Characterization of ZIC5 expression in esophageal squamous cell carcinoma and its association with patient survival

BO YANG[1*], BAIGE WANG[1*], HAIQUAN DENG[1], YONG LUO[1], SHENGHAO WU[1], HUAXIN YIN[1], HONGDIAN ZHANG[2], YUNCHENG ZHANG[3] and ZHEN ZHANG[1]

[1]Department of Cardiothoracic Surgery, The First People's Hospital of Wanzhou District, Chongqing 404100, P.R. China; [2]Department of Esophageal Cancer, Tianjin Medical University Cancer Institute and Hospital, Tianjin 300060, P.R. China; [3]Department of Thoracic Surgery, Jiangsu Provincial People's Hospital Chongqing Hospital, Chongqing 404100, P.R. China

Received December 17, 2025; Accepted July 23, 2026

DOI: 10.3892/ol.2026.15834

Abstract. Esophageal squamous cell carcinoma (ESCC) is a prevalent malignancy known for its aggressive nature and poor prognosis. The present study aimed to investigate the expression levels and clinical importance of the Zic family member 5 (ZIC5) gene in ESCC. Gene expression data and survival information obtained from The Cancer Genome Atlas and Gene Expression Omnibus were utilized. In 176 patients with surgically resected ESCC, immunohistochemical analysis was conducted to validate the expression of ZIC5 protein in cancerous and adjacent tissues. The findings of the present study revealed a significant upregulation of ZIC5 in ESCC compared with normal tissues (P<0.05), which was further corroborated by immunohistochemistry exhibiting a notable association between ZIC5 expression and clinical parameters such as tumor size, invasion depth, lymph node metastasis and TNM staging (P<0.05). Survival analysis further indicated that high ZIC5 expression was an independent prognostic factor for poor outcomes in patients with ESCC (hazard ratio=1.519; 95% CI: 1.017-2.269; P<0.05). In addition, bioinformatic analyses predicted that hsa-microRNA-212-5p may regulate ZIC5 mRNA and gene enrichment analysis suggested that ZIC5 may facilitate ESCC progression through involvement in the cell cycle and DNA repair pathways. In conclusion, ZIC5 is highly expressed in ESCC and associated with a poor prognosis, indicating its potential as a therapeutic target and biomarker for ESCC management. Further studies are warranted to elucidate the precise mechanisms underlying the role of ZIC5 in ESCC progression.

Correspondence to: Professor Zhen Zhang, Department of Cardiothoracic Surgery, The First People's Hospital of Wanzhou District, 388 Tiancheng East Road, Wanzhou, Chongqing 404100, P.R. China
E-mail: zhangzhwz@163.com

Professor Yuncheng Zhang, Department of Thoracic Surgery, Jiangsu Provincial People's Hospital Chongqing Hospital, 54 Tuowan Branch Road, Gunan Street, Qijiang, Chongqing 404100, P.R. China
E-mail: 513725689@qq.com

*Contributed equally

Abbreviations: ESCC, esophageal squamous cell carcinoma; ZIC5, Zic family member 5; TCGA, The Cancer Genome Atlas; GEO, Gene Expression Omnibus; OS, overall survival; GSEA, Gene Set Enrichment Analysis; KEGG, Kyoto Encyclopedia of Genes and Genomes

Key words: ESCC, ZIC5, prognosis, transcriptional regulation, pathway

Introduction

Esophageal cancer is the most common malignant tumor of the digestive system. Globally, the number of new cases of esophageal cancer ranks 7th and 13th among malignant tumors in male and female patients, respectively. China accounts for 53.7 and 55.3% of the global incidence and mortality, rates respectively (1). The mortality rate of esophageal cancer in China is second only to gastric cancer among malignant tumors of the digestive system. There are two main histological types of esophageal cancer: Esophageal squamous cell carcinoma (ESCC), which is the predominant type (accounting for ~90% of cases) present in China, and esophageal adenocarcinoma. Despite the effectiveness of surgical treatment, radiotherapy, chemotherapy and immunotherapy for esophageal cancer having improved, the majority of patients are diagnosed at an advanced stage. In addition, the high rates of postoperative recurrence and metastasis contribute to poor prognosis, with a 5-year overall survival rate of ~25% (2). Therefore, identifying specific molecular markers and investigating their molecular mechanisms in the occurrence and progression of esophageal cancer are of great importance for improving diagnosis, treatment outcomes and long-term survival rates.

Zic family members (ZIC) are transcriptional regulatory factors containing highly conserved C2H2 zinc finger motifs. A total of five ZIC genes (ZIC1-5) have been identified and studied during embryonic development in vertebrates (3,4). These identified genes are vertebrate homologs of the *Drosophila* odd-paired genes, structurally similar to each other and partially functionally identical (5). As a member of the Zic family, ZIC5 has been reported to be involved

in the malignant progression of numerous types of human cancer, such as cervical cancer (6), prostate cancer (7), melanoma (8), liver cancer (9), colon cancer (10) and gastric cancer (11). However, there have been a limited number of studies regarding the expression and underlying mechanism of ZIC5 in ESCC.

Therefore, the present study used bioinformatics methods to analyze the expression levels of ZIC5 in ESCC and normal esophageal tissue, as well as analyze its association with the survival of patients with ESCC. The possible functional mechanism underlying this was also explored. Immunohistochemistry was used to determine the expression of ZIC5 protein in ESCC and further analysis was conducted regarding its association with clinical characteristics and prognosis. The present findings therefore provide a theoretical basis for the diagnosis and prognosis of ESCC and lay the foundation for improving the survival rate of patients.

Materials and methods

Data collection and analysis. Firstly, the expression levels of the ZIC5 gene in various pan-cancer samples from The Cancer Genome Atlas (TCGA; portal.gdc.cancer.gov/) on the University of California, Santa Cruz Xena platform (xenabrowser.net) were extracted. An unpaired Student's t-test was used to compare the expression of the ZIC5 gene between tumor and normal tissues in various cancer types and box plots were drawn to observe the differential expression of the ZIC5 gene in different cancer types. To observe the expression of the ZIC5 gene in ESCC samples, samples with the pathological type of ESCC from TCGA esophageal cancer dataset (96 squamous cell carcinomas and 11 adjacent tissues) were extracted. Box plots were plotted showing the expression of the ZIC5 gene in ESCC and normal tissues. The GSE53625 dataset for ESCC was downloaded from National Center for Biotechnology Information Gene Expression Omnibus (GEO; ncbi.nlm.nih.gov/geo), which contains gene expression profiles of cancer tissues and paired adjacent tissues from 179 ESCC samples (12). Further box plots were plotted comparing the expression of the ZIC5 gene between ESCC and control groups in GSE53625. A paired Student's t-test was used to calculate the significance P-value.

Prognostic value analysis of ZIC5 in ESCC. Overall survival (OS) of the samples in the TCGA-ESCC and GSE53625 datasets and the expression values of the ZIC5 gene in the corresponding samples were extracted. Survival analysis was performed using the R 'survival' package (version 3.8-9, CRAN.R-project.org/package=survival) on TCGA-ESCC and GSE53625 ESCC samples. A log-rank $P<0.05$ was considered to indicate a statistically significant difference and survival curves were subsequently plotted using the same R package.

Transcriptional regulation analysis of ZIC5. miRWalk (version 3.0; http://mirwalk.umm.uni-heidelberg.de) was used to predict microRNA (miRNA/miR) binding sites on ZIC5. Default parameters were applied, specifically a binding probability ≥0.95 and binding site localization within the 3'-untranslated region, to identify miRNA-mRNA interactions. The online tool miRNA Target Prediction Database (miRDB; https://mirdb.org/) was used for miRNA and target gene prediction, selecting an miRDB score ≥50 as the threshold. Similarly, the TargetScan 8.0 database (https://www.targetscan.org/vert_80/) was accessed for miRNA-target gene prediction, applying a total context++ score <0 as the cut-off criterion. At least two of the three databases were identified as having miRNAs predicted to target ZIC5 as potential regulators of ZIC5 expression.

