Current Science
CS 5 (2), 1510-1523 (2025)
Received 15 **Dec** 2024 | **Revised 22 Jan** 2025 | **Accepted 18 Feb**. 2025 | **Online Available 12 March** 2025
https://doi.org/ 10.52845/CS/2025-5-2-19

Current Science

Current Science

OPEN ACCESS

ISSN(O)2795-8639

ORIGINAL ARTICLE

PGK1 Could Function as a Prognostic Marker and Correlate with Immune Infiltration in Head and Neck Squamous Cell Carcinoma

Hesen Huang[1], Kaiqin Chen[2], Yu Du[1], Jing Gao[1], Yixian Ye[3]

[1]Department of Otolaryngology-Head and Neck Surgery, Xiang'an Hospital of Xiamen University, Xia Men, Fu Jian, 361100, China

[2]Department of Neurosurgery, Xiang'an Hospital of Xiamen University, Xia Men, Fu Jian, 361100, China

[3]Department of Otolaryngology, Zhongshan Hospital (Xiamen), Fudan University, Fu Jian, 361006, China

[*]Corresponding Author: Yixian Ye

Abstract

Objectives: Head and neck squamous cell carcinoma (HNSC) is one of the most prevalent cancers, therefore it is crucial to identify HNSC biomarkers. Phosphoglycerate kinase 1 (PGK1) has a role in the development and progression of certain types of cancer. The function of PGK1 in HNSC remains unknown. This is the first study on the expression of PGK1 in HNSC patients, as well as its prognostic significance, putative biological roles, and influence on the immune system.

Methods: The Cancer Genome Atlas (TCGA) data served as the foundation for gene expression and clinicopathological analysis, enrichment analyses, and immune infiltration investigations. Utilizing TIMER and ssGSEA, the immunological response to PGK1 expression in HNSC was analyzed statistically. Additionally, Gene Expression Omnibus (GEO), Kaplan-Meier (K-M) survival analysis, and Human Protein Atlas (HPA) data were utilized to confirm the results.

Results: PGK1 was an important independent prognostic factor for HNSC patients. According to GSEA, PGK1 is responsible for increasing DNA replication and inhibiting immunological activity. Co-expressed with immune-related genes and immunological checkpoints, PGK1 expression was substantially linked to B cell and dendritic cell invasion.

Conclusion: The expression of PGK1 is increased in HNSC, and the high expression of PGK1 is associated with a poor prognosis. PGK1 may affect tumor development by regulating tumor-infiltrating cells in the tumor microenvironment (TME). PGK1 may be a potential target for immunotherapy.

Keywords: PGK1, HNSC, prognosis, tumor microenvironment, tumor immune cell infiltration

Foreword

Head and neck squamous cell carcinoma (HNSC) ranks sixth worldwide [1]. More than 600,000 new cases of HNSC are diagnosed yearly, accounting for 5.7% of all cancer-related deaths worldwide [2,3]. HNSCs are malignant tumors of the sinuses, pharynx, larynx, and oral mucosa [4]. Despite established risk factors including infection, HNSC pathophysiology is complex and needs additional study [5]. HNSC focuses on disease prevention, early screening, target molecule identification, and innovative therapies. We believe HNSC prognostic prediction has flaws. Biomarkers with high specificity and sensitivity are needed to improve HNSC diagnosis and prognosis and to assess tailored therapeutic regimens. Surgery, chemotherapy, and radiation are used to treat HNSC [6]. Locally progressed, recurrent, and metastatic patients need better care. HNSC is also treated with immunotherapy and targeted treatment [7]. Cetuximab is the first

FDA-approved HNSC therapy. Cetuximab increased survival by 2.7%. Immunotherapy is popular because targeted treatment is ineffective [6]. To enhance the diagnosis and prognosis of HNSC, the key components that contribute to tumor formation and progression must be identified. Valuable immune molecules [8,9].

Phosphoglycerate kinase 1 (PGK1) is needed for glycolysis and mitochondrial metabolism [10]. The Warburg effect [11], in which tumor cells have enhanced glycolysis and decreased mitochondrial metabolism, is a potential cancer treatment target. PGK1 generates ATP during glycolysis [12]. It converts 1,3-diphosphoglycerate to 3-phosphoglycerate, producing ATP [13]. According to research, PGK1 is involved in many carcinogenic signaling pathways. HIF-1/PGK1 and PGK1/AKT/mTOR are common cancer pathways [13-15]. HIF-1 regulates the PGK1 gene, which is up-regulated during hypoxia and stimulates glycolytic metabolism [14]. PGK1/AKT/mTOR pathway may increase glycolysis, metastasis, and cancer [13,15]. As cancer is a metabolic disease, targeting the glycolytic enzyme PGK1 has been a focus of study. In addition to its role in glycolysis, PGK1 is a polymerase alpha cofactor protein that influences the tricarboxylic acid cycle (TCA), DNA replication, and repair [16]. PGK1 expression affects carcinogenesis and cancer development. Unknown is PGK1's involvement in HNSC.

We checked TCGA for PGK1 expression in human HNSC samples. We used R to explore the relationship between PGK1 expression, clinical markers, and HNSC prognosis (version 3.6.3). We performed Gene Set Enrichment Analysis(GSEA) coupled with improved Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) studies to better comprehend the biological processes linked with the PGK1 regulatory network, which may underlie HNSC formation. We evaluated the connection between PGK1 and tumor-infiltrating immune cells using TIMER and ssGSEA (TIICs). K-M survival analysis, the HPA, and GEO were used to examine the relationship between PGK1 and HNSC prognosis. This is the first study to examine the relationship between PGK1 and HNSC. Accurate prognosis can help cancer patients make health care decisions, and effective models can predict prognosis [6]. This study explored HNSC risk factors and built a prognostic model.

2 Materials and Methods

2.1 Information from the TCGA Database

We acquired data on immune cell infiltration, gene expression (workflow type: HTSeq-TPM), and relevant clinical information using the HNSC TCGA database (https://portal.gdc.cancer.gov/, data type: Clinical Supplement) [17]. This study excluded samples with missing or inadequate age, TNM stage, OS time, distant and lymph node metastases, and local invasion data. Future studies will use RNA-Seq and clinical data. Our study meets TCGA standards. ROC curves were used to test PGK1 expression as a diagnostic marker.

2.2 Gene Set Enrichment Analysis

We performed GSEA (https://www.gsea-msigdb.org/gsea/downloads.jsp) analysis by obtaining normalized RNA-Seq data from TCGA [18]. The number of possible permutations is 1000. Using GSEA, possible biological activities and pathways of high and low PGK1 expression groups were identified. A P value 0.05 and a normalized enrichment score (NES) 25% were considered significant in the enrichment results.

2.3 Immune Infiltration Analysis

The TIMER2.0 database (http://timer.cistrome.org/) is a comprehensive resource that provides gene expression and immune infiltration studies for 33 kinds of cancer [19]. Based on the TIMER algorithm, the web-based platform calculates the abundance levels of six tumor-infiltrating immune cells (TIICs), including B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells. Spearman correlation analysis was used to examine the link between the mRNA expression of PGK1 and the quantity of six lymphocytes in HNSC samples. The graph created by TIMER depicts the relationship between immune cell infiltration levels and HNSC cumulative survival, as well as the influence of gene expression levels on tumor purity [20]. Using ssGSEA, 24 immune cell types were examined in malignancies. Spearman correlation was used to compare immune cell infiltration between groups with high and low PGK1 expression. We compared 24 immune cell types using a heatmap.

