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ORIGINAL RESEARCH

Comprehensive Analysis of the Prognostic Significance of ZHX Family Members in Esophageal Carcinoma

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Background: Zinc-finger and homeobox (ZHX) family members have been identified as valuable predictors of survival outcomes in several types of cancers. Nevertheless, the prognostic implications of ZHX factors in esophageal carcinoma (EC) remain unclear.
Materials and Methods: Bioinformatic analyses were conducted to evaluate the transcription levels of ZHX factors and their corresponding prognostic significance in EC. Immunohistochemistry was further utilized to assess the association between ZHX3 protein expression and clinicopathologic variables as well as survival outcomes in patient with esophageal squamous cell carcinoma (ESCC).
Results: Data from online databases showed no association between ZHX mRNA expression levels and overall survival (OS) in EC patients. However, subgroup analyses revealed significant associations between the expression of ZHX factors and survival outcomes in selected patient cohorts. Immunohistochemical analysis supported that high ZHX3 protein expression was associated with advanced histological grade and unfavorable OS in ESCC patients. Multivariate analysis revealed that ZHX3 expression was an independent prognostic predictor for patient survival.
Conclusion: These observations by means of combining bioinformatics and immunohistochemical analyses suggest that ZHX factors is involved in disease progression and may be potential biomarkers for predicting more accurate prognosis for EC patients.
Keywords: zinc-fingers and homeoboxes, esophageal carcinoma, prognosis, bioinformatics analysis, immunohistochemistry

Introduction

Esophageal carcinoma (EC) is one of the most common malignancies with increasing incidence and mortality rates. According to data from the Global Cancer Statistics 2020 Survey, approximately 600,000 new cases and 540,000 deaths due to EC in 2020.[1] EC is histologically classified as esophageal adenocarcinoma (EAC) and esophageal squamous cell carcinoma (ESCC). Notably, approximately half of the existing EC cases occur in China and among them ESCC is the more frequent subtype.[1–4] Despite the development of novel technologies and precision medicine, the molecular mechanisms involved in the emergence, development, and progression of EC remain poorly understood. Thus, discovery and validation of novel biomarkers having prognostic and therapeutic values is urgently required.

Zinc-fingers and homeoboxes (ZHX) family members have been characterized as ZHX1, ZHX2, and ZHX3.[5–8] It has been reported that ZHX factors have similar protein structures, ie, containing two amino-terminal C2-H2 zinc-finger motifs and five carboxy-terminal DNA-binding domains that reciprocally form homodimers and heterodimers reciprocally.[9] ZHX factors function as transcriptional repressors through interacting with the A subunit of the nuclear transcriptional factor Y (NY-FA).[10–13] Recent studies have demonstrated important biological functions of ZHX factors in multiple disorders.[14–19] Notably, dysfunction or impaired expression of ZHX factors has been found to be involved in

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the initiation and progression of multiple types of cancer,[20–22] raising the possibility that they may serve as potential valuable biomarkers for cancer screening, diagnosis and survival prediction. However, the detailed mechanisms underlying the biological functions and prognostic implications of ZHX family members in EC have not been well documented. Hence, the present study investigated the transcriptional expression profile and prognostic significance of ZHX factors in EC using bioinformatic data mining with several online databases. In addition, immunohistochemical analysis of ESCC specimens was performed to further clarify the association between ZHX3 protein expression and clinicopathologic variables as well as patient survival.

Materials and Methods

Tumor Immune Estimation Resource (TIMER) Database Analysis

The TIMER online database (https://cistrome.shinyapps.io/timer) has been recognized as a web-based platform for comprehensive analysis of immune infiltration across different types of cancer and dynamically produces relevant graphics.[23] In this study, the mRNA expression levels of ZHX factors in both cancer tissues along with normal tissues were examined using the TIMER database.

Cancer Cell Line Encyclopedia (CCLE) Database Analysis

The CCLE online database (https://portals.broadinstitute.org/ccle) is an open-source encyclopedia system containing approximately 1,500 cell line models across 30 distinct cancer types.[24] Data on gene expression and genomic alterations including DNA mutations and copy numbers are continuously updated in the CCLE online database. In the present study, the mRNA expression information of cell lines from diverse cancer types including EC was analyzed using the CCLE database.

Kaplan-Meier Plotter Database Analysis

The prognostic significance of ZHX mRNA expression levels on survival of EC patients was calculated using the Kaplan-Meier Plotter online database (http://kmplot.com/analysis).[25] To identify overall survival (OS), cancer patients were grouped into high and low expression subsets by auto-selection of the best cut-off for gene expression analysis. Kaplan-Meier plots were generated using survival analyses following the target probe entering into the database.

cBioPortal Cancer Genomics Database Analysis

The cBioPortal Cancer Genomics online database (http://www.cbioportal.org) has been recognized as a platform for investigating and assessing genomic data from various cancer datasets.[26] cBioPortal has 147 distinct research areas across 31 different kinds of cancer types. The impacts of genomic alterations in ZHX factors, such as copy-number variance, gene deletion, and mutations on OS in EC patient were detected using the cBioPortal database in the present study.

Immunohistochemical Analysis

One ESCC tissue microarray chip including 105 primary tumor samples and 66 paired adjacent non-cancerous samples was purchased from Outdo Biotech Co., Ltd. (Shanghai, China). The tissue samples were obtained from patients undergoing curative surgery between February 2008 and June 2010. ZHX3 protein expression was detected by immunohistochemical staining using the standard EnVision assay as described previously (20). Briefly, paraffin-embedded specimens were cut into 4-µm sections, processed in a water bath, adhered to microscope slides, and incubated at 65°C for 2 h to avoid detachment. Tissue chips were deparaffinized and hydrated. Antigens were thermally repaired using 0.01 mol/L citrate buffer at 95°C for 15 min, and incubated with 3% H_2O_2 for 20 min at room temperature to inhibit endogenous peroxidase activity. Samples were incubated with ZHX3 primary antibody (catalog no. ab84677; dilution,1:500; Abcam, Cambridge, MA, USA) and an EnVision antibody complex (anti-mouse/rabbit) from an Envision™ Detection kit (Gene Tech., Shanghai, China). Rabbit IgG was used as the negative control. The samples were reheated to 37°C for 45 min and rinsed three times with PBS for 2 min each time. 3,3'-Diaminobenzidine was used as the chromogen substrate and the nuclei were counterstained with hematoxylin.