Analysis of downstream pathways regulated by ZIC5 gene. Molecular Signatures Database (version 7.1; http://software.broadinstitute.org/gsea/msigdb/index.jsp) was used for pathway analysis. Kyoto Encyclopedia of Genes and Genomes (KEGG; genome.jp/kegg/) pathway gene sets, specifically the 'c2.cp.kegg.v7.4.symbols.gmt' was used as the enrichment background. Based on the expression values of all genes in all tumor samples of TCGA-ESCC and GSE53625, using the Pearson correlation coefficient ranking between each gene and ZIC5, Gene Set Enrichment Analysis (GSEA) was conducted using local GSEA software (version 4.3.1, Broad Institute; https://www.gsea-msigdb.org/gsea/index.jsp) to identify the positive and negative pathways associated with ZIC5 expression. Significant enrichment was defined as normalized enrichment score >1.5 with enrichment $P<0.05$. Subsequently, the 'ggplot2' package (version 4.0.3, ggplot2.tidyverse.org/) within R software was used to construct a GSEA enrichment analysis bubble plot.

Clinical data collection and follow-up. A total of 20 paired fresh ESCC and adjacent non-cancerous tissue specimens from patients presented at Tianjin Medical University Cancer Institute and Hospital (Tianjin, China) from January 2025 to June 2025 were collected prospectively for western blotting analysis. Among the 20 patients, 16 were male and four were female, with a median age of 61 years (range: 55-74 years). Furthermore, 176 specimens of ESCC and normal esophageal tissue >5 cm away from the cancer periphery were collected from patients that underwent surgical treatment in Tianjin Medical University Cancer Institute and Hospital from January 2009 to December 2010 for immunohistochemistry analysis. The inclusion criteria were as follows: i) Histological diagnosis of ESCC; ii) no history of other malignant tumors and; iii) specimens had not received radiotherapy, chemotherapy or immunotherapy before surgery. The exclusion criteria were as follows: i) Specimens with a histological diagnosis other than ESCC or with an uncertain diagnosis; ii) cases with incomplete clinical or follow-up data; and iii) specimens with poor quality or adjacent non-cancerous tissues located less than 5 cm from the tumor margin. Among the 176 patients, 144 were male and 32 were female, with a median age of 68 years (range, 39-85 years). Clinical data of patients were collected and followed up on patient recurrence and survival status via telephone. The samples were accessed from the institutional biobank between January 2023 and March 2023. The collection of both the 20 paired samples and the 176 tissue specimens in the present study was approved by the Tianjin Medical University Cancer Institute and Hospital ethics committee (approval no. bc2021340) and written informed consent was obtained from all patients.

Western blotting. Total protein was extracted from ESCC tumor tissue using RIPA lysis buffer (Beyotime

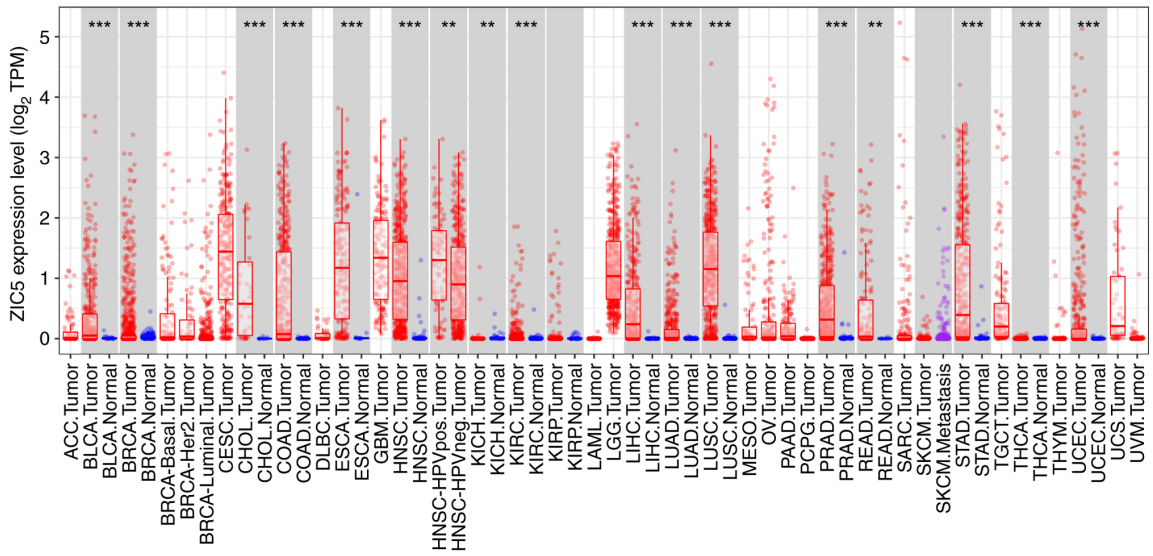

Figure 1. Expression of ZIC5 gene in pan-cancer. The ZIC5 expression profile of tumor tissues and paired normal tissues analyzed through The Cancer Genome Atlas datasets. **P<0.01, ***P<0.001. ZIC5, Zic family member 5; TPM, transcripts per million.

Biotechnology) supplemented with protease inhibitor cocktail and 1 mM PMSF. Subsequently, a BCA protein assay kit (Pierce; Thermo Fisher Scientific, Inc.) was utilized for protein quantitation. Equal amounts of protein (20 μg per lane) were separated by 10% SDS-PAGE, transferred onto PVDF membranes and blocked with TBS-Tween containing 5% skimmed milk for 1 h at ambient temperature. Subsequently, the membrane was incubated with primary antibodies overnight at 4˚C, followed by incubation with secondary antibody for 2 h at room temperature. The primary antibodies were as following: ZIC5 (1:500; cat. no. bs-12147R; BIOSS), and GAPDH (1:5,000; cat. no. 10494-1-AP; Proteintech Group, Inc.). The secondary antibody was HRP-conjugated goat anti-rabbit IgG (1:1,000; cat. no. 7074S; Cell Signaling Technology, Inc.). Enhanced chemiluminescence reagent (Merck Millipore) was used to visualize the immunoreactive bands. ImageJ (version 1.53e; National Institutes of Health) was used to measure and analyze the optical density.

Immunohistochemical detection. ZIC5 protein levels were assessed through immunohistochemistry. Briefly, tissue samples were fixed in 10% neutral-buffered formalin for 24 h at room temperature and embedded in paraffin. Sections were cut at a thickness of 4 μm, deparaffinized in xylene and rehydrated through a descending ethanol series. Antigen retrieval was performed by heating the sections in 10 mM sodium citrate buffer at 95-100˚C for 15 min. Endogenous peroxidase activity was quenched using 0.3% hydrogen peroxide and the sections were blocked in 5% goat serum (Vector Laboratories) at room temperature for 1 h. Sections were incubated overnight at 4˚C with an anti-ZIC5 polyclonal antibody (1:100; cat. no. bs-12147R; BIOSS), then exposed to a biotinylated goat anti-rabbit IgG secondary antibody (cat. no. BA-1000; Vector Laboratories; 1:200) for 20 min at room temperature. Diaminobenzidine served as the chromogenic substrate and slides were initially examined under a light microscope (Olympus BX53, Japan) and digitized using an automated slide scanning system.

Results were independently evaluated in a double-blind manner by two experienced pathologists who were blinded to the clinicopathological information. Immunohistochemistry scoring was performed as previously described (13). Staining intensity was graded as 0 (negative), 1 (weak, light yellow), 2 (moderate, yellow brown) or 3 (strong, brown). The extent of staining was scored as follows: 0, <10%; 1, 10-25%; 2, 26-50%; 3, 51-75%; and 4, >75%. The immunoreactivity score was calculated by multiplying the intensity score by the extent score. Patients with a total score <4 were considered to have low expression, and those with a total score of ≥4 were considered to have high expression.

