Journal of Pharmaceutical and Medical Research
Print ISSN: 2663-1954
Online ISSN: 2663-1962
DOI: https://doi.org/10.61784/jpmr3065

EXPRESSION AND CLINICAL SIGNIFICANCE OF LHPP IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA

ZhiGang Zhai[1], Yuan Fang[2], ZhenYu Xue[3], Jian Guo[4], Xiang Tang[2*]
[1]*Department of Thoracic Oncology, Affiliated Hospital of Jiangsu University, Zhenjiang 212013, Jiangsu, China.*
[2]*Department of Abdominal Oncology, Affiliated Hospital of Jiangsu University, Zhenjiang 212013, Jiangsu, China.*
[3]*Department of Head and Neck & Comprehensive Oncology, Affiliated Hospital of Jiangsu University, Zhenjiang 212013, Jiangsu, China.*
[4]*Department of Pathology, Affiliated Hospital of Jiangsu University, Zhenjiang 212013, Jiangsu, China.*
[*]*Corresponding Author: Xiang Tang*

Abstract: Objective: To investigate the expression characteristics, clinical significance and underlying molecular mechanism of phospholysine phosphohistidine inorganic pyrophosphate phosphatase (LHPP) in esophageal squamous cell carcinoma (ESCC), and to provide novel potential targets for the diagnosis, prognostic evaluation and clinical treatment of ESCC. **Methods:** The expression difference of LHPP mRNA between ESCC tissues and adjacent normal tissues was analyzed from the Gene Expression Omnibus (GEO) database including GSE20347, GSE38129 and GSE77861. A total of 15 pairs of ESCC tumor tissues and matched adjacent normal tissues, serum samples from 40 newly diagnosed ESCC patients and 20 healthy volunteers were collected. The expression level of LHPP was detected by quantitative real-time polymerase chain reaction (qRT-PCR) and enzyme-linked immunosorbent assay (ELISA). Western blot was used to determine the protein expression of LHPP in ESCC cell lines (TE-1, KYSE-30, KYSE-150) and the normal esophageal epithelial cell line Het-1A. The correlation between LHPP expression and survival prognosis of ESCC patients was analyzed based on The Cancer Genome Atlas (TCGA) database. Differentially expressed genes (DEGs) between LHPP high and low expression groups were screened, followed by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses. The characteristics of tumor-infiltrating immune cells, the expression profiles of immune checkpoint genes and the sensitivity to five clinical common chemotherapeutic drugs including paclitaxel were further detected and compared between the two groups. **Results:** LHPP was significantly down-regulated in ESCC tissues, peripheral serum of ESCC patients and ESCC cell lines ($P<0.05$). ESCC patients with low LHPP expression had a markedly shortened median survival time (1.5 years vs 2.8 years), and the area under the curve (AUC) values of LHPP for predicting the 1-year, 2-year and 3-year overall survival rates of ESCC patients were 0.574, 0.615 and 0.671, respectively. DEGs in the LHPP low expression group were significantly enriched in biological processes including immune disorder, cell invasion and metastasis, and also enriched in classic tumor-promoting signaling pathways such as Wnt and TNF. In contrast, DEGs in the LHPP high expression group were enriched in physiological processes including cell junction, epidermal development and tissue homeostasis regulation, without enrichment of classic tumor-promoting or metastasis-promoting signaling pathways. The LHPP low expression group showed high infiltration of pro-tumor immune cells such as macrophages and B cells, and significantly up-regulated expression of core immune checkpoint genes including SIGLEC15 and CTLA4 ($P<0.001$). The LHPP high expression group had significantly higher sensitivity to paclitaxel, cisplatin, gemcitabine, 5-fluorouracil and irinotecan than the low expression group ($P<0.05$). **Conclusion:** LHPP is expressed deficiently in ESCC and acts as a tumor suppressor gene in the occurrence and development of ESCC. Low LHPP expression is closely associated with the poor prognosis of ESCC patients, and it may participate in the malignant progression of ESCC by regulating the activation of tumor-promoting signaling pathways, remodeling the tumor immunosuppressive microenvironment and reducing the sensitivity of tumor cells to chemotherapeutic drugs. LHPP can serve as a potential early diagnostic marker, prognostic evaluation indicator and therapeutic target for ESCC.
Keywords: Esophageal squamous cell carcinoma; LHPP; Tumor suppressor gene; Tumor immune microenvironment; Chemotherapeutic sensitivity; Prognostic marker

1 INTRODUCTION

Esophageal cancer is one of the most common malignant tumors with high incidence and mortality worldwide, and its incidence has shown a significant increasing trend in recent years, becoming one of the leading causes of cancer-related death [1, 2]. Despite extensive in-depth studies on the pathogenesis, risk factors and clinical treatment of esophageal cancer by scholars at home and abroad, the core molecular mechanisms inducing the occurrence and malignant progression of esophageal cancer remain unclear to date. Existing studies have confirmed that the occurrence of esophageal cancer is a multi-factor and multi-step pathological process, which is closely related to demographic characteristics, smoking, alcohol consumption, gastroesophageal reflux disease, unhealthy dietary structure, obesity and high body mass index [3, 4]. These risk factors interact with each other and jointly promote the initiation and development of esophageal cancer.

Esophageal squamous cell carcinoma (ESCC) is the most predominant pathological subtype of esophageal cancer,

accounting for more than 90% of all esophageal cancer cases in Asian countries, especially in China, which seriously threatens the life and health of Chinese residents [5]. With the continuous advancement of medical technology, comprehensive treatment strategies such as radical surgery, chemotherapy, radiotherapy and targeted therapy have been widely applied in the clinical treatment of ESCC [6]. However, due to the insidious early clinical symptoms and the lack of specific early diagnostic markers, most ESCC patients are diagnosed at the middle and advanced stages when they seek medical treatment, resulting in poor therapeutic effect, high postoperative recurrence and metastasis rate, and an overall 5-year survival rate of less than 20% [7]. Therefore, exploring specific and sensitive early diagnostic markers, clarifying the key molecular mechanisms regulating the malignant progression of ESCC, and developing novel targeted therapeutic drugs are of great clinical significance and research value for improving the diagnostic efficiency, optimizing individual treatment regimens and enhancing the prognostic level of ESCC patients.

