Original Article

Decreased zinc-fingers and homeoboxes family expression was associated with unfavorable outcomes and immune infiltration in lung adenocarcinoma

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Background: The zinc-fingers and homeoboxes (ZHX) family is a group of nuclear homodimeric transcriptional repressors that play an essential role in developing and progressing diverse malignancies. However, the association of ZHX family expression with prognosis and immune infiltration in lung adenocarcinoma (LUAD) is still unclear. The current study aimed to investigate the relationship between ZHX family expression and clinical outcomes and immune infiltration in LUAD patients.

Methods: ZHXs family expression was determined by using the Oncomine database and Cancer Cell Line Encyclopedia (CCLE). The impact of ZHXs family expression on prognosis was analyzed by using the Kaplan-Meier-plotter online database. The Search Tool for the Retrieval of Interacting Genes (STRING) database was utilized to construct the interaction network based on the selected differentially expressed genes associated with ZHXs. The Database for Annotation, Visualization, and Integrated Discovery (DAVID) was used to perform the enrichment of the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. The functional state of the ZHXs family in diverse types of malignancies was determined by CancerSEA. The Tumor Immune Estimation Resource (TIMER) database was used to evaluate the association of the ZHXs family with immune cell infiltrates. ZHXs family expression was validated by the Gene Expression Omnibus (GEO) database and real-time polymerase chain reaction (RT-PCR) in 10 paired tumors and normal tissues.

Results: ZHX1-3 expression level significantly decreased in LUAD compared with normal tissues. Attenuated ZHXs expression was significantly associated with unfavorable overall survival in LUAD patients. ZHX family members were positively associated with immune infiltration of monocytes, tumor-associated macrophages (TAMs), M1 and M2 macrophages in LUAD. ZHX family expression was also significantly related to a variety of immune marker sets in LUAD. GEO analysis and RT-PCR validated the significant decrease of ZHXs expression level in LUAD.

Conclusions: The current study revealed that ZHX family expression was significantly correlated with unfavorable outcomes and immune infiltration in LUAD. The findings herein provide a promising basis for further study into the potential biological function of the ZHX family in LUAD and lay a foundation for developing therapeutic targets for LUAD patients.

Keywords: Zinc-fingers and homeoboxes family (ZHX family); prognosis; immune infiltration; lung adenocarcinoma (LUAD)

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Introduction

Lung cancer is the world's second most often diagnosed cancer and the primary cause of cancer-related death, accounting for approximately 2.2 million new cases and 1.8 million deaths in 2020 (1). Non-small-cell lung cancer (NSCLC) accounts for 80% of all cases (2). Lung adenocarcinoma (LUAD) is the most common histological type of NSCLC and accounts for approximately 40% of all diagnosed lung cancers (3). The vast majority of patients (approximately 75%) are diagnosed at an advanced stage (stage III/IV) with a poor prognosis (4). Despite considerable advances in surgery, radiotherapy, chemotherapy, target therapy, and immunotherapy over the previous few decades, the long-term prognosis of lung cancer remains dismal, with a 5-year survival rate of only 15% (5). Over the last decade, many prognostic markers have been suggested for these patients, but only very few have been proven to be clinically relevant (6). Thus, a simple and effective prognostic marker is in urgent need for clinicians to better predict the prognosis of LUAD patients so that individualized therapy can be implemented as soon as possible.

The zinc-fingers and homeoboxes (ZHX) family is a group of nuclear homodimeric transcriptional repressors, which contain two zinc-finger motifs and five homeobox DNA-binding domains, including ZHX1, ZHX2, and ZHX3 (7). ZHX family members play a crucial role in neural progenitor maintenance as well as hematopoietic cell development and differentiation (7). Dysfunction of ZHX family members is closely associated with the development and progression of diverse types of cancers, including gastric cancer (8), hepatocellular carcinoma (HCC) (9), renal cell carcinoma (10), and breast cancer (11). Previous research has established that the abnormal expression of ZHX family members is a significant predictor of clinical outcomes in several cancers (8,11,12). The abovementioned studies demonstrated that the ZHX family might serve an essential role in cancer initiation and progression, which gives the reason for the ZHX members as biomarker candidates that may be utilized for cancer diagnosis, survival prediction, and therapeutic surveillance (11). However, the prognostic significance and biological function of ZHX family members expression in LUAD patients remain unclear. Furthermore, although epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) and immunotherapy have dramatically improved the outcome of LUAD, the impact of ZHX family members expression on EGFR pathway and immune infiltration remains largely unexamined.

In the current study, the ZHX family members' expression and its association with the clinical outcomes of LUAD patients were investigated. Moreover, the relationship of ZHX family members with immune infiltration was explored. We present this article in accordance with the REMARK reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-2793/rc).

Highlight box

Key findings

- ZHXs were associated with unfavorable OS and immune infiltration in LUAD.

What is known and what is new?

- ZHXs are significant predictors of clinical outcomes in several cancers.
- The association of ZHXs with OS and immune infiltration in LUAD was firstly discovered in this study.

What is the implication, and what should change now?

- The findings herein lay a foundation for developing therapeutic targets for LUAD patients.

Methods

Validation of ZHXs family expression by Oncomine analysis

The mRNA expression levels of ZHXs in various cancer types were analyzed by using Oncomine gene expression array datasets (www.oncomine.org), which is an online

microarray database including 19 cancer types, 715 datasets, and 86,733 samples (13). When ZHX mRNA levels in cancer tissues were compared to those in normal tissues, a fold-change of at least two and a probability of P≤0.01 was defined as clinically significant.

Evaluation of ZHXs family mRNA levels by Cancer Cell Line Encyclopedia (CCLE) database analysis

The CCLE (https://portals.broadinstitute.org/ccle) provides public access to genomic data, analysis, and visualization for 1,457 cell lines representing 37 distinct cancer types (14). There are several datasets and data identifiers for each gene. The five major dataset types, including mRNA expression (RNAseq), mRNA expression (Affy), copy number, reduced representation bisulfite sequencing (RRBS), and reverse phase protein array (RPPA) CCLE analysis, were performed to explore the mRNA levels of the ZHXs family in a number of cancer cell lines. To determine whether and/or to what extent ZHXs family were expressed in lung cancer cell lines, the gene expression data were analyzed from human cancer cell lines.

Prognostic value assessment of ZHXs family expression

The Kaplan-Meier-plotter online database (http://kmplot.com/analysis/) was used to evaluate the prognostic significance of the mRNA expression levels of the ZHX family. This database contains survival information and gene expression of lung cancer patients (15). The prognostic value of the gene expression levels of the ZHX family in lung cancer was examined by Kaplan-Meier-plotter survival analysis. Based on the median expression levels for the ZHXs family, individual subjects in the study were divided into either the high- or low-risk subgroups. Kaplan-Meier survival plot was used to analyze the overall survival (OS) of lung cancer patients. The hazard ratio (HR) with a 95% confidence interval (CI) was estimated. P values were two-sided, and a value <0.05 was considered statistically significant.

Functional analysis of ZHXs

To identify enriched functional categories of ZHXs and related genes, the online database Search Tool for the Retrieval of Interacting Genes (STRING) (https://string-db.org/) was used to explore the functional relationships for the top 100 genes that most relevant with ZHXs. The Database for Annotation, Visualization, and Integrated Discovery (DAVID) (https://david.ncifcrf.gov) was used to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. ZHXs and related genes were uploaded to generate the function charts. The groups having a P value less than 0.05 and more than two gene counts were studied.

CancerSEA analysis

CancerSEA (http://biocc.hrbmu.edu.cn/CancerSEA/) was used to examine the functional status of the ZHX family in several cancer types. CancerSEA is the first comprehensive database devoted to understanding the distinct functional states of cancer cells at the single-cell level (16). CancerSEA depicts a cancer single-cell functional state atlas, with 41,900 cancer single cells from 25 cancer types covering 14 functional states (including inflammation, DNA repair, DNA damage, differentiation, cell cycle, apoptosis, angiogenesis, epithelial-mesenchymal transition (EMT), proliferation, metastasis, invasion, stemness, hypoxia, and quiescence) (16). To filter correlations between the gene of interest and functional state in distinct single-cell datasets, a correlation strength >0.3 and a false discovery rate (FDR) (Benjamini & Hochberg) of 0.05 were used.

Tumor Immune Estimation Resource (TIMER) database analysis

To unveil the molecular characterization of tumor-immune interactions across different cancer types, the TIMER database (http://cistrome.org/TIMER/) was used (17). TIMER uses a deconvolution statistical approach to estimate the abundance of six tumor-filtered immune cells from The Cancer Genome Atlas (TCGA), including B cells, $CD8^+$ T cells, $CD4^+$ T cells, $CD8^+$ T cells, macrophages, neutrophils, and dendritic cells (DCs). ZHXs family expression in different types of cancer and the relationship between ZHXs family expression and the abundance of immune infiltrates were examined by using the gene module. We further analyzed the association of ZHXs family expression with gene markers of tumor infiltrates, including T-helper 1 (Th1) cells, T-helper 2 (Th2) cells, T-helper 17 (Th17) cells, follicular helper T (Tfh) cells, T cells (general), $CD8^+$ T cells, B cells, M1 macrophages, M2 macrophages, monocytes, tumor-associated macrophages (TAMs), neutrophils, natural killer (NK) cells, DCs, Tregs and exhausted T cells, as reported in previous studies (18,19). The log2 RNA-Seq by Expectation Maximization (RSEM)

was used to display the gene expression level.