Statistical analysis. Statistical analysis was performed using SPSS (version 25.0; IBM Corp.) and R software (version 4.2.1; R Foundation for Statistical Computing). The 'limma' package (v.3.21) was used to identify differentially expressed genes between ZIC5-high and ZIC5-low groups, and the resulting gene ranking was subsequently used for GSEA. Categorical data are presented as numbers and percentages. Parametric continuous data are presented as the mean ± SD). Independent sample Student's t-tests were used for comparisons between two groups, while multiple-group comparisons were performed using one-way ANOVA. The associations between ZIC5 expression and clinicopathological factors were assessed using the χ^2 test or Fisher's exact test. Survival rates were calculated using the Kaplan-Meier method and differences in survival curves were assessed using log-rank tests. Cox regression analysis was conducted in both univariate and multivariate models to determine independent factors affecting prognosis. All statistical tests were two-sided and P<0.05 was considered to indicate a statistically significant difference.

Results

Expression of ZIC5 gene in ESCC. ZIC5 expression was significantly increased in head and neck squamous cell carcinoma, breast cancer, esophageal cancer, gastric cancer and bladder cancer, amongst others (Fig. 1). To examine ZIC5 expression in

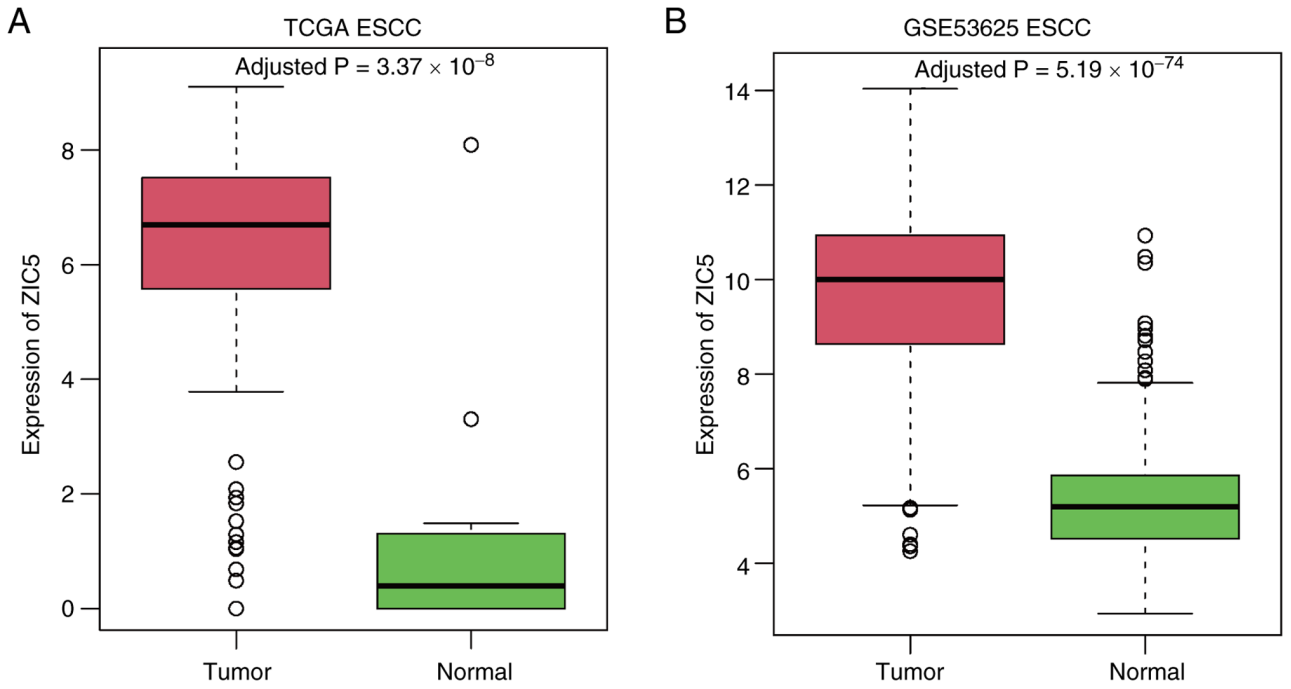

Figure 2. Expression of ZIC5 mRNA in ESCC. Assessment and quantification of ZIC5 gene expression in ESCC primary tumor and normal tissues from the (A) TCGA-ESCC database and (B) GSE53625-ESCC dataset. ESCC, esophageal squamous cell carcinoma; ZIC5, Zic family member 5; TCGA, The Cancer Genome Atlas.

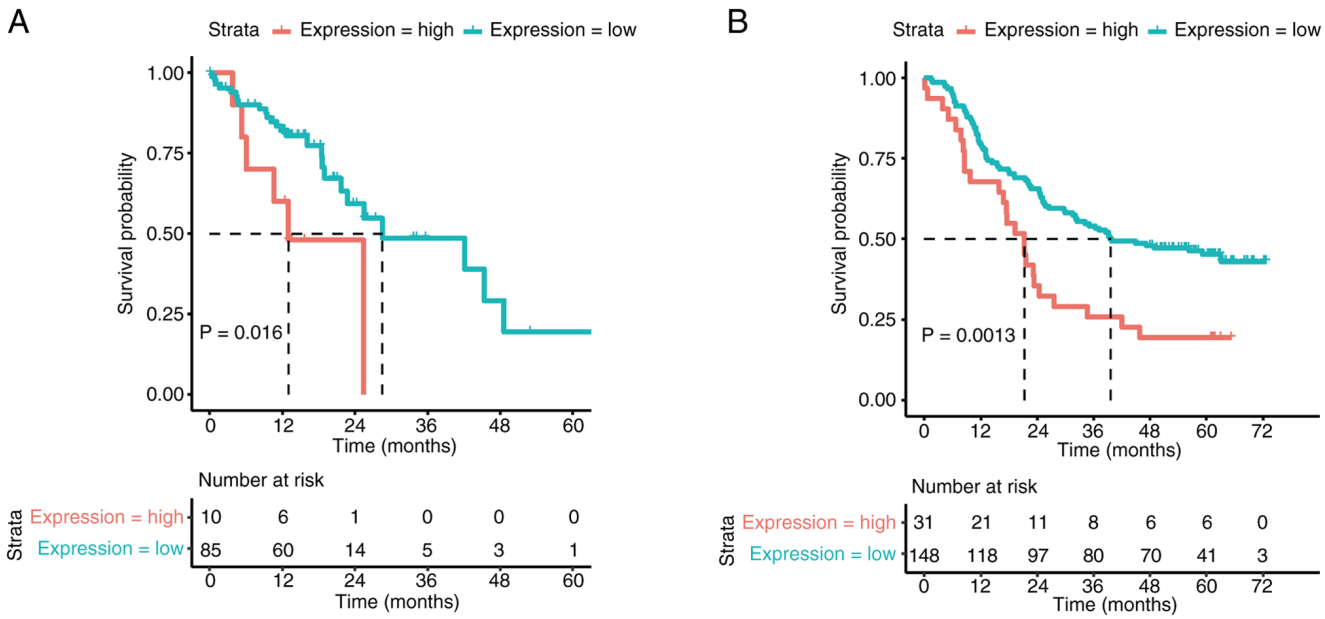

Figure 3. Impact of ZIC5 expression on the prognosis of patients with ESCC. Survival analysis of patients with ESCC with high or low ZIC5 levels, determined using data from (A) The Cancer Genome Atlas-ESCC (P=0.016) and (B) GSE53625-ESCC (P=0.0013) datasets. ESCC, esophageal squamous cell carcinoma; ZIC5, Zic family member 5.