Phospholysine phosphohistidine inorganic pyrophosphate phosphatase (LHPP) is a putative histidine phosphatase belonging to an evolutionarily highly conserved phosphatase family, which is widely distributed in archaea, bacteria and eukaryotes [8]. This protein was first discovered and purified from porcine brain tissue, and subsequent studies have confirmed that LHPP is highly expressed in brain tissue and plays an important role in the physiological activities of the nervous system [9, 10]. In addition to its regulatory role in the nervous system, LHPP has also been reported in the field of mental diseases. For example, in patients with major depressive disorder, those carrying the rs35926514 locus variation of the LHPP gene exhibit obvious abnormalities in resting brain activity and neural network regulation [11].

In recent years, LHPP has attracted extensive attention from oncology researchers as a novel tumor suppressor factor for its important regulatory role in the occurrence and development of malignant tumors. A pioneering study reported that LHPP is specifically down-regulated in hepatocellular carcinoma driven by the mechanistic target of rapamycin (mTOR), and its low expression level is closely associated with the poor prognosis of hepatocellular carcinoma patients, which can serve as a potential molecular marker for judging the survival rate of patients [12]. In cervical cancer, LHPP can significantly inhibit the proliferation, migration and invasion abilities of cervical cancer cells by blocking the abnormal activation of the AKT signaling pathway and promoting the expression of the tumor suppressor gene p53 [13]. In glioblastoma, LHPP can inhibit the malignant biological behavior of tumor cells by degrading pyruvate kinase M2 (PKM2), reducing the glycolysis and mitochondrial respiration of tumor cells [14].

Although LHPP has been confirmed to exert a significant tumor suppressive effect in a variety of malignant tumors such as hepatocellular carcinoma, cervical cancer and glioblastoma, its expression level, specific biological function and underlying molecular regulatory mechanism in ESCC remain unclear. Based on this, this study aimed to systematically investigate the expression characteristics and clinical significance of LHPP in ESCC, and to explore its potential molecular mechanism regulating the malignant progression of ESCC, so as to provide new theoretical basis and potential molecular targets for the early diagnosis, prognostic evaluation and clinical treatment of ESCC.

2 MATERIALS AND METHODS

2.1 Bioinformatics Analysis

Public ESCC gene expression profiles and clinical follow-up data were downloaded from The Cancer Genome Atlas (TCGA) database and the Gene Expression Omnibus (GEO) database. The expression difference of LHPP mRNA between ESCC tissues and adjacent normal tissues was analyzed from three GEO datasets including GSE20347, GSE38129 and GSE77861. TCGA database was used for subsequent survival analysis, differential gene screening, immune cell infiltration analysis and immune checkpoint gene expression analysis.

2.2 Patient Serum and Tumor Tissue Samples

A total of 15 pairs of ESCC tumor tissues and matched adjacent normal tissues (more than 2 cm away from the tumor edge) were collected from ESCC patients who underwent radical surgical resection at the Affiliated Hospital of Jiangsu University (Zhenjiang, China) from 2023 to 2025. All enrolled patients had not received any anti-tumor therapy such as chemotherapy, radiotherapy or targeted therapy before surgery, and all tissue samples were pathologically confirmed as ESCC by two independent senior pathologists. The tissue samples were quickly frozen in liquid nitrogen immediately after intraoperative sampling, and then stored in a -80℃ ultra-low temperature refrigerator for subsequent qRT-PCR experiments. Peripheral venous blood samples were collected from 40 newly diagnosed ESCC patients and 20 healthy volunteers who underwent physical examination in our hospital during the same period. The serum was separated by centrifugation at 3000 r/min for 10 min, and stored in a -80℃ refrigerator for subsequent ELISA experiments. All research objects signed the written informed consent, and this study was approved by the Medical Ethics Committee of the Affiliated Hospital of Jiangsu University (Approval No.: KY201903).

2.3 Cell Lines and Culture Conditions

Human ESCC cell lines TE-1, KYSE-30 and KYSE-150 were purchased from Shanghai GeneChem Co., Ltd. The immortalized human normal esophageal epithelial cell line Het-1A was purchased from Kunshan Beno Cell Bank, Jiangsu Province, China. TE-1, KYSE-30 and KYSE-150 cells were cultured in RPMI 1640 medium (Gibco, Thermo Fisher Scientific, USA), and Het-1A cells were cultured in DMEM medium (Gibco, Thermo Fisher Scientific, USA). All culture media were supplemented with 10% fetal bovine serum (FBS, Biological Industries, Israel) and 100 IU/mL

penicillin-streptomycin double antibody (Gibco, Thermo Fisher Scientific, USA). All cells were cultured in a constant temperature and humidity incubator at 37℃ with 5% CO_2, and the culture medium was replaced every 2-3 days. Cells in the logarithmic growth phase were used for subsequent experiments.

2.4 ELISA Experiment

The frozen serum samples were taken out and thawed to room temperature naturally. The concentration of LHPP protein in serum was detected by ELISA kit strictly according to the manufacturer's instructions. The anti-LHPP antibody was diluted with coating buffer and added to the 96-well enzyme-labeled plate, and incubated overnight at 4℃. After discarding the coating buffer, the plate was washed 3 times with washing buffer for 5 min each time, and then blocked with blocking solution at room temperature for 2 h. After blocking, gradient-diluted LHPP standard products and processed serum samples were added to the plate with 3 replicate wells for each sample, and incubated at room temperature for 1 h. The HRP-labeled secondary antibody was added after washing the plate, and incubated at room temperature for 1 h. TMB color developing solution was added to the plate for color development in the dark for 15 min, and the reaction was terminated with sulfuric acid stop solution. The optical density (OD) value of each well was measured at 450 nm with a microplate reader. The standard curve was drawn according to the OD values of the standard products, and the concentration of LHPP protein in serum samples was calculated according to the standard curve. The experiment was independently repeated 3 times, and blank controls were set to eliminate system errors.