2.4 Immune Checkpoint Analysis

Select TIGIT, NRP1, LAIR1, BTLA, CD200, TNFRSF14, TNFSF4, CD244, CD40LG, LAG3, ICOS, CTLA4, CD28, CD48, CD80, CD276, CD200R1, HAVCR2, ADORA2A, KIR3DL1, PDCD1, LGALS9, CD160, TNFSF14, IDO2 , ICOSLG, TMIGD2, VTCN1, IDO1, PDCD1LG2, HHLA2, TNFSF18, BTNL2, CD70, TNFSF9, TNFRSF8, CD27, TNFRSF25, VSIR, TNFRSF4, CD40, TNFRSF18, TNFSF15, CD274, CD44, CD86, and TNFRSF9 are associated with immune checkpoints transcripts. These 47 gene expression data were retrieved. We examined immunological checkpoints and PGK1 co-expression. This research uses the Tumor Immune Dysfunction and Exclusion (TIDE) algorithm to predict immune checkpoint blockade responses using expression profiling data [21].

2.5 Comprehensive Analysis

The Kapan-Meier curve (http://kmplot.com/ analysis/) was utilized to examine the association between PGK1 expression and the overall survival (OS), disease-free period (DSS), and progression-free interval (PFI) of HNSC patients [10]. To retrieve the KM survival graph, enter PGK1 into the database. Calculated were log-rank p-values and hazard ratios.

To determine the 95% CI and HR, we utilized multivariate Cox analysis and evaluated the impact of PGK1 expression and other pathological and clinical variables (sex, age, grade, lymph node, distant metastasis, tumor status, stage, etc.) on OS. Based on the outcomes of a multivariate Cox proportional hazards analysis, very accurate nomograms were developed to forecast the overall recurrence rates at 1, 3, and 5 years.

Gene Expression Profile Interaction Analysis (GEPIA) is comprised of a web server and an online database (http://gepia.cancer-pku.cn/index.html). It is a publicly accessible database [22] created by Peking University, China, which contains 9,736 tumor samples and 8,587 normal samples from the TCGA and GTEx studies that were examined for RNA-sequencing expression. To evaluate the difference in PGK1 expression between HNSCs and normal tissues, we constructed OS curves using GEPIA to validate our data and validate our results.

Researchers upload their study data to GEO (https://www.ncbi.nlm.nih.gov/gds/) so that it can be freely shared with other researchers worldwide. From the GEO database included in GPL19061 Platforms (Agilent-043965 custom human array oelinc_xw), we retrieved the GSE61218 [23] series. To evaluate variations in PGK1 expression between HNSCs and normal tissues, we validated our results using GEO boxplots [24].

Immunohistochemical (IHC) staining Immunohistochemical images from the Human Protein Atlas database (HPA) (www.proteinatlas.org) for the analysis of protein expression analysis of PGK1 between head and neck normal tissues, HNSC tissues, evaluated the protein Expression of PGK1 at the level, antibody CAB010065 was used for IHC [25]. In addition, HPA gives RNA level measurements.

2.6 Statistical Analysis

All statistical analyses were conducted using R (version 3.6.3). Normalization of gene expression data using log2 transformation Using a two-group t-test, normal and malignant tissues were compared. For comparisons between more than or equal to three groups, Kruskal-Wallis one-way ANOVA was applied. For all survival studies, Cox proportional hazards models, KM analysis, and log-rank testing were applied. The correlation between the two variables was analyzed using Spearman's test, with a statistically significant P value of 0.05. Due to the absence of complete clinical information in the TCGA database and the fact that not all samples included clinical baseline information, such as age, TNM stage, and treatment outcome, a detailed analysis of each clinical category was not possible. In the Results section table, there is a difference between the total number of samples and the number in clinical categories.

3. Results

3.1 Survival Outcomes and Variable Analysis

Using RNA-seq data from the TCGA database, this study analyzed any differences in PGK1 mRNA expression between normal and tumor tissues in pan-cancer. Excluding malignancies without normal tissue data, we identified 18 of 33 cancers with substantially altered PGK1 expressions, including BLCA, BRCA, CESC, CHOL, COAD, ESCA, GBM, HNSC, KICH, KIRC, LIHC, LUAD, LUSC, PRAD, READ, STAD, THCA, and UCEC. Tumors with highly elevated PGK1 expression include BLCA, BRCA,

CESC, CHOL, COAD, ESCA, GBM, HNSC, KIRC, LIHC, LUAD, LUSC, READ, STAD, and UCEC; tumors with significantly decreased PGK1 expression include KICH, PRAD, and THCA.

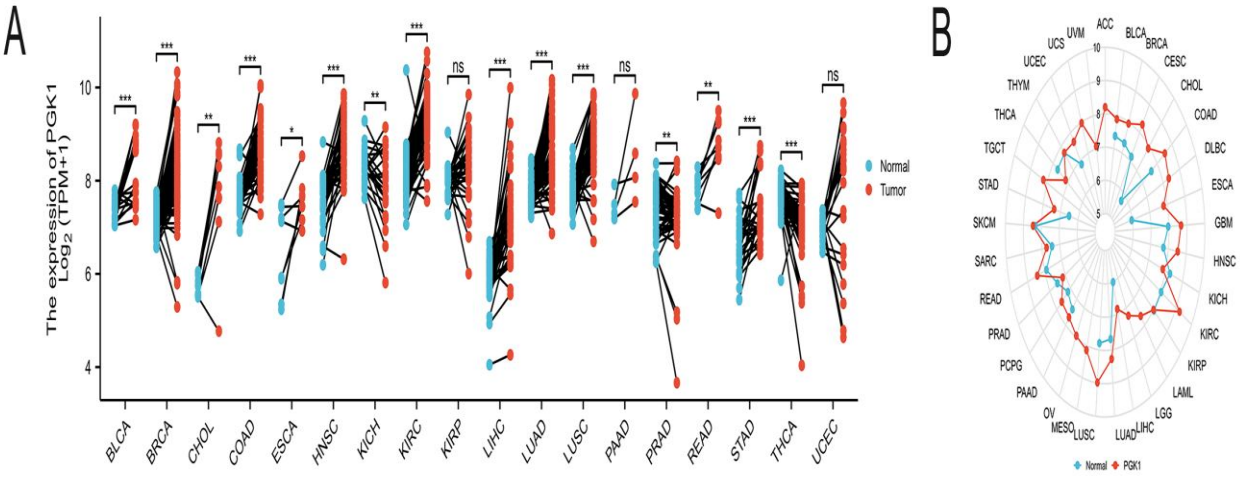


Figure 1. PGK1 expression in normal and pan-cancer tissues. (A) In paired tissues (B) Unpaired tissues.

(Figure 1) In TCGA, 502 HNSC tumors and 44 normal tissues were compared further for unpaired samples, and the median expression of PGK1 in normal and HNSC samples was 0.888 (0.721 - 1.063), P < 0.001. (Figure 2A).

In contrast to paired samples, the difference between the two groups was 1.094 (0.87 - 1.318) and statistically significant (t = 9.855, P < 0.001) (Figure 2B). Our study demonstrated that PGK1 expression is prevalent in HNSC tumor tissues.