ESCC, box plots of ZIC5 expression were generated based on the TCGA-ESCC and GSE53625-ESCC datasets to compare ESCC samples with controls. ZIC5 expression was significantly upregulated in ESCC samples when compared with the control group, with statistically significant differences (Fig. 2).

Impact of ZIC5 mRNA on the prognosis of ESCC. Survival analysis was performed on TCGA-ESCC and GSE53625-ESCC samples using the optimal cut-off value determined by X-tile for grouping. As shown in Fig. 3, ZIC5 expression was associated with the prognosis of patients with ESCC. Patients with high ZIC5 expression exhibited significantly shorter survival times when compared with those with low expression.

Transcriptional regulation analysis of ZIC5. As aforementioned, miRNA target prediction for ZIC5 was performed.

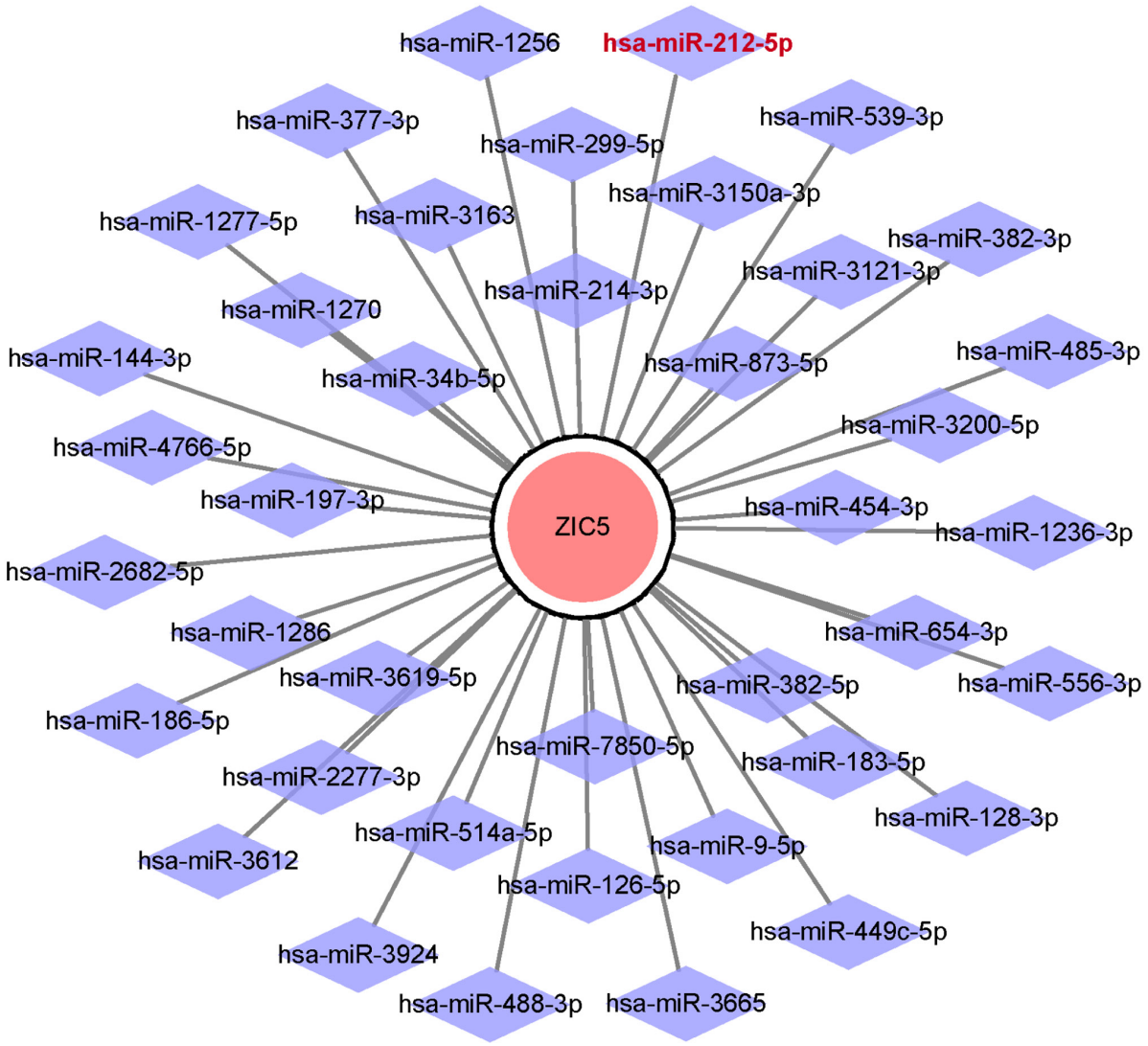

Figure 4. miRNAs that target and regulate the expression of the ZIC5 gene. ZIC5, Zic family member 5; miRNA/miR, micro-RNA.

The miRNAs predicted by at least two of the three databases were identified, as summarized in Table SI. Analysis revealed that 40 miRNAs potentially regulate ZIC5, among which hsa-miR-212-5p was predicted by all three databases (Fig. 4).

Analysis of downstream pathways regulated by ZIC5. KEGG pathway enrichment analysis for ZIC5 was conducted by GSEA on TCGA-ESCC and GSE53625-ESCC datasets. From each dataset, the top pathways exhibiting positive and negative association with ZIC5 were selected and visualized as a bubble plot (Fig. 5).

Enrichment score profiles revealed that KEGG_CELL_CYCLE and KEGG_NUCLEOTIDE_EXCISION_REPAIR were consistently enriched in ZIC5-high tumors in both datasets (Fig. 6). In both cohorts, the maximum enrichment was reached near the leading edge of the ranked gene list, indicating that the enrichment was driven by a coordinated set of core pathway genes rather than by a few outliers. Functionally, the enrichment of the cell-cycle pathway suggests that ZIC5 may promote tumor proliferation by accelerating cell division, whereas the enrichment of the nucleotide excision repair pathway implies an enhanced DNA damage repair capacity that helps maintain genomic stability and evade apoptosis.

Expression of ZIC5 protein in ESCC tissue and its association with prognosis. First, the significant upregulation of ZIC5 in 20 paired fresh ESCC tissues compared with adjacent paracancerous tissues was validated through western blotting (Fig. 7A). To further explore the expression of ZIC5 protein in ESCC tissues and its association with clinicopathological characteristics and prognosis, 176 treatment-naïve ESCC tissue specimens and normal esophageal tissue specimens were collected from the Tianjin Medical University Cancer Institute and Hospital, and ZIC5 protein expression was detected. Immunohistochemical results showed that ZIC5 protein expression was primarily localized in the nucleus of cancer cells, and the expression rate was significantly higher in ESCC tissues than in normal tissues (68.2% vs. 44.3%). Based on ZIC5 expression levels, all ESCC patients were divided into a low-expression group (n=56) and a high-expression group (n=120). Representative images are shown in Fig. 7B-E).

