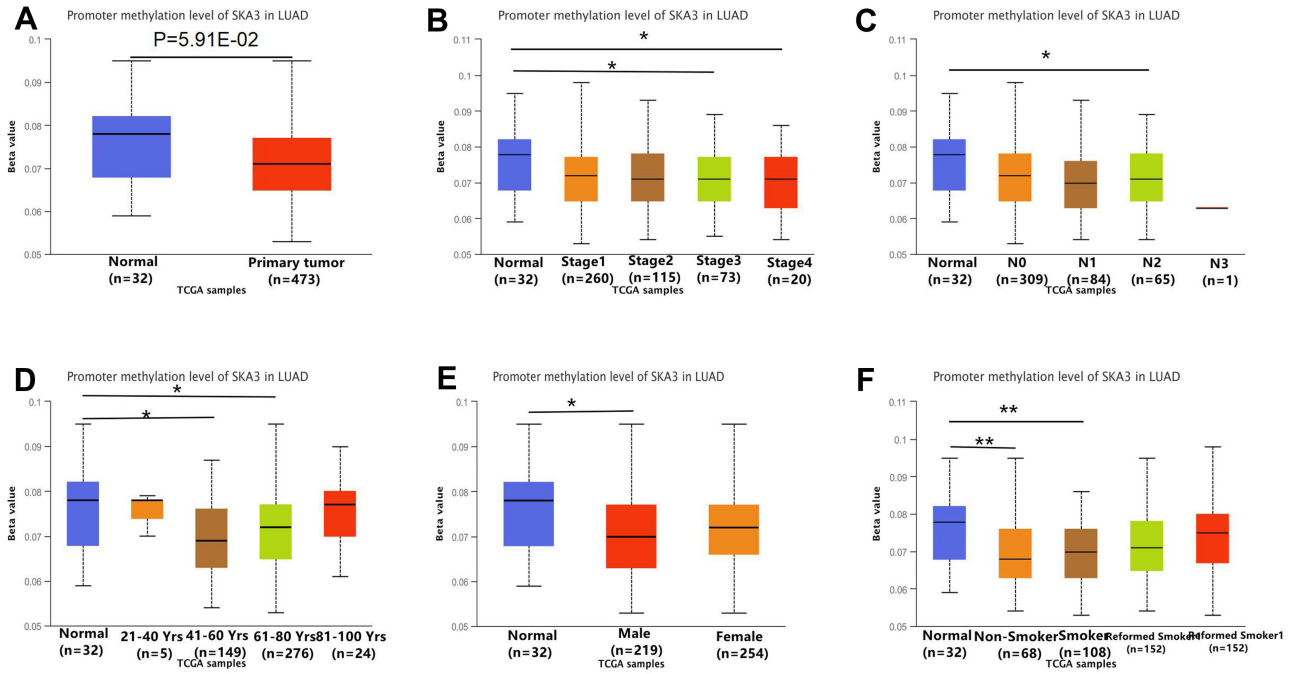

Figure 5 SKA3 promoter methylation level in LUAD, stratified based on types, stage, nodal metastasis, age, gender and smoking (UALCAN). (**A**) DNA methylation and mRNA expression of SKA3 from TCGA. (**B**) SKA3 promoter methylation profile based on individual cancer stages. (**C**) SKA3 promoter methylation profile based on nodal metastasis status. (**D**) SKA3 promoter methylation profile based on patients' gender. (**E**) SKA3 promoter methylation profile based on patients' age. (**F**) SKA3 promoter methylation profile based on smoking status. The difference of SKA3 expression among groups through the *t*-test. *, $p < 0.05$; **, $p < 0.01$.

