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Comprehensive analysis of the relationship between cuproptosis-related genes and esophageal cancer prognosis

Xu H *et al*. Based on 151 samples

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Abstract

BACKGROUND

Esophageal cancer was one of the most common malignant tumors of the digestive system, with a 5-year survival rate of 15% to 50%. Cuproptosis, a unique kind of cell death driven by protein lipoylation, was found to be strongly connected to mitochondrial metabolism. The clinical implications of cuproptosis-related genes in esophageal cancer, however, are mainly unknown.

AIM

To identify cuprotosis-related genes that are differently expressed in esophageal cancer and investigate their prognostic significance.

METHODS

With |log FC| > 1 and false discovery rate < 0.05 as criteria, Wilcox-test was used to evaluate the differentially expressed genes between 151 tumor tissues and 151 normal esophageal tissues. Cuproptosis-related genes were selected to be linked with prognosis using univariate Cox regression analysis. Genes were separated into high- and low-expression groups based on their cutoff value of gene expression, and the correlation between the two groups and overall survival or progression free survival was investigated using the log-rank test. The C index, calibration curve, and receiver operator characteristic (ROC) curve were used to assess a nomogram containing clinicopathological characteristics and cuproptosis-related genes.

RESULTS

PDHA1 was found to be highly correlated with prognosis in a univariate Cox regression analysis (hazard ratio = 22.96, 95% confidence interval = 3.09-170.73, P = 0.002). According to the Kaplan-Meier survival curves, low expression of PDHA1 was associated with a better prognosis (log-rank P = 0.0007). There was no significant correlation between PDHA1 expression and 22 different types of immune cells. TNFSF15 (P = 3.2 × 10^{-06}; r = 0.37), TNFRSF14 (P = 8.1 × 10^{-08}; r = 0.42), HHLA2 (P = 6.0×10^{-08}; r = 0.42) and LGALS9 (P = 3.1 × 10^{-06}; r = 0.37) were all found to be considerably greater in the high PDHA1 expression group, according to an analysis of genes related with 47 immunological checkpoints. The low PDHA1 expression group had significantly lower levels of CD44 (P = 0.00028; R = -0.29), TNFRSF18 (P = 1.2 × 10^{-05}; R = -0.35), PDCD1LG2 (P = 0.0032; R = -0.24), CD86 (P = 0.018; R = -0.19) and CD40 (P = 0.0047; R = -0.23), and the differences were statistically significant. We constructed a prognostic nomogram incorporating pathological type, TNM stage, and PDHA1 expression, and the C index, calibration curve, and ROC curve revealed that the nomogram's predictive performance was good.

CONCLUSION

Cuproptosis-related genes could be used as a prognostic predictor for esophageal cancer patients, providing novel insights into cancer treatment.

Key Words: Esophageal cancer; Cuproptosis; PDHA1; Overall survival; Nomogram

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Core Tip: Esophageal carcinoma was a kind of cancer that had a poor prognosis and was one of the major causes of cancer mortality worldwide. Despite recent advanced in surgical and pharmacological treatment of esophageal cancer, the prognosis for esophageal cancer remained dismal. Copper toxicity has been linked to the incidence and progression of esophageal cancers in numerous studies. At the gene level, however, we didn't know the particular probable biochemical mechanism. We included 19 cuproptosis-related genes and screened a gene that could successfully predict the prognosis of esophageal cancer by statistical analysis to further elucidate the role of cuproptosis-related genes in impacting the prognosis of esophageal cancer.

INTRODUCTION

Esophageal cancer was a major global health issue, and its incidence was quickly increasing[1]. Esophageal cancer was classified into two types: Esophageal squamous cell carcinoma (ESCC), which accounted for 90% of all occurrences, and esophageal adenocarcinoma (EAC). In recent years, epidemiological research has revealed that the incidence of EAC has grown 3-4 times, with the proportion increasing[2,3]. Despite significant advances in the diagnosis and treatment of esophageal cancer, the mortality rate for people with the disease varied from 15% to 20%, placing it fourth among all cancer-related deaths[1].