Verification of ZHXs family expression in Gene Expression Omnibus (GEO) database

All the gene expression datasets were downloaded from GEO of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/sites/GDSbrowser). In the current study, ZHX family members' mRNA expression levels in LUAD patients were examined by using GSE19188, GSE31210, and GSE75037 datasets. The dataset was selected according to the following criteria: (I) for GEO, the query ('expression profiling by array') AND ('expression profiling by throughput sequencing') AND 'homo sapiens' [organism] was used to return a list of all potential datasets to analyze; (II) the number of samples in a single group of was ≥20; (III) the number of samples >100 included normal, and lung cancer tissues; The GSE19188 dataset included 65 freshly frozen adjacent non-cancerous samples and 45 LUAD tissues samples. The GSE31210 dataset included 20 normal lungs samples and 226 lung cancers. The GSE75037 dataset included 83 normal samples and 83 lung cancer. Unpaired *t*-test was used to analyze the expression levels of ZHXs family.

Quantitative real-time polymerase chain reaction (qRT-PCR)

To validate the expression of ZHXs family in tumor and normal tissue, the liquid nitrogen frozen specimens of 10 pairs of LUAD and normal tissues were obtained from the Chongqing University Cancer Hospital. All pathological specimens were retrieved and reviewed by two independent pathologists in Department of Clinical Pathology, Chongqing University Cancer Hospital. Total RNA isolation was achieved by using TRIzol Reagent (Invitrogen, CA, USA) according to the manufacturer's instructions. Reverse transcription (RT)-PCR was performed to generate cDNA in a total volume of 20 mL. RT-PCR was performed using Go-Taq (Promega, Madison, WI, USA) with the following conditions: initial denaturation at 95 ℃ for 2 min and 32 amplification cycles (denaturation: 95 ℃ for 30 s; annealing: 55 ℃ for 30 s; and extension: 72 ℃ for 30 s), with a final extension at 72 ℃ for 3 min. Beta-actin was used as an internal control with only 23 cycles for amplification. qRT-PCR was accomplished with a SYBR Green kit (Takara Bio Inc., Japan) and a LightCycler 480 System (Roche, Basel, Switzerland). The following primers were used for amplification: ZHX1 (for. 5'-GCAGGCGAAAATCAACAACAC-3', rev. 5'-TAAGCACAGGAGGACCTTCAT-3'); ZHX2 (for. 5'-AAAGTACGACTCCCTATCCGAC-3', rev. 5'-GGTTGGTGGTTTCGATGGACT-3'); ZHX3 (for. 5'-CTGTGGTGACCAAGTATCCAGA-3', rev. 5'-TTTTTCCGGGCATCCTCAATC-3'); β-actin (for. 5'-GTCTTCCCCTCCATCGTG-3', rev. 5'-AGGGTGAGGATGCCTCTCTT-3'). P values for each group were determined by Student's *t*-test. Each sample was assessed in three independent experiments. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Medical Ethics Committee of the Chongqing University Cancer Hospital (No. CZLS2022030-A). Written informed consent was obtained from all subjects.

Statistical analyses

Statistical analyses were performed using SPSS 17.0 statistical software package (SPSS Inc., Chicago, IL, USA). The statistical significance of differences in numerical data was calculated using Student's *t*-test. OS was defined as the time from diagnosis to the date of death or last known follow-up. Survival curves were generated using the Kaplan-Meier method with a log-rank test. P<0.05 (two-tailed) was considered statistically significant.

Results

Expression of the ZHX family in lung cancer

Transcriptional expression differences of ZHXs between cancer and normal tissues were analyzed in multiple cancer types by using the Oncomine database; 308, 434, and 416 unique analyses for ZHX1, ZHX2, and ZHX3 were included, and the results are shown in *Figure 1A*. ZHX1 mRNA levels were shown to be lower in cancer tissues than in normal tissues in three investigations, whereas two others found that ZHX1 expression was higher in cancer tissues. According to 22 researches, the quantities of downregulation and overexpression of ZHX2 are comparable. ZHX3 express decreased significantly in cancer tissues compared with normal tissues in seven different studies, while it was found to be higher in three datasets. Only three studies found reduced ZHX3 expression in lung cancer patients.

To further explore the expression of the ZHX family

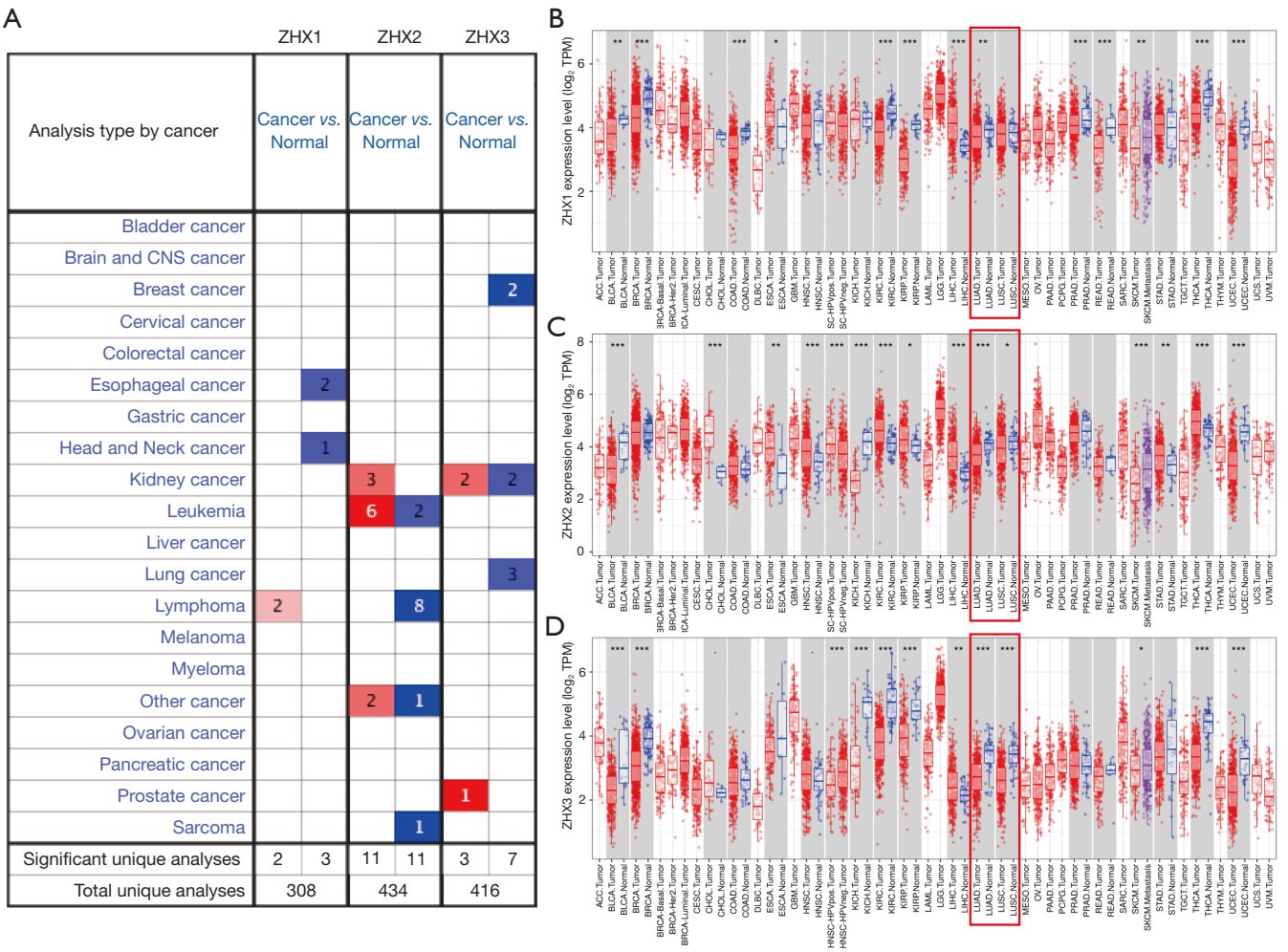

Figure 1 ZHX family expression levels in various types of human cancers. (A) Different levels of ZHX family members' expression in datasets of various malignancies in the Oncomine database. (B-D) Expression levels of ZHX family members in various malignancies analyzed by TIMER (*, P<0.05; **, P<0.01; ***, P<0.001). ZHX, zinc-fingers and homeoboxes; TPM, transcripts per million; TIMER, tumor immune Estimation Resource.

in lung cancer tissues, ZHX family expression was determined by using the RNA-seq data of lung cancers in TCGA. Compared with normal tissues, expression profile differences of ZHXs were observed in lung cancer tissues based on the TCGA database. Expression of ZHX1 was shown to be lower in LUAD than in normal tissues. In contrast, the difference between lung squamous cell carcinoma (LUSC) and normal tissues did not achieve statistical significance (*Figure 1B*). ZHX2 and ZHX3 expressions were significantly downregulated in LUAD and LUSC (*Figure 1C,1D*).

The mRNA expressions of ZHXs were examined by using the Oncomine. According to the TCGA Lung 2 and Weiss Lung datasets, ZHX1 expression significantly decreased in LUSC and LUAD tissues compared with normal tissues samples. The same results were obtained about ZHX2-3 expression differences between LUSC, LUAD, and normal tissues (*Table 1*).

