Journal of Pharmaceutical and Medical Research
Print ISSN: 2663-1954
Online ISSN: 2663-1962
DOI: https://doi.org/10.61784/jpmr3061

EXPRESSION AND CLINICAL SIGNIFICANCE OF GLYCEROL KINASE (GK) IN ESOPHAGEAL CARCINOMA

Yuan Fang[1], ZhenYu Xue[2], Jian Guo[3], Wen Sun[2], Xiang Tang[1*]
[1]*Department of Abdominal Oncology, Affiliated Hospital of Jiangsu University, Zhenjiang 212001, Jiangsu, China.*
[2]*Department of Head, Neck and Comprehensive Oncology, Affiliated Hospital of Jiangsu University, Zhenjiang 212001, Jiangsu, China.*
[3]*Department of Pathology, Affiliated Hospital of Jiangsu University, Zhenjiang 212001, Jiangsu, China.*
[*]*Corresponding Author: Xiang Tang*

Abstract: Objective: This study aimed to investigate the expression of glycerol kinase (GK) in esophageal carcinoma tissues and cell lines, and to explore its correlation with clinical characteristics and patient prognosis. Methods: Public databases (TCGA, GTEx) and clinical specimens were used to validate GK expression in esophageal carcinoma tissues. The KYSE150 cell line was selected for GK knockdown experiments. Cell proliferation and migration were assessed using CCK-8 and Transwell assays, respectively. R language was employed to analyze correlations between GK expression and immune checkpoint molecules, as well as the tumor immune microenvironment. Results: GK mRNA and protein expression levels were significantly elevated in esophageal carcinoma tissues compared to adjacent normal tissues. Patients with high GK expression had significantly shorter overall survival than those with low expression. Clinical analysis revealed that high GK expression was strongly associated with distant metastasis and tumor recurrence. In vitro experiments demonstrated that GK knockdown suppressed proliferation and migration of esophageal carcinoma cells. Bioinformatic analysis indicated that high GK expression correlated with upregulation of immune checkpoint molecules (e.g., CD274, CTLA4, LAG3) and reduced infiltration of CD8+ T cells. Conclusion: GK is highly expressed in esophageal carcinoma and promotes malignant progression by enhancing tumor cell proliferation, migration, and participation in an immunosuppressive microenvironment. GK may serve as a potential prognostic biomarker and therapeutic target.
Keywords: GK; ESCA; Immune microenvironment; Survival prognosis

1 INTRODUCTION

According to the latest Global Burden of Disease Study, malignant tumors have become the second leading cause of death globally. In 2023, there were 25.7 million new cancer cases and 10.4 million deaths worldwide[1]. Esophageal carcinoma is one of the most common malignancies, with high incidence and mortality rates. In China, there were 320,000 new cases and over 260,000 deaths in 2023, ranking fifth among all malignancies[2]. Notably, the histopathological types of esophageal carcinoma differ significantly between China and Western countries, with squamous cell carcinoma predominating in China and adenocarcinoma in the West[3]. Although multidisciplinary treatments have improved survival rates, the five-year survival rate for advanced esophageal carcinoma remains below 30%[4]. Due to the insidious onset of the disease, most patients are diagnosed at stage III or later, highlighting the importance of early diagnosis and treatment. However, reliable biomarkers for screening, diagnosis, and prognosis are limited [5]. Due to the insidious onset of the disease, most patients are diagnosed at stage III or later, highlighting the importance of early diagnosis and treatment. However, reliable biomarkers for screening, diagnosis, and prognosis are limited.

Glycerol kinase (GK) is a key metabolic enzyme that plays a central role in glycerol metabolism[6]. As a protein-coding gene, GK is expressed in multiple organs, with particularly high expression levels in the kidney, esophagus and liver [7]. The primary function of GK is to catalyze the glycerol phosphorylation reaction, converting glycerol into glycerol-3-phosphate[8, 9]. This process requires the participation of adenosine triphosphate, and the reaction products are adenosine diphosphate and glycerol-3-phosphate [10]. This reaction is a key step in the glycerol entry into the metabolic pathway, providing precursor substances for the synthesis of glycerol, lipids, proteins, and glucose [11, 12] .