Esophageal cancer was the result of a complex process involving various causes and polygene alterations. Using high-throughput sequencing technologies, a comprehensive mutation catalog was evaluated, and substantial genetic alterations were discovered in the malignancies. Gene alterations were often linked to aberrant expression, and they were becoming more essential in the early diagnosis and prognosis of esophageal cancer[4]. Currently, several gene expression products were employed as indicators for esophageal cancer diagnosis and prognosis[5,6]. Somatic mutations in the tumor protein p53 (TP53) were found in more than 83 percent of ESCCs. Adenocarcinoma and squamous cell carcinoma both had TP53 point mutations[7,8]. In addition, numerous cell cycle-controlling genes were overexpressed in ESCC. For example, cyclin-dependent

kinase 4/cyclin-dependent kinase 6 (CDK4/CDK6) accounted for 23.6 percent, murine double minute2 (MDM2) 5.7 percent, and cyclin D1 (CCND1) 46.4 percent, showing that the above components were implicated in the incidence and development of ESCC[9]. As a result, there was a pressing need to uncover genetic anomalies in esophageal cancer and understand their molecular basis in order to enhance early diagnosis and minimize esophageal cancer mortality.

Previous studies have reported several types of precisely programmed cell death, including apoptosis, pyroptosis, necrosis, and ferroptosis[10]. Similar to iron, copper was a trace metal in cells that played an integral role in maintaining protein functions. Excessive copper could cause cytotoxicity, but the exact mechanism was unclear[11]. Tsvetkov *et al*[12] discovered that the copper carrier elesclomol, which was originally used to treat cancer, killed cells in excess of copper. Elesclomol did not trigger cell death on its own, suggesting that copper toxicity was to blame. Dar *et al*[13] found that patients with esophageal cancer had considerably higher mean blood copper levels than controls, with a mean copper concentration of 169 ug/dL in the cancer group and 149 ug/dL in the control group. Therefore, we hypothesized that copper shortage or excess was linked to the occurrence and progression of esophageal cancer. Copper, however, has been linked to the development and progression of esophageal cancer in few studies.

There hasn't been any research on the role of cuproptosis-related genes in the genesis, progression, and metastasis of esophageal cancer so far. Cuproptosis-related genes and their processes need to be better understood in order to improve the prognosis of malignant tumors and uncover novel treatment targets. Bioinformatics analysis was used to evaluate the expression profile of cuproptosis-related genes and its predictive significance in esophageal cancer in this study.

MATERIALS AND METHODS

The study did not include any human participants, data, or tissue, nor did it include any animals. All of the information was gathered from a public database.

Data collection

The Cancer Genome Atlas (https://portal.gdc.cancer.gov/) provided gene expression data and clinical information for 151 esophageal cancer samples, whereas the Genotype-Tissue Expression database (https://xenabrowser.net/) provided gene expression levels for 151 healthy tissue samples. The "limma" package in R was used to conduct matrix normalization. We selected 19 genes from the scientific literature that have been linked to cuproptosis in prior studies[12].

Screening of cuproptosis-related prognosis genes

In 151 patients of esophageal cancer and 151 healthy controls, a total of 55185 genes were acquired. The log2 (x+1) scale was used to standardize all of the expression data. Differentially expressed genes were screened between tumor tissue and normal esophageal tissue using the "limma" package and Wilcox-test, with the conditions of |log FC| > 1 and false discovery rate (FDR) < 0.05. Using the "limma" package, we evaluated those genes that were differently expressed between esophageal cancer and healthy control tissues based on 19 cuproptosis-related genes. The FDR less than 0.05 was used as a criterion for further investigation. The researcher employed a univariate Cox regression analysis to find predictive markers linked to overall survival (OS) and progression-free survival (PFS). The optimum cutoff value for a prognostic gene was identified using a receiver operating characteristic (ROC) curve. Patients were classified into high-risk (less than the cutoff) and low-risk (greater than the cutoff) esophageal cancer groups based on gene expression cutoff values.