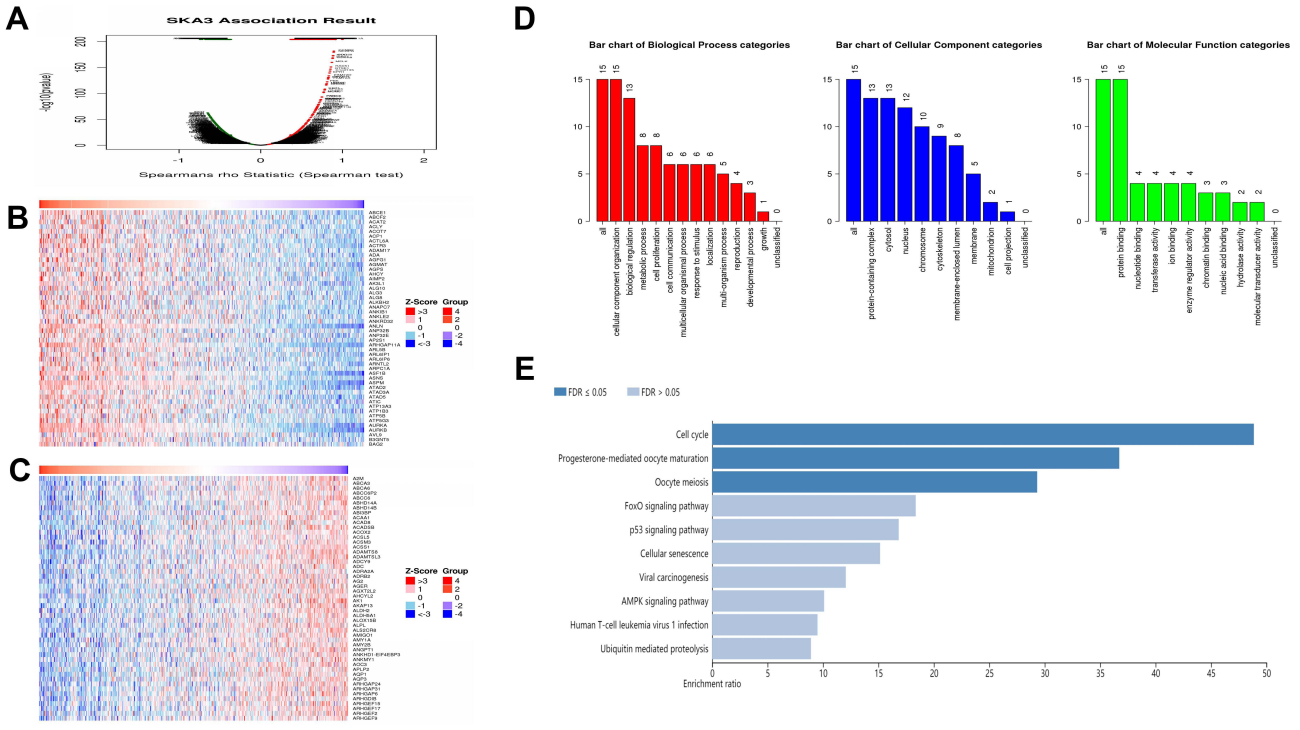

Figure 6 SKA3 co-expression genes in LUAD. (**A**) Identification of highly related genes of SKA3 by Pearson test in LUAD cohort (LinkedOmics). (**B** and **C**) Heat maps showed that the top 50 genes were positively and negatively linked to with SKA3 in LUAD. Red represents positive correlation gene, blue represents negative correlation gene (LinkedOmics). (**D** and **E**) Remarkably enriched GO annotations, as well as KEGG pathways correlated with SKA3 showing top 15 genes positively in LUAD cohort (webgestalt).

a small percentage of patients respond well to the treatment. At present, the key is to find a new biomarker, which is related to prognosis and immune infiltration, and may respond to the effectiveness of immunotherapy.

In our study, the expression of SKA3 in LUAD was analyzed by TIMER and Oncomine. We found the relationship of SKA3 expression levels with the prognoses of LUAD. Besides, we provided a possible mechanism that the elevated SKA3 expression in LUAD may influence the prognosis through immune infiltration.

Previous researchers had reported that SKA3 was highly expressed in LUAD, which was associated with poor prognosis and lymph node metastasis.[33] Our studies showed that SKA3 was elevated in LUAD through the TIMER database (Figure 1). Then, we investigated the effect of SKA3 on LUAD prognosis by the Kaplan Meier plotter and PrognoScan (Figure 2). In addition, we found that over-expression of SKA3 was linked to worse OS in both univariate analysis and multivariate analysis (Table 1). Our results were consistent with their results, suggesting that SKA3 may be an independent prognostic factor for LUAD. And SKA3 may be a novel therapeutic target, which could inhibit the metastasis of LUAD.

Previous studies had shown that the immune microenvironment is very important for tumor growth and development. Tumor cells are involved in the regulation of TME components and the progress of various stages of tumorigenesis.[34] Tumor immune cells, such as CD4 + T cells, CD8 + T cells, tumor associated macrophages and B cells, are major components of lung cancer microenvironment.[35] High expression of C-type collect domain family 3 member B (CLEC3B) was related to good overall survival, which may be related to the immune infiltration, as well as immune activation of lung cancer.[36] Many studies had reported that immune invasion was associated with the prognosis of lung cancer. We analyzed SKA3 expression correlation with immune infiltration and prognostic value in LUAD using TIMER and the Kaplan Meier plotter. Our results suggested that SKA3 expression has remarkable negative correlations with the infiltration levels of B cells, CD4+ T cells and macrophages in LUAD (Figure 4A). We investigated the relationship between the expression of SKA3 and immune cell markers through TIMER web resource. Our results demonstrated that SKA3 is negatively related to CD19 and CD79A in B cells of LUAD. And SKA3 was positively related to NOS2 and PTGS2 in macrophages of LUAD. SKA3 is negatively related to CD3E and CD2 in T cells of LUAD (Table 2). Studies have confirmed that it is a common receptor of B cell antigen receptor complex (BCR). CD19 can reduce the threshold of downstream signal pathway activation and trigger B cell response to antigen.[37] The study also demonstrated that the increased expression of CD79A protein was associated with good prognosis of LUAD patients.[38] This is consistent with our results. Previous studies showed that the long allele of NOS2 gene polymorphism is associated with a reduced risk of lung cancer, especially among non-smokers.[39] Previous studies have shown that they have determined the tumorigenic effect of CD2 on the growth of Lewis lung cancer cells transplanted in C57/BL6 mouse. The results showed that CD2 + cells could effectively accelerate tumor growth in vivo.[40] Our results also suggest that CD2 and CD3E promote tumor progression. These confirmed a strong correlation between SKA3 and a variety of immunomarkers in LUAD. Prognosis assessment based on the expression of SKA3 in LUAD had correlations with immune cells subgroup. These results showed that the elevated expression of SKA3 with LUAD in enriched B cells, enriched CD4+ T cells, enriched CD8+ T cells, enriched macrophages and Treg cells had worse prognosis, respectively (Figure 4C, E, G, I and K). These data implied that high SKA3 expression in LUAD may affect the prognosis through immune infiltration.