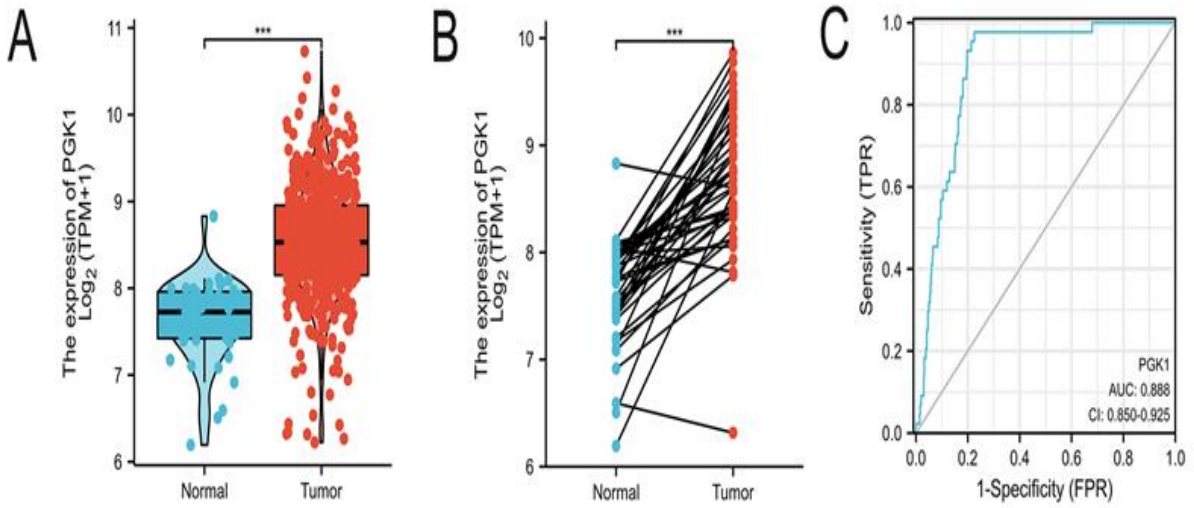
3.2 Patient Characteristics

Table S1. Baseline data on PGK1 expression in HNSC patients

Characteristic	levels	Overall	Characteristic	levels	Overall
n		502	n		502
Age, median (IQR)		61 (53, 69)	N stage, n (%)	N0	239 (49.8%)
Age, n (%)	<=60	245 (48.9%)		N1	80 (16.7%)
	>60	256 (51.1%)		N2	154 (32.1%)
Gender, n (%)	Female	134 (26.7%)		N3	7 (1.5%)
	Male	368 (73.3%)	Smoker, n (%)	No	111 (22.6%)
Histologic grade, n (%)	G1	62 (12.8%)		Yes	381 (77.4%)
	G2	300 (62.1%)	Alcohol history, n (%)	No	158 (32.2%)
	G3	119 (24.6%)		Yes	333 (67.8%)
	G4	2 (0.4%)	Lymphovascular invasion, n (%)	No	219 (64.2%)
Clinical stage, n (%)	Stage I	19 (3.9%)		Yes	122 (35.8%)
	Stage II	95 (19.5%)	Lymphnode neck dissection, n (%)	No	90 (18%)
	Stage III	102(20.9%)		Yes	409 (82%)
	Stage IV	272(55.7%)	Primary therapy outcome, n (%)	PD	41 (9.8%)
T stage, n (%)	T1	33 (6.8%)		SD	6 (1.4%)
	T2	144(29.6%)		PR	6 (1.4%)
	T3	131(26.9%)		CR	365 (87.3%)

	T4	179(36.8%)	Radiation therapy, n (%)	No	154 (34.9%)
M stage, n (%)	M0	472 (99%)		Yes	287 (65.1%)
	M1	5 (1%)			

As shown in Table S1, the baseline data table shown was collected from TCGA in February 2022 and included 502 primary tumors with clinical and gene expression data. The median age was 61 years, and the patients were divided into two groups by age, the percentage of patients under 60 years old was 48.9%, and the percentage of patients over 60 years old was 51.1%. Gender included 134 women (26.7%) and 368 men (73.3%). Histologic grade was divided into 62 cases (12.8%) of G1, 300 cases (62.1%) of G2, 119 cases (24.6%) of G3, and 2 cases (0.4%) of G4. The clinical stage was divided into 19 cases (3.9%) of Stage I, 95 cases (19.5%) of Stage II, 102 cases (20.9%) of Stage III, and 272 cases (55.7%) of Stage IV. Among the 487 patients with T stage, 33 (6.8%) were T1, 144 (29.6%) were T2, 131 (26.9%) were T3, and 179 (36.8%) were T4. There were 239 cases (49.8%) of N0, 80 cases (16.7%) of N1, 154 cases (32.1%) of N2, and 7 cases (1.5%) of N3. According to M staging, M0 was 472 cases (99%) and M1 was 5 cases (1%). 381 cases (77.4%) were smoking and 333 cases (67.8%) were drinking. Lymphovascular invasion was present in 122 cases (35.8%), and Lymph node neck dissection was present in 409 cases (82%). In primary outcome therapy, PD was 41 (9.8%), SD and PR were both 6 (1.4%), and CR was 365 (87.3%). There were 287 patients (65.1%) who received radiotherapy. PGK1 expression showed promising prognostic power, as the ROC curve showed that the AUC of PGK1 expression for predicting survival was 0.888 (Fig. 2C). At the same time, this study also showed the distribution of high and low expression of PGK1 in terms of risk score (Figure 2D). The results indicated that the high-risk patients with HNSC had high PGK1 expression levels, while the low-risk patients had low PGK1 expression levels. As shown in Figure 2E, we used Cox analysis to explore the link between PGK1 expression and OS and other multivariate characteristics of HNSC patients. Finally, our multivariate analysis revealed that some factors included M stage (HR = 10.208, p = 0.048), Lymphovascular invasion (HR = 0.602, p = 0.045), Primary therapy outcome (HR = 0.223, p < 0.001), Radiation therapy (HR = 1.912, p = 0.02), PGK1 (HR = 1.671, p = 0.026) were independent risk factors for HNSC.



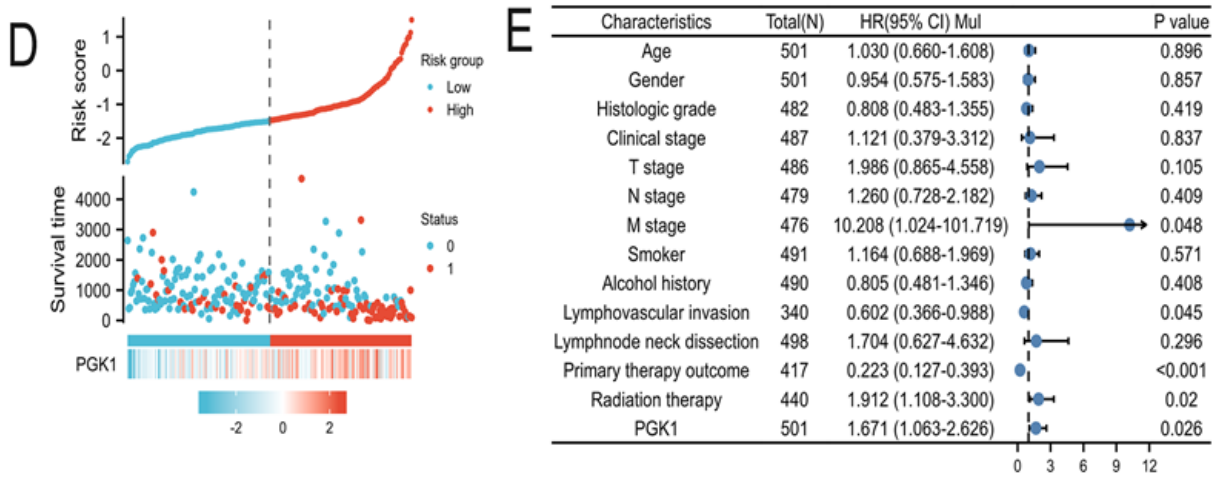


Figure 2. PGK1 expression in HNSC tissues. (A) PGK1 expression in normal and unpaired tissues. (B) PGK1 expression in paired tissues. (C) ROC curve of PGK1. (D) Risk factors for OS of PGK1 expression . (E) Forest plot of multivariate Cox analysis of PGK1 expression and other clinicopathological variables.

Figure 3 shows that the expression levels of PGK1 were significantly different in different Histologic grades (Fig. 3A) and Clinical stages (Fig. 3B) of HNSC patients compared with the corresponding paracancerous tissues.