2.5 Quantitative Real-time PCR (qRT-PCR) Assay

Total RNA was extracted from ESCC tissues, adjacent normal tissues and cultured cells using TRIzol reagent (Solarbio Biotechnology Co., Ltd., China) strictly according to the kit instructions, and the whole operation was carried out on ice. The concentration and purity of total RNA were detected by a Bio Mate 3S nucleic acid analyzer (Thermo Fisher Scientific, USA), and RNA samples with A260/A280 ratio between 1.8 and 2.0 were used for subsequent experiments. A total of 1 μg of qualified total RNA was reverse-transcribed into cDNA using a reverse transcription kit (Nanjing Vazyme Biotech Co., Ltd., China). The qRT-PCR reaction was performed in the Mx3000P real-time fluorescent quantitative PCR system (Agilent Technologies, USA) using Cham Q SYBR qPCR Master mix (Nanjing Vazyme Biotech Co., Ltd., China). The specific primers of LHPP and internal reference gene β-Actin were synthesized by Nanjing Kingsray Biotech Co., Ltd., China. The primer sequences were as follows: LHPP forward primer 5'-ACACGTCACTTGCCAGTCTCAC-3', reverse primer 5'-CACAGGCTGTATGTCGCGGA-3'; β-Actin forward primer 5'-TCACCCACACTGTGCCCATCTACGA-3', reverse primer 5'-CAGCGGAACCGCTCATTGCCAATGG-3'. The qRT-PCR reaction system was 20 μL, and the reaction conditions were as follows: pre-denaturation at 95℃ for 30 s, denaturation at 95℃ for 10 s, annealing and extension at 60℃ for 30 s, a total of 40 cycles. The 2-ΔΔCq method was used to calculate the relative expression level of LHPP mRNA, with β-Actin as the internal reference gene. The experiment was set with 3 replicate wells for each sample, and independently repeated 3 times.

2.6 Western Blot Assay

ESCC cells (TE-1, KYSE-30, KYSE-150) and Het-1A cells in the logarithmic growth phase were collected, and the culture medium was discarded. The cells were washed twice with pre-cooled PBS, and then lysed on ice with RIPA lysis buffer containing 1% protease inhibitor and 1% phosphatase inhibitor (Beyotime Biotechnology Co., Ltd., China) for 30 min with repeated pipetting. The cell lysate was transferred to a 1.5 mL centrifuge tube, and centrifuged at 12000 r/min for 15 min at 4℃. The supernatant was collected as the total protein extract. The concentration of total protein was detected by BCA protein quantitative kit (Beyotime Biotechnology Co., Ltd., China). The protein concentration of each group was adjusted to be consistent with lysis buffer, and 5×loading buffer was added, then the protein was denatured by boiling at 100℃ for 10 min. A total of 30 μg of denatured total protein was subjected to SDS-PAGE gel electrophoresis, and then the protein was transferred to a PVDF membrane (Millipore, USA) by wet transfer method. The PVDF membrane was blocked with 5% skimmed milk at room temperature for 2 h, and then incubated with rabbit anti-human LHPP polyclonal antibody (1:1000 dilution, Abcam, UK) and rabbit anti-human GAPDH polyclonal antibody (1:5000 dilution, Beyotime Biotechnology Co., Ltd., China) at 4℃ overnight with gentle shaking. The next day, the membrane was washed 3 times with TBST for 10 min each time, and then incubated with HRP-labeled goat anti-rabbit secondary antibody (1:3000 dilution, Beyotime Biotechnology Co., Ltd., China) at room temperature for 1 h with gentle shaking. The membrane was washed again with TBST for 3 times, and then the protein bands were developed with ECL chemiluminescence kit (Millipore, USA). The gel imaging system (Bio-Rad, USA) was used to take photos of the protein bands. GAPDH was used as the internal reference, and ImageJ software was used to analyze the gray value of the bands to calculate the relative expression level of LHPP protein. The experiment was independently repeated 3 times.

2.7 Screening and Enrichment Analysis of Differentially Expressed Genes

According to the median expression level of LHPP mRNA in ESCC patients from TCGA database, the patients were divided into LHPP high expression group and low expression group. R language (version 4.2.1) limma package was used to screen the differentially expressed genes (DEGs) between the two groups with the screening criteria of |log2

fold change (log2FC)|>1 and adjusted P<0.05. The clusterProfiler package of R language was used to perform Gene Ontology (GO) functional enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis on the DEGs, with the screening criterion of P<0.05. The GO analysis included three dimensions: cellular component (CC), molecular function (MF) and biological process (BP). The ggplot2 package of R language was used to draw the visualization maps of GO and KEGG enrichment analyses to intuitively show the main biological functions and enriched signaling pathways of DEGs.

2.8 Analysis of Tumor-infiltrating Immune Cells and Immune Checkpoints

The CIBERSORT algorithm of R language was used to analyze the relative infiltration abundance of 22 types of immune cells in ESCC patients from TCGA database, and the samples with low confidence (P>0.05) were excluded. The Wilcoxon rank-sum test was used to compare the difference in immune cell infiltration abundance between LHPP high and low expression groups, and the immune cells with significant intergroup differences were screened out. The expression data of immune checkpoint-related genes were extracted from the gene expression profile of ESCC patients, and the Wilcoxon rank-sum test was used to compare the expression differences of immune checkpoint genes between the two groups with the screening criterion of P<0.05. The pheatmap package of R language was used to draw the heatmaps of immune cell infiltration and immune checkpoint gene expression to show the intergroup difference characteristics.

2.9 Analysis of Chemotherapeutic Drug Sensitivity

The gene expression data of ESCC cell lines and the half-maximal inhibitory concentration (IC50) values of five clinical common chemotherapeutic drugs (paclitaxel, cisplatin, gemcitabine, 5-fluorouracil, irinotecan) were downloaded from the Genomics of Drug Sensitivity in Cancer (GDSC) database. According to the median expression level of LHPP mRNA, ESCC cell lines were divided into LHPP high expression group and low expression group. The Wilcoxon rank-sum test was used to compare the difference in IC50 values of the five chemotherapeutic drugs between the two groups, with P<0.05 as the statistically significant difference. The ggplot2 package of R language was used to draw box plots to show the difference in sensitivity of LHPP high and low expression groups to different chemotherapeutic drugs.

2.10 Survival Analysis

According to the median expression level of LHPP mRNA in ESCC patients from TCGA database, the patients were divided into LHPP high expression group and low expression group. The overall survival (OS), disease-free survival (DFS) and survival status information of the two groups were extracted. The Kaplan-Meier method was used to draw the survival curve, and the Log-rank test was used to compare the differences in OS and DFS between the two groups to evaluate the correlation between LHPP expression level and the prognosis of ESCC patients. The pROC package of R language was used to draw the receiver operating characteristic (ROC) curve of LHPP expression for predicting the 1-year, 2-year and 3-year overall survival rates of ESCC patients, and the area under the curve (AUC) value was calculated to evaluate the prognostic prediction value of LHPP. The closer the AUC value is to 1, the higher the prediction value is.