Ten random 400× microscopic fields per slide for each specimen were examined by two independent pathologists who were blinded to the clinical data. Global immunostaining was scored using a semi-quantitative integration method combining the percentage and the staining intensity of positive cells. The mean percentage of positively stained cells was scored as 0–100%. Staining intensity was scored as follows: absent (0), weak (0.5), weak-moderate (1), moderate (2), or strong (3). The multiplication of these parameters was used as the final staining score (0–300%). Patients were separated into low and high-expression groups according to the median value (<90%) of the staining scores. For the purpose of statistical evaluation, tumor specimens displaying a final score of <90% were classified as low ZHX3 expression, and those with a score ≥90% were classified as high expression.

Statistical Analysis

SPSS statistical software (version 24.0; SPSS, Inc., Chicago, IL, USA) was used for the statistical analyses. Continuous variables are presented as mean ± SEM and data on counts are expressed as frequency or rate (%). Unpaired Student's t-tests with Welch's correction and Kruskal–Wallis tests were used to examine differential ZHX3 protein expression in ESCC and adjacent healthy tissues. The association between different clinicopathological parameters and ZHX3 protein expression was determined using the chi-square test. Log rank tests were used to compare survival curves generated using Kaplan-Meier plotter analysis with a Log rank test. The prognostic significance of all clinicopathological parameters and ZHX3 expression was determined using a univariate analysis, and the factors that achieved significance in the univariate analysis was further examined by multivariate regression analysis with a Cox proportional-hazards regression model. P<0.05 was considered statistically significant.

Results

mRNA Expression Levels of ZHX Factors in Human Cancer

The TIMER online database analysis provided the mRNA expression levels of ZHX factors in various cancer types. We observed that the expression levels of ZHX1 and ZHX2 were significantly higher in esophageal carcinoma (ESCA) tissues than in the normal tissues (Figure 1). Regarding ZHX3, however, this difference was not significant. Additionally, analysis from the CCLE database demonstrated that the mRNA expression levels of ZHX1, ZHX2, and ZHX3 in EC cells were listed in the 20th, 9th, and 38th highest positions across various cancer types (Figure 2).

Association Between mRNA Expression of ZHX Factors and Patient Outcomes

We then explored whether ZHX factors may be valuable in predicting survival outcomes in EC patients using the Kaplan-Meier Plotter online database. Although no significant association was observed between ZHX1/ZHX3 mRNA expression and OS (Figure 3A and C), increased ZHX2 expression appeared to predict a better OS in EC patients (Figure 3B).

Next, we performed subgroup analyses in selected cohorts of EC patients. As shown in Figure 4A and B, ZHX1 mRNA expression was not significantly associated with OS either in EAC patients or in ESCC patients. Similarly, no association was observed between ZHX1 expression and OS in EC patients with either Grade II or III tumors (Figure 4C and D). However, high ZHX1 mRNA level suggested a prolonged OS in EC patients with high mutation burden but not in those with low mutation burden (Figure 4E and F). High ZHX1 expression also exhibited a favorable OS in EC patient with high neoantigen load (Figure 4G). In addition, increased ZHX1 mRNA expression revealed an improved OS in EC patients with Stage III tumors but not in those with Stage II tumors (Figure 4H and I).

Subgroup analyses demonstrated that increased ZHX2 mRNA expression was associated with a favorable OS in EAC patients but not in ESCC patients (Figure 5A and B). Increase ZHX2 expression also indicated a favorable OS rate in EC patients with Grade II tumors but not in those with Grade III tumors (Figure 5C and D). ZHX2 mRNA expression was not associated with OS in EC patients with high or low mutation burden (Figure 5E and F). Notably, high ZHX2 expression represented a favorable OS in EC patients with high neoantigen load (Figure 5G). High ZHX2 expression also implied an improved OS rate in EC patients with Stage III tumors but not in those with Stage II tumors (Figure 5H and I).

Subgroup analyses illustrated that ZHX3 mRNA expression was not significantly associated with OS in EAC patients or in ESCC patients (Figure 6A and B). Moreover, no association was observed between ZHX3 expression and OS in EC