Prognostic significance of ZHXs in lung cancer

By Kaplan-Meier-plotter survival analysis, the relationship between ZHXs expression and prognosis in lung cancer was examined. The attenuated mRNA expression levels of ZHXs were significantly associated with poor prognosis in LUAD. In LUAD patients, higher ZHX1 expression was

Table 1 Datasets of *ZHX* family members in lung cancer (Oncomine database)

Gene	Dataset	Cases [number]		P	*t*-test	Fold change
		Normal	Tumor			
ZHX1	TCGA Lung 2	Lung [390]	Squamous cell lung carcinoma [348]	1.47E-53	18.397	1.176
		Lung [390]	Lung adenocarcinoma, mixed subtype [67]	9.35E-10	6.965	1.146
		Lung [390]	Lung adenocarcinoma [261]	1.02E-31	13.378	1.172
	Weiss Lung	Lung [59]	Squamous cell lung carcinoma [155]	4.72E-23	11.312	1.137
		Lung [59]	Lung adenocarcinoma [77]	2.97E-9	6.509	1.133
ZHX2	TCGA Lung 2	Lung [390]	Squamous cell lung carcinoma [348]	9.96E-54	18.438	1.177
		Lung [390]	Lung adenocarcinoma, mixed subtype [67]	1.07E-9	6.932	1.160
		Lung [390]	Lung adenocarcinoma [261]	1.96E-34	14.159	1.163
	Weiss Lung	Lung [59]	Squamous cell lung carcinoma [155]	1.27E-24	11.843	1.141
		Lung [59]	Lung adenocarcinoma [77]	1.46E-11	7.664	1.122
ZHX3	TCGA Lung 2	Lung [390]	Squamous cell lung carcinoma [348]	4.52E-25	11.116	1.092
		Lung [390]	Lung adenocarcinoma, mixed subtype [67]	0.195	0.864	1.017
		Lung [390]	Lung adenocarcinoma [261]	5.64E-8	5.457	1.048
		Lung [390]	Acinar lung adenocarcinoma [6]	0.442	0.152	1.008
	Weiss Lung	Lung [59]	Squamous cell lung carcinoma [155]	8.46E-6	4.416	1.043
		Lung [59]	Lung adenocarcinoma [77]	5.68E-5	4.028	1.040
	Hou Lung	Lung [65]	Squamous cell lung carcinoma [27]	0.007	2.552	1.098

ZHX, zinc-fingers and homeoboxes; TCGA, The Cancer Genome Atlas.

associated to a better OS (HR =0.48, 95% CI: 0.37–0.62, P=1.1e-08) (*Figure 2A*). Similarly, elevated ZHX2 and ZHX3 mRNA levels predicted a longer OS of LUAD patients (HR =0.42, 95% CI: 0.33–0.54, P=1.3e-12; HR =0.76, 95% CI: 0.6–0.97, P=0.025) (*Figure 2B*,*2C*). However, no significant difference was observed between ZHXs mRNA levels and OS in the LUSC patients (*Figure 2D-2F*). In LUAD patients, the lower expression of ZHX2 and ZHX3 was associated with poor progression free survival (PFS). The LUAD patients with lower expression of ZHX1 showed a shorter PFS, but the difference did not achieve a statistical significance (Figure S1A-S1C). In LUSC patients, no significant difference in PFS was observed between different expression level of ZHXs mRNA in the LUSC patients (Figure S1D-S1F).

Functional enrichment and pathway analysis of ZHXs

STRING database was used to construct the protein-protein interaction network between ZHXs and the related genes. As shown in *Figure 3A*, ZHXs play the role of the central hub. Biological processes (BP) of GO analysis showed that the most frequent assignment was "signal transduction". The analysis of cell components (CC) showed that they were highly enriched in the nucleus. GO molecular function (MF) analysis showed that the most significantly enriched GO-term was "protein binding" (*Figure 3B*). We found that the relevant genes were highly clustered in the Erb-b2 receptor tyrosine kinases (ERBB) signaling pathway, EGFR tyrosine kinase inhibitor resistance, endometrial cancer, colorectal cancer, and other cancers using the KEGG database (*Figure 3C*).

Functional state of ZHX family in multiple cancer types

To further understand the relevance and underlying mechanisms of ZHX expressions in cancer, we determined the functional state of ZHX factors in several cancer types using the CancerSEA database. As shown in *Figure 4A*, ZHX1 was found to have a favorable relationship with

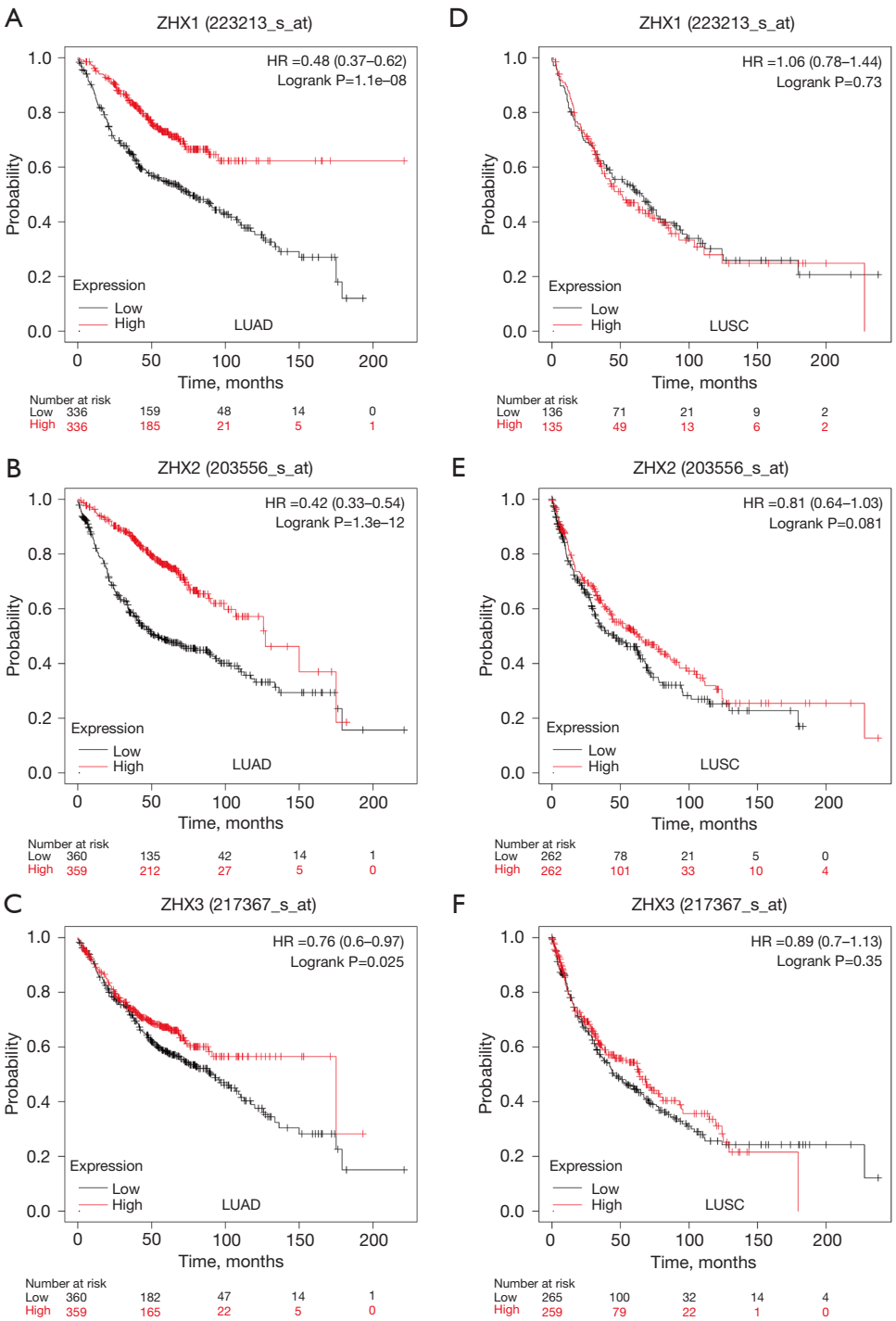

Figure 2 Overall survival analysis in Kaplan-Meier plotter database for mRNA levels of ZHX factors in LUAD patients. (A-C) The overall survival curves of ZHX1, ZHX2 and ZHX3 in LUAD patients, respectively. (D-F) The overall survival curves of ZHX1, ZHX2, and ZHX3 in LUSC patients, respectively. ZHX, zinc-fingers and homeoboxes; HR, hazard ratio; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma.