A study reported that genomic alteration occurs frequently in human cancer, such as somatic mutations. Methylation contributes to the regulation of gene expression, DNA replication and DNA repair.[41,42] In our study, we analyzed the promoter methylation and genomic alterations of SKA3 in LUAD by UALCAN database. We stratified LUAD based on types, stage, nodal metastasis, age, gender and smoking status (Figure 5). However, the results suggested that promoter methylation and genomic alterations may have little effect on SKA3 expression.

SKA3 is located in the spindle microtubules and outer kinetochore, and its exhaustion in human cells led to mitotic arrest.[43–45] SKA3, phosphorylated by CDK1, binds to Ndc80 and recruits SKA to the centromere to promote mitosis.[46,47] GO terms enriched in 15 positive co-expressed genes of SKA3, GO terms showed that they were related to mitotic cell cycle, mitosis, mitosis, chromosome segregation and cell division. KEGG pathway analysis showed enrichment in the cell cycle, progesterone mediated oocyte maturation, as well as oocyte meiosis cascades. The findings were consistent with the functional of SKA3 that it was associated with mitosis arrest or mitosis progression. It helps to understand the changes in the cell cycle that are essential for ensuring transcription, leading to major dysfunction and cancer.

Recent study revealed that miRNA-455-3p can directly regulate SKA3, and miRNA-455-3p/SKA3 axis led to cancer progression.[16] Our result showed that it had 54 miRNA-target of SKA3. Combined with previous studies, we think that miRNA-455-3p/SKA3 may be another mechanism of LUAD. Further research should test and verify this hypothesis.

Studies have shown that the expression of SKA3 is dysregulated in various tumors and is a key regulator of tumor progression. Overexpression of SKA3 upregulated the activation of Wnt/β-catenin signaling.[16] Brain metastasis of breast cancer is a pivotal cause of morbidity, as well as mortality in breast cancer patients. SKA3 is related to the OS of breast cancer patients.[10,48] SKA3 promotes cancer progression by regulating CDK2/P53 phosphorylation in hepatocellular carcinoma (HCC).[10,48] SKA3 promotes cell cycle progression in cervical cancer (CC) by activating the PI3K/Akt pathway.[9] Most studies have shown that SKA3 over-expression has a poor prognosis for a variety of different human tumor, such as LUAD; However, the correlated possible immune mechanisms of SKA3 in LUAD are not clear. Herein, we discovered that SKA3 may influence the prognosis of LUAD through a new mechanism of immune infiltration. Nevertheless, our study also had several limitations, first, because of the database, we cannot further explore the in-depth relationship between SKA3 and tumor infiltration. Secondly, in our study, in vitro and in vivo experiments are needed for further verification.

Conclusion

In summary, our study suggested that SKA3 was highly expressed in LUAD and SKA3 might function as a prognostic biomarker in LUAD. Besides, SKA3 may be a candidate oncogene, which correlates with poor prognosis and immune infiltration in lung adenocarcinoma.

Abbreviations

LUAD, lung adenocarcinoma; PFS, progression-free survival; SKA3, spindle and kinetochore-associated complex subunit 3; OS, overall survival; CI, confidence intervals; RFS, relapse free survival; CNV, Copy-Number Variance; HR, hazard ratio; TCGA, The Cancer Genome Atlas.

Ethics Approval and Consent to Participate

This study was approved by the Experimental Animal Ethics Committee, Suzhou Institute of Biomedical Engineering and Technology, Chinese Academy of Sciences [Approval Number: 2021-B25]. The study was conducted in accordance with the International Council for Laboratory Animal Science (ICLAS) and Measures of Jiangsu Province for the Administration of Animal Experiments.

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Disclosure

The authors declare that they have no competing interests.

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