Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Expression and prognostic analysis of PFKFB4 in oral squamous cell carcinoma

Shuting Chen[1,#], Jiusong Han[2,#], Wei Wang[2], Zhengzheng Wu[1], Juan Wang[1,*], Bin Cheng[1,*]

[1] Hospital of Stomatology, Guangdong Provincial Key Laboratory of Stomatology, Sun Yat-sen University, Guangzhou 510055, Guangdong, People's Republic of China

[2] Stomatological Hospital, School of Stomatology, Southern Medical University, S366 Jiangnan Boulevard, Guangzhou 510280, Guangdong, People's Republic of China

Article Info

Article history:

Received: March 21, 2024
Accepted: May 11, 2024
Published: July 31, 2024

Use your device to scan and read the article online

Abstract

Fructose-6-phosphate 2-kinase/fructose-2,6-bisphosphatase 4 (PFKFB4) is a crucial enzyme in the glycolysis pathway, possessing both kinase and phosphatase capabilities. Although it has emerged as an important oncogene in various cancer types, its function in oral squamous cell carcinoma (OSCC) is still not well understood. In our research, PFKFB4 expression was assessed via immunohistochemical (IHC) staining of tissue microarrays and OSCC patient specimens. The transcriptional expression of PFKFB4 in OSCC was analyzed by utilizing The Cancer Genome Atlas (TCGA) dataset. Correlation between PFKFB4 expression and clinicopathological features was examined using the $\chi 2$ test. Prognostic investigation of PFKFB4 was conducted via Kaplan-Meier and Cox analyses. PFKFB4 levels were notably elevated in OSCC samples in comparison to adjacent normal tissues ($P < 0.001$). Elevated PFKFB4 expression was associated with higher histologic grade ($P = 0.0438$), higher T stage ($P = 0.031$), and more advanced clinical stage ($P = 0.0063$). The ROC curve demonstrated the diagnostic potential of PFKFB4 (AUC = 0.827). Increased levels of PFKFB4 were linked to decreased overall survival (OS) ($P = 0.04$), poorer disease-specific survival (DSS) ($P = 0.04$), and shorter progression-free interval (PFI) ($P < 0.001$). PFKFB4 expression was identified as an independent risk factor for OS based on Cox regression analysis [hazard ratio (HR) = 1.517, $P = 0.044$)]. An OS nomogram was constructed with a concordance index of 0.690. Our findings reveal that upregulated PFKFB4 expression in OSCC tissues could serve as a potential prognostic biomarker.

Keywords: PFKFB4, Oral squamous cell carcinoma, Prognosis, Metabolism.

1. Introduction

OSCC ranks among the most prevalent malignancies affecting the head and neck region. Typically, most OSCC patients are at an advanced stage when first diagnosed [1], contributing to a relatively low 5-year survival rate, which has remained around 60% [2]. Poor prognosis can mainly be attributed to late diagnosis, lack of personalized targeted therapies, and the development of drug resistance [3]. Therefore, there is a critical need to identify new markers that can facilitate the diagnosis and treatment of OSCC, ultimately improving patient outcomes.

Cancer is characterized by the alteration of glucose metabolism [4]. Cancer cells tend to use glycolysis over oxidative phosphorylation for energy production, even when oxygen is available. This is a phenomenon known as the Warburg effect or aerobic glycolysis [5]. The rewiring of glucose metabolism is also essential in OSCC tumorigenesis [6]. PFKFB4 is a critical metabolic enzyme that plays a key role in controlling glucose metabolism by dynamically mediating glycolysis [7]. The PFKFB4 gene encodes a PFKFB isoenzyme which was first discovered in the testes [8]. Growing proof indicates that PFKFB4 is crucial in the glucose metabolism of tumor cells by shifting glucose to the pentose phosphate pathway. What's more, PFKFB4 facilitates ROS detoxification as well as lipid and nucleotide synthesis in cancer cells [9,10].

PFKFB4 has been found to be upregulated in various cancers, including glioblastoma, gastric, breast, thyroid, bladder, lung, and colon cancer [10-12]. Moreover, its upregulation has been linked to tumorigenesis, cell proliferation, metastasis, and poorer survival outcomes [13-16], underscoring its pivotal role in cancer initiation and progression.

Previous research has shown the importance of PFKFB4 in various human cancers. However, clinical implications of PFKFB4 in OSCC remain elusive. Thus, our research examined PFKFB4 expression using immunohistochemistry in normal and malignant oral tissues. Additionally, we evaluated PFKFB4 mRNA expression in OSCC and its clinicopathology correlation using publicly available transcriptomic databases. Our results showed a notable upregulation of PFKFB4 in OSCC tissues compared to normal tissues. Upregulated expression of PFKFB4 was significantly associated with advanced tumor stage

* Corresponding author.
E-mail address: wangj255@mail.sysu.edu.cn (J. Wang); chengbin@mail.sysu.edu.cn (B. Cheng).
# These authors contributed equally
Doi: http://dx.doi.org/10.14715/cmb/2024.70.7.28

and histologic grade. Moreover, PFKFB4 overexpression predicted poor prognosis. Our research indicates that PFKFB4 could be a useful diagnostic and prognostic biomarker for OSCC patients.