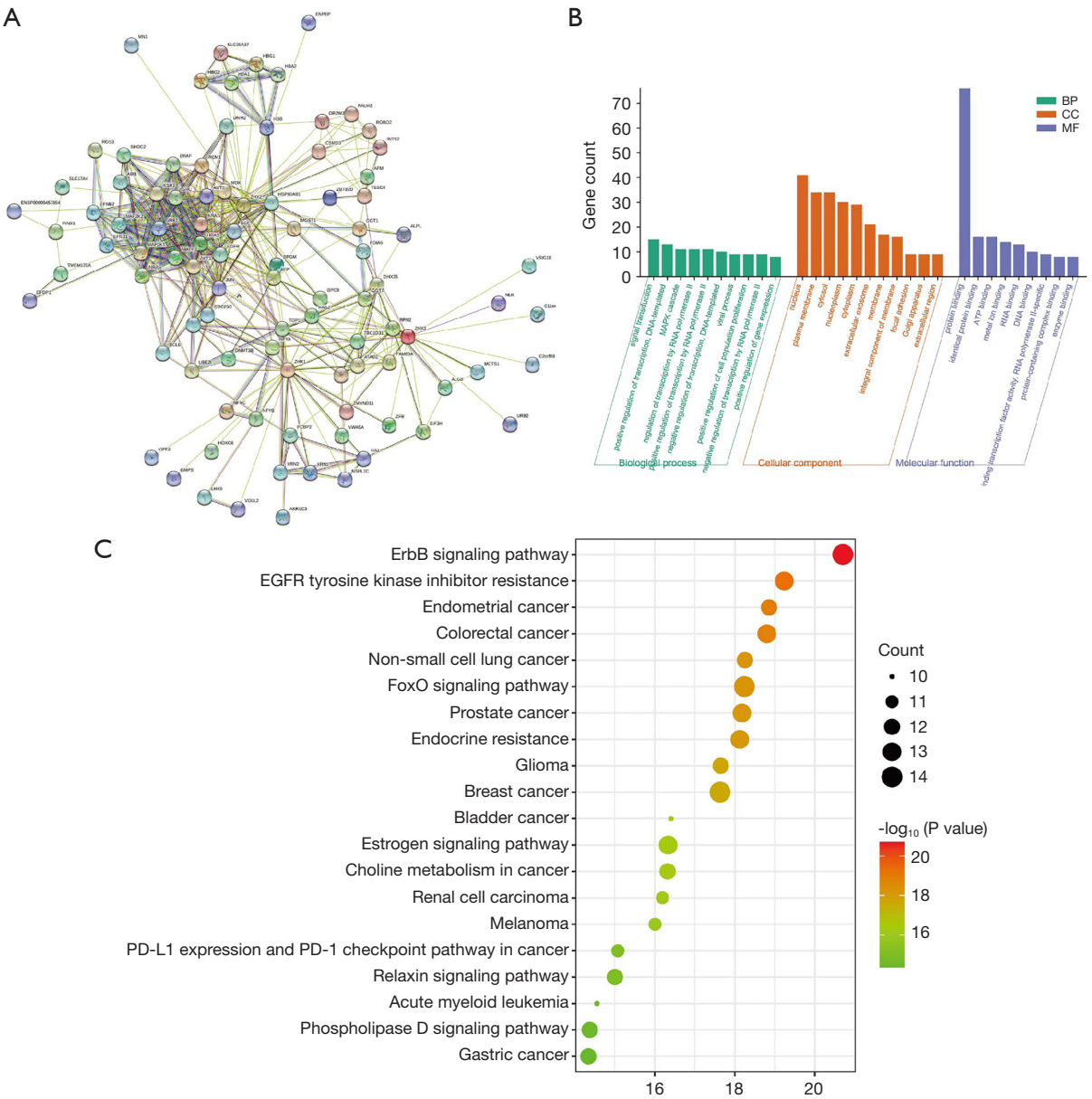

Figure 3 GO annotation and KEGG pathways enrichment analysis for ZHX family members and relevant genes. (A) The interaction network among the selected DEGs that are most relevant to ZHXs was analyzed by the STRING database. (B) BP, CC, and MF were the three main categories. The y-axis represents the enrichment score in each category, and the x-axis represents different GO terms. (C) Scatter plot of enriched KEGG pathways statistics. The top 10 KEGG enriched pathways are shown in the figure. ZHX, zinc-fingers and homeoboxes; BP, biological process; CC, cellular component; MF, molecular function; ATP, adenosine triphosphate; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differentially expressed genes; STRING, Search Tool for the Retrieval of Interacting Genes.

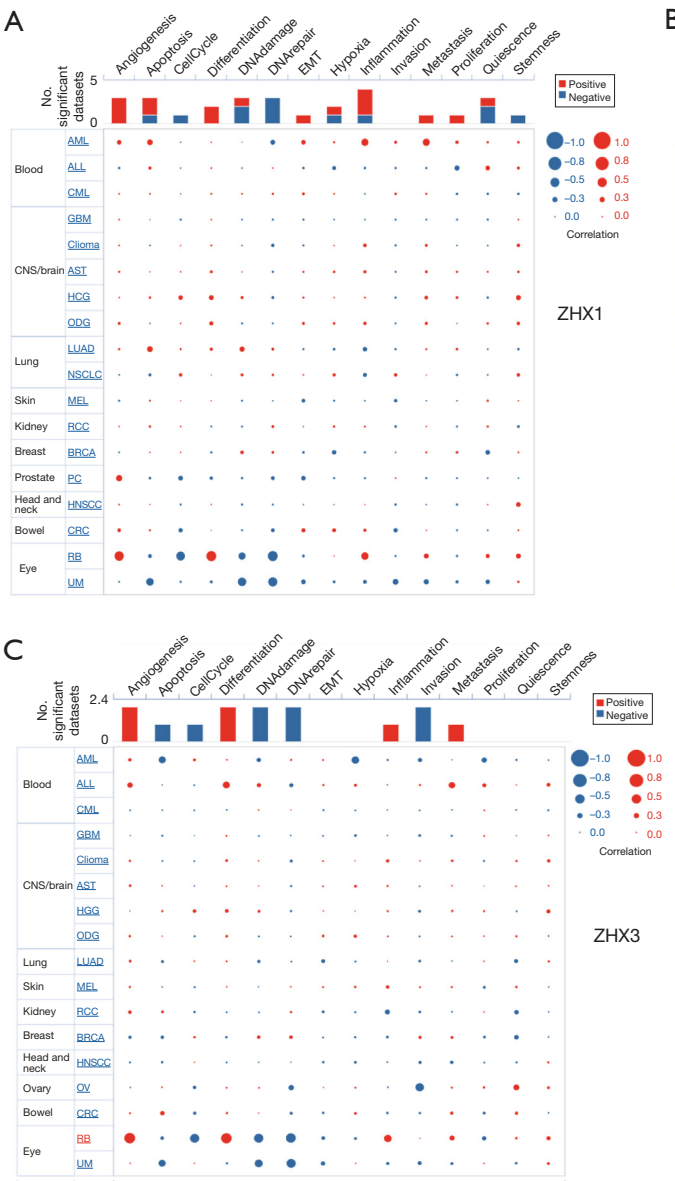

Figure 4 The functional state of ZHX family in fifteen types of cancer. (A-C) Represented the functional state of ZHX1, ZHX2, and ZHX3, respectively. The blue plots represent that ZHX family member expression was negatively correlated with the functional state, while the red plots represent that ZHX family member expression was positively correlated with the functional state determined by CancerSEA. EMT, epithelial-mesenchymal transition; CNS, central nervous system; AML, acute myeloid leukemia; ALL, acute lymphocytic leukemia; CML, chronic myelogenous leukemia; GBM, glioblastoma; AST, astrocytoma; HGG, high grade glioma; ODG, oligodendroglioma; LUAD, lung adenocarcinoma; NSCLC, non-small-cell lung cancer; MEL, melanoma; RCC, renal cell carcinoma; BRCA, breast cancer; PC, prostate cancer; HNSCC, head and neck squamous cell carcinoma; CRC, colorectal cancer; RB, retinoblastoma; UM, uveal melanoma; OV, ovarian cancer; ZHX, zinc-fingers and homeoboxes.

stemness (ρ=0.277, P=0.001) and inflammation (ρ=0.547, P=0.031) in acute myeloid leukemia (AML), EMT (ρ=0.101, P=0.003) and invasion (ρ=0.094, P=0.005) in chronic myelogenous leukemia (CML), apoptosis in breast cancer (BRCA) (ρ=0.135, P=0.028) and LUAD (ρ=0.631, P=0.012), EMT (ρ=0.060, P=0.002) in astrocytoma (AST), metastasis in high grade glioma (HGG) (ρ=0.164, P=0.001) and LUAD (ρ=0.2, P=0.025), DNA damage (ρ=0.115, P=0.004) in NSCLC, angiogenesis (ρ=0.32, P=0.019) in prostate cancer (PC), concurrently negatively correlated with proliferation (ρ=–0.25, P=0.018) in acute lymphocytic leukemia (ALL), quiescence (ρ=–0.354, P=0.023) in AML, DNA repair (ρ=–0.102, P=0.009) in glioma. We then examined ZHX2 functional state, and found that ZHX2 was positively correlated with, invasion (ρ=0.208, P=0.031) in ALL, EMT (ρ=0.048, P=0.019) in oligodendroglioma (ODG), angiogenesis (ρ=0.368, P=0.001) in renal cell carcinoma (RCC), metastasis (ρ=0.146, P=0.038) in melanoma (MEL), negatively correlated with DNA repair in ALL (ρ=–0.212, P=0.031) and AST (ρ=–0.055, P=0.001) (*Figure 4B*). ZHX3 was positively correlated with angiogenesis (ρ=0.268, P=0.044), differentiation (ρ=0.357, P=0.006) and metastasis (ρ=0.326, P=0.013) in ALL (*Figure 4C*).