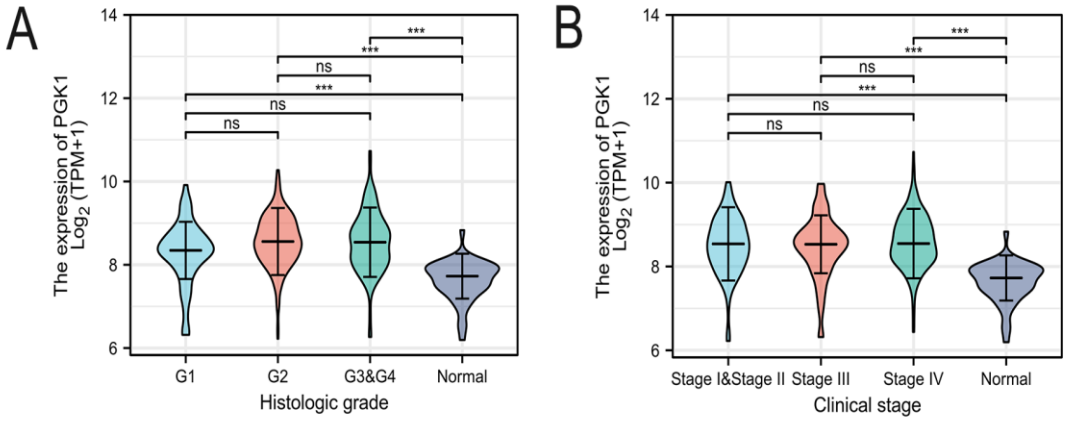


Figure 3. Expression of PGK1 correlated significantly with histological grade (A), and clinical stage (B).

3.3. GSEA Investigation of PGK1

GO term and KEGG pathway analyses were performed to explore the potential biological functions of PGK1. GSEA showed that the enrichment of the KEGG pathway and GO terms were significantly different in samples with high PGK1 levels (FDR < 0.25, p-value < 0.050) (Fig. 4). We selected the most enriched signaling pathways based on their normalized enrichment scores (NES). KEGG pathway analysis showed five pathways with the strongest positive correlation with PGK1 expression (Fig. 4A): Small cell lung cancer, homologous recombination, mismatch repair, pentose phosphate pathway, ecm receptor interaction. The five pathways with the strongest negative correlation (Figure 4B) are primary immunodeficiency, an intestinal immune network for IgA production, linoleic acid metabolism, asthma, and arachidonic acid metabolism. As shown in Figure 4C, GO annotation revealed five categories positively correlated with high levels of PGK1: ncRNA export from the nucleus, tRNA transport, hemidesmosome assembly, DNA replication origin binding, and spindle midzone. GO analysis also found five categories with the strongest negative correlations (Fig. 4D):

immunoglobulin complex, complement activation, humoral immune response mediated by circulating immunoglobulin, immunoglobulin complex circulating, and immunoglobulin receptor binding.

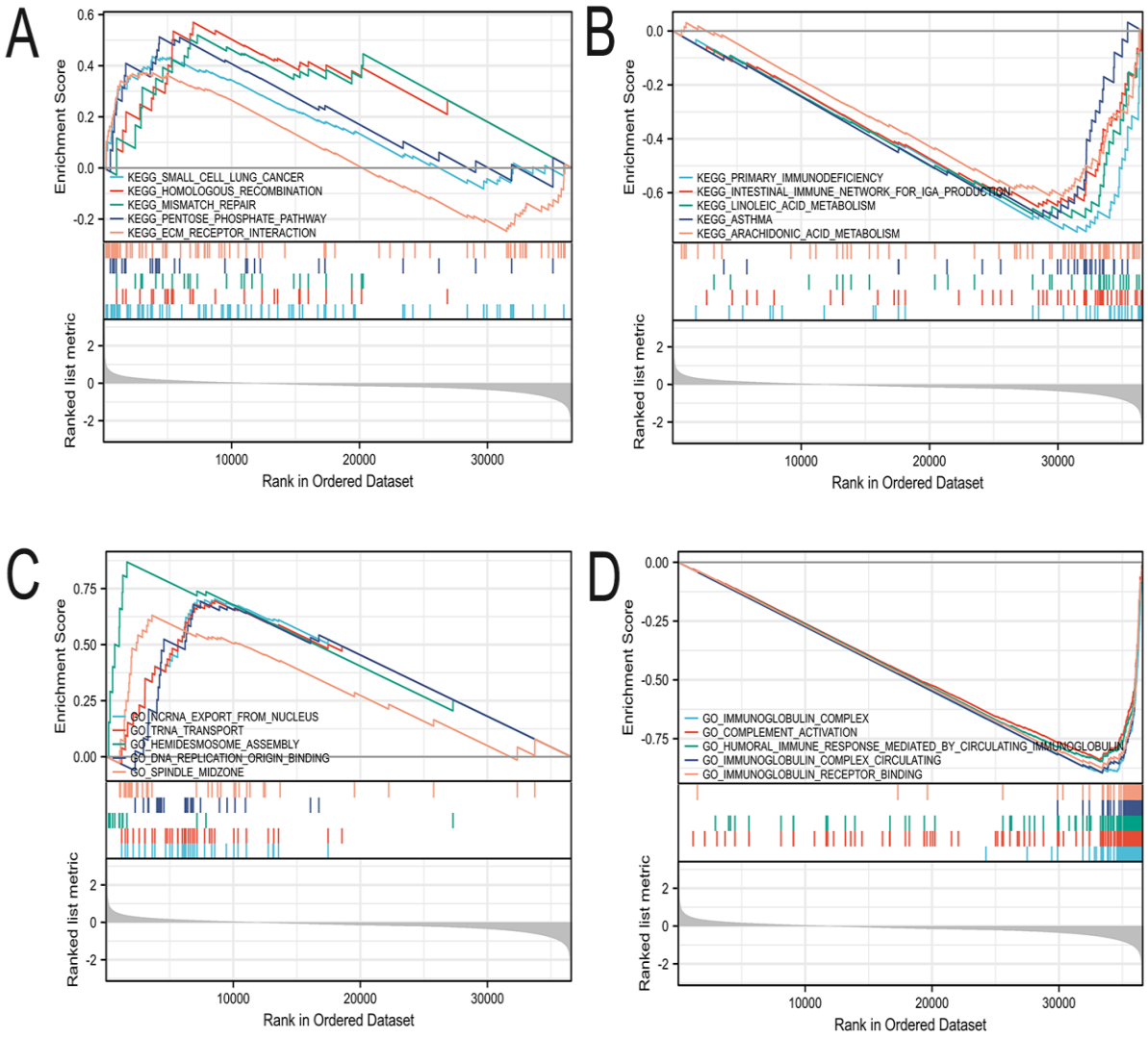


Figure 4. KEGG pathway showed five positively correlated groups (A), and five negatively correlated groups (B). GO term analysis revealed five positively correlated groups (C), and five negatively correlated groups (D).