2. Materials and methods

2.1. Clinical specimens and tissue microarray

45 patients with primary OSCC tumors were included in the research conducted at the Stomatological Hospital, Southern Medical University. 45 OSCC tissues and 11 adjacent normal tissues were collected postoperatively. Informed consent was given by all participants. Additionally, the human OSCC tissue microarray (HN192Oc01, Biotech Company, Xi'an, China) was utilized, comprising 175 tumor tissues and 12 adjacent normal tissues. The Ethics Committee of Stomatological Hospital, Southern Medical University approved the study protocol (AFSQ-0105).

2.2. Immunohistochemical analysis

OSCC samples embedded by paraffin were deparaffinized using xylene. Then, they were rehydrated with gradient concentrations of alcohol. The antigen retrieval process involved heating in EDTA buffer. Then endogenous peroxidase activity was inhibited and the slides were blocked, followed by overnight incubation with anti-PFKFB4 antibody (Proteintech, Rosemont, IL, USA). Afterward, the slides were treated with HRP-conjugated secondary antibody.

The reactions for PFKFB4 were visualized by 3,3-diaminobenzidine (DAB, Vector Laboratories, Burlingame, CA, USA) and the nuclei were visualized by counterstaining using hematoxylin. Stained slides were scanned and digital images were generated by the Aperio ePathology eIHC IVD System (Leica, Leica Biosystems Imaging, Inc., Wetzlar, Germany). PFKFB4 expression was measured utilizing the Imagescope software according to Aperio ePathology eIHC Cytoplasm Image Analysis. The image analysis was conducted based on the following algorithm: quantify the pixel intensity of the selected area, identify the nuclei and cytoplasm, detect the intensity of nuclei and cytoplasm and set threshold. The algorithm provides an H-Score, a final co-localized result and a histogram for the IHC staining of the cytoplasm and nuclei. The H-scores ranged from 0 to 105. Five representative fields were evaluated (400×magnification) for each slide. Then, the final score was assigned to each sample. The median score was employed as the threshold to categorize PFKFB4 expression into high and low levels in the samples.

2.3. Cell culture

The HSC6, SCC4, SCC9, CAL33, CAL27, and CAL27 cell lines were maintained in our laboratory. The CAL27, SCC4, and SCC9 cell lines were obtained from the American Type Culture Collection. HSC6 was generously provided by Prof. J Silvio Gutkind at the National Institutes of Health in the USA. CAL33 cells were obtained from the DSMZ (German Collection of Microorganisms and Cell Cultures). HOKs were acquired from ScienCell Research Laboratories through commercial purchase. CAL27, CAL33, and HSC6 cells were grown in DMEM (Invitrogen, Carlsbad, CA, USA) with 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA). SCC4 and SCC9 cells were cultivated in DMEM/F12 1:1 (Gibco, Rockville, MD, USA) supplemented with 10% FBS and 400 ng/ml hydrocortisone (Solarbio, Beijing, China). HOKs were maintained in Keratinocyte-SFM (Gibco, Rockville, MD, USA) medium supplemented with penicillin-streptomycin (100 U/mL).

2.4. Western blot assay

Cellular proteins were fully lysed using RIPA buffer with protease inhibitor (Roche, Basel, Switzerland) and qualified with BCA protein assay kit (Sigma-Aldrich, St. Louis, MO, USA). After performing SDS-PAGE, the extracted proteins were transferred onto PVDF membranes (Millipore, Billerica, MA, USA). Subsequently, the membranes were blocked using nonfat milk and then subjected to incubation with primary antibodies targeting PFKFB4 (Proteintech, Rosemont, IL, USA) and GAPDH (Proteintech, Rosemont, IL, USA). Then, secondary antibodies were used to incubate the membranes and they were detected using enhanced chemiluminescence (ECL) assay (Thermo, Waltham, MA, USA).

2.5. Gene expression analysis of PFKFB4 in OSCC

The TCGA database was used to obtain the transcriptional expression levels of PFKFB4 in patients with OSCC and HNSC. The OSCC data were extracted from the HNSC dataset. The analysis included 504 TCGA-HNSC samples and 330 TCGA-OSCC samples. The predictive sensitivity and specificity of PFKFB4 gene were evaluated by ROC analysis using pROC package in R. The AUC value was calculated to assess the effectiveness of the ROC analysis.