2.11 Statistical Analysis

All experimental data and bioinformatics analysis data were statistically analyzed by SPSS 26.0 statistical software and R language (version 4.2.1). Measurement data were expressed as mean ± standard deviation (x±s). The independent sample t-test or Wilcoxon rank-sum test was used for comparison between two groups, and one-way analysis of variance (ANOVA) was used for comparison among multiple groups. Count data were expressed as rate (%), and the χ^2 test was used for intergroup comparison. Survival analysis was performed by Kaplan-Meier method combined with Log-rank test, and prognostic prediction was evaluated by ROC curve analysis. P<0.05 was considered as statistically significant difference, and all experiments were independently repeated 3 times to ensure the reliability of the results.

3 RESULTS

3.1 The Expression of Tumor Suppressor Gene LHPP is Significantly Decreased in Serum and Tissues of ESCC Patients

Bioinformatics analysis of three GEO datasets (GSE20347, GSE38129, GSE77861) showed that the transcription level of LHPP mRNA in ESCC tissues was significantly lower than that in adjacent normal tissues (P<0.05, Fig.1A-C). ELISA results showed that the concentration of LHPP protein in the peripheral serum of ESCC patients was significantly lower than that of healthy volunteers (P<0.05, Fig.1D). qRT-PCR results of paired tissue samples of patients also showed that the expression level of LHPP mRNA in ESCC tissues was significantly down-regulated compared with adjacent normal tissues (P<0.05, Fig.1E). Western blot results revealed that the protein expression level of LHPP in three ESCC cell lines (TE-1, KYSE-30, KYSE-150) was significantly lower than that in the normal

esophageal epithelial cell line Het-1A (P<0.05, Fig.1F).

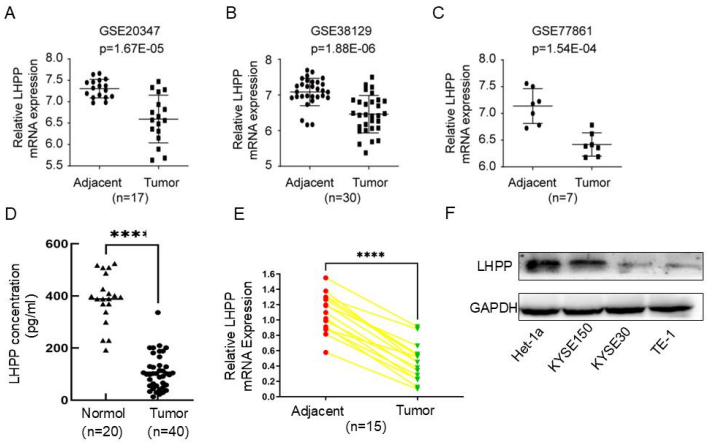

Figure 1 Expression of LHPP in Tissues and Serum of Esophageal Cancer Patients

Note: A-C: Comparison of LHPP mRNA transcription levels between ESCC tissues and adjacent normal tissues in GEO datasets; D: Concentration of LHPP protein in serum of ESCC patients and healthy volunteers; E: LHPP mRNA transcription levels in ESCC tissues and adjacent normal tissues of patients; F: Protein expression of LHPP in ESCC cell lines and normal esophageal epithelial cell line.

3.2 Deletion of LHPP Expression Shortens the Overall Survival and Disease-free Survival of ESCC Patients

Survival analysis results based on TCGA database showed that ESCC patients with low LHPP expression had a markedly shortened median survival time compared with patients with high LHPP expression (1.5 years vs 2.8 years, P<0.05, Fig.2A-B). ROC curve analysis results showed that LHPP had a certain prediction value for the prognosis of ESCC patients, and the AUC values of LHPP for predicting the 1-year, 2-year and 3-year overall survival rates of ESCC patients were 0.574, 0.615 and 0.671, respectively (Fig.2C), indicating that LHPP has a higher prediction value for the long-term prognosis of ESCC patients.

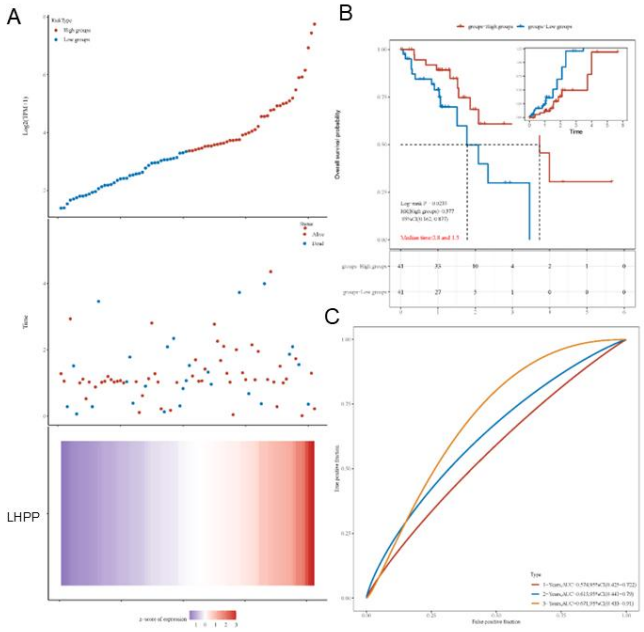

Figure 2 Survival Analysis of ESCC Patients with Different LHPP Expression Levels

Note: A: Correlation between LHPP expression and survival time and survival status in TCGA data. The top panel shows the scatter plot of LHPP expression from low to high with different colors representing different expression groups; the middle panel shows the scatter plot distribution of survival time and survival status corresponding to LHPP expression in different samples; the bottom panel shows the expression heatmap of LHPP. B: Kaplan-Meier survival curve of LHPP in TCGA data, with intergroup comparison by Log-rank test. C: ROC curve and AUC value of LHPP for predicting 1-year, 2-year and 3-year overall survival rates of ESCC patients.