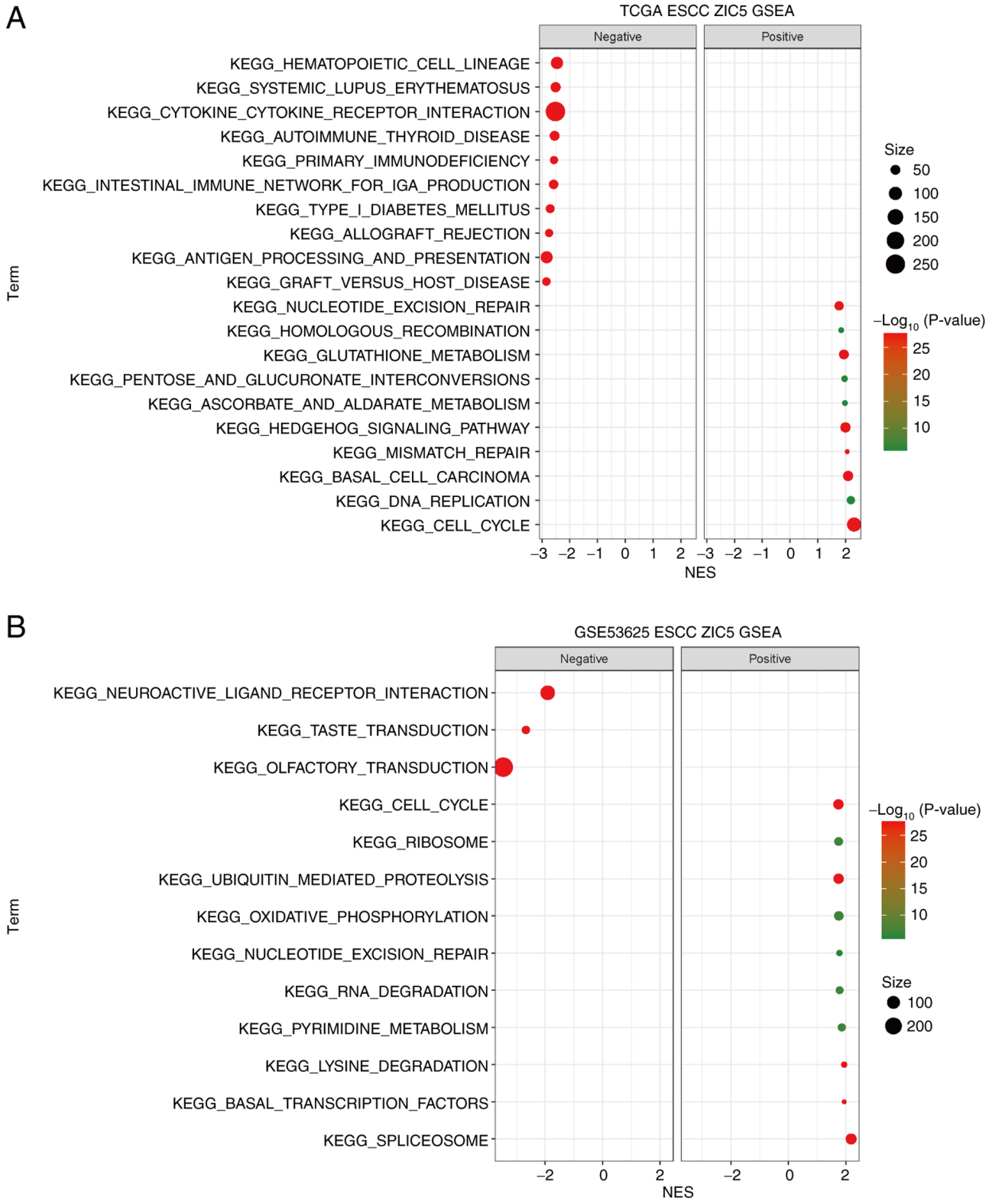

Figure 5. Bubble chart of GSEA. GSEA using the (A) TCGA-ESCC and (B) GSE53625-ESCC datasets. ESCC, esophageal squamous cell carcinoma. GSEA, Gene Set Enrichment Analysis; TCGA, The Cancer Genome Atlas; ZIC5, Zic family member 5; NES, normalized enrichment score.

The association between ZIC5 expression and different clinicopathological characteristics is shown in Table I. High ZIC5 expression was associated with tumor size, invasion depth, lymph node metastasis and TNM staging. By contrast, ZIC5 expression displayed no significant association with sex, age, smoking history, alcohol intake, tumor location or histological grade.

In addition, Kaplan-Meier survival analysis revealed that patients with high ZIC5 levels exhibited a significantly worse 5-year OS rate compared with those with low ZIC5 levels (27.5 vs. 42.7%; Fig. 8). Cox univariate analysis showed that age, tumor size, invasion depth, lymph node metastasis and ZIC5 protein expression were all associated with patient survival.

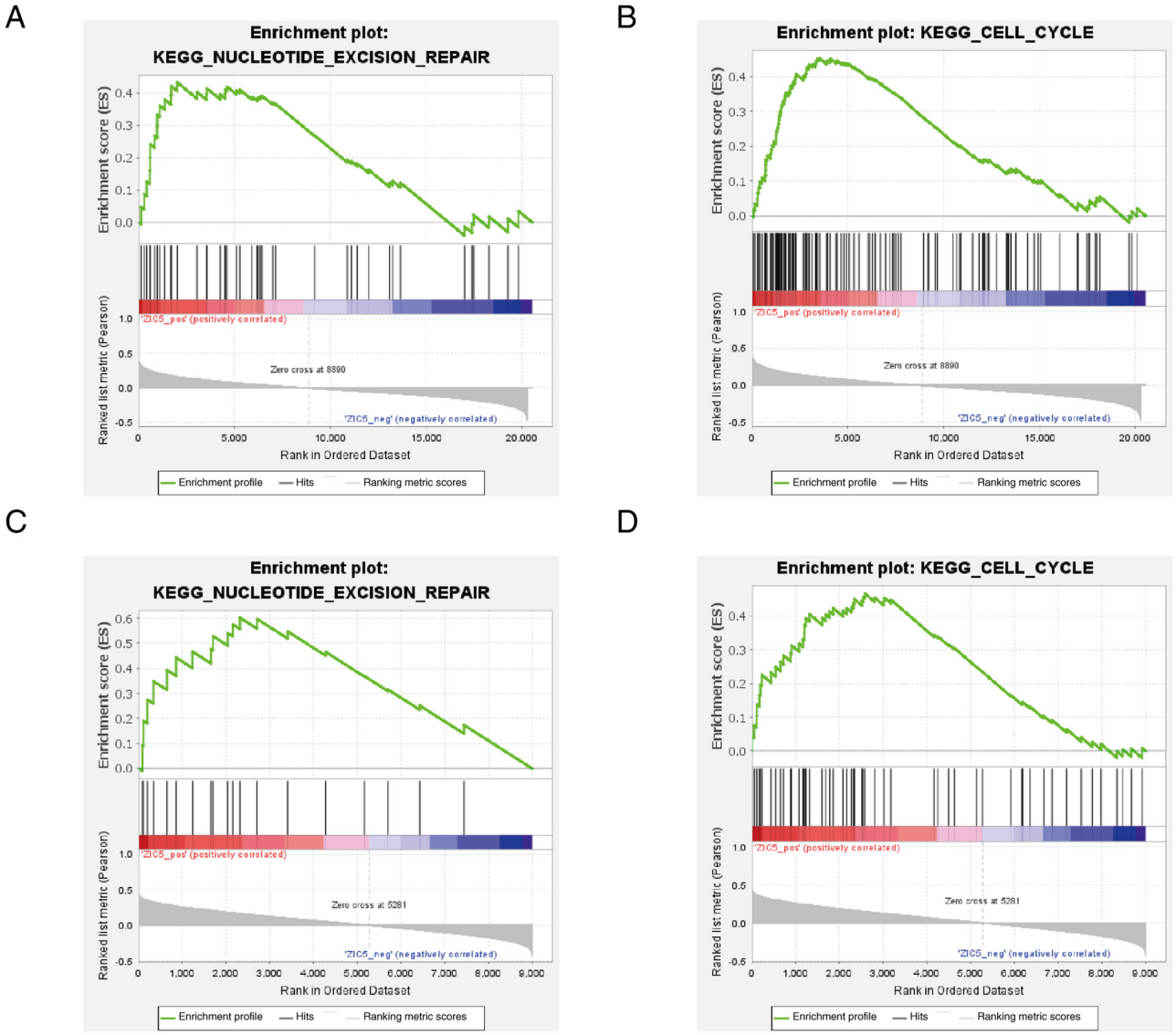

Figure 6. ZIC5 regulates identical downstream pathways in TCGA-ESCC and GSE53625 ESCC datasets. (A) Enrichment score profile of KEGG_CELL_CYCLE in ZIC5-high vs. ZIC5-low tumors in the TCGA-ESCC dataset. (B) Enrichment score profile of KEGG_NUCLEOTIDE_EXCISION_REPAIR in ZIC5-high vs. ZIC5-low tumors in the TCGA-ESCC dataset. (C) Enrichment score profile of KEGG_CELL_CYCLE in ZIC5-high vs. ZIC5-low tumors in the GSE53625 dataset. (D) Enrichment score profile of KEGG_NUCLEOTIDE_EXCISION_REPAIR in ZIC5-high vs. ZIC5-low tumors in the GSE53625 dataset. ESCC, esophageal squamous cell carcinoma; ZIC5, Zic family member 5; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Multivariate analysis indicated that age, lymph node metastasis and ZIC5 protein expression levels (hazard ratio=1.519; 95% CI: 1.017-2.269; Table II) were independent prognostic indicators affecting the survival of patients with ESCC.