As a pivotal enzyme in energy metabolism, glucose kinase (GK) plays a crucial role in maintaining the homeostasis of glucose and lipid metabolism in the human body. Alterations in GK expression can disrupt this balance, triggering various diseases. Numerous studies have shown its involvement in the pathogenesis of multiple human diseases. Some scholars have reported that GK participates in the occurrence of insulin resistance and type 2 diabetes by affecting the sensitivity of gluconeogenesis pathway and insulin signaling pathway [13, 14]. In addition, some scholars have reported that mutations in the GK gene can lead to severe hypertriglyceridemia [14]. The significant role of lipid metabolism reprogramming in tumors is receiving increasing attention. Currently, numerous scholars have reported that key enzymes involved in lipid metabolism, such as sterol regulatory element-binding proteins, stearoyl-CoA desaturases, and carnitine palmitoyl transferase, exhibit elevated expression levels in tumors and are associated with poor tumor prognosis [15-17].However，the role of GK in esophageal carcinoma remains unclear.

This study integrated public databases and clinical samples to analyze GK expression in Esophageal Carcinoma (ESCA)

and its association with clinical features and survival outcomes. Cellular experiments further investigated the functional impact of GK on ESCA cells. The results provide preliminary evidence for GK as a novel predictive marker and therapeutic target in ESCA.

2 MATERIALS AND METHODS

2.1 Clinical Sample Collection and Processing

Tumor and adjacent normal tissues were collected from 60 ESCC patients diagnosed between August 2020 and December 2025 at the Affiliated Hospital of Jiangsu University. Tissues were rinsed with PBS and stored at -80°C. The study was approved by the hospital's ethics committee (KY2023K0701).

2.2 Immunohistochemistry (IHC) and GK Expression Scoring

Tissue sections were fixed, embedded, sliced, and subjected to IHC staining. Two pathologists independently scored GK expression based on staining intensity and area. Scores were multiplied to obtain a total score, and patients were divided into low- and high-expression groups based on the median score.

2.3 Cell Culture

KYSE150 cells (purchased from Shanghai Zhongqiao Xinzhou Biotechnology) were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin at 37°C with 5% CO_2.

2.4 Cell Transfection

Cells were transfected with small interfering RNA (Si-GK) using Lipofectamine 2000. Si-GK sequences were designed and synthesized by Shanghai GenePharma.

2.5 Quantitative Real-Time PCR

Total RNA was extracted from tissues and cells, reverse-transcribed to cDNA, and amplified using SYBR Green. GK mRNA levels were normalized to β-actin and calculated using the 2^(-ΔΔCt) method. Primer sequences (β-actin, forward primer -5-TCACCCACACTGTGCCCATCTACGA-3, reverse primer 5-CAGCGGAACCGCTCATTGCCAATGG-3, GK, forward primer, 5-GAAGGAGTCGGCGTATGGAG-3, reverse primer 5-AGGGTCACCACTTTCTGGAG-3.

2.6 Western Blotting

Proteins were separated by SDS-PAGE, transferred to membranes, and incubated with anti-GK (1:1000, Chengdu Zhengneng Biotechnology) and anti-β-actin (1:1000, Santa Cruz) antibodies. Band intensities were quantified using Image J.

2.7 CCK-8 Assay

Cells were seeded in 96-well plates, and OD values were measured at 450 nm after CCK-8 incubation. Cell viability was calculated as: (OD_experimental - OD_blank) / (OD_control - OD_blank) × 100%.

2.8 Transwell Assay

Cells were suspended in serum-free medium and seeded into Transwell chambers. Migrated cells were fixed, stained, and counted under a microscope.

2.9 Statistical Analysis

Data were analyzed using GraphPad Prism 9.02. Results are presented as mean ± SD. Differences between groups were assessed using t-tests or chi-square tests, with $p < 0.05$ considered statistically significant.