Association between ZHXs expressions and immune infiltration levels in lung cancer

Further analysis revealed that there were statistically significant correlations between ZHXs expressions and immune infiltration levels in LUAD by TIMER. We found that ZHX1 expression was positively correlated with $CD8^+$ T cells (r=0.247, P=3.31E–08), $CD4^+$ T cells (r=0.099, P=2.97E–02), macrophages (r=0.204, P=6.05E–06), neutrophils (r=0.214, P=2.14E–06) and DCs (r=0.2, P=8.08E–06) immune infiltrates in LUAD. However, no obvious significant correlation was observed between ZHX1 expression and tumor purity or infiltrating levels of B cells (*Figure 5A*). The expression levels of ZHX2 was negatively related to tumor purity (r=–0.089, P=4.85E–02), positively to immune infiltration levels of B cells (r=0.215, P=1.85E–06), $CD8^+$ T cells (r=0.225, P=5.54E–07), $CD4^+$ T cells (r=0.373, P=1.87E–17), macrophages (r=0.236, P=1.45E–07), neutrophils (r=0.329, P=1.13E–13) and DCs (r=0.356, P=4.56E–16) in LUAD (*Figure 5B*). ZHX3 expression levels showed significant relationship with infiltrating levels of $CD4^+$ T cells (r =0.209, P=3.44E–06), macrophages (r=0.217, P=1.48E–06), neutrophils (r=0.223, P=7.44E–07) and DCs (r=0.225, P=4.92E–07) in LUAD (*Figure 5C*). This analysis indicates that ZHXs expressions are strongly related to immune infiltration in LUAD.

Correlation analysis between ZXH1-3 and markers of immune cells

We analyzed the relationship between ZXH1-3 expression and markers of immune cells, such as $CD8^+$ T cells, cells (general), B cells, monocytes, TAMs, M1 and M2 macrophages, neutrophils, NK cells, DCs, Th1 cells, Th2 cells, Th17 cells, Tfh cells and Tregs in LUAD, using the TIMER online database (*Table 2*). The analysis revealed a significant association between ZHX family members and monocytes, TAMs, and M1 and M2 macrophages in LUAD. We discovered that the expressions of ZHXs were highly correlated with CD86, CSF1R of monocytes, CCL2, CD68, interleukin (IL)-10 of TAMs, IRF5 of M1 macrophages, CD163, VSIG4, and MS4A4A of M2 phenotype in LUAD. Investigation was further conducted to verify the correlation between expression levels of ZHX1-3 in LUAD patients and gene markers of monocytes, TAMs, M1, and M2 macrophages by Gene Expression Profiling Interactive Analysis (GEPIA), compared to normal tissues (*Table 3*). We found that ZXH1-3 were significantly correlated with CD86, CSF1R of monocytes, and CD163 of M2 phenotype in LUAD. ZXH2 and ZXH3 showed a close relationship with CCL2 and CD68 of TAMs in tumor tissues. These analyses revealed that ZHX family members serve a critical role in macrophage function regulation and tumor formation.

Validation of expressions of ZHXs by GEO database and qRT-PCR

We examined ZHX family members' mRNA expression in LUAD patients by using GSE19188, GSE31210, and GSE75037 datasets. Three hundred and fifty-four tumor tissues of LUAD and 168 normal controls were enrolled in the analysis. Compared to normal tissues, expression levels of ZHXs were decreased significantly in LUAD patients (*Figure 6A-6G*), which might be utilized as a LUAD prognostic and immune cell infiltration marker. To further validate the expression of the ZHXs family in LUAD, ten pairs of liquid nitrogen frozen tumors and normal tissues were obtained and analyzed by qRT-PCR. The results showed that the ZHXs expression levels in LUAD were significantly lower than that in normal tissues (ZHX1, P=0.006; ZHX2, P<0.001; ZHX3, P=0.005) (*Figure 7A-7C*).

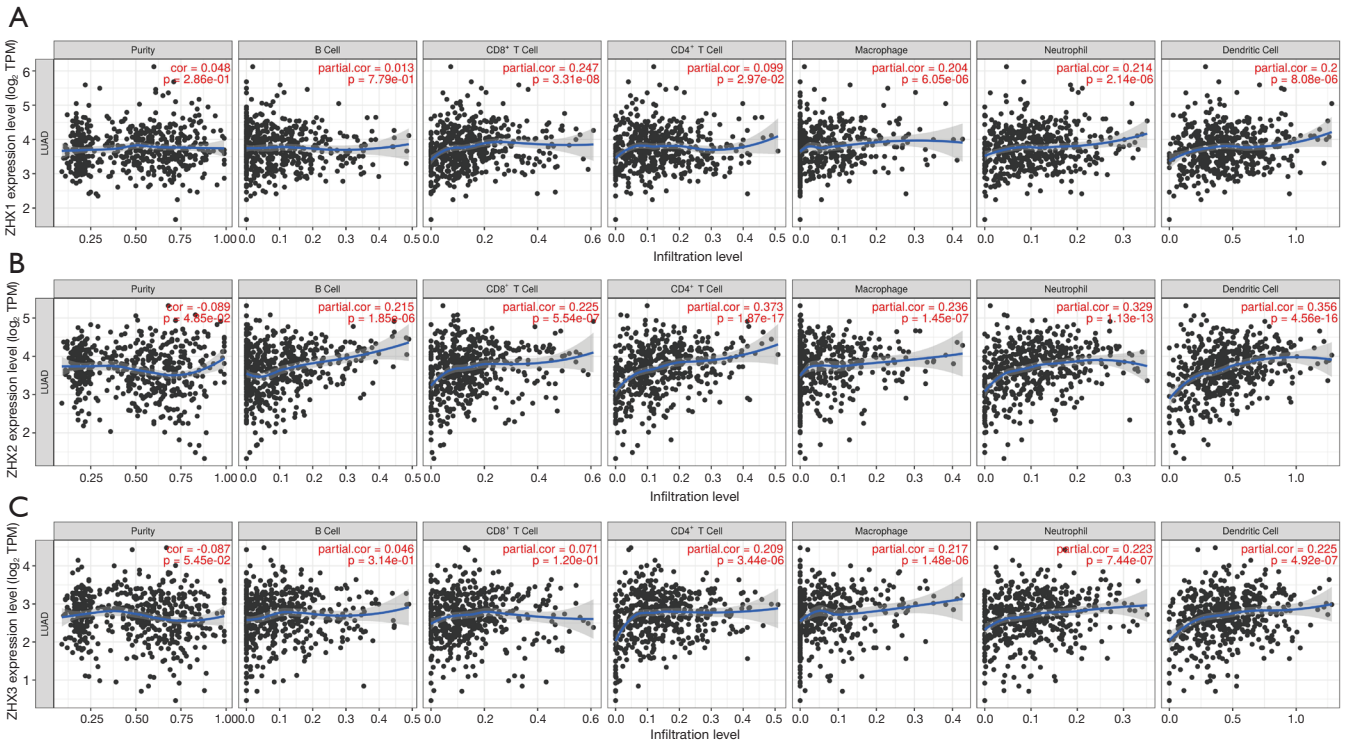

Figure 5 Correlation of expression of ZHX family member with immune infiltration levels in LUAD. (A) Expression of ZHX1 showed a significant positive correlation with infiltrating levels of dendritic cells, CD4$^+$ T cells, CD8$^+$ T cell, macrophages, and neutrophils; no significant correlations with tumor purity and infiltrating levels of B cell. (B) ZHX2 was significantly associated with infiltrating levels of dendritic cells, neutrophils, CD4$^+$ T cells, CD8$^+$ T cell, B cell and macrophages. (C) ZHX3 expression was significantly corelated with infiltrating levels of dendritic cells, neutrophils, macrophages and CD4$^+$ T cell; no significant correlation was observed with tumor purity and infiltrating levels of CD8$^+$ T cell and B cell. Cor, correlation; ZHX, zinc-fingers and homeoboxes; TPM, transcripts per million; LUAD, lung adenocarcinoma.

Table 2 Correlation analysis between ZXH1-3 and relate genes and markers of immune cells in TIMER

Description	Gene markers	LUAD (ZHX1/ZHX2/ZHX3)			
		None		Purity	
		Cor	P	Cor	P
CD8$^+$ T cell	*CD8A*	0.136/0.151/–0.014	**/***/0.75	0.177/0.118/–0.065	***/**/0.15
	CD8B	0.045/0.037/–0.1	0.305/0.401/*	0.065/0.001/–0.155	0.149/0.975/***
T cell (general)	*CD3D*	0.019/0.146/–0.06	0.664/***/0.175	0.05/0.104/–0.124	0.267/*/**
	CD3E	0.066/0.276/0.028	0.132/***/0.522	0.109/0.263/–0.019	*/***/0.671
B cell	*CD19*	–0.104/0.179/0.013	*/***/0.773	–0.084/0.148/–0.029	0.0624/***/0.517
	CD79A	–0.071/0.19/0.03	0.107/***/0.498	–0.046/0.166/–0.004	0.308/***/0.931
Monocyte	*CD86*	0.174/0.228/0.198	***/***/***	0.261/0.205/0.18	***/***/***
	CSF1R	0.22/0.317/0.321	***/***/***	0.25/0.304/0.319	***/***/***

Table 2 (*continued*)

Table 2 (*continued*)