Function enrichment analysis

The R package "ClusterProfiler" was used to analyze gene ontology (GO) and pathway enrichment [Kyoto Encyclopedia of Genes and Genomes (KEGG)]. It was deemed substantially enriched when the p value and adjusted p value were both less than 0.05. To identify the functional role of the genes, GO analysis was done on the significantly

expressed genes, and the expression levels of the genes were displayed in GO circle plots using the R package "GOplot". In order to determine tumor-infiltrating immune-cell fractions in esophageal cancer patients, we employed the CIBERSORT algorithm[14]. On samples with a CIBERSORT result of P value less than 0.05, further analysis was performed. Then, for reference, we collected 547 gene expression profiles from the CIBERSORT website (http://cibersort.stanford.edu/). A Pearson's test and the "corrplot" program were used to correlate infiltrated immune cells.

Development a nomogram for predicting OS

A cuproptosis-related gene and numerous clinicopathological variables were also subjected to univariate and multivariate Cox regression analysis. For predicting the OS, we utilized the R "rms" package to generate a nomogram including pathological categories, American Joint Committee on Cancer (AJCC)-TNM stages, and differential cuproptosis-related gene. The uniformity of the nomogram was assessed using calibration curves to anticipate various OS results. Harrell's concordance index was used to generate the C-index. The area under the receiver operating characteristic (AUC) curve and the ROC were both utilized to evaluate how predictive our nomogram was.

Statistical analysis

All statistical analyses were performed using R software, version 4.1.0 (http://www.rproject.org/). The students t-test was used to compare variables such as age at diagnosis, sex, AJCC-TNM stage, mutational status of KRAS (v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog), BRAF (v-Raf murine sarcoma viral oncogene homolog B1 genes), EGFR (Epidermal Growth Factor Receptor), PIK3CA (Phosp (unpaired *t*-test). The Cox proportional-hazards model was used to find genes and clinicopathological variables linked to survival. Based on the optimal cutoff of differential cuproptosis-related gene, the Youden index method determined that esophageal cancer patients should be divided into high-risk and low-risk groups. For

assessing survival in the high-risk and low-risk groups, Kaplan-Meier survival curves were employed, and log-rank tests were performed to compare survival rates. For two-sided *P* values, statistical significance was considered as a value less than 0.05.

RESULTS

Demographics of the 151 esophagus cancer patients

Our study comprised 151 esophageal cancer patients and 151 healthy controls. Table 1 shows the characteristics of patients with EAC (n = 74) and esophageal squamous cancer (n = 77). All esophageal cancer patients had median survival duration of 13.4 mo. EAC had a median survival time of 14.8 mo, whereas esophageal squamous cancer had a median survival time of 13 mo.

Relationship between clinical parameters and progression-free/OS

Table 2 summarizes the relationships between clinical pathological features and these individuals' OS or PFS. When compared to esophageal squamous cancer, EAC was related with a lower OS (P = 0.011). The AJCC-TNM stages III and IV were linked to OS (P = 0.021) and PFS (P = 0.013). When compared to females, males had a lower PFS (P = 0.009). Age, EGFR, BRAF, KRAS, or PIK3CA status, among other clinicopathological variables, was not linked to OS or PFS.

Identification of prognostic cuproptosis-related genes

With |log FC| > 1 and FDR < 0.05, a total of 7055 differentially expressed genes (DEGs) were identified in the healthy control group, comprising 3494 upregulated genes and 3561 downregulated genes. Then, using the FDR < 0.05 threshold, gene expression analysis was done to identify cuproptosis-related genes and 18 genes fulfilled our requirements (Supplement table 1). In the intersection of two gene sets, there were five genes: High-affinity copper uptake protein 1 (SLC31A1), ferredoxin (FDX1), lipoyl(octanoyl) transferase 2 (LIPT2), Pyruvate Dehydrogenase A1 (PDHA1), and progrmnmed cell death 6 interacting protein (PDHB) (DEGs and cuproptosis-related

genes). Furthermore, using univariate Cox regression analysis, we discovered that one gene (PDHA1) was strongly linked to prognosis [hazard ratio (HR) = 22.96, 95% confidence interval (CI) = 3.09-170.73, *P* = 0.002] (Table 3).