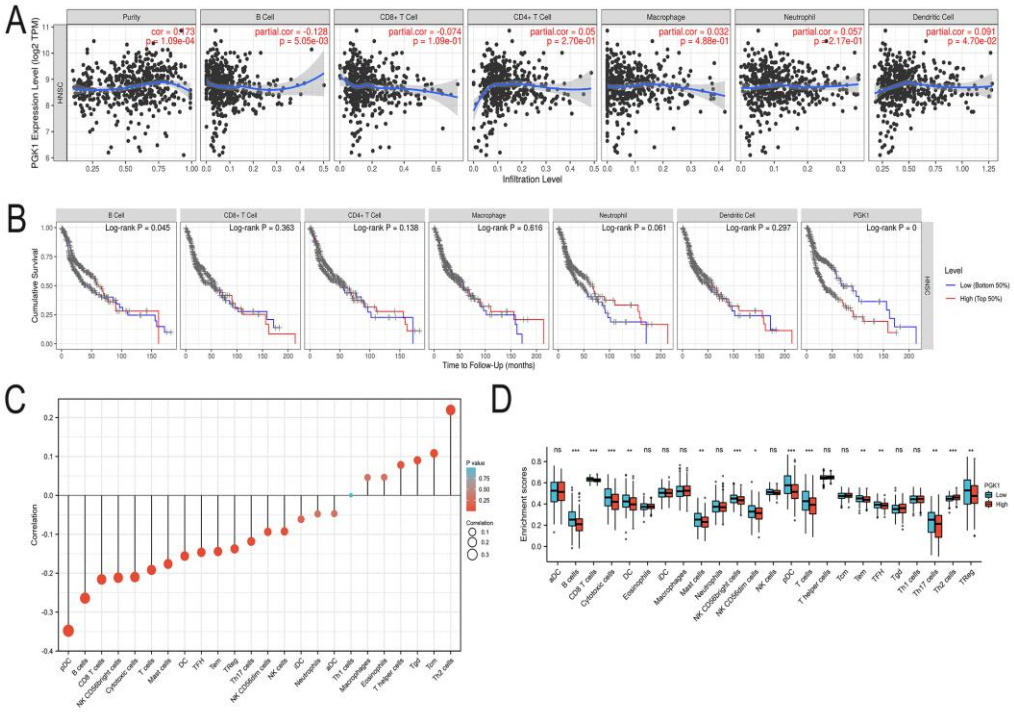
These results suggest that the occurrence and development of HNSC diseases are closely related to the following functional roles of PGK1. PGK1 expression not only promotes DNA replication-related processes and functions, but also inhibits immunoglobulin receptor binding, immunoglobulin-related cycling, intestinal immunity, and other immune-related responses, and inhibits linoleic acid and arachidonic acid metabolism. GO term and KEGG pathway analysis showed that in HNSC, PGK1 also promoted the pentose phosphate pathway and ecm receptor interaction in small cell lung cancer.

3.4 The relationship between PGK1 expression and tumor-infiltrating immune cells

Independent tumor-infiltrating lymphocytes have a crucial role in determining overall survival and the status of sentinel lymph nodes [26]. We analyzed the possible correlation between PGK1 expression and the level of immune infiltration in HNSCs using TIMER. As shown in Figure 5A, PGK1 expression was significantly correlated with B cells (r = −0.128, p-value = 5.05e-03) and dendritic cells (r = 0.091, p-value = 4.70e-02). We found that HNSC patients with high infiltration levels of B cells (p-value=0.045) had higher

cumulative survival, and the infiltration levels of the other five immune cell subtypes had no significant correlation with cumulative survival. At the same time, this study also verified that HNSC patients with high PGK1 expression had a low cumulative survival rate (Figure 5B). These results suggest that PGK1 expression level is associated with poor prognosis and immune infiltration level in HNSC patients. PGK1 plays a key role in the immune infiltration of HNSCs.

Next, the relationship between PGK1 expression and 24 different immune cell types was assessed in HNSCs. PGK1 expression was significantly positively correlated with Tgd, Tcm, Th2 cells, pDC, B cells, CD8 T cells, NK CD56bright cells, Cytotoxic cells, T cells, Mast cells, DC, TFH, Tem, Treg, Th17 cells, NK CD56dim cells, and NK cells were significantly negatively correlated (Fig. 5C). Further studies showed that there were significant differences in the expression levels of PGK1 among infiltrating immune cells, including Th2 cells, B cells, CD8 T cells, Cytotoxic cells, DC, Mast cells, NK CD56bright cells, NK CD56dim cells, Tem, TFH , Th17 cells, TReg were affected by the expression of PGK1. Compared with the PGK1 low expression group, these immune cells increased in the PGK1 high expression group, including Th2 cells, the difference was statistically significant ($P<0.05$); while Eosinophils, Macrophages, T helper cells, Tcm, Tgd, Th1 cells The differences were not statistically significant ($P > 0.05$). Compared with the PGK1 low expression group, these immune cells decreased in the PGK1 high expression group, including B cells, CD8 T cells, Cytotoxic cells, DC, Mast cells, NK CD56bright cells, NK CD56dim cells, Tem, TFH, Th17 cells, TReg, the difference was statistically significant ($P < 0.05$); but aDC, iDC, Neutrophils, NK cells, pDC, T cells, the difference was not statistically significant ($P > 0.05$) (Figure 5D). We also assessed possible correlations between 24 immune cells (Fig. 5E). The resulting heatmap indicated that the ratios of different tumor-infiltrating immune cell subsets were strong to moderately correlated. In the low and high expression groups of PGK1 in HNSC, the expression of 47 immune checkpoints such as TIGIT was further studied. The results showed that compared with the PGK1 low expression group of HNSC, in the PGK1 high expression group, TIGIT, NRP1, BTLA, TNFRSF14, TNFSF4, CD244, CD40LG, LAG3, CTLA4, CD48, CD80, CD276, CD200R1, PDCD1, LGALS9, The 26 immune checkpoints, IDO2, TMIGD2, PDCD1LG2, HHLA2, CD27, TNFRSF25, VSIR, TNFRSF4, TNFRSF18, CD274, and CD44, were significantly up-regulated (Fig. 5F). Furthermore, we revealed that in HNSCs, patients with high versus low PGK1 expression had higher TIDE scores, poorer response to immune checkpoint blockade (ICB), and shorter survival after receiving ICB (Fig. 5G).



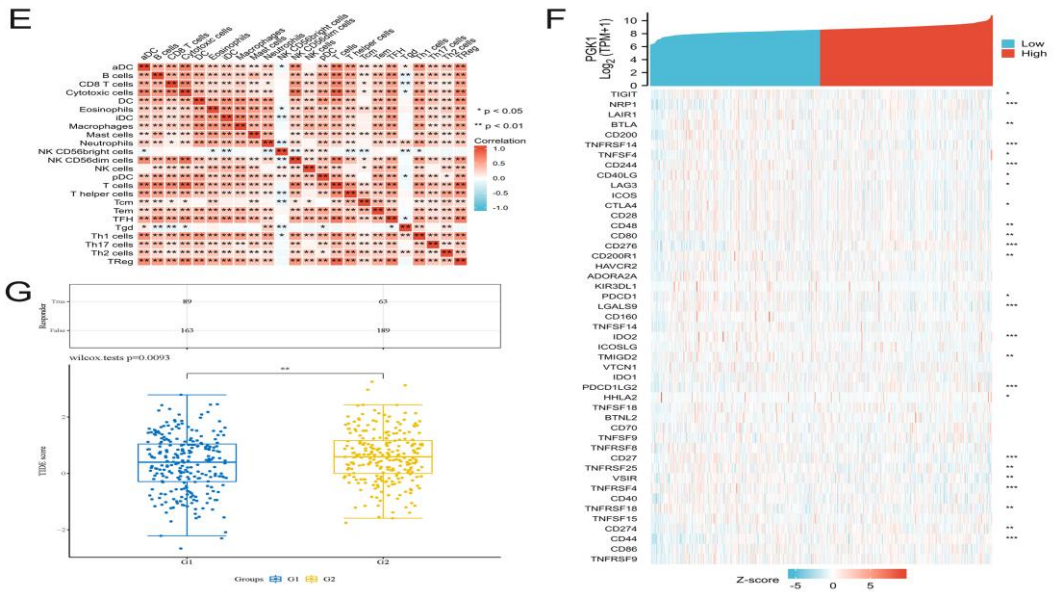
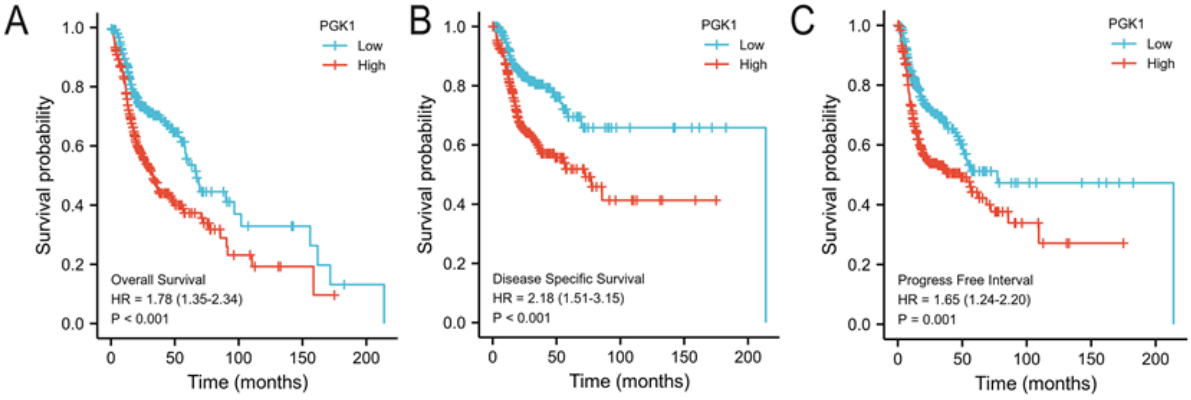