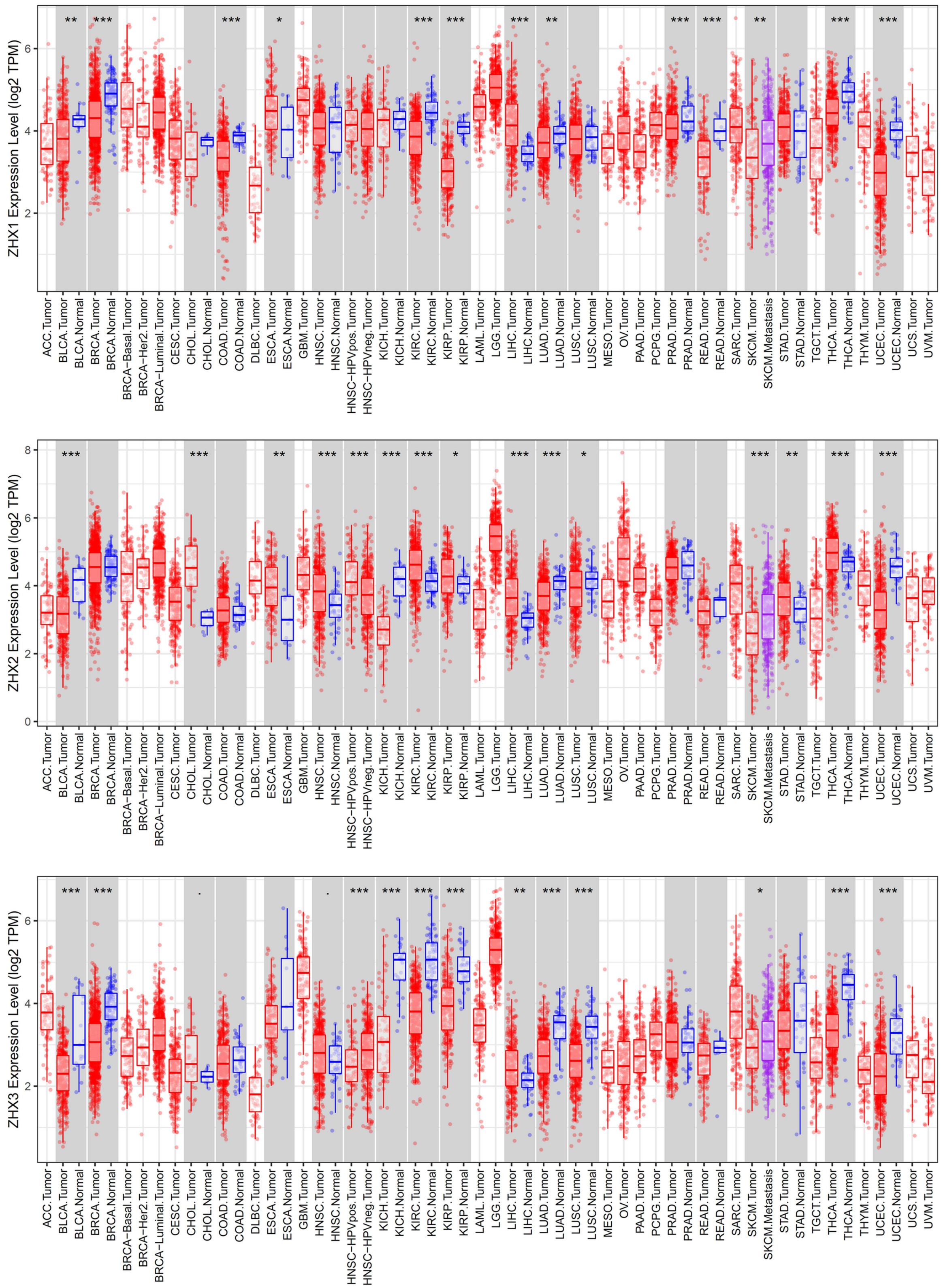

Figure 1 The mRNA levels of ZHX factors in various cancer types according to the TIMER database analysis. A graphic taken obtain from the TIMER online database show the transcriptional levels of ZHX factors in cancer tissues and normal tissues. *P<0.05; **P<0.01, ***P<0.001.

Abbreviations: ZHX, zinc-fingers and homeoboxes; Timer, Tumor Immune Estimation Resource.

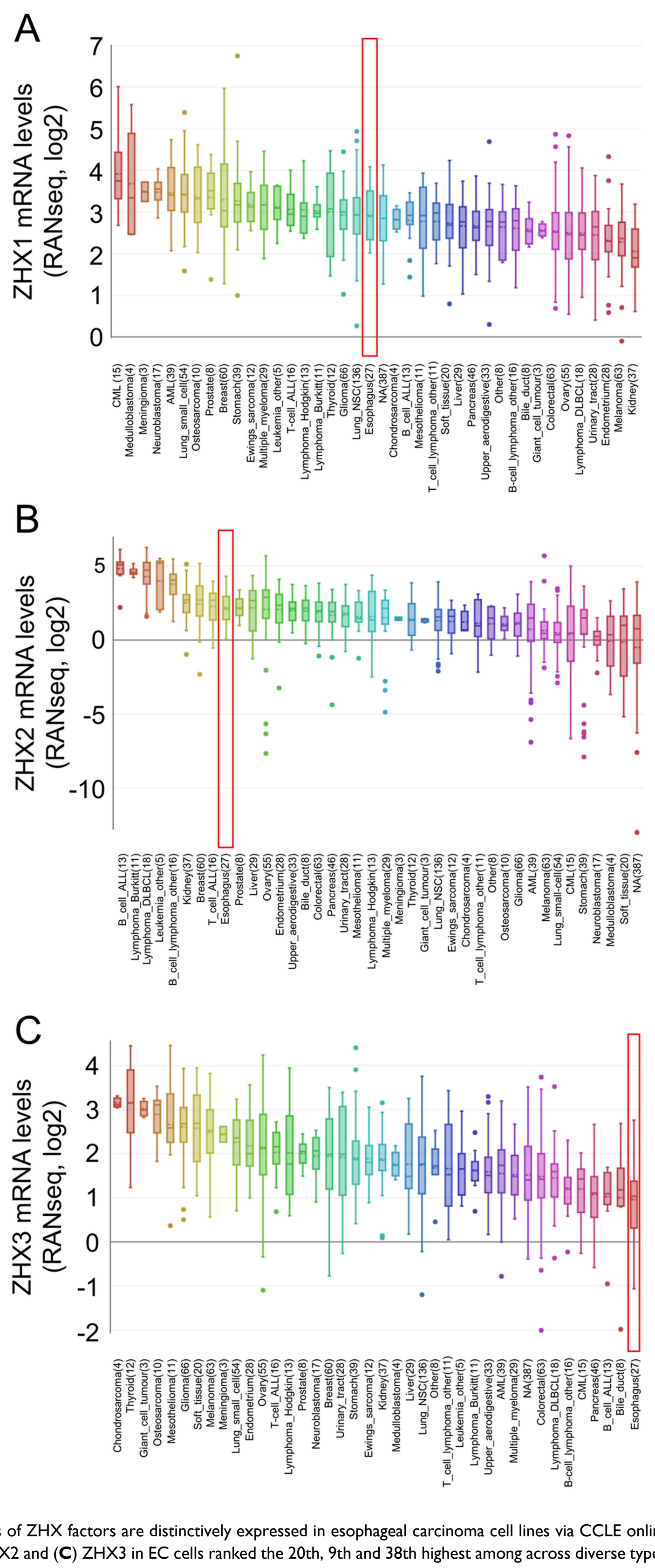

Figure 2 The mRNA expression levels of ZHX factors are distinctively expressed in esophageal carcinoma cell lines via CCLE online database. The transcriptional mRNA expression levels of (**A**) ZHX1, (**B**) ZHX2 and (**C**) ZHX3 in EC cells ranked the 20th, 9th and 38th highest among across diverse types of cancer types (shown in red frame).
Abbreviations: ZHX, zinc-fingers and homeoboxes; CCLE, Cancer Cell Line Encyclopedia.