2.6. Prognostic value of PFKFB4 gene in OSCC

Prognostic analysis was conducted using Kaplan-Meier analysis. R packages "survival" and "survminer" were utilized to generate Kaplan-Meier curves. Patients were categorized into high and low-expression groups using the optimal threshold of PFKFB4 expression [17]. The effects of clinical characteristics on patient outcomes were assessed through Cox regression analyses. Then, a nomogram was established using independent prognostic factors, with the aim of predicting overall survival probability. Additionally, calibration plots were utilized to assess the nomogram's performance.

2.7. Statistical analysis

The correlation between PFKFB4 gene expression and various clinicopathological characteristics was assessed using the $\chi 2$ test. PFKFB4 gene expression was also compared between OSCC and normal tissues utilizing t-test. Statistic Package for Social Science (SPSS) 22.0 (IBM, Armonk, NY, USA) was used to analyze the data and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Upregulation of PFKFB4 protein expression in OSCC tissues

The study included a total of 220 OSCC tissues and 23 normal tissues. Among these specimens, 190 cases were male, and 30 cases were female, with ages ranging from 27 to 78 years old and an average age of 53.4 ± 9.913 years old (Table 1).

To investigate the clinical significance of PFKFB4 in OSCC, we conducted IHC staining to assess its expression in tumor samples and normal tissues. Positive staining for PFKFB4 protein was predominantly observed in the

Table 1. Analysis of PFKFB4 protein expression and clinical features (n=220).

Characteristics	Cases	Low expression of PFKFB4	High expression of PFKFB4	P value
Age, n (%)				0.874
<60	168	84 (50.0%)	84 (50.0%)	
≥60	52	27 (51.9%)	25 (48.1%)	
Gender, n (%)				1
Male	190	96 (50.5%)	94 (49.5%)	
Female	30	15 (50.0%)	15 (50.0%)	
T stage, n (%)				0.031*
T1 + T2	112	65 (58.0%)	47 (42.0%)	
T3 + T4	108	46 (42.6%)	62 (57.4%)	
N stage, n (%)				0.686
N0	115	60 (52.2%)	55 (47.8%)	
N+	105	51 (48.6%)	54 (51.4%)	
TNM stage, n (%)				0.074
I + II	63	38 (60.3%)	25 (39.7%)	
III + IV	157	73 (46.5%)	84 (53.5%)	
Histologic grade, n (%)				0.547
Well	159	77 (48.4%)	82 (51.6%)	
Moderate + poor	61	33 (54.1%)	28 (45.9%)	

* $P < 0.05$; T, tumor; N, node.

cytoplasm of OSCC cells, whereas the majority of stromal cells displayed negative staining for PFKFB4 (Figure 1A, B, C).

Moreover, PFKFB4 was overexpressed in OSCC samples relative to adjacent normal tissues (Figure 1D, $P < 0.0001$). PFKFB4 levels were correlated with the T stage (Table 1, $P = 0.031$) and showed a potential link with TNM stage (Table 1, $P = 0.074$). Besides, PFKFB4 protein levels in OSCC cell lines were also determined by Western blot. OSCC cell lines (CAL27, CAL33, and SCC9) exhibited higher levels of PFKFB4 protein than HOKs (Figure 1E).

3.2. Expression pattern of PFKFB4 transcript in TCGA OSCC patients

To further validate our observations on PFKFB4 in OSCC, UALCAN transcriptome analysis was conducted using TCGA database. A significant upregulation of PFKFB4 transcripts was observed in HNSCC samples in comparison with normal tissues ($P < 0.001$, Figure 2A). Transcriptional expression analysis revealed elevated levels of PFKFB4 in OSCC specimens compared to adjacent normal tissues, as indicated by both unpaired and paired sample analyses. ($P < 0.001$, Figure 2B, 2C).

To assess the diagnostic performance of PFKFB4, a ROC curve was used, demonstrating high predictive accuracy in distinguishing OSCC tissues from adjacent normal tissues (AUC = 0.827, 95% CI = 0.751-0.902, Figure 2D).

3.3. Correlation between PFKFB4 expression and clinical characteristics of patients with OSCC

The $\chi 2$ test was employed to investigate the correlation between PFKFB4 levels and clinical features (Table 2). Elevated levels of PFKFB4 were linked to clinical stage ($P = 0.0063$, Figure 3A) and histologic grade ($P = 0.0438$, Figure 3B). However, no significant correlations were observed between PFKFB4 expression and pathologic stage, pathologic T stage, age, and alcohol history (Figure 3C-F).