Functional enrichment analysis

Using GO enrichment and KEGG pathway analysis, we explored into the biological processes, cellular components, and molecular activities of DEGs. Energy metabolism and Glycolysis/Gluconeogenesis signaling pathways were highly enriched among DEGs with cuproptosis relevance. The phrases "tricarboxylic acid cycle," "acetylCoA metabolic process", and "acetylCoA biosynthetic process from pyruvate" are considerably enriched in Figure 1A. "Carbon metabolism", "tricarboxylic acid cycle", "Pyruvate metabolism", "Glycolysis/Gluconeogenesis", "Platinum drug resistance", "Biosynthesis of cofactors", "Mineral absorption", and "Central carbon metabolism in cancer" were the most substantially enriched pathways (Figure 1B).

Prognostic value of PDHA1

Patients in the 151 esophageal cancer patients were divided into two groups based on the cut-off value: Those with high PDHA1 expression and those with low PDHA1 expression. In the analysis of OS, high PDHA1 expression was linked to considerably lower OS rates than low expression (*P* = 0.007, Figure 2A). However, there was no link between PDHA1 expression and PFS (Figure 2B). Within a multivariate context, we investigated the link between PDHA1 expression and survival result (Table 4). When we controlled for clinical prognostic indicators that were significant (*P* < 0.05) in univariate Cox regression models, we discovered that overexpression of the PDHA1 gene might predict poor clinical outcomes. The expression of PDHA1 (HR: 1.67, 95% CI: 1.03-2.73; *P* = 0.0386) and AJCC TNM stage (HR: 2.30, 95% CI: 1.58-3.35; *P* < 0.001) were independent risk factors for OS, but not for PFS, according to the multivariate Cox regression analysis.

Different infiltration levels of immune cells between low- and high-PDHA1 groups

Our study used the CIBERSORT algorithm to examine the ratio of tumor-infiltrating immune cells (TICs) in esophageal cancer to further verify the correlation between PDHA1 and the immune cells (Figure 3A). *P* value less than 0.05 was used to classify samples as statistically different. The ratio of TICs in the low-risk group was represented by the first 108 of 151 esophageal cancer patients, while the ratio of TICs in the high-risk group was represented by the last 43 samples. The correlation between 22 immune cells was represented using a heatmap (Figure 3B). Neutrophils and activated mast cells, macrophages M1 and activated NK cells, plasma cells and naive B cells, activated mast cells and activated dendritic cells, and macrophages M1 and naive $CD4^+$ T cells were the top five results with a positive correlation. $CD8^+$ T cells and resting memory $CD4^+$ T cells, on the other hand, were the immune cells that were most negatively associated. Furthermore, the bar graph revealed that the immune infiltration of 22 immune cell types in high- and low-PDHA1 esophageal cancer patients did not differ statistically significantly (Figure 3C).

PDHA1 expression and immune checkpoint correlation analysis

We also investigated at the relationships between PDHA1 and 47 genes associated with immunological checkpoints that have been reported in the literature[15]. The results showed that TNFSF15 ($P = 3.2 \times 10^{-06}$; $r = 0.37$), TNFRSF14 ($P = 8.1 \times 10^{-08}$; $r = 0.42$), HHLA2 ($P = 6.0 \times 10^{-08}$; $r = 0.42$) and LGALS9 ($P = 3.1 \times 10^{-06}$; $r = 0.37$), were significantly higher than that of low-expression group of PDHA1; CD44 ($P = 0.00028$; R = -0.29), TNFRSF18 ($P = 1.2 \times 10^{-05}$; R = -0.35), PDCD1LG2 ($P = 0.0032$; R = -0.24), CD86 ($P = 0.018$; R = -0.19) and CD40 ($P = 0.0047$; R = -0.23) were significantly lower than that of low-expression group of PDHA1 (Figure 4A). The expression profiles of 47 immune checkpoint genes involved in cuproptosis were investigated further. In addition, we categorized the above genes into high and low expression groups based on their cutoff values. Kaplan-Meier analysis and log-rank testing were used to assess OS. The findings

revealed that low HHLA2, TNFRSF18, and CD44 overexpression were all linked to a significantly shorter OS and a worse prognosis (Figure 4B-D).