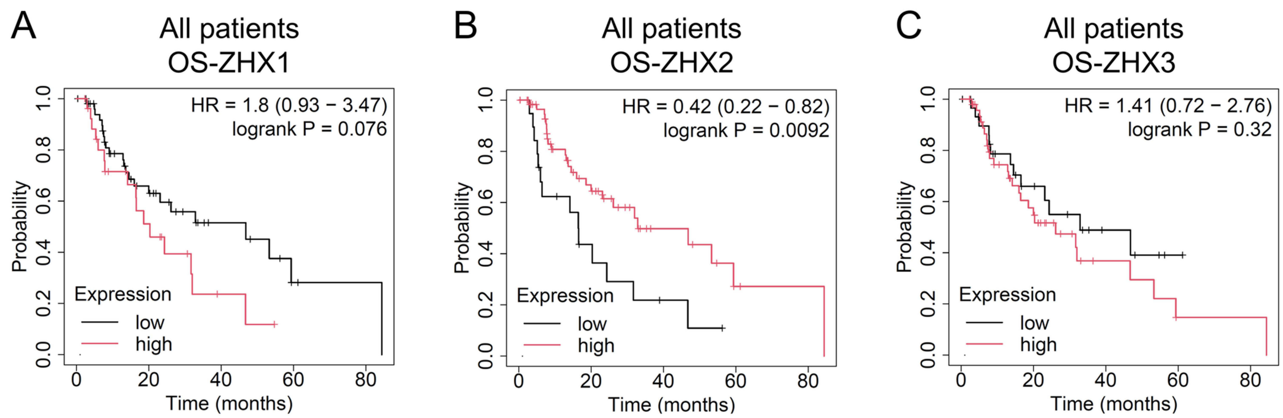

Figure 3 Correlation between mRNA expression of ZHX factors and survival outcomes in EC patients with via Kaplan-Meier Plotter survival analysis. (**A**) OS analysis of ZHX1 in EC patients. (**B**) OS analysis of ZHX2 in EC patients. (**C**) OS analysis of ZHX2 in EC patients.
Abbreviations: ZHX, zinc-fingers and homeoboxes; EC, esophageal cancer; OS, overall survival.

patients with either Grade II or Grade III tumors (Figure 6C and D). Nevertheless, high ZHX3 expression level illustrated a favorable OS rate in EC patients with high mutation burden and neoantigen load (Figure 6E–G). High ZHX3 expression also displayed an improved OS rate in EC patients with Stage III tumors but not in those with Stage II tumors (Figure 6H and I).

Association Between ZHX Genomic Alterations and Patient Survival

We next characterized the prognostic correlation between genomic alterations of ZHX factors and outcomes of EC patients using the cBioPortal database. The genomic alteration rates for ZHX1, ZHX2, and ZHX3 were 10, 9, and 5%, respectively (Figure 7A). No significant association was found between genomic alterations of ZHX1 and OS in ESCC patients or in EAC patients (Figure 7B and C). Notably, we found that the genomic alterations of ZHX2 exhibited a better OS in ESCC patients but indicated a worse OS in EAC patients (Figure 7D and E). The genomic alterations of ZHX3 were not associated with OS either in ESCC patients or in EAC patients (Figure 7E–G).

ZHX3 expression is an independent predictive indicator in patients with ESCC

We further assessed the ZHX3 protein expression status in 105 ESCC samples and 66 paired adjacent noncancerous tissues by immunohistochemistry. We observed that positive ZHX3 immunostaining was predominantly expressed in the cytoplasm of cancer cells (Figure 8A–C). ZHX3 expression level was significantly higher in ESCC tissues than in adjacent noncancerous tissues (Figure 9A). High ZHX3 expression was found to be significantly associated with advanced histological grade (Table 1). Kaplan-Meier survival curves showed that patients with high ZHX3 expression had a worse OS than those with low ZHX3 expression (Figure 9B). The univariate analysis revealed that patient sex, tumor size, histological grade, N stage, clinical stage and ZHX3 expression were determined to be associated with an unfordable OS (Table 2). After adjusting for the prognostic parameters obtained from univariate analysis, patient sex, tumor size, and ZHX3 expression were defined as independent predictors for OS in multivariate analysis (Table 2).

Discussion

The present study comprehensively estimated the potential association between the expression profiles of ZHX family members and survival outcomes in patients with EC, with the aim of identifying novel diagnostic molecular targets and prospective prognostic biomarkers. Our results revealed significant associations between the mRNA expression of ZHX factors and patient survival in selected EC patient cohorts and confirmed that ZHX3 protein expression may serve as an independent prognostic factor for ESCC.

ZHX1 has been defined as the first factor member of the ZHX family and has been reported as a tumor suppressor in several types of cancer.[27,28] Nevertheless, recent studies have suggested that ZHX1 may act as an oncogene, and its