3 RESULTS

3.1 GK Expression Profile in Pan-Cancer Analysis

Pan-cancer analysis of TCGA data revealed significant differences in GK expression across 33 cancer types**.** The expression level of GK exhibits significant differences in nine types of human tumors, including glioblastoma multiforme (GBM), esophageal carcinoma (ESCA), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), breast invasive carcinoma (BRCA), kidney chromophobe carcinoma (KICH), kidney renal clear cell carcinoma (KIRC), and kidney renal papillary cell carcinoma (KIRP)(Figure 1A). After integrating GTEx data, GK

expression remained significantly different only in GBM, ESCA, and LUSC (Figure 1B).

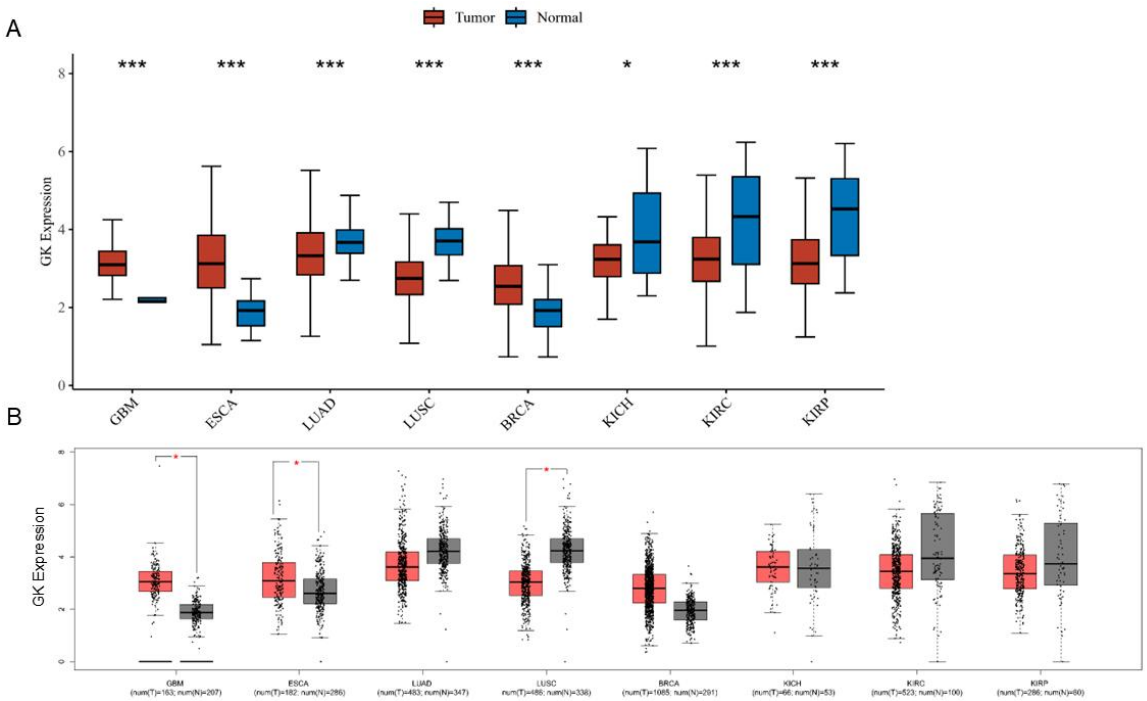

Figure 1 The Expression of GK in Pan-cancer (A, Boxplot comparing the expression of GK in 33 tumor tissues and adjacent non-tumor tissues from the TCGA database. B, Boxplot comparing the expression of GK in the TCGA+GTEx database).

3.2 Survival Analysis Based on TCGA Data

Cox univariate analysis showed a significant negative correlation between GK expression and overall survival in ESCA (Figure 2A). Kaplan-Meier analysis confirmed that high GK expression was associated with shorter survival (mOS: 1.8 years vs. 3.5 years for low expression) (Figure 2C). No significant correlation was found for GBM or LUSC (Figures 2B, D).