Constructing and evaluating a predictive nomogram

The 1-, 2-, and 3-year OS probability was calculated using a nomogram that included the pathological type, AJCC-TNM stage, and PDHA1 expression (Figure 5A). The actual observed *vs* anticipated rates of the 1-, 2-, and 3-year OS demonstrated near to the ideal 45° oblique line on the calibration curve (Figure 5B). Furthermore, the AUC of ROC curves for 1-, 2-, and 3-year survival were 0.725, 0.776, 0.619, and 0.810, respectively (Figure 5C). With a C-index of 0.703 for OS, the nomogram showed promising discrimination.

DISCUSSION

In the present study, we investigated the expression signature of 19 cuproptosis-related genes in esophageal cancer tissues and explored their relationships with OS and PFS. A prognostic nomogram involving gene expression and clinicopathological parameters was constructed for the first time. Functional analysis showed that differentially expressed genes were enriched in energy metabolism, especially in pathways related to the tricarboxylic acid cycle. Cuproptosis-related genes were also confirmed to be associated with immune checkpoint genes.

In our study, 151 patients with esophageal cancer and 151 healthy controls were included. In univariate Cox regression analysis, PDHA1 was selected from 19 cuproptosis-related genes to be associated with the prognosis of esophageal cancer. At the same time, PDHA1 expression was also different in tumor tissues and healthy tissues. The results of the present study suggested that the PDHA1 expression was relatively low in cancer tissues, which was consistent with previous studies[16,17]. Li *et al*[17] also reported that high expression of PDHA1 in ovarian cancer cells was significantly correlated with better OS and PFS. Our results suggested that the high expression of PDHA1 expression was associated with poor OS, but not with PFS.

Li *et al*[18] showed that PDHA1 knockout inhibited glucose entering the tricarboxylic acid cycle, resulting in the reconnection of glutamine metabolism by increasing glutaminase 1 (GLS1) and glutamate dehydrogenase 1 (GLUD1) expression, thus increasing the survival rate of glutamine dependent cells. Consistent with our results of functional enrichment, these differentially expressed genes were significantly enriched in tricarboxylic acid cycling-related pathways. We hypothesize that PDHA1 gene knockdown or low expression causes mitochondrial malfunction, resulting in aberrant generation of intracellular reactive oxygen species (ROS) and adenosine triphosphate (ATP), which is compatible with the cuproptosis mechanism.

TNM staging was linked with OS and PFS in esophageal cancer, while pathological types were associated with OS in esophageal cancer, according to our findings. Through univariate and multivariate Cox analysis, Zhang *et al*[19] also claimed that pathological stage was an independent risk factor for OS and PFS in patients with operable esophageal cancer. Therefore, pathological types and TNM stages were included in the prognostic nomogram. Men and women had different rates of esophageal cancer, with men having nearly twice as many cases as women[20]. We did not include gender in our nomogram because, while our analysis indicated that gender impacted PFS, the OS of esophageal cancer was not connected with gender.

CIBERSORT and ssGSEA techniques were used to analyze the composition of tumor-infiltrating immune cells in each sample. Treg and $CD8^+$ T cells have been shown to play an important role in anti-tumor immunity in previous studies[21-23]. Studies have demonstrated that PDHA1 mediates metabolic reprogramming in macrophages[24,25]. However, there was no significant difference between the 22 types of immune cells in the high and low PDHA1 expression groups, according to our findings.

Immune checkpoint inhibitors have recently been studied in a variety of cancers, and they provide a novel therapy option[26]. However, no evidence of a link between cuproptosis and immune checkpoint genes has been found. TNFSF15, TNFRSF14, HHLA2, LGALS9, CD44, TNFRSF18, PDCD1LG2, CD86, and CD40 have all been demonstrated to have a strong relationship with PDHA1 expression in our recent

research. The link between immune checkpoint-related genes expression and prognosis in certain esophageal cancer patients is still debated[27,28]. The immune checkpoint-related genes associated with PDHA1 expression were analyzed using the log-rank test, and the results revealed that HHLA2, TNFRSF18, and CD44 were substantially correlated with prognosis.

