The role of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 in esophageal squamous cell carcinoma

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Abstract
6-phosphofructo-kinase-2/fructose diphosphatase-2 isoenzyme 3 (PFKFB3) is closely related to the growth of many types of cancer cells. Glycolysis not only provides Adenosine triphosphate for the growth of tumor cells, but also protects them from acid products, which is beneficial to the invasion and metastasis of tumors. However, PFKFB3 expression in esophageal squamous cell carcinoma (ESCC) has been scarcely reported. In this study, the role of PFKFB3 was studied in 120 ESCC samples using immunohistochemistry technique (IHC), western blotting, and reverse transcriptase-polymerase chain reaction (RT-PCR). Both PFKFB3 protein and gene expression in ESCC tissues were significantly higher than in adjacent non-tumor tissues ($P < .05$). Single factor analysis showed that both PFKFB3 protein and gene expression are related to infiltration depth, stage, tumor metastasis, and the degree of tumor differentiation in ESCC. Multivariate Cox survival analysis revealed that PFKFB3 protein expression, tumor location, tumor metastasis, tumor differentiation degree, and tumor stage were independent factors affecting the overall survival of postoperative patients. Multivariate Cox survival analysis showed that PFKFB3 mRNA has a good performance for predicting 3-year survival of patients with ESCC 0.89 (0.79–0.99), with a sensitivity of 0.85 and specificity of 0.77. Encouragingly, the sensitivity and specificity of PFKFB3 in the diagnosis of early ESCC (stage I and stage II) can reach 87.8% and 91.5%. In conclusion, high PFKFB3 protein and gene expression may be associated with the occurrence, development, and prognosis of ESCC. PFKFB3 could be used to help develop new therapeutic and diagnostic strategies for ESCC patients.

Abbreviations: EAC = esophageal adenocarcinoma, ESCC = esophageal squamous cell carcinoma, IHC = immunohistochemistry technique, PFKFB3 = 6-phosphofructo kinase-2/fructose diphosphatase-2 isoenzyme 3, RT-PCR = reverse transcriptase-polymerase chain reaction.

Keywords: 6-phosphofructokinase-2/fructose diphosphatase-2 isoenzyme 3, esophageal squamous cell carcinoma, glycolysis, prognosis

1. Introduction

Esophageal cancer (EC) is a common malignant tumor of the digestive tract.¹,² EC ranks 8th in incidence and 6th in fatality worldwide. China is a high incidence area of EC.³ The incidence of EC in high incidence areas is about 121/10,000, which is >20 times higher than that in low incidence areas. Every year, there are over 270,000 new cases of EC, resulting in 200,000 deaths, with a mortality rate that ranks in fourth places.⁴⁻⁵ There are 2 main tissue subtypes of EC, namely esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). In China, ESCC is the main subtype of EC.⁶

ESCC is a highly malignant subtype of tumor. At present, only about 1/3 of patients with ESCC qualify for surgical resection, with the majority requiring adjuvant chemotherapy postoperation.⁷ The prognosis of ESCC is poor, with an average 5-year survival rate of only 35% to 45%. The treatment and prognosis associated with the different stages of ESCC vary greatly.⁸ Patients with early ESCC have a good prognosis, particularly individuals who meet the requirements for endoscopic resection, which involves a minimally invasive endoscopic resection that avoid seriously damaging the patients’ quality of life, unlike surgery and chemotherapy. Contrarily, patients with advanced ESCC have a poor prognosis that is often associated with a high medical burden.⁹⁻¹⁰ Therefore, investigating the mechanism of the occurrence and development of ESCC may provide a scientific
basis for its diagnosis and treatment, especially due to the fact that an early diagnosis of ESCC is of high clinical significance.

6-phosphofructo kinase-2/fructose diphosphatase-2 isoenzyme 3 (PFKFB3) is a subtype of phosphofructokinase (PFK), which exists widely in various biological cells and plays an important role in the proliferation, migration, invasion, and metastasis of certain types of cancer cells.\textsuperscript{[11,12]} PFKFB3 is a key rate-limiting enzyme in the process of glucose decomposition into adenosine triphosphate, which has dual activities of kinase and phosphatase.\textsuperscript{[13]} The activity of PFKFB3 kinase is much higher than that of phosphatase, which can increase the rate of glycolysis. Several studies have shown that inhibiting the expression of PFKFB3 can significantly reduce the glycolysis rate and the growth of cancer cells,\textsuperscript{[14,15]} which indicated that the PFKFB3 involved in glycolysis may be an important target for the treatment of malignant tumors. However, the expression of PFKFB3 in ESCC and their role in the development of ESCC have not been reported.