3.3 Screening of Differentially Expressed Genes between LHPP High and Low Expression Groups in ESCC

Differential gene screening results showed that there were a large number of differentially expressed genes (DEGs)

between LHPP high and low expression groups in ESCC (Fig.3), among which LHPP was the core DEG with significant expression difference between the two groups. Meanwhile, multiple significantly up-regulated or down-regulated DEGs were screened out, including IGFBP5, GPC4, IGFBP4, PTGER4, CTAG2, CDKN2B, ACKR3 and MMP7. Functional annotation showed that these DEGs are widely involved in multiple biological processes such as cell proliferation, cell adhesion, immune regulation and intracellular signal transduction, which may be the key molecular targets of LHPP regulating the malignant progression of ESCC.

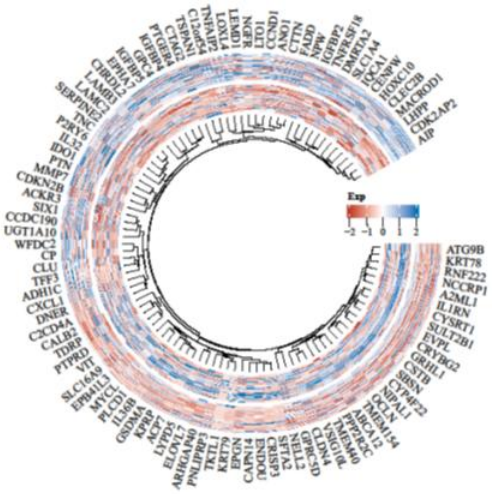

Figure 3 Differential Genes between LHPP High and Low Expression Groups in Esophageal Squamous Cell Carcinoma

Note: The heatmap shows the expression profile of differentially expressed genes between LHPP high and low expression groups. The sample groups are distributed from outside to inside as LHPP low expression group and LHPP high expression group, respectively.

3.4 Enrichment Analysis of Differentially Expressed Genes in LHPP Low Expression Group of ESCC

GO and KEGG enrichment analysis results showed that the up-regulated DEGs in ESCC patients with low LHPP expression were significantly enriched in biological functions including immune disorder, inflammatory response activation, abnormal tumor microenvironment and cell adhesion/invasion (Fig.4A). KEGG pathway enrichment analysis showed that these up-regulated DEGs were also significantly enriched in classic tumor-promoting signaling pathways such as Wnt and TNF (Fig.4B), which are the core regulatory pathways of ESCC cell proliferation, invasion and metastasis. In contrast, the DEGs in ESCC patients with high LHPP expression were mainly enriched in physiological processes such as cell junction, epidermal development and tissue homeostasis regulation (Fig.4C), without enrichment of classic tumor-promoting or metastasis-promoting signaling pathways (Fig.4D).

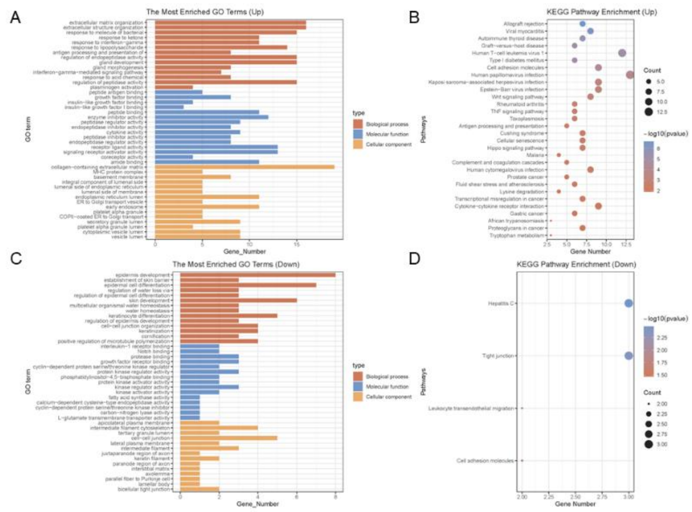

Figure 4 Enrichment Analysis of Differentially Expressed Genes in LHPP Low Expression Group in Esophageal Squamous Cell Carcinoma

Note: A-B: GO term and KEGG pathway enrichment analysis of up-regulated differentially expressed genes in LHPP low expression group; C-D: GO term and KEGG pathway enrichment analysis of down-regulated differentially expressed genes in LHPP low expression group.

3.5 Analysis of Immune Cell Infiltration Characteristics and Immune Checkpoint Genes in LHPP High and Low

Expression Groups of ESCC

Tumor-associated immune cell infiltration analysis results showed that there were obvious intergroup specificities in the infiltration abundance and differential significance of immune cells between the LHPP low expression group (G1) and the high expression group (G2) (Fig.5A). Among them, the infiltration abundance of B cells, endothelial cells and macrophages showed extremely significant differences between the two groups (P<0.001), while the infiltration abundance of CD8+ T cells, NK cells, uncharacterized cells and CD4+ T cells had no significant difference between the two groups (P>0.05). Immune checkpoint gene expression analysis results showed that the expression levels of 5 core immune checkpoint genes including SIGLEC15, CTLA4, PDCD1, TIGIT and HAVCR2 were extremely significantly up-regulated in the LHPP low expression group (P<0.001, Fig.5B).

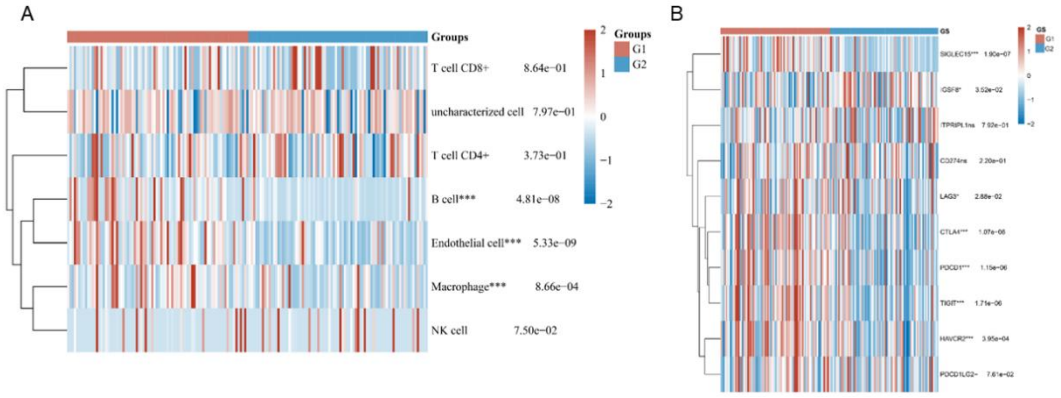

Figure 5 Analysis of Immune Cell Infiltration Characteristics and Immune Checkpoint Gene Expression in LHPP High and Low Expression Groups of ESCC