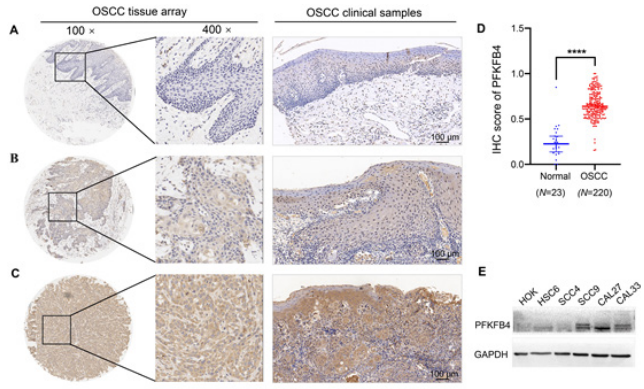

Fig. 1. PFKFB4 levels are increased in both OSCC tissues and cell lines. (A-C) Representative immunohistochemistry images of PFKFB4 staining in normal and OSCC tissues. Staining intensity: (A) Negative, (B) Weak, (C) Strong. (D) Statistical analysis of PFKFB4 expression in normal and OSCC tissues based on staining scores. (E) Relative protein levels of PFKFB4 in human oral keratinocyte HOKs and OSCC cell lines were determined via western blot analysis, with GAPDH serving as an endogenous control. **** indicates $P < 0.0001$.

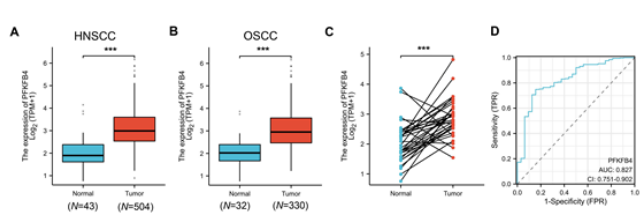

Fig. 2. The transcriptional expression of PFKFB4 was compared between OSCC and normal tissues in TCGA cohort. (A) Transcriptional expression analysis revealed up-regulation of PFKFB4 in HNSCC tissues. (B) Transcriptional expression of PFKFB4 was higher in OSCC tissues than in non-matched normal tissues. (C) The expression of PFKFB4 was elevated in OSCC tissues compared to normal tissues. (D) The ROC curve was utilized to assess PFKFB4's diagnostic performance in OSCC patients. *** indicates $P < 0.001$.

Table 2. The association between PFKFB4 levels and clinicopathological parameters in patients with OSCC in the TCGA dataset.

Characteristics	Low expression of PFKFB4	High expression of PFKFB4	P value
Age, n (%)			0.912
<= 60	83 (25.2%)	82 (24.8%)	
> 60	82 (24.8%)	83 (25.2%)	
Gender, n (%)			0.341
Male	110 (33.3%)	118 (35.8%)	
Female	55 (16.7%)	47 (14.2%)	
Pathologic T stage, n (%)			0.265
T1&T2	71 (23.4%)	58 (19.1%)	
T3&T4	85 (28.0%)	90 (29.5%)	
Pathologic N stage, n (%)			0.187
N0	55 (19.9%)	65 (23.6%)	
N1&N2&N3	84 (30.4%)	72 (26.1%)	
Pathologic stage, n (%)			0.331
Stage I &Stage II	41 (13.7%)	33 (10.7%)	
Stage III &Stage IV	110 (36.8%)	115 (38.8%)	
Histologic grade, n (%)			0.038*
G1	33 (10.2%)	19 (5.9%)	
G2&G3&G4	129 (40.1%)	141 (43.8%)	
Clinical stage, n (%)			0.037*
Stage I	9 (2.8%)	2 (0.6%)	
Stage II &Stage III &Stage IV	154 (48.1%)	155 (48.4%)	
Alcohol history, n (%)			0.251
No	48 (14.9%)	57 (17.7%)	
Yes	114 (35.4%)	103 (32%)	
Smoker, n (%)			0.152
No	38 (11.7%)	50 (15.4%)	
Yes	123 (38%)	113 (34.9%)	
Race, n (%)			0.079
Asian	2 (0.6%)	7 (2.2%)	
Black or African American	14 (4.4%)	7 (2.2%)	
White	145 (45.5%)	144 (45.1%)	
Radiation therapy, n (%)			0.963
No	58 (19.7%)	58 (19.7%)	
Yes	90 (30.5%)	89 (30.2%)	

* $P < 0.05$; T, tumor; N, node.