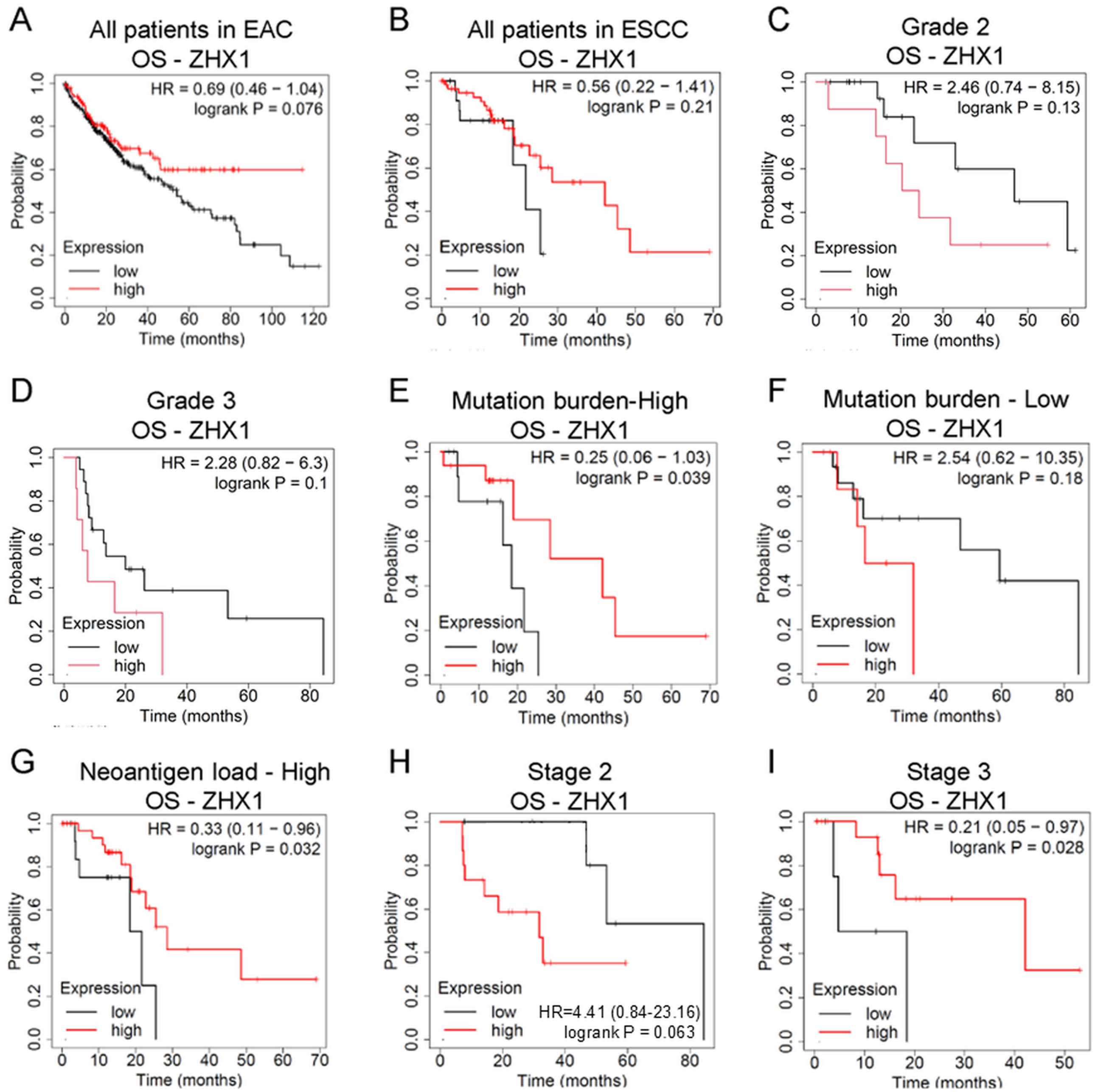

Figure 4 Subgroup analysis of the association between ZHX1 mRNA expression and OS in EC patients via Kaplan-Meier Plotter survival analysis. OS analysis of ZHX1 in (**A**) EAC patients and (**B**) ESCC patients. OS analysis of ZHX1 in EC patients with (**C**) Grade II and (**D**) Grade III tumors. OS analysis of ZHX1 in EC patients with (**E**) high and (**F**) low mutation burden. (**G**) OS analysis of ZHX1 in EC patients with high neoantigen load. OS analysis of ZHX1 in EC patients with (**H**) Stage II and (**I**) Stage III tumors.

Abbreviations: ZHX, zinc-fingers and homeoboxes; EC, esophageal cancer; EAC, esophageal adenocarcinoma; ESCC, esophageal squamous cell carcinoma; OS, overall survival.

overexpression is correlated with worse prognosis in cancers.[22,28,29] Thus, the prognostic impact of ZHX1 on various cancer types appears contradictory. We recently reported that high ZHX1 expression predicts an unfavorable OS rate in breast cancer but presents a better favorable OS for gastric cancer, confirming its different roles in the development of different cancer types.[20,30] Although no significant association was observed between ZHX1 expression and survival outcomes in EC patients in the present study, subgroup analyses revealed that increased ZHX1 expression represented a favorable OS rate in EC patients with Stage III tumors. Nevertheless, high ZHX1 levels displayed improved PPS in patients with high mutation burden and neoantigen load. These data suggest that dysregulated ZHX1 mRNA expression