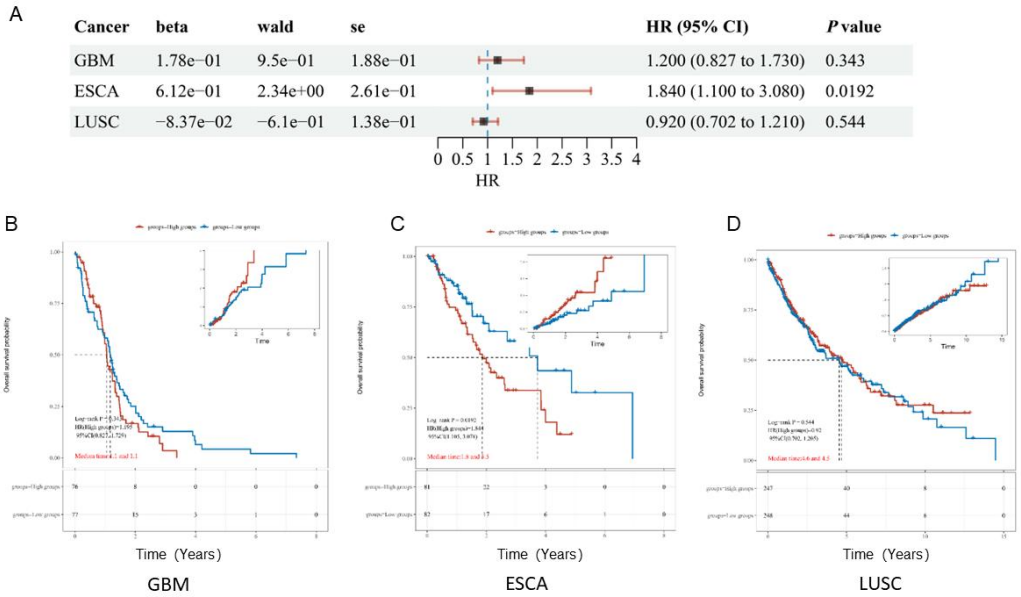

Figure 2 Conduct Survival Analysis of GK, GBM, LUSC, and ESCA Based on the TCGA Database (A, Cox univariate analysis comparing the impact of GK on the survival of GBM, LUSC, and ESCA. B-D, Kaplan-Meier survival analysis of the relationship between GK and overall survival rates in GBM, LUSC, and ESCA).

3.3 GK Expression in Clinical Samples and Prognostic Analysis

IHC and qPCR confirmed higher GK expression in tumor tissues compared to normal tissues (Figures 3A-B). Survival analysis showed that high GK expression reduced mOS by 9.5 months (Figure 3C).