Figure 5. The correlation between PGK1 expression and immune cell infiltration in tumor microenvironment and the expression of immune checkpoints in HNSC. (A) The relationship between PGK1 expression and the infiltration level of six types of immune cells. (B) The relationship between immune cell infiltration level and Correlation of cumulative survival of HNSCs. (C) Relationship between PGK1 expression and 24 immune cell types. (D) Change ratio of 24 immune cell subtypes in high and low PGK1 expression groups in tumor samples. (E) Tumor samples Heat map of 24 immune-infiltrating cells in . (F) Differential expression of immune checkpoints with low and high expression of PGK1. (G) Differences of immune checkpoint blockade with low expression (G1) and high expression (G2) of PGK1 reactio

3.5. Data verification

The KM survival plot revealed that the group with high PGK1 expression had shorter OS (HR = 1.78 (1.35−2.34), p-value < 0.001, Figure. 6A), DSS (HR = 2.18 (1.51−3.15), P-value < 0.001, Figure. 6B) and PFI (HR = 1.65 (1.24−2.20), P = 0.001, Figure. 6C) based on the association with HNSC prognosis Independent of risk factors, we plotted the nomogram to estimate OS of the disease (Figure 6D), and the calibration curve showed good predictive value (Figure. 6E).



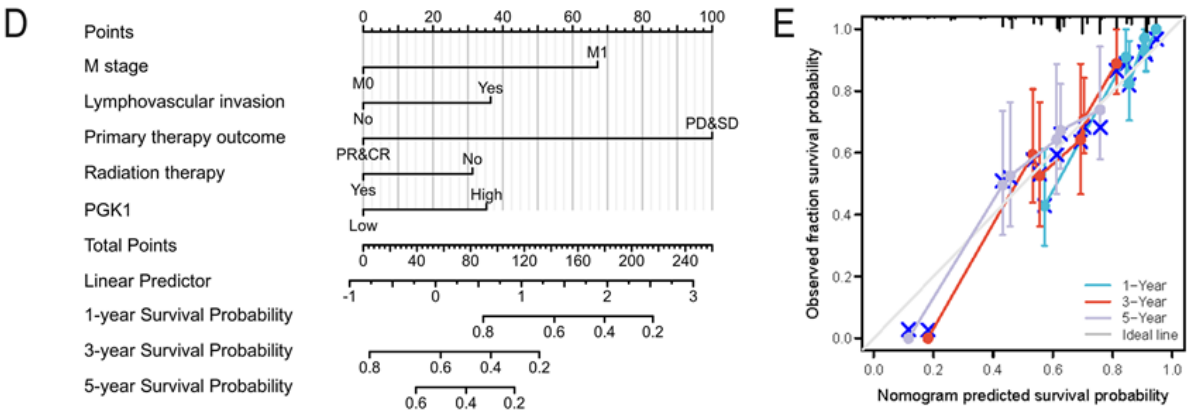


Figure 6. Prognostic analysis of PGK1 expression. Compared with patients with low PGK1 expression, patients with high PGK1 expression had poorer prognosis, including (A) overall survival (OS) (P < 0.001) , (B) disease-specific survival (DSS) (P < 0.001) , (C) Progress Free Interval (PFI)（P = 0.001）. (D) Multivariate analysis nomogram of clinical features based on PGK1 expression. (E) Calibration plot showing the predictive performance of the model constructed using multivariate Cox regression analysis.

Using the GEO database, we determined that PGK1 mRNA expression was considerably elevated in HNSC compared to the normal group (p-value = 0.0058, Figure S1.A) . In addition, HNSC tumor tissue expressing PGK1 was obtained from HPA. HNSC tumor tissue with medium and high PGK1 expression stained more intensely (Figure S1.C, Figure S1.D) compared to normal head and neck tissue (Figure S1.B).

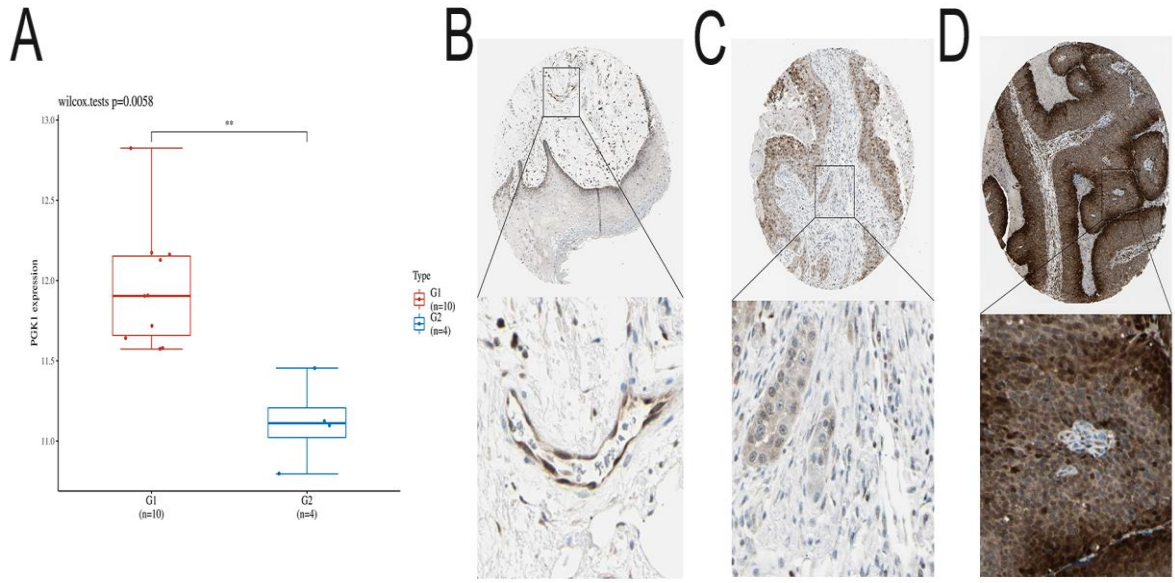


Figure S1. (A) Expression levels of PGK1 mRNA in HNSC(G1) and normal(G2) tissues obtained from GEO. Observation of PGK1 expression using HPA immunohistochemistry, normal head and neck tissue (B) , HNSC tumor tissue with medium (C) and high PGK1 (D)expression stained more intensely.