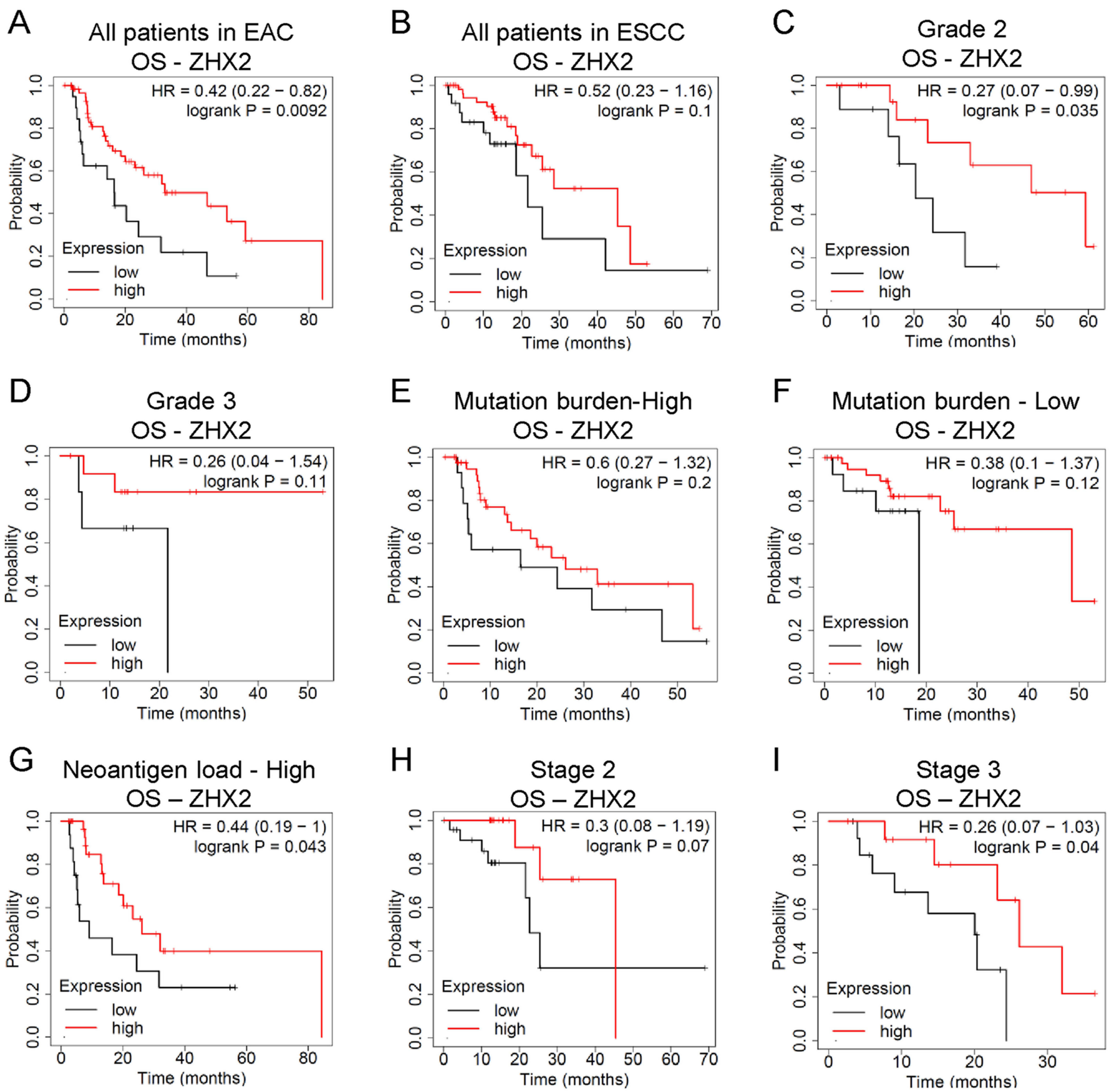

Figure 5 Subgroup analysis of the association between ZHX2 mRNA expression and OS in EC patients via Kaplan-Meier Plotter survival analysis. OS analysis of ZHX2 in (**A**) EAC patients and (**B**) ESCC patients. OS analysis of ZHX1 in EC patients with (**C**) Grade II and (**D**) Grade III tumors. OS analysis of ZHX2 in EC patients with (**E**) high and (**F**) low mutation burden. (**G**) OS analysis of ZHX2 in EC patients with high neoantigen load. OS analysis of ZHX2 in EC patients with (**H**) Stage II and (**I**) Stage III tumors.

Abbreviations: ZHX, zinc-fingers and homeoboxes; EC, esophageal cancer; EAC, esophageal adenocarcinoma; ESCC, esophageal squamous cell carcinoma; OS, overall survival.

may be of value in predicting survival outcomes in EC patients with advanced-stage tumors and in direct clinical therapy with neoantigen vaccines.

ZHX2 mRNA expression was found to be significantly associated with a better OS in EC patients. Subgroup analyses revealed that high ZHX2 levels were associated with a favorable OS rate in EAC patients. Elevated ZHX2 expression also indicated better OS in EC patients with Stage III tumors. This finding demonstrates the tumor-suppressive role of ZHX2, consistent with another report on lung cancer suggesting that ZHX2 inhibits proliferation and promotes apoptosis of tumor cells via inactivation of p38MAPK signaling.[31] More recently, it has been reported that ZHX2 suppresses

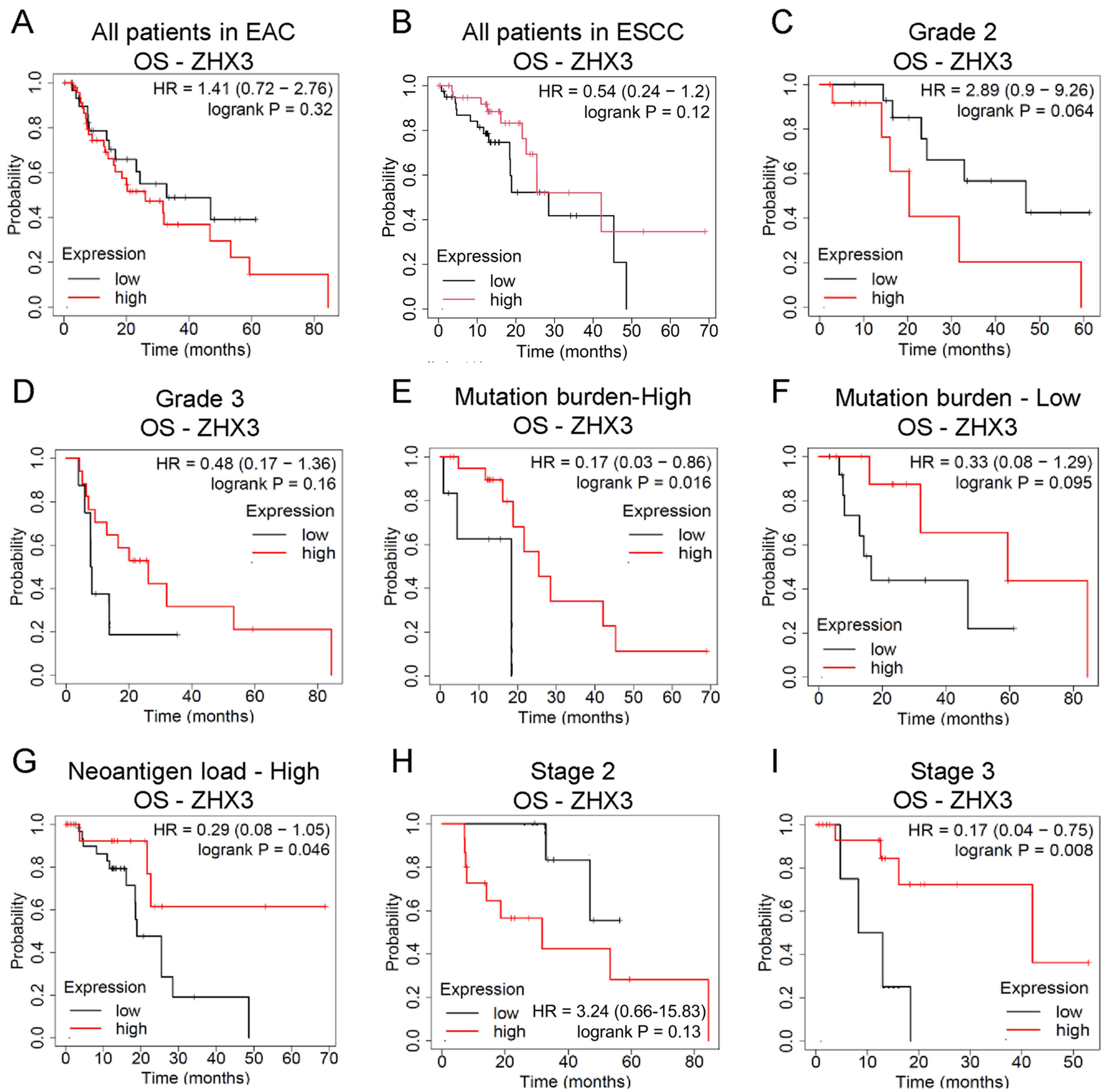

Figure 6 Subgroup analysis of the association between ZHX3 mRNA expression and OS in EC patients via Kaplan-Meier Plotter survival analysis. OS analysis of ZHX3 in (**A**) EAC patients and (**B**) ESCC patients. OS analysis of ZHX3 in EC patients with (**C**) Grade II and (**D**) Grade III tumors. OS analysis of ZHX3 in EC patients with (**E**) high and (**F**) low mutation burden. (**G**) OS analysis of ZHX3 in EC patients with a high neoantigen load. OS analysis of ZHX3 in EC patients with (**H**) Stage II and (**I**) Stage III tumors.