Discussion

Esophageal cancer is a common malignant tumor of the upper gastrointestinal tract. Due to the atypical symptoms of early esophageal cancer, the majority of patients seek medical attention at middle to late stages, with a 5-year survival rate of only 15-25% (14-16). Therefore, identifying effective biomarkers is of great clinical importance for the early diagnosis of esophageal cancer. In the present study, the expression of the ZIC5 gene in pan-cancer was first analyzed, revealing that ZIC5 expression was significantly increased in head and neck squamous cell carcinoma, breast cancer, esophageal cancer, gastric cancer and bladder cancer. To investigate ZIC5 expression in ESCC, ZIC5 mRNA expression levels in TCGA and GEO databases were analyzed, revealing that ZIC5 was highly expressed in ESCC. In addition, elevated ZIC5 protein expression level was significantly associated with poor prognosis in patients with ESCC.

Members of the Zic family serve important roles in neural development and tumorigenesis. As a member of the cerebellar zinc finger family, ZIC5 has been reported as a transcription factor involved in regulating the expression of target genes (5). A number of studies have found that ZIC5 is highly expressed in certain malignant tumors, including lung cancer, prostate cancer and glioma (17-19). Zeng *et al* (20) found that ZIC5 is highly expressed in lung adenocarcinoma tissue. In A549 and H1299 cells, silencing ZIC5 suppressed its expression, inhibited cell proliferation and attenuated

Table I. Association between ZIC5 expression and clinical pathological characteristics in patients with esophageal squamous cell carcinoma.

		ZIC5 expression			
Variable	n	Low expression	High expression	χ^2	P-value
Total	176	56 (31.8%)	120 (68.2%)		
Sex				0.838	0.360
Male	144	48 (33.3%)	96 (66.7%)		
Female	32	8 (25.0%)	24 (75.0%)		
Age, years				2.728	0.099
≤68	82	21 (25.6%)	61 (74.4%)		
>68	94	35 (37.2%)	59 (62.8%)		
Smoking history				0.274	0.600
Yes	121	37 (30.6%)	84 (69.4%)		
No	55	19 (34.5%)	36 (65.5%)		
Alcohol intake				1.265	0.261
Yes	105	30 (28.6%)	75 (71.4%)		
No	71	26 (36.6%)	45 (63.4%)		
Tumor location				5.497	0.064
Upper	7	0 (0%)	7 (100.0%)		
Middle	148	49 (33.1%)	99 (66.9%)		
Lower	21	7 (33.3%)	14 (66.7%)		
Tumor size, cm				4.949	0.026[a]
<3.5	73	30 (41.1%)	43 (58.9%)		
≥3.5	103	26 (25.2%)	77 (74.8%)		
Histological grade				2.023	0.364
G1	9	3 (33.3%)	6 (66.7%)		
G2	131	45 (34.4%)	86 (65.6%)		
G3	36	8 (22.2%)	28 (77.8%)		
Invasion depth				6.706	0.035[a]
pT1-2	30	14 (46.7%)	16 (53.3%)		
pT3	95	32 (33.7%)	63 (66.3%)		
pT4	51	10 (19.6%)	41 (80.4%)		
Lymph node metastasis				5.315	0.021[a]
Yes	107	41 (38.3%)	66 (61.7%)		
No	69	15 (21.7%)	54 (78.3%)		
TNM staging				11.865	0.001[a]
I-II	68	32 (47.1%)	36 (52.9%)		
III	108	24 (22.2%)	84 (77.8%)		

[a]Statistically significant. ZIC5, Zic family member 5; G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated/undifferentiated; TNM, tumor-nodes metastasis.

metabolic activity as evidenced by reduced glucose uptake, lactate production and ATP levels. Associated research has also been conducted regarding ZIC5 in digestive system tumors. Satow *et al* (21) found that ZIC5 was upregulated in colon cancer and promoted the proliferation and primary drug resistance of colon cancer cells. The OS rate of patients with pancreatic cancer with high ZIC5 expression was markedly lower compared with that of patients with low ZIC5 expression. Targeting ZIC5 therefore enhances the efficacy of gemcitabine treatment by effectively reducing tumor volume in pancreatic cancer *in vivo* (22). ZIC5 can markedly inhibit the proliferation of liver cancer cells (9) and induce apoptosis of colon cancer, pancreatic cancer and cholangiocarcinoma cells after being silenced (21,23).

ZIC5 may serve a role in the occurrence and development of tumors through the following pathways: i) ZIC5 may act as a