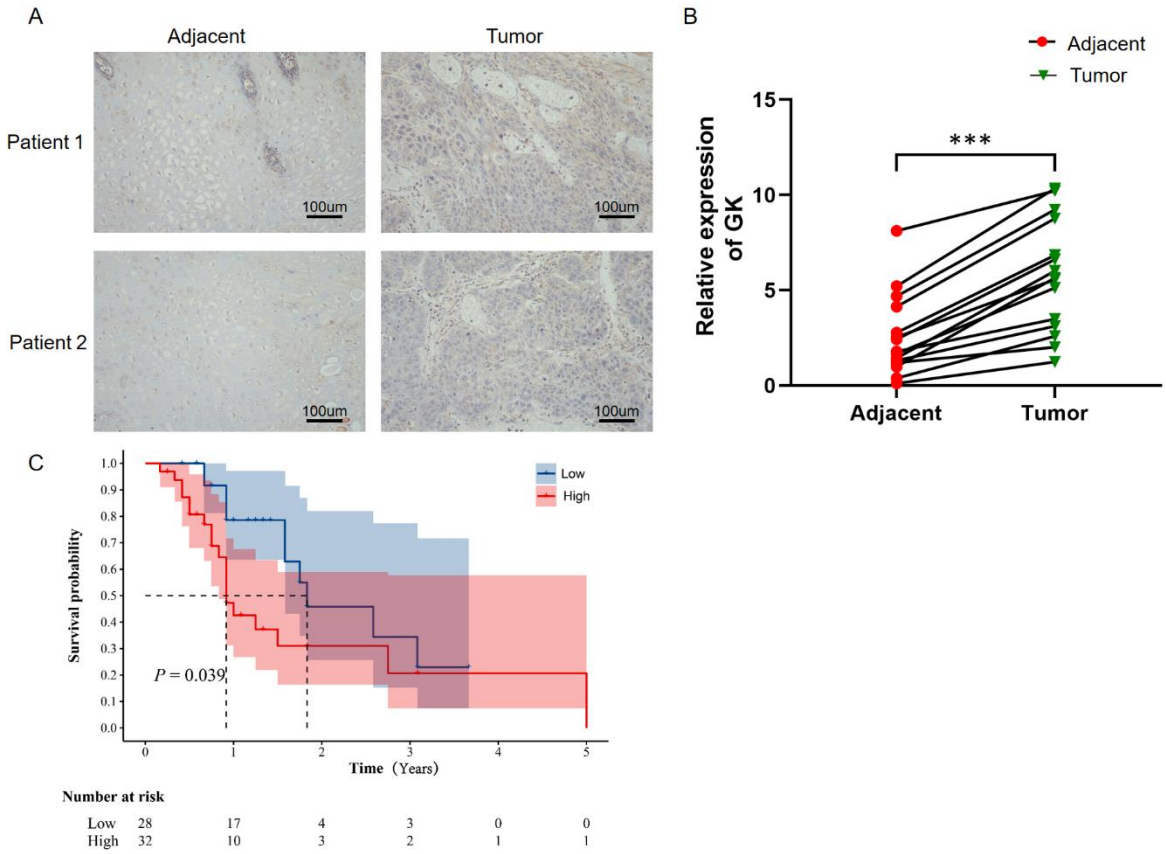

Figure 3 Expression of GK in Clinical Samples from Esophageal Cancer Patients and its Relationship with Patient Survival (A, Immunohistochemistry (IHC) was used to compare the expression of GK in cancerous tissues and adjacent non-cancerous tissues (magnification x200). B, Polymerase chain reaction (PCR) was used to compare the mRNA levels of GK in cancerous and adjacent non-cancerous tissues. C, Survival analysis was conducted to compare the differences in survival time between the low and high GK expression groups).

3.4 Correlation Between GK Expression and Clinical Characteristics

GK expression was not associated with age, gender, tumor differentiation, stage, or lymph node metastasis but was significantly correlated with distant metastasis and recurrence (Table 1).

Table 1 Comparison of General Information and Clinical Characteristics among Different Subgroups of Esophageal Cancer Patients

		GK expression			
Variable	Totaln = 60	Lown=31	High n=29	χ^2/t	*p*
Age	66.22 ± 8.34	65.87 ± 8.40	66.59 ± 88.42	-0.33	0.743
Gender				0.01	0.927
Male	35	18	17		
Female	25	13	122		
Differentiation				0.50	0.778
High	25	12	13		
Median	18	9	9		
Low	17	10	7		
Stages				0.65	0.421
I/II	33	16	17		
III/IV	27	15	12		

		GK expression			
Variable	Totaln = 60	Lown=31	High n=29	χ^2/t	*p*
Lymph node metastasis				1.01	0.315
No	40	23	17		
Yes	20	8	12		
Distant metastasis				5.36	0.021
No	35	23	12		
Yes	25	8	17		
Recurrence				4.22	0.040
No	32	21	11		
Yes	28	10	18		

3.5 GK Knockdown Suppresses Cell Proliferation and Migration

Si-GK significantly reduced GK mRNA and protein levels (Figures 4A-B). GK knockdown inhibited cell proliferation and migration (Figures 4C-D).