Note: (A) Heatmap of immune cell infiltration in LHPP low-expression (G1) and high-expression (G2) groups. The color gradient represents the relative abundance of immune cell infiltration, with red indicating high infiltration and blue indicating low infiltration. *** indicates an extremely significant difference in infiltration between groups (P < 0.001). The analyzed immune cells include CD8+ T cells, uncharacterized cells, CD4+ T cells, B cells, endothelial cells, macrophages, and NK cells. (B) Heatmap of immune checkpoint gene expression in G1 and G2 groups. The color gradient represents the relative expression level of genes, with red indicating high expression and blue indicating low expression. *** indicates an extremely significant difference in gene expression between groups (P < 0.001), and * indicates a significant difference (P < 0.05). The detected immune checkpoint genes include SIGLEC15, IGSF8, LAG3, ITPRIPL1, CD274, CTLA4, PDCD1, TIGIT, HAVCR2, and PDCD1LG2.

3.6 LHPP Expression Affects the Sensitivity of ESCC Cells to Common Chemotherapeutic Drugs

The Wilcoxon rank-sum test was used to compare the difference in sensitivity (IC50 value) to five clinical common chemotherapeutic drugs between the LHPP low expression group (G1) and the high expression group (G2) in ESCC. The results showed that the IC50 values of paclitaxel, cisplatin, gemcitabine, 5-fluorouracil and irinotecan in the LHPP high expression group were significantly lower than those in the low expression group (P<0.05, Fig.6A-E), indicating that the LHPP high expression group had significantly higher sensitivity to the five chemotherapeutic drugs than the low expression group, and the expression level of LHPP was positively correlated with the sensitivity of ESCC cells to chemotherapeutic drugs.

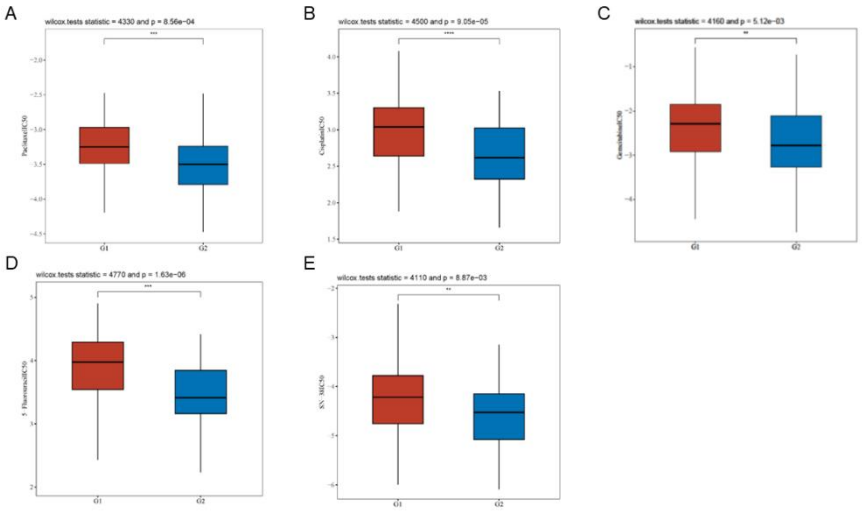

Figure 6 Chemotherapeutic Drug Sensitivity in ESCC with Different LHPP Expression Levels

Note: Box plots showing the sensitivity of LHPP low-expression group (G1, red) and LHPP high-expression group (G2, blue) to five

chemotherapeutic agents: (A) Paclitaxel, (B) Gemcitabine, (C) Gimeracil, (D) 5-Fluorouracil, and (E) Irinotecan. Wilcoxon rank-sum tests were used to assess the significance of differences between groups. The lower the IC50 value, the higher the sensitivity of tumor cells to chemotherapeutic drugs.

4 DISCUSSION

This study systematically investigated the expression characteristics, clinical significance and underlying molecular mechanism of phospholysine phosphohistidine inorganic pyrophosphate phosphatase (LHPP) in esophageal squamous cell carcinoma (ESCC), and first confirmed that LHPP, as a tumor suppressor gene, is significantly down-regulated in the peripheral serum, tumor tissues and ESCC cell lines of ESCC patients. Moreover, the expression level of LHPP is closely associated with the prognosis of ESCC patients, and patients with high LHPP expression have significantly prolonged overall survival and disease-free survival. Meanwhile, LHPP participates in the malignant progression of ESCC by regulating the tumor-associated molecular network, remodeling the tumor immune microenvironment and affecting the sensitivity of tumor cells to chemotherapeutic drugs, which provides new theoretical basis and experimental support for the screening of early diagnostic markers, prognostic evaluation indicators and therapeutic targets of ESCC.

Through bioinformatics analysis and in vitro experimental verification, this study found that the transcription level of LHPP mRNA and the protein expression level in ESCC tissues were significantly lower than those in adjacent normal tissues, and the concentration of LHPP protein in the peripheral serum of ESCC patients was also much lower than that of healthy volunteers. In addition, the expression level of LHPP protein was significantly down-regulated in ESCC cell lines such as TE-1, KYSE-30 and KYSE-150 compared with the normal esophageal epithelial cell line Het-1A. This result is consistent with the expression characteristics of LHPP in other malignant tumors such as hepatocellular carcinoma and cervical cancer, suggesting that the deletion of LHPP expression may be a common molecular event in the occurrence and development of malignant tumors, and its down-regulated expression may serve as a potential molecular marker for the early diagnosis of ESCC. Survival analysis results based on TCGA database further confirmed that low LHPP expression is an independent risk factor for the poor prognosis of ESCC patients, and patients with low LHPP expression have a significantly shortened median survival time. LHPP has a certain predictive value for the 1-year, 2-year and 3-year overall survival rates of ESCC patients, and the AUC value increases with the extension of time, indicating that the expression level of LHPP has a higher predictive value for the long-term prognosis of ESCC patients and can serve as an effective molecular indicator for the clinical evaluation of the prognosis of ESCC patients.