Discussion

Changes in cell metabolism, growth, adhesion, and proliferation are among the processes by which cancer cells arise [27]. PGK1 increases tumor cell proliferation, migration, and invasion, and is critical to the progression of many cancers. The oncogene PGK1 is highly related with a poor prognosis in breast cancer, endometrial cancer, colon cancer, glioma, lung cancer, and liver cancer [28-32]. These findings match published research. PGK1 has a major role in oral cancer

tumor formation, especially in local recurrence and lymph node recurrence [33]. Gallbladder cancer patients had lower PGK1 expression than healthy ones [34]. Clinical cancer identification and therapy are critical as cancer rates grow. PGK1 is a diagnostic and therapeutic target for tumors [35].

In this study, KEGG pathway and GO term analysis showed that up-regulated PGK1 mainly promotes important ncRNA export from the nucleus, tRNA transport, hemidesmosome assembly, DNA replication origin binding, spindle mid zone, and inhibits immunoglobulin complex, complement activation, humoral immune response mediated by circulating immunoglobulin, immunoglobulin complex circulating, immunoglobulin receptor binding are closely related. At the same time, PGK1 inhibits primary immunodeficiency, an intestinal immune network for IgA production, linoleic acid metabolism, asthma, and arachidonic acid metabolism. In HNSC, increased PGK1 expression also promotes Small cell lung cancer, homologous recombination, mismatch repair, pentose phosphate pathway, ecm receptor interaction. We infer that overexpression of PGK1 in HNSC patients is likely to achieve HNSC proliferation, migration and metastasis by promoting DNA replication-related processes and functions and inhibiting immune function-related effects. In HNSC patients, PGK1 overexpression also inhibited the metabolism of linoleic acid and arachidonic acid. To determine how PGK1 acts in HNSCs, more research is needed. Our data increase knowledge of PGK1's biology and function, which makes overexpression particularly harmful in HNSCs. PGK1 inhibitors aren't being studied or used for HNSC. Our study will guide future development and usage of PGK1 inhibitors in HNSC.

Malignancies depend on the immunological microenvironment [36]. Tumor microenvironment (TME) signatures can be indicators for tumor cell immunotherapy response and prognosis [37]. Numerous studies have shown that the HNSC tumor microenvironment contributes to immunological changes during HNSC progression [38-40]. Our work is the first to establish PGK1 as an immune-related prognostic marker in HNSC tumor microenvironments. Using TIMER, we compared PGK1 expression and immune infiltration in HNSCs. Figure 5A shows that PGK1 correlated negatively with B cells and positively with dendritic cells. Patients with B-cell infiltration had a higher cumulative survival rate. This study compared the invasion of 24 immune cell types between high and low PGK1 HNSC patients. These data imply PGK1 regulates HNSC immunological activity. To understand the relationship between PGK1 and immune cells in vivo, controlled research and multicenter clinical trials are needed.

PGK1 was co-expressed with 26 immunological checkpoints, including TIGIT, NRP1, BTLA, TNFRSF14, TNFSF4, CD244, CD40LG, LAG3, CTLA4, CD48, CD80, CD276, CD200R1, PDCD1, LGALS9, IDO2, TMIGD2, PDCD1LG2, HHLA2, CD27, TNFRSF25, VSIR, TNFRSF4, TNFRSF18, High-PGK1 patients have greater immune checkpoint expression than low-PGK1 patients. High PGK1 expression in HNSCs reduced the efficiency of immune checkpoint blockade therapy. Patients with increased PGK1 in HNSCs exhibited lower survival after immune checkpoint blockade (ICB). Based on the GO enrichment analysis, we expected that patients with high PGK1 expression in HNSC had more inhibition of immunoglobulin receptor binding, immunoglobulin-related circulation, intestinal immunity, and other immune-related responses. We believe that PGK1 modulates the immune system and immunological function to cause and promote HNSC.

Our research contains flaws. First, this study's data originate from internet sources that are constantly updated, thus the results may change. Second, our study lacked issue and therapy information. Third, neither in vivo nor in vitro experiments were undertaken to validate PGK1's function in HNSC proliferation, migration, and invasion, nor its molecular mechanism in HNSC immunity. In future studies, we'll pay more attention to baseline patient information and conduct more testing to validate expected outcomes.

Conclusion

This is the first research to correlate PGK1 with immune function in HNSC. With a greater understanding of its functional breadth, PGK1 might be used to diagnose and treat HNSC and contribute to the development of biomarker

therapy as a future treatment option. How PGK1 promotes tumor development and metastasis in HNSCs needs additional explanation.

Conflict of interest statement

All authors declared that there was no conflict of interest.

Funding Statement

This work was sponsored by the grand "Xiamen Health High Quality Development Project (2024GZL-QN087)", "Young Investigator Research Program of Xiang'an Hospital of Xiamen University (XAH23003)", "Guidance in Medical and Health Program of Xiamen, China" (Grant number: 3502Z20224ZD1155) .

Authors' contributions

Hesen Huang: Writing - original draft, Investigation, Conceptualization, Visualization, Formal analysis. Kaiqin Chen and Yu Du: Writing – original draft, Investigation, Software. Jing Gao: Writing -original draft, Software. Yixian Ye: Resources, Writing - review & editing, Project administration, Supervision.

Acknowledgments

The author thanks all the medical staff who contributed to the maintenance of the medical record database.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors have agreed to the publication.

References

1. Chaturvedi AK, Anderson WF, Lortet-Tieulent J, et al. Worldwide trends in incidence rates for oral cavity and oropharyngeal cancers. J Clin Oncol. 2013;31(36):4550-4559. doi:10.1200/JCO.2013.50.3870.
2. Liu S, Shi C, Wang X, Ma X, Gao P. Low expression of RalGAPs associates with the poorer overall survival of head and neck squamous cell carcinoma. Transl Cancer Res. 2021;10(12):5085-5094. doi:10.21037/tcr-21-1489.
3. Jing F, Wang J, Zhou L, Ning Y, Xu S, Zhu Y. Bioinformatics analysis of the role of CXC ligands in the microenvironment of head and neck tumor. Aging (Albany NY). 2021;13(13): 17789-17817. doi:10.18632/aging.203269.
4. Johnson DE, Burtness B, Leemans CR, Lui VWY, Bauman JE, Grandis JR. Head and neck squamous cell carcinoma. Nat Rev Dis Primers. 2020;6(1):92. Published 2020 Nov 26. doi:10.1038/s41572-020-00224-3.
5. Kitamura N, Sento S, Yoshizawa Y, Sasabe E, Kudo Y, Yamamoto T. Current Trends and Future Prospects of Molecular Targeted Therapy in Head and Neck Squamous Cell Carcinoma. Int J Mol Sci. 2020;22(1):240. Published 2020 Dec 29. doi:10.3390/ijms22010240.
6. Zhou F, Chen AX, Lv HY, Liang DH, Yu HS. Establishment of an immune-related gene prognostic model for head and neck tumors. J Biol Regul Homeost Agents. 2021;35(3):975-986. doi:10.23812/21-14-A.
7. Lecerf C, Kamal M, Vacher S, et al. Immune gene expression in head and neck squamous cell carcinoma patients. Eur J Cancer. 2019; 121:210-223. doi:10.1016/j.ejca. 2019.08.028.
8. Lin B, Li H, Zhang T, Ye X, Yang H, Shen Y. Comprehensive analysis of macrophage-related multigene signature in the tumor microenvironment of head and neck squamous cancer. Aging (Albany NY). 2021;13(4):5718-5747. doi:10.18632/aging.202499.
9. Hu K, Yao L, Zhou L, Li J. Diverse Chromobox Family Members: Potential Prognostic Biomarkers and Therapeutic Targets in Head and Neck Squamous Cell Carcinoma. Int J Gen Med. 2022;15:2463-2474. Published 2022 Mar 4. doi:10.2147 /IJGM.S350783.
10. Li L, Bai Y, Gao Y, et al. Systematic Analysis Uncovers Associations of PGK1 with Prognosis and Immunological Characteristics in Breast Cancer. Dis Markers. 2021;2021:7711151. Published 2021 Nov 8. doi:10.1155/2021/7711151.
11. van Weverwijk A, Koundouros N, Iravani M, et al. Metabolic adaptability in metastatic breast cancer by AKR1B10-dependent balancing of glycolysis and fatty acid oxidation. Nat Commun. 2019;10(1):2698. Published 2019 Jun 20. doi:10.1038/s41467-019-10592-4.
12. Chu Z, Huo N, Zhu X, et al. FOXO3A-induced LINC00926 suppresses breast tumor growth and metastasis through inhibition of PGK1-mediated Warburg effect. Mol Ther.