Description	Gene markers	LUAD (ZHX1/ZHX2/ZHX3)			
		None		Purity	
		Cor	P	Cor	P
TAM	*CCL2*	0.104/0.166/0.175	*/***/***	0.123/0.142/0.162	**/**/***
	CD68	0.099/0.256/0.167	*/***/***	0.125/0.243/0.155	**/***/***
	IL10	0.111/0.209/0.146	*/***/***	0.14/0.185/0.118	**/***/**
M1 macrophage	*NOS2*	0.121/0.078/0.172	**/0.0769/***	0.131/0.062/0.152	**/0.172/***
	IRF5	0.124/0.14/0.147	**/**/***	0.142/0.116/0.127	**/**/**
	PTGS2	0.027/0.084/0.221	0.534/0.0561/***	0.045/0.095/0.222	0.32/*/***
M2 macrophage	*CD163*	0.299/0.303/0.335	***/***/***	0.348/0.295/0.335	***/***/***
	VSIG4	0.145/0.168/0.147	***/***/***	0.168/0.147/0.135	***/**/**
	MS4A4A	0.179/0.195/0.15	***/***/***	0.215/0.175/0.13	***/***/**
Neutrophils	*CEACAM8*	0.093/0.163/0.077	*/***/0.0825	0.095/0.159/0.081	*/***/0.072
	ITGAM	0.194/0.308/0.272	***/***/***	0.229/0.297/0.277	***/***/***
	CCR7	0.017/0.326/0.103	0.707/***/*	0.046/0.314/0.073	0.307/***/0.108
	KIR2DL1	0.018/0.036/0.013	0.688/0.411/0.772	0.024/0.021/−0.003	0.588/0.645/0.95
	KIR2DL3	0.14/0.085/0.035	**/0.0542/0.435	0.161/0.059/0.018	***/0.195/0.691
	KIR2DL4	0.076/0.043/0.017	0.0832/0.328/0.704	0.094/0.02/−0.01	*/0.661/0.822
	KIR3DL1	0.073/0.004/0.039	0.0997/0.927/0.374	0.074/−0.019/0.02	0.0992/0.667/0.661
	KIR3DL2	0.102/0.091/0.045	*/*/0.303	0.119/0.065/0.018	**/0.15/0.698
	KIR3DL3	0.053/0.018/0.031	0.229/0.686/0.488	0.046/0.011/0.026	0.309/0.805/0.557
	KIR2DS4	0.117/0.086/0.107	**/0.0521/*	0.121/0.066/0.093	**/0.142/*
Dendritic cell	*HLA-DPB1*	0.014/0.271/0.086	0.76/***/0.0505	0.025/0.254/0.061	0.575/***/0.176
	HLA-DQB1	0/0.169/0.119	0.995/***/**	0.01/0.142/0.103	0.816/**/*
	HLA-DRA	0.032/0.22/0.054	0.47/***/0.223	0.048/0.194/0.025	0.288/***/0.584
	HLA-DPA1	0.104/0.28/0.119	*/***/**	0.121/0.269/0.095	**/***/*
	CD1C	0.01/0.241/0.083	0.825/***/0.0599	0.013/0.215/0.07	0.777/***/0.12
	NRP1	0.437/0.186/0.382	0/***/***	0.433/0.178/0.381	***/***/***
	ITGAX	0.08/0.268/0.23	0.0695/***/***	0.112/0.256/0.225	*/***/***
Th1	*TBX21*	0.117/0.234/0.064	**/***/0.15	0.161/0.209/0.034	***/***/0.452
	STAT4	0.075/0.261/0.051	0.0873/***/0.245	0.105/0.246/0.013	*/***/0.782
	STAT1	0.291/0.263/0.166	***/***/***	0.33/0.254/0.145	***/***/**
	IFN-γ (IFNG)	0.095/0.044/−0.078	*/0.315/0.0785	0.126/0.012/−0.112	**/0.794/*
	TNF-α (TNF)	0.034/0.162/0.135	0.436/***/**	0.051/0.126/0.124	0.254/**/**

Table 2 (*continued*)

Table 2 (*continued*)

Description	Gene markers	LUAD (ZHX1/ZHX2/ZHX3)			
		None		Purity	
		Cor	P	Cor	P
Th2	*GATA3*	0.106/0.355/0.133	*/***/**	0.138/0.354/0.114	**/***/*
	STAT6	0.171/0.378/0.195	***/***/***	0.176/0.384/0.213	***/***/***
	STAT5A	0.158/0.395/0.294	***/***/***	0.185/0.398/0.287	***/***/***
	IL13	–0.036/0.088/–0.092	0.41/*/*	–0.018/0.061/–0.095	0.689/0.173/*
Tfh	*BCL6*	0.2/0.33/0.356	***/***/***	0.201/0.345/0.355	***/***/***
	IL21	0.159/0.105/0.052	***/*/0.238	0.17/0.091/0.038	***/*/0.397
Th17	*STAT3*	0.412/0.404/0.458	***/***/***	0.412/0.418/0.457	***/***/***
	IL17A	0.016/0.008/–0.107	0.723/0.864/*	0.016/–0.018/–0.138	0.717/0.695/**
Treg	*FOXP3*	0.116/0.271/0.122	**/***/**	0.156/0.261/0.1	***/***/*
	CCR8	0.26/0.361/0.188	***/***/***	0.307/0.356/0.172	***/***/***
	STAT5B	0.333/0.416/0.414	***/***/***	0.328/0.417/0.402	***/***/***
	TGFβ (TGFB1)	0.165/0.24/0.317	***/***/***	0.176/0.231/0.304	***/***/***
T cell exhaustion	*PD-1 (PDCD1)*	0.064/0.148/0.029	0.145/***/0.508	0.094/0.116/–0.019	*/**/0.68
	CTLA4	0.065/0.188/0.068/	0.141/***/0.125	0.104/0.16/0.037	*/***/0.418
	LAG3	0.027/0.1/0.073	0.535/*/0.0992	0.052/0.076/0.035	0.246/0.0904/0.436
	TIM-3 (HAVCR2)	0.151/0.194/0.138	***/***/**	0.193/0.17/0.117	***/***/**
	GZMB	0.046/–0.006/–0.059	0.299/0.889/0.184	0.077/–0.051/–0.106	0.0859/0.257/*

P<0.05 were considered to be statistically significant (*, P<0.05; **, P<0.01; ***, P<0.001). ZHX, zinc-fingers and homeoboxes; TIMER, tumor immune estimation resource; LUAD, lung adenocarcinoma; Cor, correlation; TAM, tumor-associated macrophages.

Table 3 Correlation analysis between ZXH1-3 and relate genes and markers of monocyte and macrophages in LUAD in GEPIA

Description	Gene markers	LUAD (ZHX1/ZHX2/ZHX3)			
		Tumor		Normal	
		R	P	R	P
Monocyte	*CD86*	0.14/0.16/0.13	**/***/**	–0.22/–0.21/–0.41	0.09/0.11/**
	CSF1R	0.17/0.23/0.26	***/***/***	–0.15/0.069/0.12	0.27/0.61/0.36
TAM	*CCL2*	0.058/0.14/0.2	0.2/**/***	0.11/0.35/–0.024	0.43/**/0.86
	CD68	0.074/0.22/0.13	0.1/***/**	–0.34/–0.087/–0.076	**/0.51/0.57
	IL10	0.033/0.12/0.066	0.47/**/0.15	–0.23/–0.074/–0.22	0.075/0.58/0.1
M1 macrophage	*NOS2*	–0.035/–0.028/0.016	0.44/0.54/0.72	0.48/0.4/0.54	***/**/***
	IRF5	0.083/0.071/0.051	0.069/0.12/0.26	–0.37/–0.21/–0.14	**/0.11/0.3
	PTGS2	0.041/–0.04/0.13	0.37/0.38/**	0.18/0.43/0.23	0.16/***/0.075
M2 macrophage	*CD163*	0.13/0.1/0.18	**/*/***	–0.38/–0.16/–0.25	**/0.24/0.061
	VSIG4	0.086/0.086/0.087	0.059/0.058/0.057	–0.48/–0.37/–0.31	***/**/*
	MS4A4A	0.11/0.13/0.096	*/**/*	–0.41/–0.41/–0.39	**/**/**

P<0.05 were considered to be statistically significant (*, P<0.05; **, P<0.01; ***, P<0.001). ZHX, zinc-fingers and homeoboxes; LUAD, lung adenocarcinoma; GEPIA, gene expression profiling interactive analysis; TAM, tumor-associated macrophages.