To explore the molecular mechanism of LHPP regulating the occurrence and development of ESCC, this study screened the differentially expressed genes (DEGs) between LHPP high and low expression groups and performed functional enrichment analysis. The results showed that the up-regulated DEGs in the LHPP low expression group were significantly enriched in tumor-promoting signaling pathways such as Wnt and TNF, as well as biological functions related to immune disorder, inflammatory response activation and cell invasion/metastasis [15, 16]. These signaling pathways are the core regulatory pathways of the malignant biological behavior of ESCC cells, which can promote the proliferation, invasion and metastasis of ESCC cells by regulating the expression of downstream target genes, and remodel the tumor microenvironment to promote the malignant progression of ESCC. In contrast, the DEGs in the LHPP high expression group were mainly enriched in normal physiological processes such as cell junction, epidermal development and tissue homeostasis regulation, without enrichment of classic tumor-promoting or metastasis-promoting signaling pathways. This suggests that the deletion of LHPP expression can activate the tumor-promoting molecular network and abnormal signaling pathways in ESCC cells, while the high expression of LHPP can maintain the normal molecular regulatory mode of esophageal tissue cells, inhibit the activation of tumor-associated signaling pathways, and thus exert a tumor suppressive effect. Meanwhile, the DEGs such as IGFBP5, MMP7 and CDKN2B screened in this study are all involved in cell proliferation, extracellular matrix degradation and immune regulation [17-19], and their expression changes may be important molecular targets for LHPP to regulate the malignant progression of ESCC, which provides a key research direction for further analyzing the downstream regulatory network of LHPP in the follow-up study.

The remodeling of the tumor immune microenvironment is an important factor affecting the occurrence, development and prognosis of malignant tumors, and the imbalance of immune cell infiltration and the abnormal expression of immune checkpoint genes are the key characteristics of the tumor immunosuppressive microenvironment. This study analyzed the characteristics of immune cell infiltration and immune checkpoint gene expression in LHPP high and low expression groups of ESCC, and found that the LHPP low expression group had high infiltration of pro-tumor immune cells such as macrophages and B cells, while the infiltration abundance of endothelial cells which maintain vascular structural integrity and inhibit tumor angiogenesis was significantly reduced. This suggests that the deletion of LHPP expression can lead to the transformation of the ESCC immune microenvironment to a pro-tumor direction, and accelerate the malignant progression of ESCC by promoting the recruitment of pro-tumor immune cells, activating the inflammatory response and destroying the structural integrity of tumor tissues. In contrast, the immune microenvironment of the LHPP high expression group is relatively stable without obvious abnormal enrichment of pro-tumor immune cells. Further immune checkpoint gene expression analysis showed that the core immune checkpoint genes such as SIGLEC15, CTLA4, PDCD1 and TIGIT were extremely significantly up-regulated in the LHPP low expression group. The abnormal activation of these immune checkpoint genes can bind to their corresponding ligands, inhibit the activation and proliferation of T lymphocytes, induce the immune escape of tumor cells, and further shape

the tumor immunosuppressive microenvironment. However, the expression level of immune checkpoint genes in the LHPP high expression group is generally low without obvious activation of immunosuppressive signaling pathways. This indicates that LHPP can maintain the body's anti-tumor immune function by regulating the expression of immune checkpoint genes and the infiltration balance of immune cells, thereby inhibiting the immune escape process of ESCC cells and exerting a tumor suppressive effect.

Chemotherapy is an important means for the clinical comprehensive treatment of ESCC, especially for middle and advanced ESCC patients and patients with postoperative recurrence and metastasis [20]. However, the emergence of chemoresistance of tumor cells is the main cause of chemotherapy failure and poor prognosis of patients. This study found through chemotherapeutic drug sensitivity analysis based on GDSC database that the LHPP high expression group had significantly higher sensitivity to a variety of clinical common chemotherapeutic drugs including paclitaxel, cisplatin and gemcitabine than the low expression group, indicating that the expression level of LHPP is closely associated with the chemotherapeutic sensitivity of ESCC cells. The high expression of LHPP can significantly improve the response efficiency of ESCC cells to chemotherapeutic drugs, while the deletion of LHPP expression may be an important molecular mechanism of chemoresistance in ESCC. This result provides a new idea for the optimization of clinical chemotherapy regimens for ESCC, and the individualized selection of chemotherapeutic drugs can be realized by detecting the expression level of LHPP in ESCC patients, which is conducive to improving the effect of chemotherapy and the prognostic level of patients.

This study still has certain limitations that need to be improved in the follow-up research. First, the sample size of clinical specimens in this study is relatively small, and it is necessary to expand the sample size and carry out multi-center clinical research to further verify the expression characteristics and clinical significance of LHPP in ESCC. Second, this study only initially explored the tumor suppressive mechanism of LHPP in ESCC through bioinformatics analysis and in vitro cell experiments, and has not carried out in vivo animal experiments and molecular interaction experiments to verify its specific regulatory targets and downstream signaling pathways. Finally, this study did not conduct an in-depth analysis of the upstream regulatory mechanism of LHPP expression deletion in ESCC, such as epigenetic modification, transcription factor regulation and non-coding RNA regulation, which still needs further in-depth research in the future.

In conclusion, this study confirmed that LHPP is expressed deficiently in ESCC and acts as a tumor suppressor gene in the occurrence and development of ESCC. Low LHPP expression is closely associated with the poor prognosis of ESCC patients. LHPP exerts a significant tumor suppressive effect by inhibiting the activation of classic tumor-promoting signaling pathways, maintaining the normal tumor immune microenvironment and improving the sensitivity of tumor cells to chemotherapeutic drugs. LHPP is a potential early diagnostic marker, prognostic evaluation indicator and therapeutic target for ESCC. In the future, in-depth research can be carried out on the upstream and downstream molecular regulatory network of LHPP and its in vivo tumor suppressive effect in ESCC, and novel gene therapy and immunotherapy strategies targeting LHPP can be explored, which will provide a new direction for the precision diagnosis and treatment of ESCC.