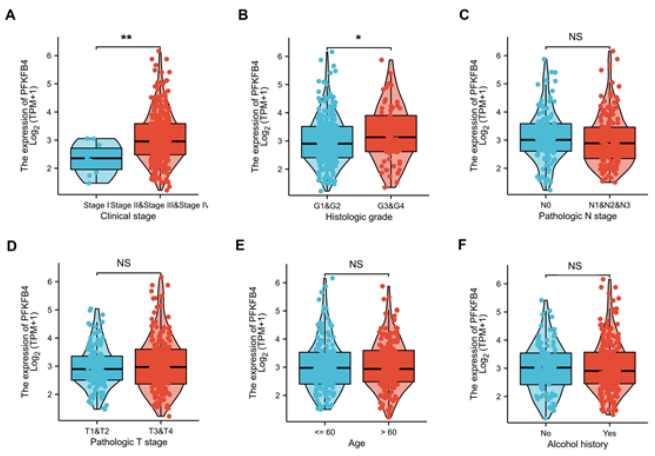

Fig. 3. Analysis of PFKFB4 expression levels was conducted in various subgroups, (A) Clinical stage, (B) Histologic grade, (C) Pathologic N stage, (D) Pathologic T stage, (E) Age, and (F) Alcohol history.

3.4. Prognostic value of PFKFB4 in patients with OSCC

The Kaplan-Meier analysis showed that increased levels of PFKFB4 were associated with lower overall survival (OS) rates (HR = 1.44, 95% CI = 1.02-2.03, $P = 0.04$), worse disease-specific survival (DSS) (HR = 1.57, 95% CI = 1.02-2.41, $P = 0.04$), and shorter progression-free interval (PFI) (HR = 1.83, 95% CI = 1.30-2.59, $P < 0.001$) (Figure 4A-C), suggesting a connection between elevated PFKFB4 expression and poor OSCC prognosis.

Univariate Cox analysis demonstrated that high PFKFB4 expression (HR = 1.633, 95% CI = 1.143-2.334, $P = 0.007$), along with pathologic N1-3 stage (HR = 1.785, 95% CI = 1.223-2.605, $P = 0.003$), pathologic T2-4 stage (HR = 3.057, 95% CI = 1.347-6.936, $P = 0.008$), and pathologic stage III/IV (HR = 2.174, 95% CI = 1.388-3.405, $P < 0.001$) were associated with shorter OS time (Table 3). Multivariate analysis further identified PFKFB4 expression (HR = 1.517, 95% CI = 1.011-2.275, $P = 0.044$) and

Table 3. Cox analysis of clinicopathological features and PFKFB4 expression for overall survival.

Characteristics	Cases	Univariate analysis		Multivariate analysis	
		HR (95% CI)	P value	HR (95% CI)	P value
Age					
<=60	158	Reference			
>60	172	1.261 (0.912 - 1.744)	0.161		
Gender					
Male	225	Reference			
Female	105	1.101 (0.786 – 1.544)	0.576		
Pathologic stage					
Stage I & Stage II	74	Reference		Reference	
Stage III & Stage IV	225	2.174 (1.388 - 3.405)	< 0.001*	2.107 (1.051 - 4.223)	0.036*
Pathologic N stage					
N0	120	Reference		Reference	
N1&N2&N3	156	1.785 (1.223 - 2.605)	0.003*	1.281 (0.823 - 1.994)	0.272
Pathologic T stage					
T1	31	Reference		Reference	
T2&T3&T4	273	3.057 (1.347 - 6.936)	0.008*	3.008 (0.936 - 9.669)	0.065
PFKFB4					
low	247	Reference			
High	83	1.633 (1.143 – 2.334)	0.007*	1.517 (1.011 – 2.275)	0.044*
Histologic grade					
G1	52	Reference			
G2&G3&G4	269	1.517 (0.946 - 2.432)	0.084		

* $P < 0.05$; T, tumor; N, node.

pathologic stage (HR = 2.107, 95% CI = 1.051-4.223, P = 0.036) as independent prognostic factors (Table 3).

We establish a nomogram to account for the predictive capacity of PFKFB4 expression and pathologic stage. Total score of each individual was calculated and patients with a higher score exhibited poorer survival (Figure 4D). The calibration curves demonstrated the powerful predictive ability of PFKFB4 gene for overall survival at 1, 3, and 5 years (Figure 4E-G). The nomogram was constructed with a concordance index 0.690 (95% CI = 0.659-0.721), indicating that the Cox proportional hazard model had moderate accuracy in predicting OS.

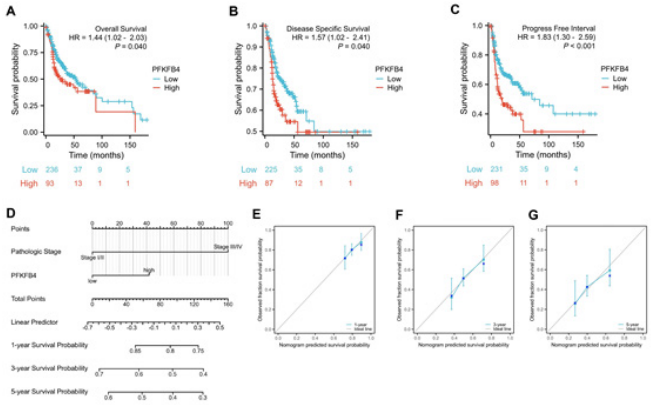

Fig. 4. Prognostic value of PFKFB4 expression in OSCC patients measured by Kaplan-Meier analysis. OS in (A), DSS in (B), PFI in (C) for OSCC patients with high versus low PFKFB4. (D) Nomogram was generated to predict OS probability. (E-G) Calibration curves demonstrated good predictive ability for overall survival at 1, 3, and 5 years.