Defining the relationship between PFKFB3 and the occurrence and development of ESCC could provide an insight into the diagnosis, treatment, or prevention of ESCC. In this study, we evaluated the role of PFKFB3 expression in ESCC using immunohistochemistry (IHC), western blotting, and reversed transcriptase-polymerase chain reaction (RT-PCR) with the aim of proposing a potential prognostic diagnostic indicator for patients with ESCC.

2. Materials and methods

2.1. Patients and controls

We retrospectively analyzed a cross-sectional group of 120 eligible ESCC patients selected from the First People’s Hospital of Yancheng between January 2015 and January 2018. All patients underwent surgical resection. The inclusion criteria for diagnosing ESCC was as follows: patients had primary esophageal cancer, excluding recurrence, metastasis, and other malignant tumors; cases that received radiotherapy, chemotherapy, or other anti-cancer treatment before operation were excluded; patients were diagnosed with ESCC by pathologists in our hospital; complete clinical and pathological data were available.

Among the 120 ESCC patients, 85 were male and 35 were female (aged 32–87 years, mean age of 58 years). Tumor location: upper segment 40 cases, middle segment 49 cases, lower segment 31 cases. Infiltration depth: T1+T2 56 cases, T3+T4 64 cases. Poor differentiation: 58 cases, moderate differentiation: 27 cases, high differentiation: 35 cases. Forty-two cases were grade I and grade II, and 78 cases were grade III and grade IV. Paired adjacent tissues were collected from the 120 eligible ESCC patients as a control group.

The condition and survival of 120 ESCC patients were followed up by telephone. The total survival time (OS) is defined as the time from the date of operation to the last follow-up or death, and is calculated in terms of months. Postoperative follow-ups were performed every 2 months in the first year, every 3 months in the second year, every 6 months in the third year, and every 8 months until mortality. Informed consent was obtained from all patients prior to extraction of specimens. This study was approved by the Ethics Committee of the First People’s Hospital of Yancheng (Identification No. HMU [Ethics] 2015004).

2.2. Detection of PFKFB3 expression in ESCC tissues by immunohistochemistry

IHC was performed to determine the distribution of PFKFB3. The Envision and DAB chromogenic reagent kits (antibody diagnostic inc., ADI) were used for immunohistochemical staining. Briefly, the specimens were cut into small pieces of tissue, fixed with 4% paraformaldehyde, and made into conventional sections (4 μm). The sections were dewaxed and gradient ethanol infused before performing high-pressure antigen repair by incubating in sheep serum 37°C for 20 minutes. This was followed by drop serum and the addition of PFKFB3-1 antibody (1: 200; Abcam Company, USA) before incubating the samples overnight. The sections were then incubated with second antibody labeled with corresponding horseradish peroxidase at 37°C for 20 minutes. After rinsing with phosphate buffer saline (PBS) buffer, streptavidin-peroxidase was added to the sections and incubated at 37°C for 20 minutes. The samples were then rinsed with PBS buffer and the color was developed under a light microscope. The samples were then dyed with hematoxin, dehydrated, and sealed.

All staining results were determined by 2 independent pathologists blinded to the experiment. The cell staining reactions were evaluated based on the immunoreactive score (IRS) as follows:\textsuperscript{[16]}: IRS = staining intensity score × percentage of positive tumor cells. The score of the staining intensity was defined as follows: 0, no expression; 1, weak expression, 2, medium expression, and 3, strong expression. The percentage of positive stained cells was defined as follows: 0, no expression; 1, 1% to 10% positive tumor cells; 2, 11% to 50% positive tumor cells; 3, 51% to 80% tumor cells; 4, >80% positive tumor cells. An IRS value ≥4 was considered as high-PFKFB3-1 expression, whereas an IRS value <4 was considered low-PFKFB3-1 expression.