5 CONCLUSION

This study confirms that LHPP is significantly downregulated in esophageal squamous cell carcinoma (ESCC) and functions as a tumor suppressor gene in ESCC oncogenesis and progression. Low LHPP expression correlates closely with poor prognosis in ESCC patients, with such patients exhibiting shorter median survival. Mechanistically, LHPP may modulate malignant progression by inhibiting tumor-promoting signaling pathways like Wnt/TNF, remodeling the tumor immunosuppressive microenvironment, and reducing chemotherapeutic sensitivity. LHPP has predictive value for ESCC prognosis and can serve as a potential early diagnostic marker, prognostic indicator and therapeutic target for ESCC, providing new insights for precise diagnosis and treatment of this malignancy.

COMPETING INTERESTS

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

REFERENCES

[1] Xu YF, Dang Y, Kong WB, et al. Regulation of TMEM100 expression by epigenetic modification, effects on proliferation and invasion of esophageal squamous carcinoma. World J Clin Oncol, 2024, 15(4): 554-565. DOI: 10.5306/wjco.v15.i4.554.

[2] Yuan MX, Cai QG, Zhang ZY, et al. Application of neoadjuvant chemoradiotherapy and neoadjuvant chemotherapy in curative surgery for esophageal cancer: A meta-analysis. World J Gastrointest Oncol, 2024, 16(1): 214-233. DOI: 10.4251/wjgo.v16.i1.214.

[3] Liu Y, Xia GH, Liu SS, et al. Comparing suspension laryngoscopic mucosal dissection and plasma resection for laryngeal leukoplakia: prognostic outcomes. Am J Transl Res, 2024, 16(2): 515-523. DOI: 10.62347/NXGS1826.

[4] Yang YL, Qin HW, Chen ZY, et al. Detachable string magnetically controlled capsule endoscopy for the noninvasive diagnosis of esophageal diseases: A prospective, blind clinical study. World J Gastroenterol, 2024, 30(9): 1121-1131. DOI: 10.3748/wjg.v30.i9.1121.

[5] Yu HY, Si G, Si FC. Mendelian Randomization Validates the Immune Landscape Mediated by Aggrephagy in Esophageal Squamous Cell Carcinoma Patients from the Perspectives of Multi-omics. J Cancer, 2024, 15(7): 1940-1953. DOI: 10.7150/jca.93376.
[6] Zhang X, Yu CT, Zhou SW, et al. Risk model based on genes regulating the response of tumor cells to T-cell-mediated killing in esophageal squamous cell carcinoma. Aging (Albany NY), 2024, 16(3): 2494-2516. DOI: 10.18632/aging.205495.
[7] GBD 2019 Asia and All Cancers Collaborators. Temporal patterns of cancer burden in Asia, 1990-2019: a systematic examination for the Global Burden of Disease 2019 study. Lancet Reg Health Southeast Asia, 2024, 21, 100333. DOI: 10.1016/j.lansea.2023.100333.
[8] Liu SS, Gao WZ, Lu YP, et al. As a Novel Tumor Suppressor, LHPP Promotes Apoptosis by Inhibiting the PI3K/AKT Signaling Pathway in Oral Squamous Cell Carcinoma. Int J Biol Sci, 2022, 18(2): 491-506. DOI: 10.7150/ijbs.66841.
[9] Lin D, Li LH, Chen WB, et al. LHPP expression in neurons. Mol Psychiatry, 2023, 28(2): 517. DOI: 10.1038/s41380-022-01923-x.
[10] Zhang LP, Gao WJ, Chen YB, et al. LHPP in Glutamatergic Neurons of the Ventral Hippocampus Mediates Depression-like Behavior by Dephosphorylating CaMKIIα and ERK. Biol Psychiatry, 2023, 95(5): 389-402.
[11] Stephen Rush Fuhs, Tony Hunter. pHisphorylation: the emergence of histidine phosphorylation as a reversible regulatory modification. Curr Opin Cell Biol, 2017, 45, 8-16. DOI: 10.1016/j.ceb.2016.12.010.
[12] Sravanth K H, Marco C, Stephen R F, et al. The protein histidine phosphatase LHPP is a tumour suppressor. Nature, 2018, 555(7698): 678-682. DOI: 10.1038/nature26140.
[13] Zheng JL, Dai X, Chen HL, et al. Down-regulation of LHPP in cervical cancer influences cell proliferation, metastasis and apoptosis by modulating AKT. Biochem Biophys Res Commun, 2018, 503(2): 1108-1114. DOI: 10.1016/j.bbrc.2018.06.127.
[14] Chen WJ, Chen LH, Wang J, et al. LHPP impedes energy metabolism by inducing ubiquitin-mediated degradation of PKM2 in glioblastoma. Am J Cancer Res, 2021, 11(4): 1369-1390.
[15] Mohammad Mahdi F, Shirin A, Sima a K, et al. MAML1 promotes ESCC aggressiveness through upregulation of EMT marker TWIST1. Mol Biol Rep, 2020, 47(4): 2659-2668. DOI: 10.1007/s11033-020-05356-z.
[16] Ashish K. SBS 3.1, a novel natural product small molecule regulates TNF-α-induced NF-κB activation and key signals of inflammation to promote apoptosis in lung cancer tumour microenvironment. J Biomol Struct Dyn, 2023, 42(19): 10091-10100. DOI: 10.1080/07391102.2023.2255288.
[17] Huang J, Ge SD, Zhao LL, et al. The FOSB-IGFBP5-IGF-1 axis: a novel regulatory pathway that suppresses prostate cancer growth. Cancer Gene Ther, 2026. DOI: 10.1038/s41417-026-01012-z.
[18] Chen JH, Pang HX, Wang JD, et al. Multi-scale systems toxicology defines a KLF5-centered adverse outcome pathway linking DEHP exposure to pancreatic cancer progression and signaling programs relevant to therapy tolerance. Front Cell Dev Biol, 2026, 14. DOI: 10.3389/fcell.2026.1769023.
[19] Wajeeha T, Ali Raza A, Asim Khalid M, et al. Altered expression and mutations in cell cycle genes CCND1, CDK4, CDKN2B and TP53 are associated with Pakistani breast cancer patients. Mol Biol Rep, 2026, 53(1): 439. DOI: 10.1007/s11033-026-11610-7.
[20] Zhu YR, Zhou BL, Hu XY, et al. LncRNA LINC00942 promotes chemoresistance in gastric cancer by suppressing MSI2 degradation to enhance c-Myc mRNA stability. Clin Transl Med, 2022, 12(1): e703. DOI: 10.1002/ctm2.703.