4. Discussion

OSCC poses a significant global healthcare challenge due to late diagnosis and poor survival rates. Early detection and diagnosis of early-stage OSCC are crucial for improving patient outcomes. Therefore, it is essential to delve into the oncology and pathogenesis of OSCC and discover novel molecular biomarkers, in the hope of facilitating early detection and enhancing personalized therapies. PFKFB4, a critical regulator of glycolysis, has been reported to function as an important oncogene in various malignancies [15,18-22]. Hence, we explored the diagnostic and prognostic role of PFKFB4 in OSCC patients. Our findings showed that PFKFB4 expression was elevated in OSCC. Besides, up-regulation of PFKFB4 was correlated with unfavorable clinicopathological variables, such as higher histologic grade and advanced tumor stage. Moreover, relevance between upregulated PFKFB4 expression and poor survival was proven. According to Cox analysis, PFKFB4 may act as an independent predictor of prognosis in OSCC.

Upregulation of PFKFB4 expression has been reported in prostate, breast, gastric, bladder, and colorectal cancers [10,14,20,23]. Consistent with these findings, our IHC results demonstrated a notable increase in PFKFB4 protein expression in OSCC tissues. In addition, the protein level of PFKFB4 was higher in OSCC cells than in oral keratinocytes. Subsequently, we analyzed PFKFB4 transcript expression in OSCC using the TCGA database, revealing an upregulation of PFKFB4 mRNA in OSCC tissues. Our investigation unveiled PFKFB4 overexpression in OSCC tissues at both transcriptional and translational levels.

Moreover, high levels of PFKFB4 were found to be linked to more advanced pathologic tumor stage (T3-T4)

and higher histologic grade (grade 2-4) in OSCC patients. Similar correlation between PFKFB4 expression and tumor stage has been reported in lung cancer [24]. Additionally, upregulation of PFKFB4 expression was observed in gastric cancer tissues and PFKFB4 upregulation was correlated with age, pathologic tumor stage, and tumor-node-metastasis stage [25]. Collectively, these studies indicated that PFKFB4 overexpression was significantly relevant to clinicopathological characteristics in various solid cancers.

Multiple studies have shown that increased expression of PFKFB4 promotes cancer cell proliferation, migration and invasion, as well as plays a role in developing resistance to chemotherapy [11,14,26]. Silencing or inhibiting PFKFB4 gene was shown to suppress glycolysis and proliferation in various types of human cancer cells [14,21,23]. In a preclinical animal model, depletion of PFKFB4 results in tumor growth arrest [27]. Besides, PFKFB4 has been demonstrated to promote tumor progression by regulating glycolytic flux or glycolysis-independent mechanism. Alterations in PFKFB4 expression affect glucose metabolism, release of lactate, and ROS production in breast cancer cells [28]. PFKFB4 plays a role in promoting cancer tumorigenesis and progression by phosphorylating and activating steroid receptor coactivators (SRCs) in breast cancer and lung adenocarcinoma [18,21]. Furthermore, PFKFB4 affects chemoresistance in lung cancer through regulation of autophagy [26]. PFKFB4 also stimulates synthesis of hyaluronan through activation of p38 pathway, thereby promoting breast cancer metastasis [14]. Further research is needed to clarify the functional profiles and precise mechanisms of PFKFB4 upregulation in OSCC.

We analyzed the diagnostic capacity of PFKFB4 in OSCC through ROC analysis, revealing its high accuracy in distinguishing OSCC tissue from normal tissue. Subsequently, the prognostic significance of PFKFB4 was assessed using Kaplan-Meier analysis. The results revealed that PFKFB4 overexpression was found to be associated with reduced OS, DSS, and PFI. Similar correlations between PFKFB4 overexpression and poor clinical outcomes have been observed in several other cancers. Besides, patients with decreased PFKFB4 expression exhibited notably improved overall survival in gastric cancer [25]. Moreover, our multivariate Cox analysis identified PFKFB4 expression and pathologic stage as independent predictors in OSCC. Calibration curves indicated that PFKFB4 gene had high predictive accuracy in OSCC. Taken together, we unravel the role of PFKFB4 in OSCC progression and indicates that it can be used as an independent prognostic indicator.