2.3. Detection of PFKFB3 protein expression by western blotting

The ESCC tissue samples were homogenized and lysed with cell lystate before centrifuging. The resulting supernatant was harvested as total protein, the concentration of which was determined using the bicinchoninic acid method. After calculating the volume of the sample and NaCl, 5 times buffer solution was measured according to the protein concentration, mixed with the sample, and placed in a 95°C water bath for 5 minutes. A polyacrylamide gel was prepared for protein electrophoresis. The protein sample was run at 80 V under constant pressure, then at 120 V at the interface between the separation gel and the concentrated glue. The resulting protein bands were transferred onto the polyvinylidene fluoride membrane under 200 mA for 2 hours with a constant current. After 2 hours of blocking, the gels were incubated with PFKFB3 (1:1000) and β-actin (1:1000) antibodies overnight at 4°C. Tris-Buffered Saline Tween (TBST) was washed 3 times, for 10 minutes each time. The second antibody was added to the samples and incubated for 2 hours, after which the TBST was washed 3 more times. The relative content of target protein in the sample was analyzed by visualizing using a protein gel imaging system after coloring with chemiluminescence agent.

2.4. Detection of PFKFB3 mRNA by RT-PCR

Fresh frozen ESCC tissues were ground using glass grinder. The total RNA was extracted and reverse transcribed using a reverse transcription kit (Invitrogen Qiagen, Germany) to synthesize
cDNA. β-actin was used as the internal reference. A PCR amplification kit was used for the amplification. The sequences of the primers used are provided in Table 1. The thermal cycling conditions were as follows: pre-denaturation at 95°C for 3 minutes, 95°C for 50 seconds, and 55°C for 40 seconds, for a total of 40 cycles; extension at 72°C for 60 seconds. The results demonstrated that the relative expression of PFKFB3 mRNA was expressed by 2–ΔΔCt, obtained by averaging the results of 3 independent experiments.

2.5. Statistical methods

GraphPad (IBM, NY, USA) Prism 5 software and SPSS13.0 were used for the statistical analysis. The normal distribution data were expressed as the mean ± standard deviation (SD). The chi-square test was used to compare the association between the expression status of PFKFB3 and clinicopathological parameters in normal adjacent and cancer tissues. Kaplan–Meier survival analysis was used to analyze disease-specific and disease-free survival rates. The log-rank test was performed to analyze the difference in survival curves. Multivariable regression analysis was performed to detect prognostic factors using the Cox proportional hazards model. A value of $P < .05$ was considered statistically significant. The logistic regression model was used to draw receiver operator characteristic curve (ROC) and calculate the area under curve (AUC) to evaluate the efficiency of diagnosis.

3. Results

3.1. PFKFB3 protein expression in ESCC tissues and adjacent tissue

PFKFB3 protein is mainly expressed in the nucleus of ESCC tissue cells. The expression of PFKFB3 protein in ESCC tissues was significantly higher than in the normal adjacent tissues ($P < .05$; Fig. 1A–D). The positive expression rate of PFKFB3 protein in ESCC tissues was 90.83% (109/120) and 54.17% (65/120) in the adjacent tissues.

Further analysis showed that the immunostaining intensity ($t = 3.165, P < .05$) and the percentage of positive staining cells ($t = 6.776, P < .05$) of PFKFB3 in ESCC tissues were higher than those in adjacent tissues (Fig. 1E, F). The staining score of PFKFB3 in ESCC tissues was significantly higher than that in adjacent tissues ($t = 6.122, P < .05$; Fig. 1G).

Western blotting revealed that the expression of PFKFB3 protein in 120 ESCC tissues was significantly higher than that in the adjacent tissues ($P < .05$; Fig. 2A).

3.2. PFKFB3 mRNA expression in ESCC and adjacent tissues

Next, we evaluated the expression of PFKFB3 mRNA in 102 ESCC and adjacent tissues by RT-PCR. The results revealed a positive rate of PFKFB3 mRNA in ESCC tissues of 86.67% (104/
120), which was significantly higher than that in adjacent tissues, of 49.17% (59/120; P < .05; Fig. 2B).

3.3. Relationship between PFKFB3 mRNA and protein expression and clinicopathological features of ESCC

PFKFB3 mRNA and protein in ESCC tissues was consistently highly expressed. Single factor analysis showed that both PFKFB3 mRNA and protein are not associated with sex, age, or tumor location (all P > .05), but are related to infiltration depth, stage, tumor metastasis, and the degree of tumor differentiation (all P < .05; Table 2).