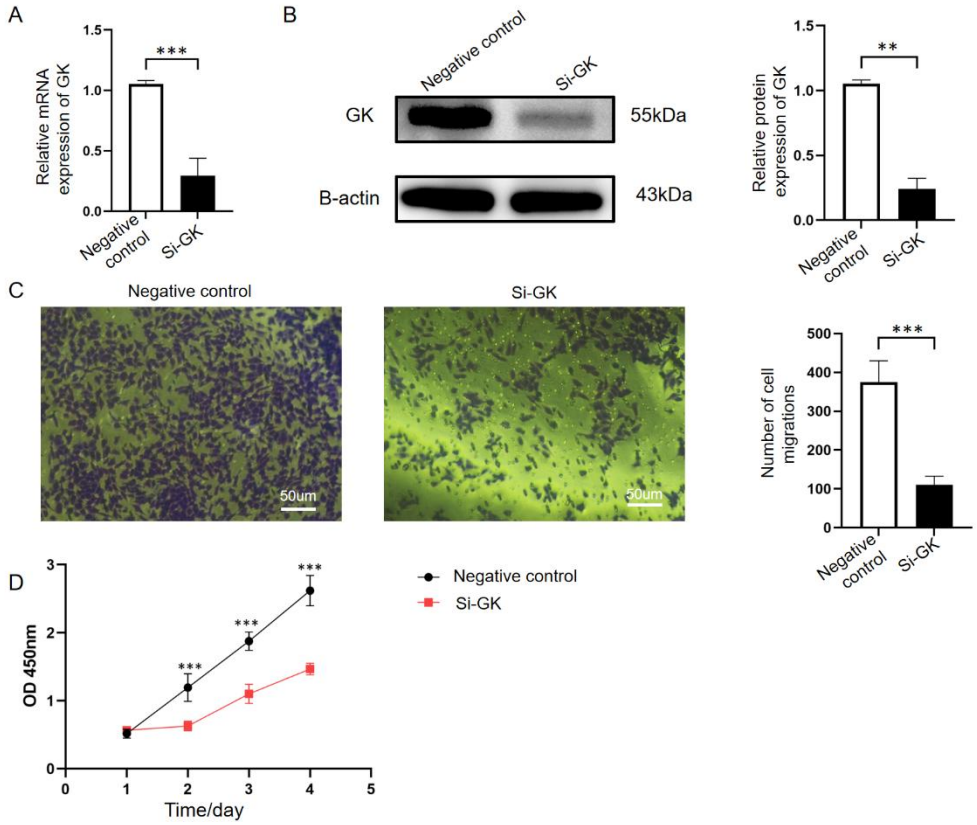

Figure 4 Interfering with GK Affects the Proliferation and Migration of KYSE150 Cells (A-B: Verification of the inhibitory effect of small interfering RNA (siRNA) on GK expression by polymerase chain reaction (PCR) and Western blot (WB). C: Comparison of the difference in cell migration numbers between the Si-GK group and the Negative control group using the Transwell assay. D: Comparison of the difference in cell viability between the Si-GK group and the Negative control group using the CCK-8 assay).

3.6 GK Involvement in Immunosuppressive Microenvironment

High GK expression correlated with upregulation of immune checkpoint molecules (CD274, CTLA4, LAG3, etc.) and reduced infiltration of CD4+ and CD8+ T cells (Figures 5A-B).

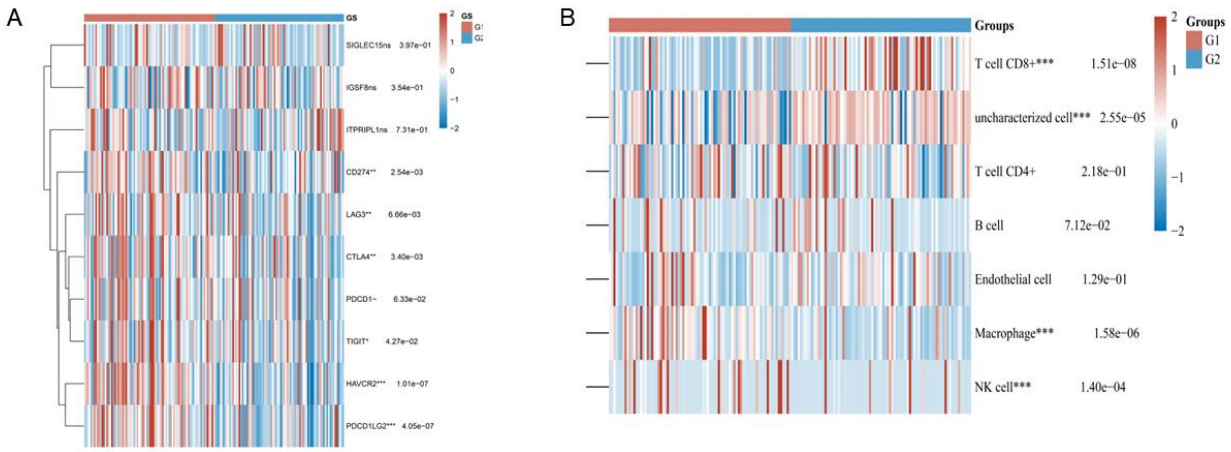

Figure 5 GK Participates in the Formation of the Immune Microenvironment of Esophageal Cancer (G1, Group with high GK expression; G2,Group with low GK expression).