However, our study had a number of limitations. To begin, the sample size must be increased in order to analyze EAC and ESCC individually. Second, if the sample size was high enough, the treatment procedures and stages of the esophagus must be unified. Finally, given that prognostic characteristics were generated and analyzed using data from public databases, further biological evidence, in addition to the statistical evidence we present, was required.

CONCLUSION

This study analyzed the association between cuproprosis-related genes and the prognosis of esophageal cancer in a systematic method. Cuproprosis-related genes, especially PDHA1, can be used as prognostic predictors in esophageal cancer patients, providing additional information on how to treat the disease.

ARTICLE HIGHLIGHTS

Research background

Despite many breakthroughs in treatment, the general prognosis for esophageal cancer, one of the least responsive malignancies to cancer therapy, remained dismal. As a result, identifying biomarkers and understanding the molecular mechanisms of esophageal cancer were critical for improving patient outcomes.

Research motivation

A nomogram for predicting the prognosis of esophageal cancer would be developed by evaluating cuproprosis-related genes features and their correlation with prognosis in order to predict the prognosis of esophageal cancer.

Research objectives

Considering cuproprosis-related genes expression was linked to patient prognosis, we intended to develop a nomogram to predict prognosis based on cuproprosis-related genes characteristics and evaluate its prediction performance.

Research methods

Cuproprosis-related genes were found to be linked with esophageal cancer prognosis using univariate COX regression analysis on 151 esophageal cancer samples. The C index, calibration curve, and receiver operator characteristic (ROC) curve were used to evaluate the prediction ability of a prognostic nomogram created by combining clinicopathological variables and cuproprosis-related genes.

Research results

Univariate COX regression analysis of 19 Cuproprosis-related genes revealed that the expression of pyruvate dehydrogenase A1 (PDHA1) was associated with the prognosis of esophageal cancer. The low PDHA1 expression group had a better prognosis of esophageal cancer, according to the log-rank test. There was no statistical correlation between PDHA1 expression and 22 immune cells, however PDHA1 expression and several immune checkpoint genes did. The C index, calibration curve, and ROC curve were used to confirm the predictive ability of the esophageal cancer prognostic nomogram, which was developed by combining pathological type, TNM stage, and PDHA1 expression.

Research conclusions

Cuproprosis-related genes were correlated to esophageal cancer prognosis, and a deep understanding of its molecular mechanism might contribute to novel cancer treatments in the future.

Research perspectives

To enhance the overall survival of esophageal cancer patients, researchers must investigate cuproprosis biomarkers and anticipate possible therapy targets.

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Figure 1 Esophageal cancer functional enrichment analysis of cuproptosis-related genes. A: The expression of cuproptosis-related genes was represented by the outer circle in each enriched GO terms; red dots on each GO term showed up-regulated cuproptosis-related genes, and the inner circle indicated the importance of GO terms (log10 adjusted P-values). The down-regulated cuproptosis-related genes were shown by blue dots, and the chart on the right showed the distribution of cuproptosis-related genes in important GO terms. B: The top 16 KEGG pathway enrichment analysis findings were shown; GeneRatio denotes the number of differentially expressed genes detected in one GO pathway in proportion to the total number of differentially expressed genes.

Figure 2 The Kaplan-Meier survival of patients with esophageal cancer who were classified according to PDHA1 expression. A and B: In esophageal cancer, patients with low PDHA1 expression levels had a better overall survival (A) and progression-free survival (B) than patients with high PDHA1expression levels. The significance of the difference between high and low expression was calculated using a log-rank test. Pyruvate Dehydrogenase A1, PDHA1.