2021;29(9):2737-2753. doi:10.1016/j.ymthe.2021.04.036.
13. Li X, Jiang Y, Meisenhelder J, et al. Mitochondria-Translocated PGK1 Functions as a Protein Kinase to Coordinate Glycolysis and the TCA Cycle in Tumorigenesis. Mol Cell. 2016;61(5):705-719. doi:10.1016/j.molcel.2016.02.009.
14. De Mello RA, Aguiar PN, Tadokoro H, et al. MetaLanc9 as a novel biomarker for non-small cell lung cancer: promising treatments via a PGK1-activated AKT/mTOR pathway. J Thorac Dis. 2018;10(Suppl 17):S2076-S2078. doi:10.21037/jtd.2018.04.122.
15. Yu T, Zhao Y, Hu Z, et al. MetaLnc9 Facilitates Lung Cancer Metastasis via a PGK1-ActivatedAKT/mTOR Pathway. Cancer Res. 2017;77(21):5782-5794. doi:10.1158/0008-5472.CAN-17-0671.
16. Duncan L, Shay C, Teng Y. PGK1 : An Essential Player in Modulating Tumor Metabolism. Methods Mol Biol. 2022;2343:57-70. doi:10.1007/978-1-0716-1558-4_4.
17. Wang Z, Jensen MA, Zenklusen JC. A Practical Guide to The Cancer Genome Atlas (TCGA). Methods Mol Biol. 2016;1418:111-141. doi:10.1007/978-1-4939-3578-9_6.
18. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS. 2012;16(5):284-287. doi:10.1089/omi.2011.0118.
19. Li T, Fu J, Zeng Z, et al. TIMER2.0 for analysis of tumor-infiltrating immune cells. Nucleic Acids Res. 2020;48(W1):W509-W514. doi:10.1093/nar/gkaa407.
20. Aran D, Sirota M, Butte AJ. Systematic pan-cancer analysis of tumour purity [published correction appears in Nat Commun. 2016;7:10707]. Nat Commun. 2015;6:8971. Published 2015 Dec 4. doi:10.1038/ncomms9971.
21. Jiang P, Gu S, Pan D, et al. Signatures of T cell dysfunction and exclusion predict cancer immunotherapy response. Nat Med. 2018;24(10):1550-1558. doi:10.1038/s41591-018-0136-1.
22. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res. 2017;45(W1):W98-W102. doi:10.1093/nar/gkx247.
23. Fan C, Wang J, Tang Y, et al. Upregulation of long non-coding RNA LOC284454 may serve as a new serum diagnostic biomarker for head and neck cancers. BMC Cancer. 2020;20(1):917. Published 2020 Sep 24. doi:10.1186/s12885-020-07408-w.
24. Zhang H, Liu R, Sun L, Guo W, Ji X, Hu X. Comprehensive Analysis of Gene Expression Changes and Validation in Hepatocellular Carcinoma. Onco Targets Ther. 2021;14:1021-1031. Published 2021 Feb 15. doi:10.2147/OTT.S294500.
25. Lánczky A, Nagy Á, Bottai G, et al. miRpower: a web-tool to validate survival-associated miRNAs utilizing expression data from 2178 breast cancer patients. Breast Cancer Res Treat. 2016;160(3):439-446. doi:10.1007/s10549-016-4013-7.
26. Ohtani H. Focus on TILs: prognostic significance of tumor infiltrating lymphocytes in human colorectal cancer. Cancer Immun. 2007;7:4. Published 2007 Feb 21.
27. Gu Z, Wang H, Xia J, et al. Decreased ferroportin promotes myeloma cell growth and osteoclast differentiation. Cancer Res. 2015;75(11):2211-2221. doi:10.1158/0008-5472.CAN-14-3804.
28. Schneider CC, Archid R, Fischer N, et al. Metabolic alteration--Overcoming therapy resistance in gastric cancer via PGK-1 inhibition in a combined therapy with standard chemotherapeutics. Int J Surg. 2015;22:92-98. doi:10.1016/j.ijsu.2015.08.020.
29. Townsend MH, Ence ZE, Felsted AM, et al. Potential new biomarkers for endometrial cancer. Cancer Cell Int. 2019;19:19. Published 2019 Jan 21. doi:10.1186/s12935-019-0731-3.
30. Ameis HM, Drenckhan A, von Loga K, et al. PGK1 as predictor of CXCR4 expression, bone marrow metastases and survival in neuroblastoma. PLoS One. 2013;8(12):e83701. Published 2013 Dec 20. doi:10.1371/journal.pone.0083701.
31. Irshad K, Mohapatra SK, Srivastava C, et al. A combined gene signature of hypoxia and notch pathway in human glioblastoma and its prognostic relevance. PLoS One. 2015;10(3):e0118201. Published 2015 Mar 3. doi:10.1371/journal.pone.0118201.
32. Fu Q, Yu Z. Phosphoglycerate kinase 1 (PGK1) in cancer: A promising target for diagnosis and therapy. Life Sci. 2020;256:

117863. doi:10.1016/j.lfs.2020.117863.
33. Carnielli CM, Macedo CCS, De Rossi T, et al. Combining discovery and targeted proteomics reveals a prognostic signature in oral cancer. Nat Commun. 2018;9(1):3598. Published 2018 Sep 5. doi:10.1038/s41467-018-05696-2.
34. Lu W, Gao J, Yang J, et al. Down-Regulated Phosphoglycerate Kinase 1 Expression Is Associated With Poor Prognosis in Patients With Gallbladder Cancer. Medicine (Baltimore). 2015;94(49):e2244. doi:10.1097/MD. 0000000000002244.
35. Tekade RK, Sun X. The Warburg effect and glucose-derived cancer theranostics. Drug Discov Today. 2017;22(11):1637-1653. doi: 10.1016/j.drudis.2017.08.003.
36. Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. Nat Immunol. 2013;14 (10): 1014-1022. doi:10.1038/ni.2703.
37. Wu T, Dai Y. Tumor microenvironment and therapeutic response. Cancer Lett. 2017;387: 61-68. doi:10.1016/j.canlet.2016.01.043.
38. Caruntu A, Scheau C, Tampa M, Georgescu SR, Caruntu C, Tanase C. Complex Interaction Among Immune, Inflammatory, and Carcinogenic Mechanisms in the Head and Neck Squamous Cell Carcinoma. Adv Exp Med Biol. 2021;1335:11-35. doi:10.1007/5584_2021_626.
39. Wang J, Tian Y, Zhu G, et al. Establishment and validation of immune microenvironmental gene signatures for predicting prognosis in patients with head and neck squamous cell carcinoma. Int Immunopharmacol. 2021;97: 107817. doi:10.1016/j.intimp.2021.107817.
40. Wang G, Zhang M, Cheng M, et al. Tumor microenvironment in head and neck squamous cell carcinoma: Functions and regulatory mechanisms. Cancer Lett. 2021;507:55-69. doi:10.1016/j.canlet.2021.03.009.