Abbreviations: ZHX, zinc-fingers and homeoboxes; EC, esophageal cancer; EAC, esophageal adenocarcinoma; ESCC, esophageal squamous cell carcinoma; OS, overall survival.

thyroid cancer metastasis of thyroid cancer through transcriptional inhibition of S100 calcium-binding protein A14.[32] Although these above observations suggest that ZHX2 may might act serve as a tumor suppressor in lung cancer, ZHX2 expression has been found observed to be upregulated in clear cell renal cell carcinoma and facilitate tumorigenesis of in a hypoxia inducible factor-α (HIF-α)-independent manner.[33,34] These observations are consistent with relevant findings of ZHX2 as an oncogene in gastric cancer, including our previous report.[20,35] In addition, it has been found that ZHX2 may promote HIF1α oncogenic signaling in triple-negative breast cancer (TNBC) and hypoxia-induced phase separation

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Figure 7 Genomic alterations of ZHX genes and their corresponding associations with outcomes in EC patients using the cBioPortal online database. (**A**) The proportion and distribution of samples with alterations in ZHX factors by OncoPrint in cBioPortal. The impact of genomic alterations of ZHX1 on OS in (**B**) ESCC patients and (**C**) EAC patients. The impact of genomic alterations of ZHX2 on OS in (**D**) ESCC patients and (**E**) EAC patients. The impact of genomic alterations of ZHX3 on OS in (**F**) ESCC patients and (**G**) EAC patients.

Abbreviations: ZHX, zinc-fingers and homeoboxes; EC, esophageal cancer; EAC, esophageal adenocarcinoma; ESCC, esophageal squamous cell carcinoma; OS, overall survival.

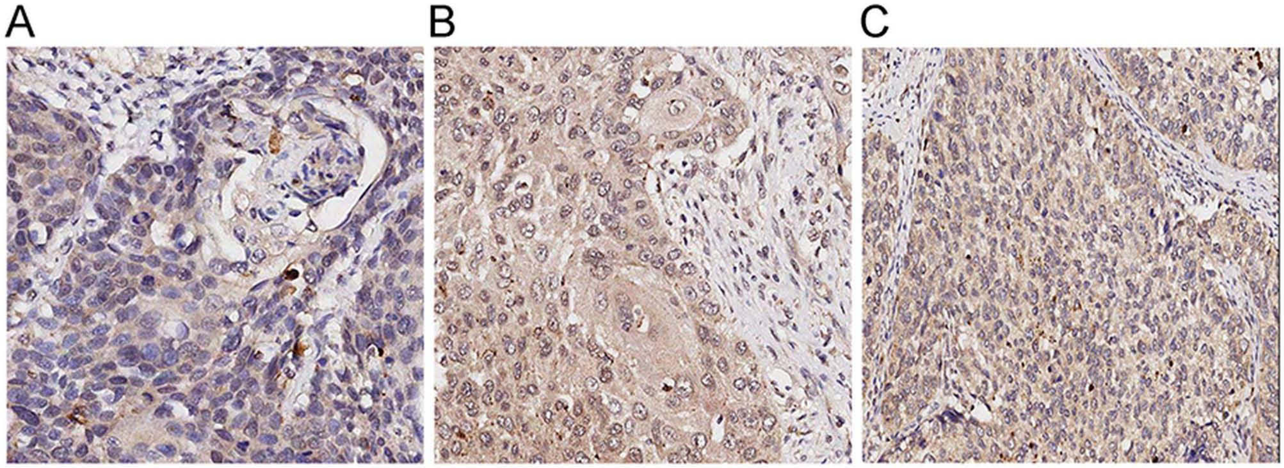

Figure 8 Representative immunohistochemical staining for ZHX3 protein in ESCC tissues. (**A**) Weak staining; (**B**) Moderate staining; and (**C**) Strong staining. Original magnification, x200.

Abbreviations: ZHX3, zinc-fingers and homeoboxes 3; ESCC, esophageal squamous cell carcinoma.

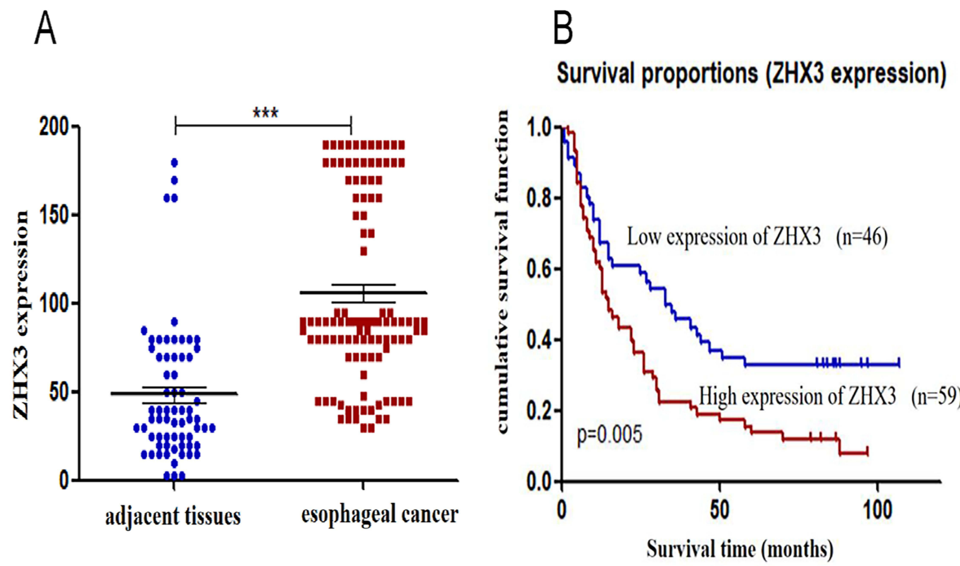

Figure 9 Kaplan-Meier curves comparing the OS rates in ESCC patients with high and low ZHX3 protein expression. (**A**) Comparison of ZHX3 protein expression levels in ESCC tissues (n=105) and normal tissues (n=66). ***P<0.001. (**B**) High ZHX3 expression was significantly correlated with an unfavorable OS in ESCC patients (P=0.005).