Figure 3 tumor-infiltrating immune cells profile. A: A barplot showed the ratio of 22 different tumor-infiltrating immune cells in esophageal cancer patients; column names (X-axis): sample ID. B: A heatmap showed the association between 22 different types of tumor-infiltrating immune cells; each dot represented the p-value of the correlation between two different cell types, and a Pearson coefficient was used to determine significance. C: A bar graph showed the difference between esophageal cancer patients with high or low expression of PDHA1 in terms of 22 different types of tumor-infiltrating immune cells; Wilcoxon rank sum was used for the significance test.

Figure 4 Correlation between PDHA1 expression and immune checkpoint-related genes. A: Barplot revealed expression of 47 immune checkpoint-related genes in high- and low-PDHA1 esophageal cancer patients; (B) HHLA2, (C) TNFRSF18, and (D) CD44 Kaplan-Meier survival curves of high and low expression of immune checkpoint-related genes. $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$.

Figure 5 Developing a predictive nomogram. A: A nomogram that predicts patients with esophageal cancer's 1-, 2-, and 3-year survival; B a calibration curve of 1-, 2-, and 3-year survival in the nomogram and ideal model; C: Receiver operating characteristic (ROC) curves and area under the curve (AUC) for the nomogram's 1, 2, and 3-year survival.

Table 1 Demographic characteristics and clinicopathological characteristics

Variables	Total (*n* = 151)	Esophagus cancer (*n* = 151)	
		Adenocarcinoma (*n* = 74)	Squamous-cell carcinoma (*n* = 77)
Age, yr			
Median	60	68.5	57
Interquartile range	(53-72)	(57-77)	(51-63.5)
Sex			
Female	22 (14.6%)	11 (14.9%)	11 (14.3%)
Male	129 (85.4%)	63 (85.1%)	66 (85.7%)
AJCC TNM Stage			
Ⅰ	18 (11.9%)	11 (14.9%)	7 (9.1%)
Ⅱ	70 (46.4%)	24 (32.4%)	46 (59.7%)
Ⅲ	51 (33.8%)	31 (41.9%)	20 (26.0%)
Ⅳ	12 (7.9%)	8 (10.8%)	4 (5.2%)
EGFR status			
Mutant	5 (3.3%)	3 (4.1%)	2 (2.6%)
Wild-type	146 (96.7%)	71 (95.9%)	75 (97.4%)
KRAS status			
Mutant	2 (1.3%)	2 (2.7%)	0 (0)
Wild-type	149 (98.7%)	72 (97.3%)	77 (100%)

BRAF status			
Mutant	1 (0.7%)	0 (0)	1 (1.3%)
Wild-type	150 (99.3%)	74 (100%)	76 (98.7)
PIK3CA status			
Mutant	14 (9.3%)	4 (5.4%)	10 (13.0%)
Wild-type	137 (90.7%)	70 (94.6%)	67 (87.0%)
OS event			
Event	58 (38.4%)	36 (48.6%)	22 (28.6%)
Non-event	93 (61.6%)	38 (51.4%)	55 (71.4%)
OS months			
Median	13.4	14.8	13.0
Range	(7.8-22.9)	(7.3-27.5)	(11.0-18.6)
PFS event			
Event	73 (48.3%)	38 (51.4%)	35 (45.5%)
Non-event	78 (51.7%)	36 (48.6%)	42 (54.5%)
PFS months			
Median	10.7	10.3	10.7
Range	(5.1-18.9)	(5.4-24.5)	(3.7-15.8)

American Joint Committee on Cancer, AJCC; v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog, KRAS; v-Raf murine sarcoma viral oncogene homolog B1 genes, BRAF; Epidermal Growth Factor Receptor, EGFR; Phosphatidylino-sitol-4,5-bisphosphate 3-kinase, catalytic subunit alpha, PIK3CA; Overall survival, OS; Progression-free survival, PFS.