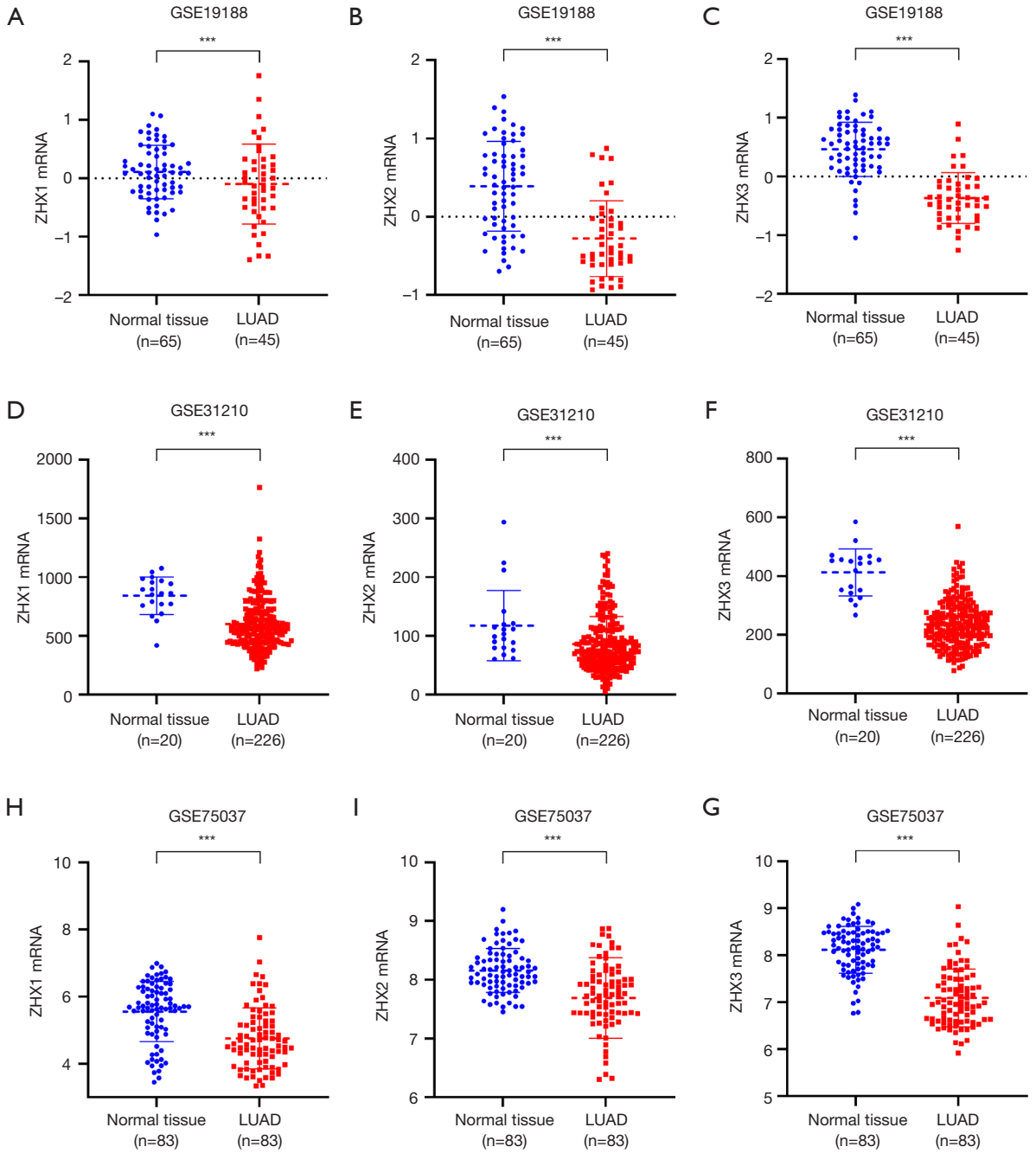

Figure 6 The validation of ZHX family member expression in LUAD and normal tissues by GEO database. (A,D,H) Indicated that the ZHX1 was significantly decreased in 354 patients of LUAD compared to 168 controls in the three datasets (GSE19188, GSE31210, and GSE75037). (B,E,I) Indicated that the ZHX2 was also significantly decreased in LUAD compared to normal tissue. (C,F,G) Showed that the ZHX3 was significantly lower in LUAD compared to normal tissue. The ZHX family member expression was analyzed using an unpaired *t*-test. P<0.05 were considered to be statistically significant (***, P<0.001). ZHX, zinc-fingers and homeoboxes; LUAD, lung adenocarcinoma; GEO, Gene Expression Omnibus.

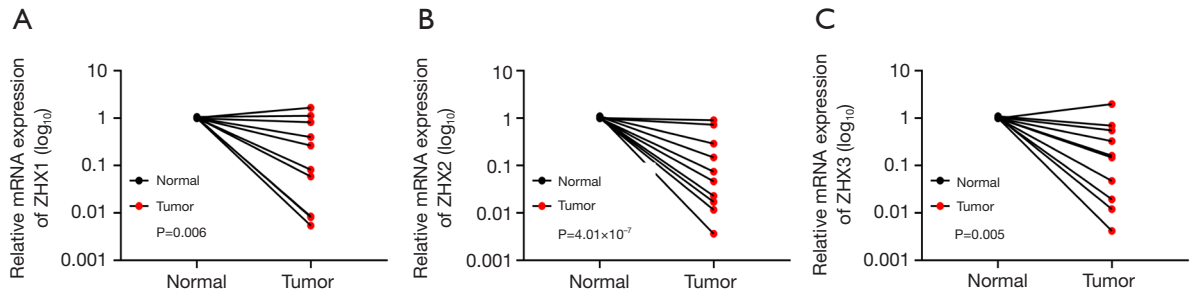

Figure 7 The validation of ZHX family member expression in LUAD and normal tissues by RT-PCR in 10 pairs of LUAD and normal tissues. (A-C) Indicated that ZHX1, ZHX2, and ZHX3 expression levels were significantly lower in LUAD than in normal tissues. ZHX, zinc-fingers and homeoboxes; LUAD, lung adenocarcinoma; RT-PCR, real-time polymerase chain reaction.

Discussion

To our knowledge, this is the first study to investigate the expression, prognosis, and immune infiltration of members of the ZHXs family in LUAD. The findings revealed that expression of ZHXs was shown to be considerably lower in LUAD tissues than in normal tissues. Attenuated expression of ZHXs was an unfavorable predictor for LUAD patients. Moreover, the findings showed that the levels of ZHXs expression were correlated to immune infiltration and various immunological marker sets. Thus, this study provides novel insights for understanding the potential role of the ZHXs family in prognosis and tumor immunology and novel guidance on developing therapeutic targets for LUAD patients.

ZHX1 gene was firstly cloned by immunoscreening with a monoclonal B92 antibody (20). Several reports have shown that ZHX1 was involved in tumorigenesis as a tumor suppressor, including in gastric cancer (21), hepatocellular cancer (9,22), breast cancer (11), and renal cell carcinoma (10). The recent two studies, however, indicated that ZHX1 might play an oncogene role in several malignant tumors (23,24). A higher expression level of ZHX1 was significantly associated with worse overall survival in glioblastoma and cholangiocarcinoma (23,24). In contrast to findings in glioblastoma and cholangiocarcinoma, the current study demonstrated that the expression of ZHX1 in LUAD considerably decreased compared to in normal tissues and was a favorable predictor for overall survival in LUAD patients. This result supported the findings of most early studies that ZHX1 served as a suppressor gene role in malignant tumors. One unanticipated result was that no difference in ZHX1 expression level was observed between LUSC and normal tissue. And the significant association of ZHX1 expression with OS was not found in the current study. A possible explanation for this might be that ZHX1 plays a different role in development and progression of various cancers.

As a member of the ZHX family, ZHX2 was firstly cloned as a ZHX1-interacting protein, which is located on 8q24.13 and contains two zinc finger domains and five homeodomains (HDs) (25). ZHX2 can form homodimers or heterodimers with the other two family members, ZHX1 or ZHX3 (25). ZHX2 was initially discovered to be a crucial transcriptional repressor for the alpha-fetoprotein regulator 1 (Afr1), which plays a critical oncogene role in liver cancer (26). Several reports have shown that ZHX2 exhibited tumor suppressor action in HCC by inhibiting cyclin A, E, and multidrug resistance 1 (MDR1) expression (27,28). It has been found that the hepatitis B virus (HBV) can inhibit ZHX2 expression to promote the proliferation of HCC through miR-155 activation (29).

Conversely, ZHX2 can restrict HBV replication via regulating HBV promoter activities and cccDNA modifications (30). You *et al.* (11) found that higher ZHX2 expression predicted prolonged OS in patients with breast cancer. These findings supported that ZHX2 functioned as a tumor suppressor. However, some reports are contrary to the abovementioned studies, which have suggested that ZHX2 plays an oncogene role in several cancers. Hu *et al.* (31) found that ZHX2 expression was present only in HCC tissues and was significantly related to higher tumor-node-metastasis (TNM)-stage and distant metastasis in HCC, which implied that ZHX2 may be involved in hepatocellular carcinogenesis and HCC progression. Zhang *et al.*'s study (32) revealed that ZHX2 served as an oncogenic driver via promoting nuclear factor κB activation in clear cell renal cell carcinoma. Another recent study also demonstrated that ZHX2 activated HIF1α oncogenic

signaling in triple-negative breast cancer (33). Results of this study demonstrated that the expression of ZHX2 was significantly lower in both LUAD and LUSC than in normal tissue, and higher ZHX2 expression was correlated to favorable overall survival in LUAD patients. Consistent with most of the prior studies, our findings supported that ZHX2 may play a tumor suppressor role in LUAD patients. The disparity of ZHX2 function among these studies is probably attributable, in part, to several factors, including differences in tumor types, molecular types of cancers, and so on. Another possible explanation for this is that ZHX2 might play dual role in the development and progression of various malignant tumors. More further studies are required to better define the real function of *ZHX2* gene in cancers.

ZHX3, another member of the ZHX family, is mapped to chromosome 20q12 (34). The encoded protein contains two C2H2-type zinc fingers and five HDs and forms a dimer with itself or with zinc fingers and homeoboxes family member 1. Yamada *et al.* (35) initially observed that the dimerized ZHX3 protein interacts with the A subunit of the ubiquitous transcription factor nuclear factor-Y and may function as a transcriptional repressor in the nucleus. In clear cell RCC, expression of ZHX3 was decreased and was significantly correlated to the clinical stage (10). You *et al.* (11) revealed that despite no significant difference in ZHX3 expression between cancer tissues and normal tissues, it had been found that a higher level of ZHX3 expression was significantly correlated to the improved OS in breast cancer patients. In contrast, in urothelial carcinoma of the bladder, ZHX3 was found to be a critical oncogenic component linked to a poor prognosis (34). Similarly, increased ZHX3 expression in gastric cancer was associated with a prolonged OS (8). In the current study, we observed that the expressions of ZHX3 in LUAD and LUSC were significantly higher than in normal tissues. Higher ZHX3 expression was associated with better OS in LUAD. Similar to the other two family members, ZHX1 and ZHX2, it was revealed that ZHX3 might also play a dual role (oncogene or tumor suppressor) in different human cancers. Further investigations are warranted to confirm and validate the pro- or anticancer role of ZHX3 in diverse human malignancies.