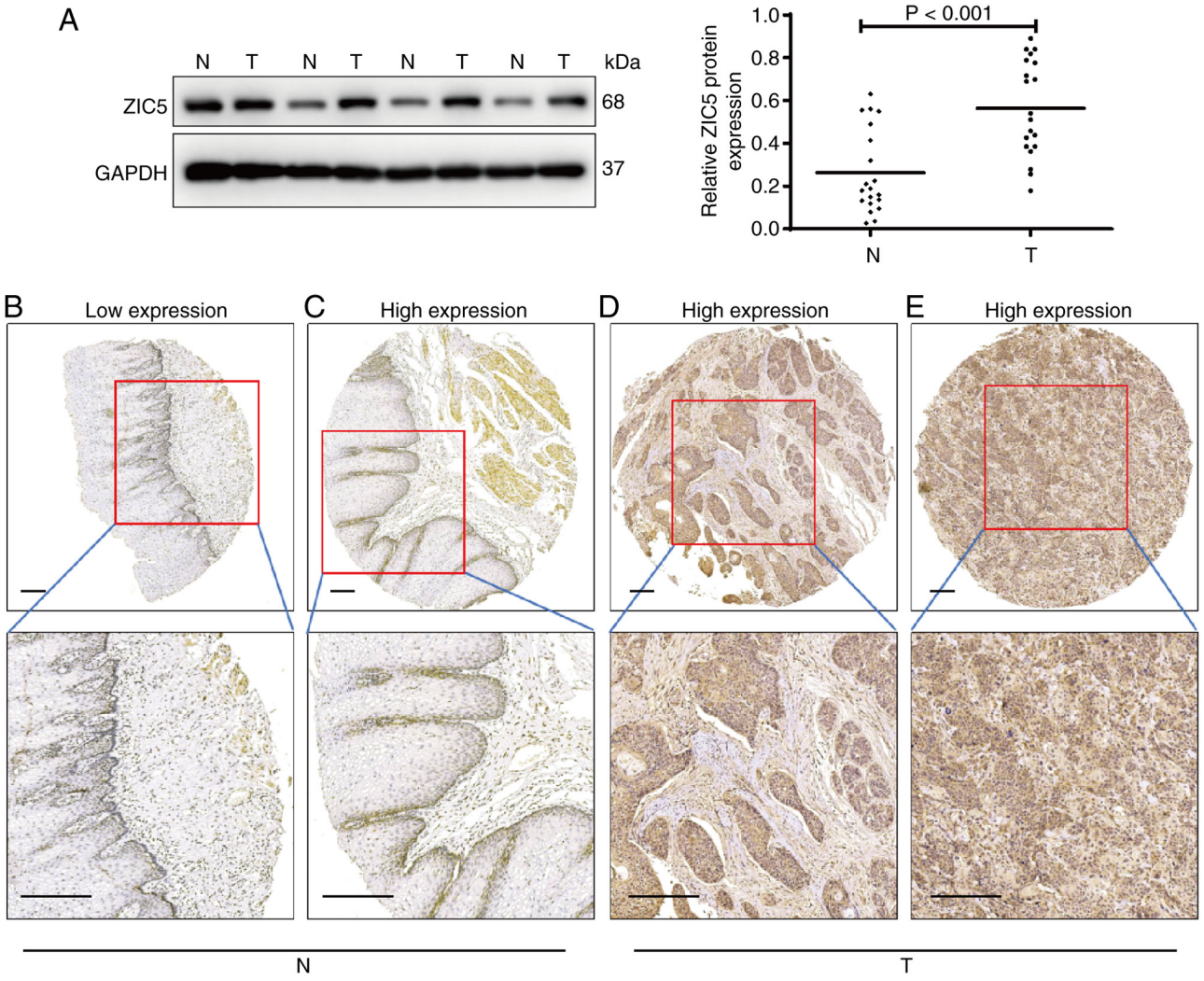

Figure 7. ZIC5 protein is highly expressed in ESCC. (A) The expression of ZIC5 protein in patients with ESCC assessed by western blotting (n=20). (B) Low ZIC5 expression in sections of non-neoplastic mucosa adjacent to tumors. (C) High ZIC5 expression in sections of non-neoplastic mucosa adjacent to tumors. (D and E) High ZIC5 expression in ESCC tissue. Scale bar, 100 μm. ESCC, esophageal squamous cell carcinoma; ZIC5, Zic family member 5; T, tumor; N, normal.

proto-oncogene to affect the expression of cyclin B1 and CDK1 complexes and participate in regulating cell proliferation (17); ii) ZIC5 promotes tumor cell proliferation and invasion by regulating the activity of certain miRNAs (19,24); iii) ZIC5 may promote tumor growth, invasion and migration by regulating the Wnt/β-catenin signaling pathway (10); iv) ZIC5 promotes malignant progression of tumor cells by regulating the CDK1/CDC25c signaling pathway (25). However, there are limited research reports on the expression and mechanism of action of ZIC5 in ESCC.

miRNA serves an important regulatory role in tumorigenesis. In recent years, numerous studies have found that miRNAs are abnormally expressed in certain types of malignant tumors and exert notable effects on cell proliferation, apoptosis, invasion and migration, amongst other processes. mRNAs, as targets of miRNAs, also serve important roles in tumor malignancy (26,27). The present study was based on analysis results from three databases. ZIC5 may be a target gene of hsa-miR-212-5p and also serves an important role in the malignant progression of ESCC. A number of studies have shown that hsa-miR-212-5p exhibits carcinogenic properties in different tumor types (28,29). Qu *et al* (30) found that circZFR

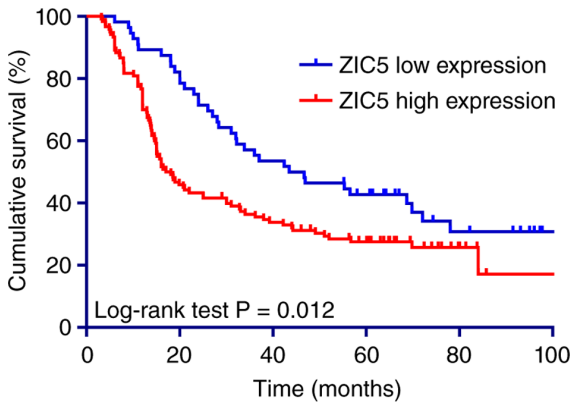

Figure 8. Survival curves of patients with high and low expression of ZIC5 in esophageal squamous cell carcinoma tissue. The 5-year survival rate of patients with high expression of ZIC5 was 27.5% and that for patients with low expression of ZIC5 was 42.7%. ZIC5, Zic family member 5.

regulates the activity of superoxide dismutase 2 by inhibiting hsa-miR-212-5p, thereby promoting the progression of ovarian cancer cells. In addition, further analysis was carried out of

Table II. Impact of ZIC5 and clinical pathological features on the prognosis of patients with esophageal squamous cell carcinoma.

	Univariate analysis			Multivariate analysis		
Variable	HR	95% CI	P-value	HR	95% CI	P-value
Sex (Female vs. Male)	0.966	0.767-1.217	0.772			
Age, years (>68 vs. ≤68)	1.459	1.022-2.083	0.038[a]	1.518	1.056-2.183	0.024[a]
Smoking history (Yes vs. No)	1.107	0.753-1.627	0.604			
Alcohol intake (Yes vs. No)	1.304	0.907-1.874	0.152			
Tumor location (Lower vs. Middle vs. Upper)	0.835	0.543-1.284	0.411			
Tumor size, cm (≥3.5 vs. <3.5)	1.497	1.040-2.156	0.030[a]	1.288	0.887-1.869	0.184
Histological grade (G3 vs. G2 vs. G1)	1.301	0.908-1.864	0.152			
Invasion depth (pT4 vs. pT3 vs. pT1-2)	1.438	1.100-1.880	0.008[a]	1.300	0.980-1.725	0.069
Lymph node metastasis (Yes vs. No)	2.049	1.438-2.919	0.000[a]	1.733	1.207-2.490	0.003[a]
ZIC5 expression (High vs. Low)	1.645	1.117-2.421	0.012[a]	1.519	1.017-2.269	0.041[a]

[a]Statistically significant. ZIC5, Zic family member 5; HR, hazard ratio.

the downstream pathways regulated by the ZIC5 gene through GSEA, with results showing that ZIC5 may promote the proliferation of ESCC cells by participating in the cell cycle and repair processes. However, these results need to be further determined through gain- and loss-of-function experiments, transcriptomic profiling and targeted molecular assays.

There are a number of limitations to the present study. Firstly, the present study was a single-institute study with a small sample size and there may be selection bias during patient and data collection. In addition, the present study did not incorporate functional experiments to validate the specific mechanisms by which ZIC5 influences ESCC progression. This lack of experimental validation may limit the robustness of the present conclusions regarding the role of ZIC5 in ESCC. Furthermore, the absence of multi-center validation may hinder the external applicability of the present results. Lastly, inherent differences between the datasets used (TCGA and GSE53625) could introduce batch effects, potentially impacting the consistency of the present findings across different cohorts.

In conclusion, bioinformatics was used to screen for differentially expressed ZIC5 associated with the prognosis of patients with ESCC through TCGA and GEO databases, with the expression of ZIC5 protein in ESCC tissues further verified through immunohistochemistry. The results showed that ZIC5 is highly expressed in ESCC tissues and is notably associated with poor prognosis in patients with ESCC. ZIC5 may be a target gene of hsa-miR-212-5p that regulates the malignant progression of ESCC cells. Further *in vivo* and *in vitro* experimental studies are needed to validate these findings. The present study highlighted that the ZIC5 gene has the potential to become a new prognostic marker for ESCC and may provide a reference for targeted therapy and prognosis of patients with ESCC.

Acknowledgements

Not applicable.

Funding

The present study was supported by the National Natural Science Foundation of China (grant no. 82002551).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

BY, BW, YZ and ZZ conceived and designed the present workflow. BY, BW, HZ and ZZ performed the experiments and analyzed the data. HD and YL conducted the bioinformatics analysis. SW and HY contributed to the histological analysis. BY and BW wrote the manuscript. BW, YZ and ZZ revised the manuscript. All authors read and approved the final manuscript. YZ and ZZ confirm the authenticity of all the raw data.