3.4. Prognostic value of PFKFB3 protein expression for the overall survival of postoperative patients with ESCC

Patients with higher levels of PFKFB3 protein expression in ESCC tissues had a significantly shorter disease-specific survival rate than other patients. Kaplan–Meier survival analysis showed that there was a significantly statistical difference between the 2 types of patients (P < .05) (Fig. 3). Among the 109 patients exhibiting higher levels of PFKFB3 protein expression in ESCC tissues, 91 patients died and 18 patients survived. Among the 11 patients with lower levels of PFKFB3 protein expression, 3 patients died and 8 patients survived. The median survival time of patients with higher and lower levels of PFKFB3 expression was 10.2 ± 1.1 months and 23.5 ± 6.4 months, respectively, which indicated that high levels of PFKFB3 expression affect the prognosis of ESCC patients.

Multivariate Cox survival analysis showed that PFKFB3 protein expression, tumor location, tumor metastasis, tumor differentiation degree, and tumor stage were independent factors affecting the overall survival of postoperative patients, while sex, age, and infiltration depth did not independently affect the overall survival of these patients (Fig. 4).

Table 2

| Characteristic                        | n   | PFKFB3 protein positive rate | χ²   | P   | PFKFB3 mRNA positive rate | χ²   | P   |
|--------------------------------------|-----|-----------------------------|------|-----|---------------------------|------|-----|
| Gender                               |     |                             |      |     |                           |      |     |
| Male                                 | 85  | 78 (91.76)                  | 0.152| .503| 74 (87.06)                | 0.021| .842|
| Female                               | 35  | 31 (88.57)                  |      |     | 30 (85.71)                |      |     |
| Age, y                               |     |                             |      |     |                           |      |     |
| <60                                  | 44  | 40 (90.91)                  | 0.190| .965| 39 (88.64)                | 0.023| .804|
| ≥60                                  | 76  | 69 (90.79)                  |      |     | 65 (85.53)                |      |     |
| Infiltration depth                   |     |                             |      |     |                           |      |     |
| T1+T2                                | 56  | 47 (83.93)                  | 0.149| .019| 44 (78.57)                | 0.112| .211|
| T3+T4                                | 64  | 62 (96.88)                  |      |     | 60 (93.73)                |      |     |
| Tumor location                       |     |                             |      |     |                           |      |     |
| Upper segment                        | 40  | 36 (90.00)                  | 0.217| .554| 34 (85.00)                | 0.129| .497|
| Middle segment                       | 49  | 46 (93.88)                  |      |     | 44 (89.80)                |      |     |
| Lower segment                        | 31  | 27 (87.10)                  |      |     | 26 (83.87)                |      |     |
| Degree of tumor differentiation      |     |                             |      |     |                           |      |     |
| High differentiation                 | 35  | 29 (82.86)                  | 0.233| .027| 27 (77.14)                | 0.239| .166|
| Moderate differentiation             | 27  | 23 (85.19)                  |      |     | 21 (77.78)                |      |     |
| Poor differentiation                 | 58  | 57 (98.28)                  |      |     | 56 (96.55)                |      |     |
| Lymph node metastasis                |     |                             |      |     |                           |      |     |
| Yes                                  | 54  | 53 (98.15)                  | 0.219| .018| 51 (94.44)                | 0.201| .021|
| No                                   | 66  | 56 (84.85)                  |      |     | 53 (80.30)                |      |     |
| Tumor stage                          |     |                             |      |     |                           |      |     |
| I–II                                 | 42  | 33 (78.57)                  | 7.129| .015| 31 (73.81)                | 7.098| .022|
| III–IV                               | 78  | 76 (97.44)                  |      |     | 73 (93.59)                |      |     |

ESCC = esophageal squamous cell carcinoma, PFKFB3 = 6-phosphofructo kinase-2/fructose diphosphatase-2 isoenzyme 3.
3.5. The accuracy of PFKFB3 mRNA for predicting 3-year survival of patients with ESCC

To further clarify the role of PFKFB3 in the overall survival of postoperative patients, area under the receiver operating characteristic (AUROCs) were calculated to determine the value of PFKFB3 mRNA for predicting the 3-year survival of patients with ESCC. The AUROC of PFKFB3 mRNA for predicting the 3-year survival of patients with ESCC was 0.89 (0.79–0.99), with a sensitivity of 0.85 and a specificity of 0.77 (Fig. 5).