4 DISCUSSION

This study systematically investigated the role of glycerol kinase (GK) in the development and progression of esophageal carcinoma (ESCA) through bioinformatics analysis, validation with clinical samples, and in vitro cellular experiments. Tumorigenesis is a multi-step, complex process involving the acquisition of several biological hallmarks, including sustained proliferation, resistance to apoptosis, invasion and metastasis, and immune escape [18-20]. Our findings reveal that aberrant GK expression is closely associated with multiple malignant features of ESCA, playing a significant role not only in the intrinsic metabolic reprogramming of tumor cells but also in shaping the extrinsic immune microenvironment.

All results clearly demonstrate that GK is significantly overexpressed in ESCA tissues, and its high expression serves as an independent risk factor for shortened overall survival in patients. Survival analysis of the TCGA database showed that the median overall survival (mOS) was significantly shorter in the high GK expression group compared to the low expression group (3.5 years vs. 1.8 years). This conclusion was further validated in our clinical cohort comprising 60 ESCC cases, where patients with high GK expression exhibited a reduction in mOS by 9.5 months. Importantly, analysis of clinical characteristics revealed that GK expression levels were not associated with conventional clinical parameters but were specifically and significantly correlated with distant metastasis and tumor recurrence. This suggests that GK is a key driver molecule promoting the invasive and metastatic potential of ESCA, rather than a mere bystander phenomenon.

Furthermore, tumor progression cannot be achieved without its ability to escape host immune surveillance. A significant finding of this study is the association between high GK expression and the formation of an immunosuppressive tumor microenvironment. Bioinformatics analysis indicated that in ESCA samples with high GK expression, the expression of several key immune checkpoint molecules (e.g., PD-L1, CTLA-4, LAG-3) was significantly upregulated, whereas cytotoxic CD8+ T cells and CD4+ T cells were markedly depleted. This reveals another potential mechanism by which GK promotes tumor progression: through GK-mediated metabolic reprogramming, tumor cells may alter the nutrient competition landscape within the tumor microenvironment or generate specific metabolites (such as lactate, kynurenine, etc.). These metabolites can directly inhibit T cell function, induce the infiltration of immunosuppressive cells like myeloid-derived suppressor cells (MDSCs), and concurrently upregulate immune checkpoint molecules, collectively fostering a "cold tumor" microenvironment conducive to tumor cell survival and immune evasion.

In summary, the innovation of this study lies in being the first to systematically link GK to poor prognosis, metastasis, recurrence, and an immunosuppressive microenvironment in ESCA, confirming its value as an independent prognostic biomarker and a potential therapeutic target. Although this study has limitations, including a limited sample size and insufficient in vivo functional validation, the results provide new experimental evidence and theoretical support for applying GK in ESCA prognosis assessment and for developing novel therapies targeting lipid metabolic reprogramming.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

FUNDING

This work was supported by Zhenjiang Social Development Guidance Science and Technology Project (FZ2024059).