Table 2 Relationship between the clinicopathological characteristics and survival outcome

Parameters	Overall survival			Progression-free survival		
	Non-event	Event	*P* value	Non-event	Event	*P* value
Age			0.758			0.628
≤65	57	37		50	44	
> 65	36	21		28	29	
Sex			0.102			**0.009**
Female	17	5		17	5	
Male	76	53		61	68	
AJCC TNM stage			**0.021**			**0.013**
I and II	61	27		53	35	
III and IV	32	31		25	38	
Pathological type			**0.011**			0.469
Adenocarcinoma	38	36		36	38	
SCC	55	22		42	35	
EGFR status						0.596
Wild-type	3	2	0.941	2	3	
Mutant	90	56		76	70	
BRAF status			0.428			0.332
Wild-type	1	0		1	0	

Mutant	92	58		77	73	
KRAS status			0.734			0.962
Wild-type	1	1		1	1	
Mutant	92	57		77	72	
PIK3CA status			0.828			0.896
Wild-type	9	5		7	7	
Mutant	84	53		71	66	

Squamous-cell carcinoma, SCC; American Joint Committee on Cancer, AJCC; v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog, KRAS; v-Raf murine sarcoma viral oncogene homolog B1 genes, BRAF; Epidermal Growth Factor Receptor, EGFR; Phosphatidylino-sitol-4,5-bisphosphate 3-kinase, catalytic subunit alpha, PIK3CA.

Table 3 The results of the univariate Cox regression analysis

Gene name	HR	HR.95L	HR.95H	*P* value
PDHA1	22.96	3.09	170.73	0.002
ATP7A	3.83	0.96	15.21	0.057
CDKN2A	0.84	0.64	1.10	0.204
PDHB	1.76	0.37	8.50	0.479
GLS	1.50	0.48	4.67	0.484
ATP7B	1.15	0.77	1.71	0.496
FDX1	1.52	0.41	5.59	0.527
DLD	1.82	0.27	12.11	0.534
LIPT2	0.87	0.46	1.64	0.662
DLST	0.58	0.05	6.70	0.662
NFE2L2	0.73	0.18	3.04	0.666
DLAT	1.43	0.23	8.89	0.702
DBT	0.83	0.27	2.54	0.744
GCSH	0.87	0.32	2.36	0.777
MTF1	0.85	0.26	2.80	0.785
LIAS	0.86	0.25	2.93	0.806
NLRP3	0.91	0.44	1.90	0.809
LIPT1	1.14	0.29	4.41	0.853
SLC31A1	1.08	0.19	6.15	0.928

Hazard ratio, HR; Low 95% confidence interval of HR, HR.95L; High 95% confidence interval of HR, HR.95H.

Table 4 Univariate and multivariate Cox regression analyses of PDHA1 expression and the clinicopathological factors in the esophagus cancer patients

Variables	Overall survival		Progression-free survival	
	HR (95% CI of HR)	*P* value	HR (95% CI of HR)	*P* value
PDHA1 low	1.00 (Reference)		1.00 (Reference)	
UVA PDHA1 high	2.34 (1.42-3.87)	0.0009	1.51 (0.95-2.38)	0.08
MVA PDHA1 high	1.67 (1.03-2.73)	0.0386		
Age < = 65	1.00 (Reference)		1.00 (Reference)	
Age > 65	0.81 (0.47-1.41)	0.46	0.97 (0.60-1.55)	0.89
Sex, Female	1.00 (Reference)		1.00 (Reference)	
UVA Male	2.12 (0.84-5.34)	0.11	2.97 (1.19-7.39)	0.02
MVA Male			2.27 (0.90- 5.73)	0.08
Pathological types, adenocarcinoma	1.00 (Reference)		1.00 (Reference)	
SCC	0.81 (0.47-1.39)	0.45	1.11 (0.70-1.77)	0.66
AJCC stage I, II	1.00 (Reference)		1.00 (Reference)	
UVA AJCC stage III, IV	2.53 (1.76-3.64)	$5.86*10^{-7}$	1.98 (1.46-2.67)	$9.48*10^{-6}$
MVA AJCC stage III, IV	2.30 (1.58-3.35)	$1.46*10^{-5}$	1.86 (1.37-2.52)	$6.98*10^{-5}$

Pyruvate Dehydrogenase A1, PDHA1; univariate, UVA; multivariate, MVA; squamous-cell carcinoma, SCC; hazard ratio, HR; confidence interval, CI.

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