3.6. Diagnostic value of PFKFB3 in early ESCC

We further studied the diagnostic ability of PFKFB3 in early ESCC (stage I and stage II). ROC curve analysis showed that the percentage of positive stained cells of PFKFB3 was 0.940 (95% CI: 0.87–1.00) for early ESCC, with a sensitivity and specificity of 87.8% and 91.5%, respectively (Fig. 6A). The AUC of the staining score of PFKFB3 for early ESCC was 0.89 (95% CI: 0.80–0.98), with a sensitivity and specificity of 83.2% and 91.3% respectively (Fig. 6B).

4. Discussion

The proliferation, invasion, and distant metastasis of malignant tumors require adequate nutrition and energy. Tumor cells are metabolically active. In order to meet the needs of rapid proliferation, invasion, and metastasis, the energy metabolism must be carefully regulated, which was denoted as metabolic reprogramming. Among them, aerobic glycolysis is the most important metabolic mode. Tumor cells are able to maintain a high level of anaerobic glycolysis under aerobic conditions. Glucose can be decomposed into lactic acid via the so-called “Warburg effect.” Therefore, inhibiting the glycolysis of tumor cells can inhibit their proliferation, differentiation, and distant metastasis. There are 4 subtypes of PFKFB family, namely PFKFB1, PFKFB2, PFKFB3, and PFKFB4, which have different expression levels and functions in tissues.

PFKFB3 was first isolated from brain tissue in 1995. It has the dual activity of a kinase and phosphatase with a kinase activity that is stronger than its phosphatase activity. It can be divided into spectrum type and induction type due to differences in the terminal sequence of PFKFB3C. The expression level of PFKFB3 in normal tissue is very low, but is highly expressed in many cancer cells, such as gastric, lung, and prostate cancer. The mechanism of PFKFB3 is as follows: an increase in PFKFB3, a phosphofructokinase-1 (PFK-1) activator, enhances the activity of PFK-1 to promote the rapid hydrolysis of glucose uptake by cancer cells, providing sufficient energy for cell proliferation; PFKFB3 is necessary for the growth of tumors.

| Survival Factor                  | HR[95%CI]   |
|----------------------------------|-------------|
| PFKFB3 protein expression        | 3.256(1.440,7.365) |
| Infiltration depth               | 1.609(0.672,3.852) |
| Tumor differentiation degree    | 2.291(1.152,4.554) |
| Tumor metastasis                | 1.820(1.048,3.159) |
| Tumor stage                     | 3.084(1.262,7.537) |
| Tumor location                  | 2.707(1.100,6.661) |
| Gender                          | 1.251(0.740,2.113) |
| Age                             | 0.977(0.950,1.004) |

Figure 4. Association of PFKFB3 expression and prognosis of ESCC patients: multivariable Cox survival. ESCC = esophageal squamous cell carcinoma, PFKFB3 = 6-phosphofructo kinase-2/fructose diphosphatase-2 isoenzyme 3.
containing Ras-transformed cells, promoting normal angiogenesis and the growth of tumor cells, as well as accelerating the occurrence and development of tumors; the activation of the HIF-1α and p38/MK2 signaling pathways and the phosphorylation of serine at 461 site of PFKFB3 upregulates the expression of PFKFB3, thus promoting the proliferation of cancer cells; PFKFB3 also plays a role in cell cycle and cell apoptosis. Therefore, PFKFB3 plays an important role in maintaining the development and progress of cancer and is a potential target for cancer treatment.

Li et al[27] found that the expression of PFKFB3 in head and neck squamous cell carcinoma (HNSCC) tissues was significantly higher than in adjacent mucosal tissues. The pharmacological inhibition of PFKFB3 via PFK15 was found to suppress tumor growth and alleviate metastasis in HNSCC. Ko et al[28] found that fibroblasts cocultured with TIGAR-overexpressing breast carcinoma cells induce hypoxia-inducible factor (HIF) activation via increased glucose uptake, increased PFKFB3 expression, and increased lactate dehydrogenase-A expression. Zhang et al[29] found that protein kinase D3 (PRKD3) upregulated PFKFB3 and activated glycolysis, as shown by an increased glucose consumption and lactate production. The knockdown of PFKFB3 suppressed glycolysis in gastric cancer (GC) cells with highly expressed PRKD3 but not in PRKD3 silenced cells. Furthermore, PRKD and PFKFB3 inhibitor suppressed the viability of GC cells. PFKFB3 is also considered an important prognostic indicator of cancer. It was found[24] that both PFKFB3 mRNA and protein expression were significantly high in lung adenocarcinoma cells. A high expression of PFKFB3 protein was an independent prognostic marker in lung adenocarcinoma. Chen et al[29] found that the levels of PFKFB3+ CD68+ cell infiltration in peritumoral tissues were negatively correlated with overall survival and could serve as an independent prognostic factor for survival in patients with hepatocellular carcinoma. Therefore, PFKFB3 not only reflects the occurrence and development of cancers, but can also be used as an alternative endpoint marker for cancer chemoprevention.