Ethics approval and consent to participate

All methods were performed in accordance with the relevant ethical regulations and were conducted under approval from the Research Ethics Committee of Tianjin Medical University Cancer Institute and Hospital (Tianjin, China; approval no. bc2021340). Written informed consent was acquired from all patients of the primary cohort for the acquisition of clinical and pathological information and the use of surgical specimens.

Patient consent for publication

The patients provided written informed consent for publication of their data.

Competing interests

The authors declare that they have no competing interests.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71: 209-249, 2021.
2. Meng P, Gu T, Xu J, Huang H, Jin H, Wang Y, Zhang H and Ruan Z: Pretreatment CALLY index as promising novel biomarker in the prediction of surgical and oncological outcomes in esophageal cancer: A multi-center retrospective cohort study. Front Immunol 16: 1605067, 2025.
3. Grinberg I and Millen KJ: The ZIC gene family in development and disease. Clin Genet 67: 290-296, 2005.
4. Houtmeyers R, Souopgui J, Tejpar S and Arkell R: The ZIC gene family encodes multi-functional proteins essential for patterning and morphogenesis. Cell Mol Life Sci 70: 3791-3811, 2013.
5. Ali RG, Bellchambers HM and Arkell RM: Zinc fingers of the cerebellum (Zic): Transcription factors and co-factors. Int J Biochem Cell Biol 44: 2065-2068, 2012.
6. Jia Q, Song J, Xu T, Liu J, Chai J, Yang Y, Li L, Li M and Yang X: ZIC5 promotes aggressiveness and cancer stemness in cervical squamous cell carcinoma. Pathol Res Pract 241: 154268, 2023.
7. Tan YF, Zhang Y, Ge SY, Zhong F, Sun CY and Xia GW: AR-regulated ZIC5 contributes to the aggressiveness of prostate cancer. Cell Death Discov 8: 393, 2022.
8. Satow R, Nakamura T, Kato C, Endo M, Tamura M, Batori R, Tomura S, Murayama Y and Fukami K: ZIC5 drives melanoma aggressiveness by PDGFD-mediated activation of FAK and STAT3. Cancer Res 77: 366-377, 2017.
9. Liu L, Hu X, Sun D, Wu Y and Zhao Z: ZIC5 facilitates the growth of hepatocellular carcinoma through activating Wnt/β-catenin pathway. Biochem Biophys Res Commun 503: 2173-2179, 2018.
10. Zhao Z, Wang L, Bartom E, Marshall S, Rendleman E, Ryan C, Shilati A, Savas J, Chandel N and Shilatifard A: β-Catenin/Tcf7l2-dependent transcriptional regulation of GLUT1 gene expression by Zic family proteins in colon cancer. Sci Adv 5: eaax0698, 2019.
11. Ma G, Dai W, Sang A, Yang X and Li Q: Roles of ZIC family genes in human gastric cancer. Int J Mol Med 38: 259-266, 2016.
12. Li J, Chen Z, Tian L, Zhou C, He MY, Gao Y, Wang S, Zhou F, Shi S, Feng X, *et al*: LncRNA profile study reveals a three-lncRNA signature associated with the survival of patients with oesophageal squamous cell carcinoma. Gut 63: 1700-1710, 2014.
13. Zhang H, Han Y, Xiao W, Gao Y, Sui Z, Ren P, Meng F, Tang P and Yu Z: USP4 promotes the proliferation, migration, and invasion of esophageal squamous cell carcinoma by targeting TAK1. Cell Death Dis 14: 730, 2023.
14. He S, Xu J, Liu X and Zhen Y: Advances and challenges in the treatment of esophageal cancer. Acta Pharm Sin B 11: 3379-3392, 2021.
15. Yang J, Liu X, Cao S, Dong X, Rao S and Cai K: Understanding esophageal cancer: The challenges and opportunities for the next decade. Front Oncol 10: 1727, 2020.
16. Domper Arnal MJ, Ferrández Arenas Á and Lanas Arbeloa Á: Esophageal cancer: Risk factors, screening and endoscopic treatment in Western and Eastern countries. World J Gastroenterol 21: 7933-7943, 2015.
17. Sun Q, Shi R, Wang X, Li D, Wu H and Ren B: Overexpression of ZIC5 promotes proliferation in non-small cell lung cancer. Biochem Biophys Res Commun 479: 502-509, 2016.
18. Wang ZY, Duan Y and Wang P: SP1-mediated upregulation of lncRNA SNHG4 functions as a ceRNA for miR-377 to facilitate prostate cancer progression through regulation of ZIC5. J Cell Physiol 235: 3916-3927, 2020.
19. Li GF, Li L, Yao ZQ and Zhuang SJ: Hsa_circ_0007534/miR-761/ZIC5 regulatory loop modulates the proliferation and migration of glioma cells. Biochem Biophys Res Commun 499: 765-771, 2018.
20. Zeng C, Huang D, Wang L, Liang H and Ma X: Silencing ZIC5 suppresses glycolysis and promotes disulfidptosis in lung adenocarcinoma cells. Cancer Biol Ther 26: 2501780, 2025.
21. Satow R, Inagaki S, Kato C, Shimozawa M and Fukami K: Identification of zinc finger protein of the cerebellum 5 as a survival factor of prostate and colorectal cancer cells. Cancer Sci 108: 2405-2412, 2017.
22. Satow R, Kashiwaba Y, Okao M, Takano S, Aiga Y, Yoneda A, Hosomichi K and Fukami K: Zic family member 5 promotes RIO kinase 3 expression to enhance pancreatic cancer survival. FEBS J 292: 4057-4068, 2025.
23. Satow R, Aiga Y, Watanabe T, Ishizuka N, Yoneda A and Fukami K: Zic family member 5 promotes survival in human pancreatic cancer and cholangiocarcinoma cells. Biochem Biophys Rep 31: 101289, 2022.
24. Yang X, Wu P, Wang Z, Su X, Wu Z, Ma X, Wu F and Zhang D: Constructed the ceRNA network and predicted a FEZF1-AS1/miR-92b-3p/ZIC5 axis in colon cancer. Mol Cell Biochem 478: 1083-1097, 2023.
25. Maimaiti A, Aizezi A, Anniwaer J, Ayitula, Ali B and Dilixiati M: Zinc finger of the cerebellum 5 promotes colorectal cancer cell proliferation and cell cycle progression through enhanced CDK1/CDC25c signaling. Arch Med Sci 17: 449-461, 2019.
26. Fu Z, Zhou Y, Zhang Y, Zhou Z, Yu Y, Yuan C, Dong J and Duan S: MicroRNA-325: A comprehensive exploration of its multifaceted roles in cancer pathogenesis and therapeutic implications (Review). Oncol Lett 28: 459, 2024.
27. Zhang N, Chi M, Pan W, Zhang C, Wang Y, Gao X, Bai C and Liu X: miR-576-3p/M-phase phosphoprotein 8 axis regulates the malignant progression of hepatocellular carcinoma cells via the PI3K/Akt signaling pathway. Oncol Lett 28: 327, 2024.
28. Chen FF, Sun N, Wang Y, Xi HY, Yang Y, Yu BZ and Li XJ: miR-212-5p exerts tumor promoter function by regulating the Id3/PI3K/Akt axis in lung adenocarcinoma cells. J Cell Physiol 235: 7273-7282, 2020.
29. Yuan M, Zhang X, Yue F, Zhang F, Jiang S, Zhou X, Lv J, Zhang Z, Sun Y, Chen Z, *et al*: CircNOLC1 promotes colorectal cancer liver metastasis by interacting with AZGP1 and sponging miR-212-5p to regulate reprogramming of the oxidative pentose phosphate pathway. Adv Sci (Weinh) 10: e2205229, 2023.
30. Qu D, Zou X and Liu Z: Propofol modulates glycolysis reprogramming of ovarian tumor via restraining circular RNA-zinc finger RNA-binding protein/microRNA-212-5p/superoxide dismutase 2 axis. Bioengineered 13: 11881-11892, 2022.