EGFR-TKIs provide favorable treatment outcomes in EGFR mutation-positive LUAD patients. However, most patients will eventually develop the progressive disease within about one year of treatment. Recently, several mechanisms of EGFR-TKI resistance have been reported, such as EGFR T790M mutation and MET amplification, but these mechanisms could not explain all resistance phenomena. It implies that there are some other unknown mechanisms in EGFR-TKI resistance. Our study revealed that ZHXs family-related genes were highly clustered in EGFR tyrosine kinase inhibitor resistance in LUAD. This interesting finding supports a general hypothesis that abnormal expression of ZHXs family might play a potential role in EGFR tyrosine kinase inhibitor resistance in LUAD. Zhu *et al.* (36) revealed that ZHX2 overexpression induces Sunitinib, a multi-targeted receptor tyrosine kinase inhibitor, resistance by activating autophagy. While the sunitinib resistance can be reversed by the combination treatment of sunitinib and chloroquine. The abovementioned study suggested that ZHXs family was involved in receptor tyrosine kinase inhibitor resistance. Currently, the relationship of the ZHXs family and EGFR tyrosine kinase inhibitor resistance in LUAD is still unclear. This finding may provide a clue to guide the exploration of EGFR-TKI resistance mechanisms in future studies.

Immune cells play a crucial role in developing and establishing the tumor microenvironment as a component of the tumor microenvironment (37). Several reports have revealed that immune cells in the tumor microenvironment have been demonstrated to serve as tumor suppressors or promoters (38). B cells infiltrating lung cancer have their own unique roles in anti-tumor immunity (39). B lymphocytes infiltrating tumors are observed in all phases of the development and progression of human lung cancer. Their presence varies depending on the stage and histological subtype (40,41), implying that B cells serve a pivotal role in lung cancer development and progression. $CD4^+$ cells are a type of T cell that is crucial to the adaptive immune system's function. By releasing cytokines, they enhance the function of other immune cells. $CD8^+$ T cells play an essential role in immunological defense and tumor surveillance. Prior studies have demonstrated that $CD8^+$ T cells infiltration is a significant predictor of better clinical outcomes in malignancies (42-44). DCs serve as a critical regulator in the adaptive immune system and are required for T cell-mediated cancer immunity. Anti-tumoral responses, in particular, are dependent on a subset of conventional DCs that carry tumor antigens to draining lymph nodes and cross-present antigen to activate cytotoxic T cells (45). Neutrophils cells play an essential role in tumor immunity and show two-faced roles in cancer development and progression (46). Working along with DCs and neutrophils, macrophages serve as a key effector to initiate and direct the host reaction and can exert a dual influence

on tumor growth and progression (47). To date, this is the first study that has focused on the relationship between ZHX family expression and tumor immunity. The current study indicated that ZHX family might be associated with $CD4^+$ cells, $CD8^+$ T cells, B cells, macrophage, neutrophil, and DCs infiltration.

However, the influences of ZHX1, ZHX2, and ZHX3 on the kind of immune cells were different, implying that ZHX1, ZHX2, and ZHX3 might regulate tumor immunity through different mechanisms. It is worth noting that most immune cells were affected by ZHX2. This finding suggested that ZHX2 may have the most robust regulation function on tumor immune infiltration. Another important finding is that all members of ZHX family were significantly associated with neutrophil and DCs in LUAD. A possible explanation for this might be that ZHX family might have common mechanism to regulate neutrophil and DCs infiltration. Whether a specific immune cell type is involved in the LUAD prognosis and impacts the therapeutic effect of immunotherapy remain unclear. Thus, more studies on the mechanism are required to clarify these questions.

Several limitations need to be noted regarding the present study. First, the major limitation of this study is that the physiological and molecular mechanisms of ZHXs *in vitro* and *in vivo* were not validated. Considerably more work will need to be done to determine the expression and biological functions of ZHXs in lung cancer. Second, as this study was a bioinformatic analysis based on online database, the characteristics (age, race, sex, smoking, etc.) and clinical outcomes (survival, follow-up, metastases, recurrence, progression, etc.) were not available. This issue should be addressed by the future studies with complete clinical characteristics and clinical outcomes. Third, the mRNA levels and prognostic significance of ZHXs family were mainly determined in silico analysis. Further researches are required to explore their protein expression patterns in a large sample size. Forth, immune population evaluations are based on the database's transcriptome data as surrogates. It would be beneficial to confirm the most noteworthy distinctions in immune cell subtypes by direct pathological imaging. Direct evaluations of immune cell populations will be needed for further research.

Conclusions

In conclusion, the ZHX family expression was significantly associated with LUAD prognosis and immune infiltration. It was shown that members of the ZHX family influenced the prognosis of LUAD patients and altered their immunological condition. The findings herein offered a foundation for further study into the biological function of the ZHX family in LUAD and provided insights into developing therapeutic targets for LUAD patients.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-2793/rc

Peer Review File: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-2793/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-2793/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Medical Ethics Committee of the Chongqing University Cancer Hospital (No. CZLS2022030-A). Written informed consent was obtained from all subjects.

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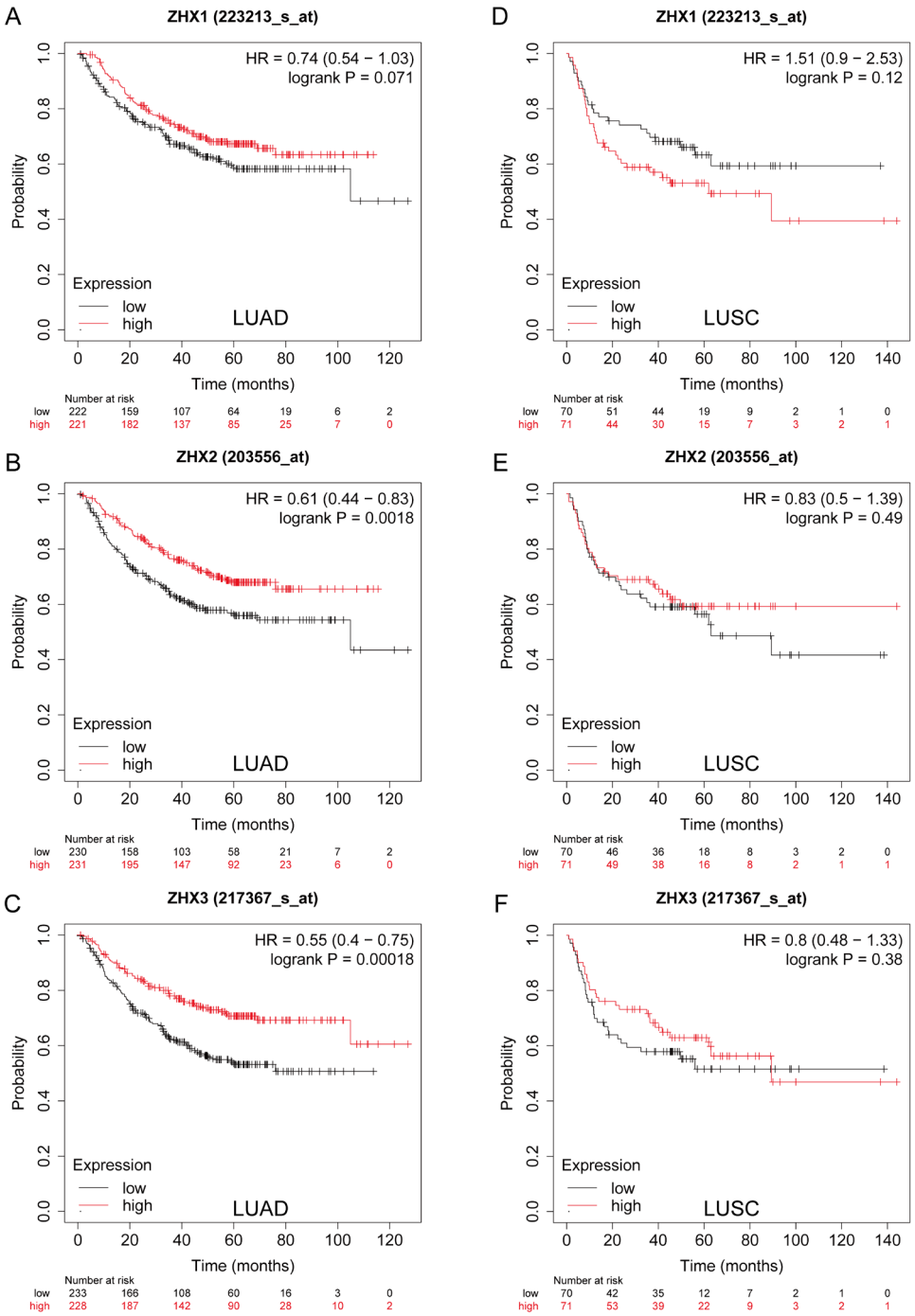

Figure S1 Progression-free survival analysis in Kaplan-Meier plotter database for mRNA levels of ZHX factors in LUAD patients. (A-C) The overall survival curves of ZHX1, ZHX2 and ZHX3 in LUAD patients, respectively. (D-F) The overall survival curves of ZHX1, ZHX2, and ZHX3 in LUSC patients, respectively. LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; HR, hazard ratio.

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