REFERENCES

[1] GBD 2023 Cancer Collaborators. The global, regional, and national burden of cancer, 1990-2023, with forecasts to 2050: a systematic analysis for the Global Burden of Disease Study 2023. Lancet, 2025, 406(10512): 1565-1586.
[2] Chinese Guidelines on Perioperative Management of Resectable Esophageal Cancer (2023 edition). Zhonghua Yi Xue Za Zhi, 2023, 103(33).
[3] Jinxian H, Gaofeng L, Hui T, et al. Wnt signaling pathway-related gene PRICKLE1 is a prognostic biomarker for esophageal squamous cell carcinoma. Front Oncol, 2023, 12.
[4] An W, Xiaojia L, Lu L, et al. Grading criteria for venous invasion in thoracic esophageal squamous cell carcinoma. J Cardiothorac Surg, 2023, 18(1).
[5] Meitong L, Yaning Z, Zhuoyun X, et al. CD39-Expressing CD8(+) T Cells as a New Molecular Marker for Diagnosis and Prognosis of Esophageal Squamous Cell Carcinoma. Cancers (Basel), 2023, 15(4): 1184.
[6] Somalisa P, Simon A M U, Christopher W H, et al. Glycerol metabolism impacts biofilm phenotypes and virulence in Pseudomonas aeruginosa via the Entner-Doudoroff pathway. mSphere, 2024, 9(4): e0078623.
[7] Shuyu O, Shu Z, Mengmei Y, et al. Glycerol Kinase Drives Hepatic de novo Lipogenesis and Triglyceride Synthesis in Nonalcoholic Fatty Liver by Activating SREBP-1c Transcription, Upregulating DGAT1/2 Expression, and Promoting Glycerol Metabolism. Adv Sci (Weinh), 2024, 11(46): e2401311.
[8] João S O, Rúben J M, Ana D M, et al. Glycerol Kinase 2 as a Metabolic Sentinel for Human Sperm Motility and Male Fertility. Biomolecules, 2025, 15(9):1249 .
[9] Karol M S, Julie A M-F. Biochemical properties of glycerol kinase from the hypersaline-adapted archaeon Haloferax volcanii. Appl Environ Microbiol, 2025, 91(8).
[10] Hyun Wook Y, A Ra K, Song Hee L, et al. A phosphodiesterase 4 (PDE4) inhibitor, amlexanox, reduces neuroinflammation and neuronal death after pilocarpine-induced seizure. Neurotherapeutics, 2024, 21(4).
[11] Lili M, Yongsheng Y, Yue L, et al. Glycerol kinase interacts with nuclear receptor NR4A1 and regulates glucose metabolism in the liver. FASEB J, 2019, 33(6): 6736-6747.
[12] Leandro José D A, Lilian Pereira S, Li L, et al. The High Osmolarity Glycerol Mitogen-Activated Protein Kinase regulates glucose catabolite repression in filamentous fungi. PLoS Genet, 2020, 16(8): e1008996.
[13] Superior S, Sumit K S, Riva M R, et al. Functional and Biochemical Analyses of Glycerol Kinase and Glycerol 3-phosphate Dehydrogenase in HEK293 Cells. Protein J, 2025, 44(2): 231-244.
[14] Cemre K, Pınar K Ç, Engin K, et al. Pseudohypertriglyceridemia as a clue: clinical and genetic spectrum of glycerol kinase deficiency in three pediatric cases. J Pediatr Endocrinol Metab, 2025, 38(12): 1361-1366.
[15] Ying H, Shasha Q, Lu C, et al. The roles and mechanisms of SREBP1 in cancer development and drug response. Genes Dis, 2024, 11(4): 100987.
[16] Sampurna G, Roman C, Sunny C, et al. Stearoyl-CoA desaturase in development and disease. Prog Lipid Res, 2026. DOI: 10.1016/j.plipres.2025.101374.
[17] Yaqin Z, Yue W, Ying L, et al. Carnitine palmitoyltransferase 1A promotes mitochondrial fission by enhancing MFF succinylation in ovarian cancer. Commun Biol, 2023, 6(1): 618.
[18] Demeng X, Shuo W, Kaiwen W, et al. Ginsenosides and Tumors: A Comprehensive and Visualized Analysis of Research Hotspots and Antitumor Mechanisms. J Cancer, 2024, 15(3): 671-684.
[19] Maoquan Y, Yan L, Shuangshuang Z, et al. Associations of PD-1 and PD-L1 gene polymorphisms with cancer risk: a meta-analysis based on 50 studies. Aging (Albany NY), 2024, 16(7): 6068-6097.
[20] Heui-Jin P, Kyuri K, Eun-Young L, et al. Methylanthranilate, a Food Fragrance Attenuates Skin Pigmentation through Downregulation of Melanogenic Enzymes by cAMP Suppression. Biomol Ther (Seoul), 2024, 32(2): 231-239.