5. Conclusions

In our study, PFKFB4 was markedly overexpressed in OSCC and significantly correlated with poor prognosis, serving as an independent adverse prognostic factor. Moreover, our research adds to a more comprehensive interpretation of the function role of PFKFB4 in OSCC. Additional comprehensive investigations are needed to fully elucidate the precise mechanisms by which PFKFB4 contributes to the tumorigenesis and progression of OSCC.

Data availability

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author upon reasonable request.

Conflicts of interest

The authors declare no conflicts of interest.

Ethics approval

The study was conducted in accordance with the Declaration of Helsinki, and approved by the institutional Ethics Committee of Stomatological Hospital, School of Stomatology, Southern Medical University (No. AFSQ-0105).

Consent to participate

Informed consent was obtained from all subjects involved in the study.

Acknowledgments

We extend our gratitude to all members of the Division of Oral and Maxillofacial Surgery, Stomatological Hospital, School of Stomatology, Southern Medical University, for their valuable assistance in this study.

Author contributions

S.C. and J.H. contributed to designing the study plan, performing the experiments, analyzing the data and drafting the manuscript. W.W. and Z.W. assisted in performing the experiments and data interpretation. B.C. and J.W. contributed to the project conception, drafting the manuscript and supervision. All authors read and approved the final manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (Grant No. 81800969), and the Major Research Plan of Science and Technology Program of Guangzhou, China (Grant No. 202206080009).

References

1. Du M, Nair R, Jamieson L, Liu Z, Bi P (2020) Incidence Trends of Lip, Oral Cavity, and Pharyngeal Cancers: Global Burden of Disease 1990-2017. J Dent Res 99:143-151. doi: 10.1177/0022034519894963
2. Koyfman SA, Ismaila N, Crook D, D'Cruz A, Rodriguez CP, Sher DJ et al (2019) Management of the Neck in Squamous Cell Carcinoma of the Oral Cavity and Oropharynx: ASCO Clinical Practice Guideline. J Clin Oncol 37:1753-1774. doi: 10.1200/JCO.18.01921
3. Chamoli A, Gosavi AS, Shirwadkar UP, Wangdale KV, Behera SK, Kurrey NK et al (2021) Overview of oral cavity squamous cell carcinoma: Risk factors, mechanisms, and diagnostics. Oral Oncol 121:105451. doi: 10.1016/j.oraloncology.2021.105451
4. Hanahan D (2022) Hallmarks of Cancer: New Dimensions. Cancer Discov 12:31-46. doi: 10.1158/2159-8290.CD-21-1059
5. Vander HM, Cantley LC, Thompson CB (2009) Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 324:1029-1033. doi: 10.1126/science.1160809
6. Wang Y, Zhang X, Wang S, Li Z, Hu X, Yang X et al (2022) Identification of Metabolism-Associated Biomarkers for Early and Precise Diagnosis of Oral Squamous Cell Carcinoma. Biomolecules 12:400. doi: 10.3390/biom12030400
7. Yalcin A, Telang S, Clem B, Chesney J (2009) Regulation of glucose metabolism by 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases in cancer. Exp Mol Pathol 86:174-179. doi:

10.1016/j.yexmp.2009.01.003
8. Sakata J, Abe Y, Uyeda K (1991) Molecular cloning of the DNA and expression and characterization of rat testes fructose-6-phosphate,2-kinase:fructose-2,6-bisphosphatase. J Biol Chem 266:15764-15770.
9. Bartrons R, Simon-Molas H, Rodriguez-Garcia A, Castano E, Navarro-Sabate A, Manzano A et al (2018) Fructose 2,6-Bisphosphate in Cancer Cell Metabolism. Front Oncol 8:331. doi: 10.3389/fonc.2018.00331
10. Yi M, Ban Y, Tan Y, Xiong W, Li G, Xiang B (2019) 6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 and 4: A pair of valves for fine-tuning of glucose metabolism in human cancer. Mol Metab 20:1-13. doi: 10.1016/j.molmet.2018.11.013
11. Feng C, Li Y, Li K, Lyu Y, Zhu W, Jiang H et al (2021) PFKFB4 is overexpressed in clear-cell renal cell carcinoma promoting pentose phosphate pathway that mediates Sunitinib resistance. J Exp Clin Canc Res 40:308. doi: 10.1186/s13046-021-02103-5
12. Zhang L, Liu Z, Dong Y, Kong L (2021) E2F2 drives glioma progression via PI3K/AKT in a PFKFB4-dependent manner. Life Sci 276:119412. doi: 10.1016/j.lfs.2021.119412
13. Goidts V, Bageritz J, Puccio L, Nakata S, Zapatka M, Barbus S et al (2012) RNAi screening in glioma stem-like cells identifies PFKFB4 as a key molecule important for cancer cell survival. Oncogene 31:3235-3243. doi: 10.1038/onc.2011.490
14. Gao R, Liu Y, Li D, Xun J, Zhou W, Wang P et al (2018) PFKFB4 Promotes Breast Cancer Metastasis via Induction of Hyaluronan Production in a p38-Dependent Manner. Cell Physiol Biochem 50:2108-2123. doi: 10.1159/000495055
15. Lu H, Chen S, You Z, Xie C, Huang S, Hu X (2020) PFKFB4 negatively regulated the expression of histone acetyltransferase GCN5 to mediate the tumorigenesis of thyroid cancer. Dev Growth Differ 62:129-138. doi: 10.1111/dgd.12645
16. Li D, Tang J, Gao R, Lan J, Shen W, Liu Y et al (2022) PFKFB4 promotes angiogenesis via IL-6/STAT5A/P-STAT5 signaling in breast cancer. J Cancer 13:212-224. doi: 10.7150/jca.66773
17. Capone M, Giannarelli D, Mallardo D, Madonna G, Festino L, Grimaldi AM et al (2018) Baseline neutrophil-to-lymphocyte ratio (NLR) and derived NLR could predict overall survival in patients with advanced melanoma treated with nivolumab. J Immunother Cancer 6:74. doi: 10.1186/s40425-018-0383-1
18. Bobarykina AY, Minchenko DO, Opentanova IL, Moenner M, Caro J, Esumi H et al (2006) Hypoxic regulation of PFKFB-3 and PFKFB-4 gene expression in gastric and pancreatic cancer cell lines and expression of PFKFB genes in gastric cancers. Acta Biochim Pol 53:789-799.
19. Dasgupta S, Rajapakshe K, Zhu B, Nikolai BC, Yi P, Putluri N et al (2018) Metabolic enzyme PFKFB4 activates transcriptional coactivator SRC-3 to drive breast cancer. Nature 556:249-254. doi: 10.1038/s41586-018-0018-1
20. Li X, Chen Z, Li Z, Huang G, Lin J, Wei Q et al (2020) The metabolic role of PFKFB4 in androgen-independent growth in vitro and PFKFB4 expression in human prostate cancer tissue. Bmc Urol 20:61. doi: 10.1186/s12894-020-00635-0
21. Cai YC, Yang H, Shan HB, Su HF, Jiang WQ, Shi YX (2021) PFKFB4 Overexpression Facilitates Proliferation by Promoting the G1/S Transition and Is Associated with a Poor Prognosis in Triple-Negative Breast Cancer. Dis Markers 2021:8824589. doi: 10.1155/2021/8824589
22. Meng J, Chen X, Han Z (2021) PFKFB4 promotes lung adenocarcinoma progression via phosphorylating and activating transcriptional coactivator SRC-2. Bmc Pulm Med 21:60. doi: 10.1186/s12890-021-01420-x
23. Minchenko OH, Tsuchihara K, Minchenko DO, Bikfalvi A, Esumi H (2014) Mechanisms of regulation of PFKFB expression in pancreatic and gastric cancer cells. World J Gastroentero 20:13705-13717. doi: 10.3748/wjg.v20.i38.13705
24. Zhou Y, Fan Y, Qiu B, Lou M, Liu X, Yuan K et al (2022) Effect of PFKFB4 on the Prognosis and Immune Regulation of NSCLC and Its Mechanism. Int J Gen Med 15:6341-6353. doi: 10.2147/IJGM.S369126
25. Wang F, Wu X, Li Y, Cao X, Zhang C, Gao Y (2021) PFKFB4 as a promising biomarker to predict a poor prognosis in patients with gastric cancer. Oncol Lett 21:296. doi: 10.3892/ol.2021.12557
26. Wang Q, Zeng F, Sun Y, Qiu Q, Zhang J, Huang W et al (2018) Etk Interaction with PFKFB4 Modulates Chemoresistance of Small-cell Lung Cancer by Regulating Autophagy. Clin Cancer Res 24:950-962. doi: 10.1158/1078-0432.CCR-17-1475
27. Kam CS, Ho DW, Ming VS, Tian L, Sze KM, Zhang VX et al (2023) PFKFB4 Drives the Oncogenicity in TP53-Mutated Hepatocellular Carcinoma in a Phosphatase-Dependent Manner. Cell Mol Gastroenter 15:1325-1350. doi: 10.1016/j.jcmgh.2023.02.004
28. Gao R, Li D, Xun J, Zhou W, Li J, Wang J et al (2018) CD44ICD promotes breast cancer stemness via PFKFB4-mediated glucose metabolism. Theranostics 8:6248-6262. doi: 10.7150/thno.28721