In our study, we investigated the role of PFKFB3 expression in ESCC tissues. Our study showed that PFKFB3 protein was localized in the nucleus of ESCC tissue cells. The expression of PFKFB3 in ESCC was higher than that in adjacent tissue. The immunostaining intensity, the percentage of positive staining cells, and the staining score of PFKFB3 in ESCC tissues were all higher than those in adjacent tissues. The expression of PFKFB3

Figure 5. AUROC of PFKFB3 mRNA for predicting 3-year survival of patients with ESCC. ESCC = esophageal squamous cell carcinoma, PFKFB3 = 6-phosphofructo kinase-2/fructose diphosphatase-2 isoenzyme 3.

Figure 6. AUROC of PFKFB3 for predicting early ESCC patients (stage I and stage II). A, The percentage of positive staining cells; B, the staining score. ESCC = esophageal squamous cell carcinoma, PFKFB3 = 6-phosphofructo kinase-2/fructose diphosphatase-2 isoenzyme 3.
mRNA in ESCC tissues was also significantly higher than that in adjacent tissues. The relationship between PFKFB3 protein and gene expression and the clinical pathological features of ESCC patients are consistent. Our study also found that the PFKFB3 protein and gene expression in ESCC were not related to sex, age, or tumor location, but were related to infiltration depth, stage, tumor metastasis, and the degree of tumor differentiation. The expression of PFKFB3 protein and gene in the poor differentiation group and the lymph node metastasis group was significantly higher than in the high and moderate differentiation group and the group without lymph node metastasis. The expression of PFKFB3 protein and gene in the stage III–IV group and infiltration depth T3+T4 group was significantly higher than that in the stage I–II group and infiltration depth T1+T2 group. These results suggest a relationship between the formation, invasion, and metastasis of ESCC and high levels of PFKFB3 expression.

Moreover, we also studied the relationship between PFKFB3 expression and the overall survival of ESCC patients. The median survival time for patients with high levels of PFKFB3 expression was significantly shorter than that of patients with lower levels of PFKFB3 expression in ESCC. Multivariate Cox survival analysis revealed that PFKFB3 protein expression, tumor location, tumor metastasis, tumor differentiation degree, and tumor stage were independent factors affecting the overall survival of ESCC patients. These results indicate that an increased PFKFB3 expression was related to the prognosis of ESCC patients. AUROC analysis revealed that PFKFB3 mRNA had a good performance for predicting 3-year survival of patients with ESCC 0.89 (0.79–0.99), with a sensitivity of 0.85 and a specificity of 0.77, which suggests that PFKFB3 mRNA can be used to effectively predict the prognosis of ESCC patients. Encouragingly, PFKFB3 showed a good AUROC 0.940 (95% CI: 0.87–1.00) in the diagnosis of early ESCC (stage I and stage II), with a sensitivity of 87.8% and a specificity of 91.5%.

The present study also had a number of limitations that warrant consideration. Firstly, this work includes the largest cohort for the study of PFKFB3 protein and gene expression in ESCC. However, more clinical patient validation will be necessary. Secondly, both the comprehensive ability of PFKFB3 mRNA as a clinical predictor for 3-year survival of ESCC patients and PFKFB3 in diagnosis of early ESCC (stage I and stage II) will need to be further confirmed and validated. Lastly, further cell and animal experiments will need to be used to further elucidate the mechanism of action of PFKFB3.

In conclusion, our study demonstrated that high levels of PFKFB3 protein and gene expression are closely related to the occurrence, development, and prognosis of ESCC. The findings presented in this study provide a basis for the development of new therapeutic and diagnostic strategies for the treatment of patients with ESCC.

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