Abbreviations: ZHX, zinc-fingers and homeoboxes; ESCC, esophageal squamous cell carcinoma; OS, overall survival.

of ZHX2 may alter chromatin looping to drive promote cancer metastasis.[36,37] These data suggest that ZHX2 may have different functions in different types of cancer.

No significant association was observed between ZHX3 mRNA expression and OS in patients with EC or in its subtypes (EC and ESCC). However, increased ZHX3 expression was found to be correlated with favorable OS in EC patients with Stage III tumors. These results suggest high ZHX3 mRNA level may be, at least in part, associated with tumor advancement and progression. Because ESCC is the most common subtype of EC in China, we performed immunohistochemistry to examine ZHX3 protein expression and its association with clinicopathologic variables and survival outcomes. Low ZHX3 expression was found to be associated with higher histological grade and might serve as an independent prognostic factor for prognostic prediction for ESCC. These findings are consistent with the oncogenic function of ZHX3 in gastric cancer and bladder urothelial carcinoma,[20,38] but contradictory to our previous reports in breast and liver cancer.[30,39]

Table 1 Correlation Between ZHX3 Expression and Clinicopathological Characteristics in ESCC Patients

Parameters	No.of Patients	ZHX3 Expression		χ^2	P value
		Low, n (%)	High, n (%)		
Age (years)					
<65	43	20 (46.5)	23 (53.5)	0.239	0.625
≥65	60	25 (41.7)	35 (58.3)		
NA	2				
Sex					
Female	25	7 (28.0)	18 (72.0)	1.486	0.223
Male	80	44 (55.0)	36 (45.0)		
Tumor size					
≤5cm	65	29 (44.6)	36 (55.4)	0.063	0.801
>5cm	36	17 (47.2)	19 (52.8)		
NA	4				
Histological grade					
I/II	90	43 (47.8)	47 (52.2)	4.030	0.045
III/IV	15	3 (20.0)	12 (80.0)		
T classification					
T1/T2	20	10 (50.0)	10 (50.0)	0.349	0.555
T3/T4	82	35 (42.7)	47 (57.3)		
NA	3				
N classification					
N0	49	25 (51.0)	24 (49.0)	1.732	0.188
N1-N3	55	21 (38.2)	34 (61.8)		
NA	1				
TNM stage					
I/II	49	25 (51.0)	24 (49.0)	2.042	0.153
III/IV	54	20 (37.0)	34 (63.0)		
NA	2				

Abbreviation: NA, not available.

Table 2 Univariate and Multivariate Analyses of Factors Correlated with Overall Survival in ESCC Patients

Parameters	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
ZHX3 (high vs low)	1.859 (1.188–2.91)	0.007	2.141 (1.296–3.537)	0.003
Sex (male vs female)	2.766 (1.524–5.02)	0.001	2.033 (1.066–3.876)	0.031
Tumor size (>5cm vs ≤5cm)	1.661 (1.05–2.628)	0.03	1.801 (1.079–3.005)	0.024
Clinical stage (III/IV vs I/II)	3.028 (1.88–4.879)	<0.001	2.447 (0.792–7.558)	0.12
T stage (III/IV vs I/II)	2.495 (1.311–4.747)	0.005	1.768 (0.828–3.774)	0.141
N stage (N1-N3vs N0)	1.992 (1.275–3.11)	0.002	0.787 (0.282–2.197)	0.647

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The different prognostic effects and biological functions of ZHX factors in diverse cancer types appear to be contradictory.[40,41] One possible reason is that, through interacting with NF-YA and forming homodimers or heterodimers with each other, ZHX factors may function as positive or negative transcriptional regulators and exhibit opposite functions of oncogene or tumor suppressors in different malignancies.[40,41] We inferred that different specimen sources, histological phenotypes, tumor micro-environment and intrinsic properties in various cancer type may be enough to explain this discrepancy. Of note is that, as important transcriptional regulators for major signaling pathways involved in physiology and pathology, ZHX factor have been identified to be modulated by multiple layers in cancer development. For example, ZHX3 has been characterized as a downstream target of RNF26/TRIM21 pathway in bladder cancer cells.[39] Similarly, ZHX1 has been observed to be directly regulated by MiR-199a-3p and miR-23b-3p in gastric cancer and glioma cells,[42,43] and ZHX2 is modified by METTL3 and IGF2BP1-mediated m6A to promote renal cell carcinoma progression.[44] The above findings may also be crucial to clarify their modulation of oncogenic and tumor-suppressive pathways across various cancers.

There are several limitations to be addressed as regarding the current study. Because the current study performed integrative bioinformatics analyses using a set of online databases, certain search parameters were not available. The limited sample size and restricted follow-up period in the immunohistochemical analysis demands enlarged sample cohort with integral information in our future work. The biological functions and underlying molecular mechanisms of ZHX factors require further validation under well-controlled conditions in cell and/or animal models.

Conclusions

The present study systematically investigated the expression profiles and prognostic implications of the ZHX family members in EC. Our results indicated that ZHX factors could serve as novel biomarkers for predicting the prognosis of this malignancy.

Data Sharing Statement

The dataset used and/or analyzed in the current study is available from the corresponding authors upon reasonable request.

Ethics Approval and Consent to Participate

Signed informed consent was obtained from the patients prior to tissue sample collection. The clinical research protocol of this study conformed to the ethical guidelines outlined in the Declaration of Helsinki, and was reviewed and approved by the Ethics Committee of People's Hospital of Ningxia Hui Autonomous Region.

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Disclosure

The authors report no conflicts of interest in this work.

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